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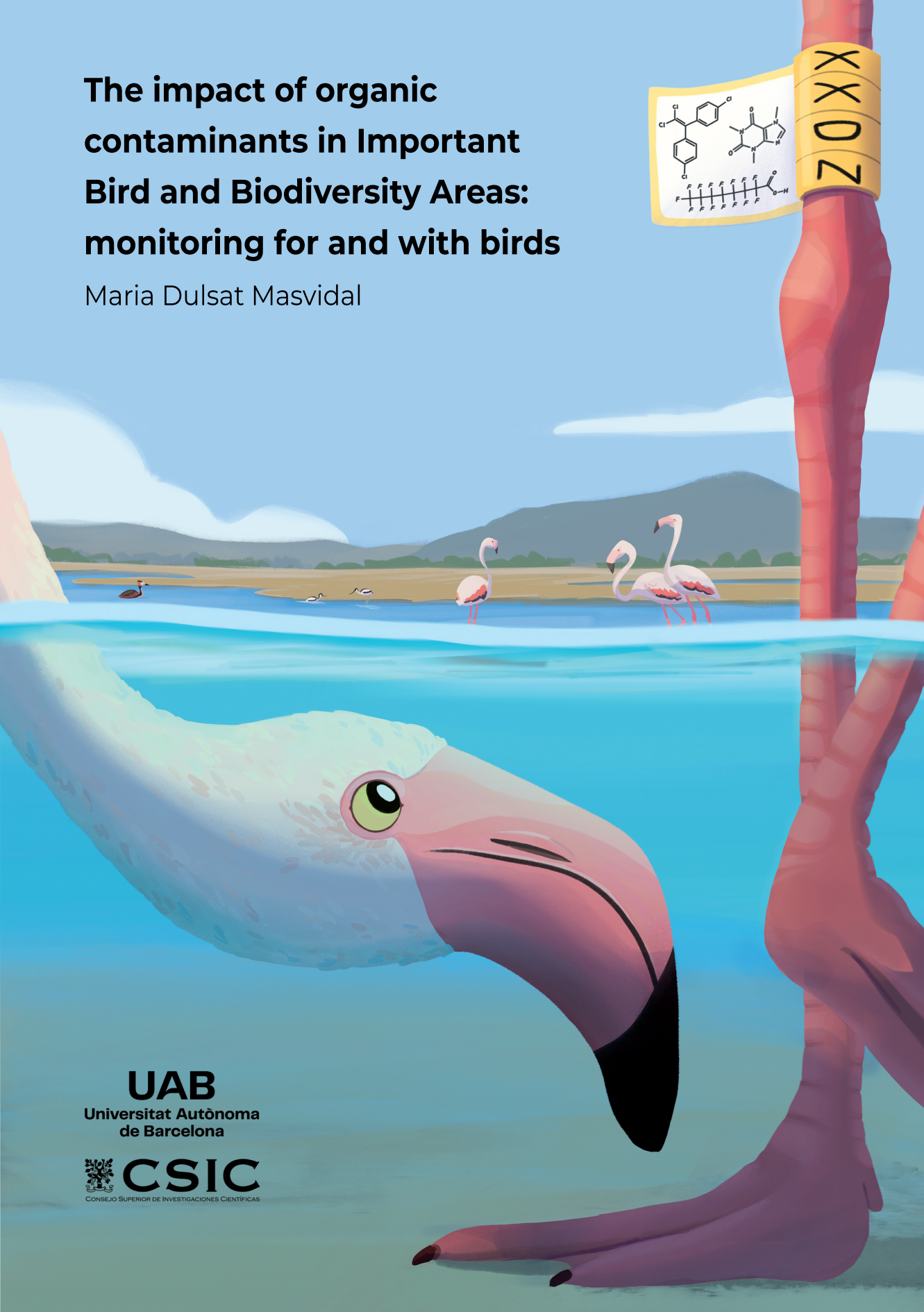

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Programa de doctorat en Ecologia Terrestre

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Barcelona, Novembre 2023

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“Only if we understand, can we care. Only if we care, we will help. Only if we help, shall all be saved”.
Jane Goodall

AGRAÏMENTS

Aquesta tesi ha sigut possible gràcies a l'esforç i suport de molta gent que m'ha acompanyat aquests quatre anys. En primer lloc, vull agrair l'oportunitat i suport dels meus directors de Tesi Sílvia Lacorte i Rafa Mateo, per haver-me permès fer recerca en un tema que m'apassiona i per tota l'ajuda i experiència compartida en aquests quatre anys. I el suport i consells del meu tutor Xavier Domene. Gràcies Sílvia per haver-me permès formar part del grup, estic profundament agraïda per la teva paciència en explicar-me cada dubte, la teva infinita dedicació en tots els treballs que hem fet, i per haver-me escoltat i valorat sempre. Al llarg d'aquests quatre anys he pogut aprendre i créixer com a persona i científica en aquest grup, i és el millor que m'emporto d'aquesta etapa.

Vull agrair a totes les persones amb qui he pogut col·laborar i que han fet possible aquesta Tesi. *Gracias a Carlos Ciudad, por el inmenso trabajo y dedicación en la recogida de muestras y correcciones, sin ti el proyecto de las IBAs no hubiera sido posible.* Gràcies a l'Albert Bertolero per l'oportunitat de poder participar a l'anellament de flamencs del Delta de l'Ebre i per tot el coneixement sobre el delta i els seus ocells compartit, sens dubte dels records més especials que m'emporto. *Thanks to Rui Lourenço for welcoming me into his group five years ago, for his trust, and for the opportunity to collaborate in the raptor studies.* També vull agrair a l'equip de masses de l'IDAEA, Roser i Àlex, per la paciència i ajuda en resoldre cada llum vermella que m'he trobat als equips, que reconec que no han sigut poques.

Vull agrair als meus companys de grup al llarg d'aquests anys que juntament amb la Sílvia, han sigut la meva sort. A en Rubén i a en Miguel per haver-me acollit al laboratori i per haver-me ensenyat totes les tècniques d'extracció i anàlisis quan jo no hi entenia pràcticament res! A en Bernat, amb qui hem fet el tàndem perfecte, hem compartit tots els quatre anys i hem crescut junts. Gràcies per l'ajuda en la química, els equips i tot el suport emocional que m'has donat. A en Pere, ha sigut un immens plaer haver compartit part del viatge amb tu, dins i fora del laboratori. A l'Ana Torres, gràcies per haver-m'ho fet passar tant bé amb les teves xerrades aquests últims mesos. Ara et toca a tu gaudir d'aquest grup! A l'Ana López, per ser la meva referència de dona científica i ocellaire del grup, gràcies per compartit la teva experiència i per haver-me transmès que tot i que és difícil, al final val la pena.

Gràcies a les amistats que m'heu acompanyat aquests anys. A la colla de Canet, els de sempre per formar part també d'aquesta etapa i de moltes altres. A la meva dona, la Marta, per ser incondicional en mi, per tot l'amor, i escolta. A l'Helena per l'amistat, suport, i per la millor extracció de sang que m'han fet mai. Gràcies a l'Èlia, l'Alba, i la Leti, per ser-hi presents cada dia tot i estar dispersades. A la Celia, per l'estima i suport constant tot i la distància. A

la Laura i en Marc per la desconnexió en les hores de jocs. I a la Laia Anducas, gràcies per la preciosa portada de tesi que tan bé reflecteix el seu contingut.

Aquesta Tesi ha estat sobretot possible a l'ajuda i suport de la meva família. Als meus pares, gràcies per haver-me transmès l'estima per la Natura i els ocells des de petita i sobretot per el vostre esforç per donar-me el privilegi d'escollir dedicar-me a allò que m'apassiona, us ho dec tot a vosaltres. Vull agrair en especial el meu pare haver-me transmès l'interès per la ciència i per compartir amb mi la passió pels ocells. I a la meva mare, per la confiança cega en mi, i per haver-me cuida't (sempre) i especialment en aquests anys de Tesi, amb les facilitats del dia a dia ha sigut tot molt més fàcil! Als meus germans, Gemma i Martí, essencials a la meva vida i també en aquesta Tesi. A la Gemma, qui ha sigut sempre el meu referent, gràcies per ajudar-me a relativitzar els problemes, ser la via d'escapament, per ser sempre sincera i compartir-ho tot a les bones i a les dolentes. A en Martí, amb qui hem compartit els estudis, tu l'enginyeria i jo el doctorat, ha sigut molt més divertit fer-ho junts i t'agraeixo totes les estones d'esbarjo mental que hem tingut, estic molt orgullosa dels dos per haver-ho aconseguit! També vull agrair a l'Adrià, per haver-me escoltat, i recordar i desitjar-me sort a totes les presentacions, entregues i reunions que he tingut en aquests anys. Gràcies a la Maria Antònia, Milena i Jeshua, per haver-me acollit i pel vostre suport, en especial a la Milena per l'ajuda i dedicació en maquetar aquesta Tesis.

I finalment, gràcies a en Darko, és molt difícil poder escriure tot l'agraïment pel teu suport constant, per cuidar-me tant, i per ser el meu company en totes les aventures, i les que ens queden per viure. Amb tu, tot ha sigut més fàcil, divertit i bonic. T'estimo!

GRÀCIES!

SUMMARY

Chemical pollution is an underestimated threat to biodiversity conservation. This Thesis aims to quantify for the first time the impact of organic contaminants in Important Bird and Biodiversity Areas (IBAs) which are sites identified to be key for the conservation of biodiversity. The monitoring of water, soils, and sediments from 140 IBAs from Spain was used to assess the pressure of chemical pollution in natural sites and to provide information to determine potential sources of contamination and distribution patterns of contaminants. Water pollution was studied by analysing 59 organic micropollutants, including lifestyle compounds, pharmaceuticals, in-use pesticides, organophosphate esters (OPEs), and perfluoroalkyl substances (PFASs) in 411 water samples. The applied methodology consisted of a single solid-phase extraction method followed by 3 analytical methods based on liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). Organic micropollutants were detected in all studied IBAs waterbodies and pharmaceuticals, lifestyle compounds and OPEs were the most ubiquitous chemical families. The presence of agricultural surfaces around the sampling points was related to significantly high concentrations for all chemical compounds, especially pesticides. Lifestyle compounds and PFASs were related to the presence of artificial surface and wastewater treatment plants (WWTPs) discharges, which were also an important source of pharmaceuticals to surface waters.

Soils and sediment samples were analysed for a total of 52 organic compounds including polycyclic aromatic hydrocarbons (PAHs), organochlorine pesticides (OCPs), organophosphorus pesticides (OPPs), polychlorinated biphenyls (PCBs), plasticizers and OPEs. The compounds were extracted in a single extraction method and determined by gas chromatography coupled to tandem mass spectrometry (GC-MS/MS). Soils from IBAs were affected by diffuse sources of contamination and OCPs were the most ubiquitous chemical group followed by PAHs. PCBs were found at background levels, with higher concentrations in samples with higher influence of artificial surface. In sediment, the most ubiquitous chemical group were PAHs followed by OCPs. The results evidence that soils and sediments from natural areas act as reservoirs of persistent contaminants. The monitoring study of contaminants in IBA has provided for the first-time evidence of the widespread threat of chemical pollution on these relevant ecological sites, evidencing the need to identify the sources of contamination in natural areas important for biodiversity conservation.

The presence of contaminants in natural areas poses a risk for organisms and biodiversity. In this Thesis, birds are proposed as sentinel species to monitor environmental contaminants. The feasibility of the biomonitoring programmes based on bird's species has been proven by identifying which

are the main constraints regarding the collection of raptor samples in Europe and proposing feasible solutions to successfully implement pan-European biomonitoring schemes based on raptor species. The usefulness of birds as sentinel species has also been experimentally proven by determining the exposure to organic micropollutants. The target compounds for biomonitoring studies were based on the list of contaminants studied in water, soils, and sediment. Three analytical methods were developed to determine OCPs, PAHs, PCBs, PFASs, pharmaceuticals, pesticides and OPEs in blood and liver samples from birds.

The exposure of 91 organic micropollutants has been assessed for the first time in the Ebro delta greater flamingos (*Phoenicopterus roseus*) breeding colony. The analysis of flamingo chicks' blood evidenced that the breeding colony is multi-exposed to contaminants from a very young age. PFASs were the chemical group found at the highest concentrations in flamingos. PFOA was the most abundant compound in flamingos' fledglings; the detected levels are considered high compared to the ones reported in other bird species worldwide. OCPs and PCBs were ubiquitous but present at trace concentrations. Their presence is related to the historical pollution of river sediments from a choro-alkali factory affecting the area. The most prevalent halogenated compound was the metabolite 4,4'-DDE. The flamingo's filtering feeding behaviour on mud and maternal ovo-transfer are the more likely routes of exposure of organic micropollutants to flamingos' chicks. However, the reported levels of micropollutants were not associated with any alteration in the flamingos' body condition.

The exposure of 81 organic micropollutants was also determined in livers from five species of road-killed nocturnal raptors including eagle owl (*Bubo bubo*), long-eared owl (*Asio otus*), tawny owl (*Strix aluco*), barn owl (*Tyto alba*), and little owl (*Athene noctua*) from Portugal. Organic micropollutants were detected in all individuals. Differences in the contamination patterns were observed according to the species habitat and trophic position. Individual differences were also assessed, and adult individuals presented significantly higher concentrations of OCPs and PCBs than young individuals.

Overall, this Thesis provides evidence of the threat of chemical pollution in natural areas and the impacts on biodiversity. The findings demonstrate the importance of contaminant monitoring studies in different environmental compartments that can help to implement successful biodiversity conservation strategies.

LIST OF ABBREVIATIONS

AChE	Acetylcholinesterase
AFFF	Aqueous film-forming foam
ASE	Accelerated Solvent Extraction
BBZP	Benzyl butyl phthalate
BPA	Bisphenol a
DBP	Dibutyl Phthalate
DDD	Dichlorodiphenyldichloroethane
DDE	Dichlorodiphenyldichloroethylene
DDT	Dichlorodiphenyltrichloroethane
DEHP	Bis(2-ethylhexyl) phthalate
DEP	Diethyl phthalate
DiBP	Diisobutyl phthalate
DMP	Dimethyl phthalate
EC	Effect Concentration
ECD	Electron Capture Detector
ECHA	European Chemicals Agency
EDTA	Ethylenediaminetetraacetic acid
EEA	Environmental European Agency
EHDPHP	2-ethylhexyl diphenyl phosphate
EMA	European Medicine Agency
EQS	Enviornmental Quality Strandard
ERA	Environmental Risk Assessment
ESI	Electrospray ionization
EU	European Union
GC	Gas chromatography
GIS	Geographic Information System
HCB	Hexachlorobenzene
HCBD	Hexachlorobutadiene
HCHs	Hexachlorocyclohexane
HR-MS	High resolution mass spectrometry
IBA	Important Bird and Biodiversity Areas
IDL	Instrumental limits of detection
IUCN	International Union for Conservation of Nature
Koc	Organic carbon-water partition coefficient
K_{ow}	Octanol-water partition coefficient
LC	Liquid chromatography

MDL	Method limits of detection
MEC	Measured Environmental Concentration
MS	Mass Spectrometry
NOEC	No Observed Effect Concentration
NP	Nonylphenol
NSAID	Non-Steroidal Anti-Inflammatory Drug
OCPs	Organochlorine pesticides
OPEs	Organophosphate esters
OPPs	Organophosphorus Pesticides
PCA	Principal Component Analysis
PCB	Polychlorinated biphenyl
PEC	Predicted Environmental Concentration
PFASs	Perfluoroalkyl substances
PFCAs	Perfluoroalkyl carboxylate
PFBA	Perfluorobutanoic acid
PFBS	Perfluorobutanesulfonic acid
PFDA	Perfluorodecanoic acid
PFDoA	Perfluorododecanoic acid
PFDS	Perfluorodecanesulfonic acid
PFHpA	Perfluoroheptanoic acid
PFHxA	Perfluorohexanoic acid
PFHxDA	Perfluorohexadecanoic acid
PFHxS	Perfluorohexanesulfonic acid
PFNA	Perfluorononanoic acid
PFOA	Perfluorooctanoic acid
PFODA	Perfluorooctadecanoic acid
PFOS	Perfluorooctanesulfonic acid
PFTeDA	Perfluorotetradecanoic acid
PFTriDA	Perfluorotridecanoic acid
PFUnA	Perfluoroundecanoic acid
PFPeA	Perfluoropentanoic acid
PFSAs	Perfluoroalkyl sulfonates
PNEC	Predicted Non-Effect Concentration
POP	Persistent Organic Pollutant
RQ	Risk Quotient
SPA	Special Protection Areas
SPE	Solid Phase Extraction
TPhP	Triphenyl phosphate

TBOEP	Tris(2-butoxyethyl) phosphate
TBP	Tributyl phosphate
TCEP	Tris(2-carboxyethyl)phosphine
TDCPP	Tris(1,3-dichloro-2-propyl)phosphate
TOC	Total Organic Content
TOF-MS	Time-of-flight mass spectrometry
UAE	Ultrasonic-Assisted Extraction
UPLC	Ultra Performance Liquid Chromatography
USEPA	United States Environmental Protection Agency
WWTP	Wastewater Treatment Plant

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1. GENERAL INTRODUCTION

1.1. Chemical pollution as a driver of biodiversity loss

Biodiversity is threatened worldwide, and its loss has been recognised as one of the major ecological challenges of our time. Biodiversity is the variety of life on earth, it plays an essential role in ecosystem functions, such as nutrient and organic matter cycling, and provides key ecosystem services to sustain life including food, water, climate regulation, and pest/disease control (Cardinale et al., 2012). Therefore, the loss of biodiversity has strong implications for the environment and human well-being, becoming an urgent issue that the current society must face.

The rate biodiversity loss is estimated to be the most accelerated since the end of the Cretaceous, 66 million years ago (Dasgupta, 2021). The Living Planet Index (LPI), which measures the state of the world's biological diversity based on the population trends of vertebrate species, stated a global loss of 69% of the wildlife abundance populations between 1970 and 2018. In Europe and Central Asia the loss is estimated at 18% (WWF, 2022). There are five main direct drivers of global biodiversity loss: I) direct exploitation of natural resources, II) land-use changes, III) pollution, IV) invasive alien species, and V) climate change (European Commission, 2021; IPBES, 2019). Pollution is ranked to be the third most important driver of biodiversity loss, only after direct exploitation and land-use change (Jaureguiberry et al., 2022). However, chemical pollution is the less studied driver. Investigations assessing the impact of pollutants on biodiversity have been traditionally focused on eutrophication (nitrogen and phosphorus) and the toxicity of a few selected chemicals, mainly pesticides and some metalloids (Sigmund et al., 2023). During the last decades, the pressure of organic contaminants in the environment has increased continuously, as new chemicals are produced and released into the environment every day. More than 350,000 chemicals and mixtures are registered for production and use worldwide, and their production is expected to increase in the following decades (Wang et al., 2020). This great amount of synthetic chemicals represents a challenge for their testing and regulation. It is estimated that using the actual regulatory methods, it will take over 100,000 years to evaluate the human and environmental safety of all existing synthetic chemicals (Naidu et al., 2021). At present, a myriad of contaminants is released into the environment, where chemical pollution has become a baseline stressor in ecosystems, exposing wildlife and humans chronically.

Chemical pollutants have the capacity to interact with organisms even at very low concentrations levels acting as endocrine disruptors, genotoxic, or neurotoxic agents. These effects are translated into alterations in the reproduction, development, immune system or behaviour of individuals, with potential implications at population levels (Aulsebrook et al., 2020; Walker et al., 2012). The adaptation to chemical exposure may also decrease

genetic diversity, and reduce resilience to environmental stressors, such as global warming and other aspects of global change (Sigmund et al., 2022). Both direct and indirect impacts of chemical pollutants can cause a decline in populations of various exposed species or even the extinction of the very sensitive ones (Sigmund et al., 2023). The IUCN Red List is a global indicator of the world's biodiversity conservation, and it is one of the most authoritative data sources in conservation (IUCN, 2023). Recent studies have pointed out that environmental contamination threatens 18.2% of the IUCN Red List, and is the main threat to 4.7% pushing them to extinction (Hogue and Breon, 2022). For instance, Europe has been recognized as a hotspot for pollution risk for amphibians and mammals (Harfoot et al., 2021).

Summarizing, to effectively conserve biodiversity, it is crucial to address the direct drivers that contribute to its loss. Chemical pollution is a significant factor that impacts ecosystems, potentially leading to habitat degradation and subsequently resulting in the loss of biodiversity and ecosystem services.

1.2. Birds as biodiversity indicators

To assess biodiversity in an effective way, it is essential to identify taxa that can act as indicators of ecosystem health. Birds are good candidates as they are sensitive to changes in the environment and become excellent indicators of biodiversity. Birds are a very diverse group of species that play multiple roles within ecosystems. There are avian species at all trophic levels: from those feeding on seeds and insects to top predators and scavengers. Thanks to this diversity, they provide multiple ecosystem services, such as seed dispersal, pollination, nutrient cycling, pest control, waste removal and disease control (Wenny et al., 2011; Whelan et al., 2015). They are relatively easy to detect and identify in the field, census methods are feasible, and many skilled volunteers are enrolled to provide count data (Fraixedas et al., 2020). In addition, the diversity of birds has fascinated humans for centuries around the world, they are unparalleled in popularity with strong links in human culture. This popularity has brought them to be the most well-known animal group, as no other taxa are better monitored than birds (BirdLife International, 2022). Bird populations have been studied for decades in many countries, and their traits such as diet, habitat, reproduction performance and movements are well documented. The existing long-term series of bird populations enable the assessment of temporal trends and changes over time (Gregory and van Strien, 2010; Sheehan et al., 2010).

Birds are also excellent sentinel species and are used to perform biomonitoring studies to assess the impact of contaminants (Smits and Fernie, 2013). Some species of birds feed on high positions of the trophic chain accumulating high levels of contaminants present in the trophic web.

Therefore, analysing contaminants in their body can reflect the contamination of an area. Also, they are generally long-lived species, meaning that they have the capacity to bioaccumulate contaminants throughout their entire life, making it possible to assess potential temporal trends or assessing the biomagnification of contaminants along the food chain. Different tissues can be analysed, spanning from internal tissues (blood and internal organs) to less intrusive samples such as feathers and eggs (Espín et al., 2016). The similarities between species and the availability of samples worldwide make it possible the comparison of contamination patterns among countries (Gómez-Ramírez et al., 2014).

Birds' counts are indicating a change in their population trends, that matches the general loss observed in wildlife populations. In Europe, between 560 and 620 million birds individuals, representing approximately 17-19% of the bird population abundance, have been lost since 1980 (Burns et al., 2021). In a recent study, Rigal et al., (2023) confirmed the birds' population decline quantifying a loss of 25% in the last 40 years. The rate loss is even higher in farmland species which have decreased by 66%, among the main drivers of its decline the study highlights the intensive agriculture associated with the application of pesticides and fertilisers.

Due to their popularity among the public, birds can act as an excellent communication tool to raise awareness of biodiversity loss. For instance, the changes in the abundance of bird populations were used by Rachel Carson in the *Silent Spring* (1962) (Carson, 1962) to describe the harmful effects of agrochemicals, such as the organochlorine pesticide DDT on the environment. These changes were not only recognized by the scientific community but also by the general population, who noticed shifts in the abundance of birds within their own gardens. This book was the spark to trigger research studies on the monitoring and risk assessment of chemicals in environmental matrices.

Considering all the above, birds are remarkable allies to enhance our comprehension on the impact of chemical pollution on the crisis of biodiversity loss.

1.2.1. Birds' species as biomonitors

The selection of the target bird species in biomonitoring studies depends on the habitat of interest. In terrestrial food webs, raptors (birds of prey) are the most used sentinel species due to their long live-span and top-predator position which makes them more prone to accumulate contaminants. Therefore, the chemical compounds detected in raptor samples represents a summary of the existent contaminants present in the terrestrial ecosystem. Raptors are a diverse group of birds including diurnal species (eagles, falcons, vultures) and nocturnal species (owls). Among raptors, the chosen species to

monitor contaminants depend on the existing monitoring schemes to provide samples and also on their geographical distribution (Gómez-Ramírez et al., 2014; Vrezec et al., 2014). Scavenger species (vultures and some eagles) are preferred to monitor pharmaceuticals as they are directly exposed to these compounds due to their feeding behaviour that includes the consumption of dead cattle treated with veterinary drugs (Herrero-Villar et al., 2023). Apart from raptors, other groups can be more representative of specific threats, for example farmland birds such as partridges are commonly used to assess the exposure to pesticides applied in agricultural fields (Fernández-Vizcaíno et al., 2022; Rodrigues et al., 2023). Another group are waterbirds which are used to monitor the presence of contaminants occurring in surface waters and sediments from wetlands and marshes, and herons (Huertas et al., 2016; Wei et al., 2023) and ducks (Sharp et al., 2021; Tomza-Marciniak et al., 2019) are among the most commonly used species. However, many other waterbirds can be used for contaminant monitoring, for example greater flamingos (*Phoenicopterus roseus*) have been used in punctual studies based on opportunistic sampling to monitor organochlorine compounds (Guitart et al., 2005) or for heavy metals monitoring (Ancora et al., 2008). Another relevant group are passerine birds which have smaller home ranges and are more appropriate than other species to monitor local contamination, for example following gradients of pollution (Custer et al., 2019; Custer et al., 2017; Lopez-Antia et al., 2019). Finally, there are numerous monitoring studies on the bioaccumulation and biomagnification of contaminants along the marine food web using seabirds top-predators. Gulls are one of the most frequently species used in biomonitoring programmes due to their widespread distribution and feasible access to their nests and samples (Colomer-Vidal et al., 2022; Pereira et al., 2019; Sebastiano et al., 2023; Zapata et al., 2018). Other marine top-predators are also monitored such as shearwaters (Szabo et al., 2021) or gannets (*Morus bassanus*) (Pereira et al., 2021, 2009).

1.3. Important Bird and Biodiversity areas

The protection of natural areas is a core strategy to halt the biodiversity loss crisis. The recent EU Biodiversity Strategy 2030 has set the ambitious goal of protecting 30% of the EU land and 30% of the seas, with 10% of land and sea areas strictly protected to restore nature (European Commission, 2021). However, the efficiency of this strategy depends on the target areas to be protected. Biodiversity is not evenly distributed, meaning that there are areas that hold a greater richness of species and are more relevant for conservation than others. As an example, the Mediterranean basin is one of the most important areas for conservation in Europe due to its richness of species. It hosts nearly 82% of the European breeding birds, 69% of the

terrestrial mammals, more than 88% of the European amphibian species and almost 74% of the terrestrial reptiles (Pascual et al., 2011). The designation of protected areas does not always match the distribution of biodiversity-relevant areas and tends to be biased toward high elevations, remote areas, and sites with lower suitability for agriculture (Joppa and Pfaff, 2009), rather than in areas where the formal protection is needed to preserve their ecological values, thus limiting the effectiveness of protected areas to conserve biodiversity (Butchart et al., 2015; Geldmann et al., 2019). To restore biodiversity, it is key to target conservation actions not only in the actual protected areas but also in those sites that should be protected for their ecological relevance.

Important Bird and Biodiversity Areas (IBAs) are sites identified by BirdLife International as being of global importance for the conservation of bird's population and biodiversity in general. The designation of global IBAs is based on international scientific agreed criteria (BirdLife International, 2023) and include areas with:

- Globally threatened species (A1): the site regularly holds significant numbers of species listed in the IUCN Red List as globally threatened.
- Restricted range species (A2): the site holds a significant population of at least two range-restricted species, which are defined to have a global range size ≤50.000 km^2.
- Biome-restricted assemblages (A3): the site is known or thought to hold a significant component of the group of species whose distributions are largely or wholly confined to one biome.
- Congregations (A4): the site is known or thought to hold congregations of ≥1% of the global population of one or more species on a regular or predictable basis.

In addition to global IBAs criteria, other IBAs have been identified using specific standards based on a regional scale. Regional IBAs criteria have been used in Europe, the Middle East, and the Caribbean (Donald et al., 2019).

The IBA programme was created in 1979 in Europe and has grown to the present day. Today, it is the largest network of conservation sites in the world, with 13,600 identified IBAs covering 9% of the world's land area and 2% of the world's marine area (BirdLife International, 2022). The designation of IBAs is internationally recognised and used by multilateral environmental agreements such as the Convention on Biological Diversity (CBD), the Ramsar Convention, and the Convention on Migratory Species (Waliczky et al., 2019). In Europe, the IBAs inventory (Figure 1.1.A) is used as a reference for the designation of Special Protection Areas (SPA) under the birds directive (Directive 2009/147/EC) as part of the Natura 2000 network of sites, which provides the most

important legal protection of habitats and species in Europe. On average, SPA covers 66% of the terrestrial IBAs surface (Kukkala et al., 2016). In Europe, the protection of IBAs can play an important role in the adaptation to climate change. For instance, IBAs are important refugees for waterbird populations, which are increasing in protected and non-protected IBAs due to the general wetlands habitat loss (Pavón-Jordán et al., 2020). Globally, it has been observed that IBAs with more than 50% of the protected surface presented a lower extinction risk of species than those with lower protected surfaces (Butchart et al., 2012).

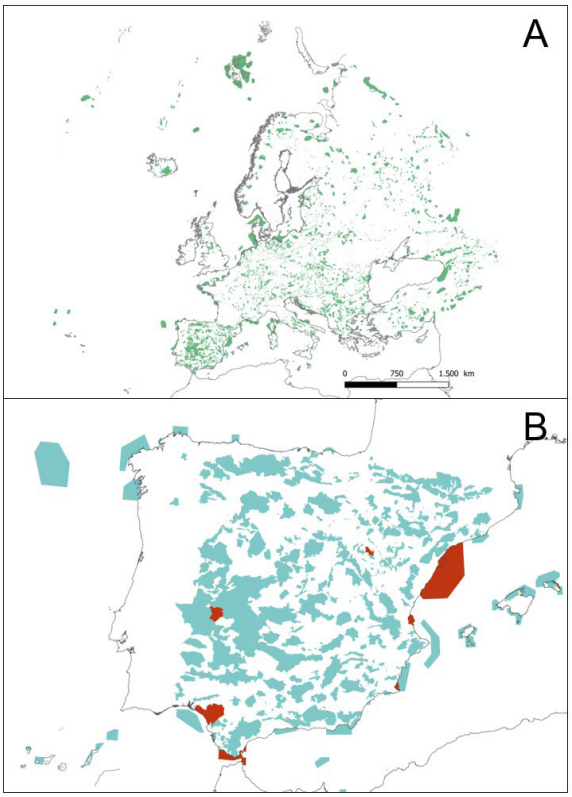

Figure 1.1. Existing IBAs in A) Europe and B) Spain. Spanish 12 IBAs in danger are coloured in red. Data source: BirdLife International, 2023b.

BirdLife International provides a systematic monitoring effort to address the conservation status of IBAs, based on evaluating the state of IBAs such as bird population counts or habitat quality, identifying pressures such as agricultural intensification, over-exploitation, or pollution, and also the response actions such as changes in their conservation (BirdLife International, 2006). Although this information is not available for all IBAs, the last data estimated that 45% of the IBAs worldwide are in an unfavourable condition. From these, 277 IBAs are qualified as "IBAs in danger", that refers to sites that need urgent conservation actions to preserve their ecological values (BirdLife International, 2022).

Spain is one of the countries with the highest number of IBAs, with 469 IBAs identified, covering almost 24 million hectares, and terrestrial IBAs occupy 36% of the total country surface (Figure 1.1B) (Infante et al., 2011). Spain is a country of international relevance for bird conservation; from the 469 IBAs, 325 were identified due to A1 criteria, related to being important sites for breeding, wintering, or passage of globally threatened or near threatened species (BirdLife International, 2023). As an example, Spain holds the major

or entire breeding population of Cinereous vulture (*Aegypius monachus*), Spanish imperial eagle (*Aquila adalberti*), Lesser kestrel (*Falco naumanni*), Great bustard (*Otis tarda*), Little bustard (*Tetrax tetrax*), Audouin's gull (*Larus audouinii*), Bolle's pigeon (*Columba bollii*), Laurel pigeon (*Columba junoniae*), Canary Islands stonechat (*Saxicola dacotiae*) and Tenerife blue chaffinch (*Fringilla teydea*) (Heath et al., 2000). Despite of this biodiversity richness, Spain has been identified as the seventh country in the world with a higher number of "IBAs in danger" and the third in Europe, with 12 IBAs in very unfavourable conditions highlighted in red in Figure 1.1B. The main reason for this critical conservation state is identified as pollution-related pressures (BirdLife International, 2023). Other identified threats ordered by importance are: agricultural practices, climate change, hunting, invasive species, dams and water management, fisheries, human intrusions, residential and commercial development, and transportation (BirdLife International, 2023). Therefore, the actual status of Spanish IBAs can have an impact on the global conservation of threatened bird species.

The management of IBAs is essential to enhance their conservation, and for this reason, it is urgent to identify and monitor the main causes that may jeopardize these significant sites. Although several efforts have been done to identify human pressures in these sites, the threat of chemical pollution is still unknown in the majority of IBAs due to the complexity to implement monitoring programmes. IBAs are an excellent framework to address the potential impact of chemical pollution in the management of biodiversity due to their importance for conservation and the anthropogenic pressures that receives.

1.4. Environmental contaminants

Environmental contaminants are chemical substances that occur in the environment where they should not exist or at higher levels than those naturally occurring, with the potential to produce adverse effects. The present Thesis is focused on the study of organic contaminants, which involve carbon-based molecules. The target compounds can be also referred to as organic micropollutants as they are found in trace amounts in different environmental matrices (Abbasi et al., 2022). The fate and toxicity of these pollutants is depending on their molecular structure, molecular weight, and functional groups.

Except for polycyclic aromatic hydrocarbons (PAHs), most compounds included in this Thesis are synthetic chemicals manufactured and released into the natural environment since the last century. This broad category includes numerous compounds extensively used in industry, agriculture, domestic applications, medicine, and for the synthesis of materials, such as

pharmaceuticals, lifestyle compounds, perfluoroalkyl substances (PFASs), pesticides, organophosphate pesticides (OPPs), phthalates, polychlorinated biphenyl (PCBs), and organochlorine pesticides (OCPs). The chemical families studied in this Thesis were selected based on their concern for human and ecosystem health, including persistent substances used in the past and in-use compounds produced in large quantities and continuously released into the environment. The next section describes the chemical families and the main compounds studied, their sources, properties, legislative status and environmental impact.

1.4.1. Pharmaceuticals and lifestyle compounds

Pharmaceuticals are highly consumed chemicals in our daily life to treat diseases and improve the quality of life. Lifestyle compounds, including substances like nicotine and caffeine, are widely used in tobacco, coffee, and numerous consumer products. With hundreds of tons consumed worldwide each year, the extensive use of pharmaceuticals and lifestyle compounds, together with their bioactive properties, has led to include these chemicals as new emerging contaminants (Patel et al., 2019).

A great number of pharmaceuticals and lifestyle compounds, including antibiotics, antidepressants, anti-inflammatory drugs, analgesics, beta-blockers, and oral contraceptives, reach wastewater through human excretion in urine and faeces, or because they are improperly dumped down in sinks or toilets and reach the sewage system. The treatment of wastewater in wastewater treatment plants (WWTPs) is not always sufficiently effective to eliminate these residues, and pharmaceuticals and similar compounds are discharged into natural surface waters. Also, pharmaceuticals are used for veterinary purposes, including aquaculture, livestock, and poultry. Residues of veterinary drugs are found in manure which is applied in agricultural fields being another important source of pharmaceuticals into the environment (Ebele et al., 2017).

Pharmaceuticals and lifestyle compounds are a group of heterogeneous contaminants as they present very different chemical structures and biological properties depending on their intended use. In general, pharmaceuticals are polar compounds that are easily metabolized or degraded in the environment. However, as a result of their constant release into the environment, they are considered “pseudo-persistent” micropollutants (Ebele et al., 2017). They are widespread in surface waters worldwide at concentrations ranging from ng/L to mg/L (Sanusi et al., 2023).

Pharmaceuticals are bioactive compounds designed to interact with organisms and produce biological effects at very low concentrations levels. Therefore, even at trace concentrations, pharmaceuticals and lifestyle

compounds can produce alterations in organisms exposed to them. A recent global study reported that approximately 43.5% of the rivers in the world have concentrations of pharmaceuticals and lifestyle compounds where ecotoxicological effects might be expected (Bouzas-Monroy et al., 2022). Perhaps, one of the most known impact of pharmaceuticals on wildlife was reported in 2004 when the Asian vulture populations collapsed almost to their extinction due to the intoxication with the non-steroidal anti-inflammatory drug (NSAID NSAID) diclofenac used in livestock veterinary treatment (Oaks et al., 2004). Diclofenac is still authorized for veterinary use in Europe and Africa, posing at risk vulture populations which feed on livestock carcasses. In fact, in 2020 the first case of diclofenac poising in a European vulture was reported in Spain (Herrero-Villar et al., 2021).

The contamination by pharmaceuticals is recognized as an emerging environmental concern. European strategies have been developed to address pharmaceutical pollution by monitoring their presence and assessing whether they pose a risk to surface water. Some pharmaceuticals including diclofenac, ibuprofen, α-estradiol, and β-ethinylestradiol, where included in the first Surface Water Watch List under the European Water Framework Directive (European Commission et al., 2018). Recently, more pharmaceuticals were added to the new Watch List to determine whether they pose a risk or not, including: metformin, antibiotics such as sulfamethoxazole, trimethoprim, clindamycin, and ofloxacin, the antidepressant venlafaxine, and the azoles antifungals clotrimazole, fluconazole, and miconazole (Commission Decision 2022/1307), also the pharmaceutical diclofenac was removed in the latest version of the Watch List due to the lack of risk in European surface waters. The United States Environmental Protection Agency (USEPA) also considers pharmaceuticals in the Contaminant Candidate List (CCL), which aims to identify compounds not regulated but that are known or anticipated to occur in public water systems. The latest CCL5 includes pharmaceuticals such as estrogenic hormones, antidepressant desvenlafaxine, and the antifungal fluconazole, among others (USEPA, 2022).

However, the number of monitored pharmaceuticals is still very scarce compared to the approximately 3000 total active ingredients listed by the European Medicine Agency (EMA). In fact, the EMA indicates the need to monitor pharmaceuticals in water and evaluate the risk when concentrations are higher than 10 ng/L. In addition, most monitoring studies include wastewaters and river waters, and very little information is available on the presence of pharmaceuticals in natural or protected areas, where their presence could represent a threat for many aquatic species.

In this Thesis, 2 lifestyle compounds, nicotine, and caffeine, were selected for their widespread presence in consumer products and 19 pharmaceuticals were studied based on their high consumption in Spain or for their low

degradability (Gómez-Canela et al., 2019), which increases their likelihood of being found into the environment. Compounds studied are indicated in Figure 1.2.

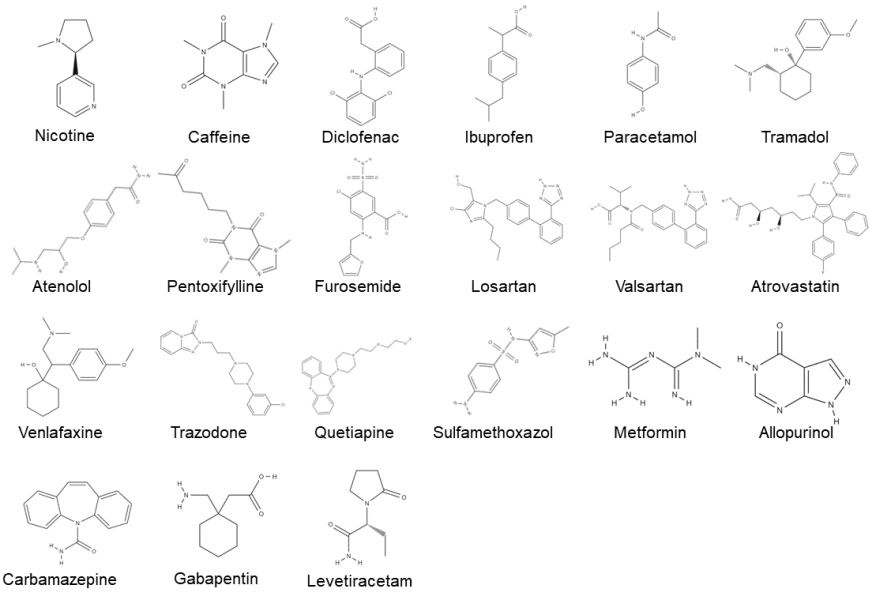

Figure 1.2. Target pharmaceuticals and lifestyle compounds studied.

1.4.2. Perfluoroalkyl substances (PFASs)

Perfluoroalkyl substances (PFASs) are a broad group of over 4000 synthetic compounds with linear, branched, or cyclic structures characterized by having a perfluoroalkyl group (Zarębska and Bajkacz, 2023). Two of the most well-known PFASs families are perfluoroalkyl sulfonates (PFSAs) and perfluoroalkyl carboxylates (PFCAs). PFSAs are compounds which contain a sulfonic acid group (-SO_3H) attached to the perfluorinated carbon chain. Among the most well-known PFSAs are perfluorooctanesulfonic acid (PFOS) and perfluorohexanesulfonic acid (PFHxS). PFCAs presents a carboxylic group (-COOH), and perfluorooctanoic acid (PFOA) and perfluorononanoic acid (PFNA) are the most common and well-known compounds.

PFASs were synthesised for the first time in the 1940s, and since then are used in numerous products and industrial applications due to their chemical stability, and oil and water-repellent properties. Some of the best-known applications are in non-stick coatings such as the polytetrafluoroethylene (PTFE) known for the commercial name of Teflon, in aqueous film-forming foam (AFFF), food packaging, stain and water repellent in textiles, production of plastics and rubber, and electronics and machinery manufacture. Also, they are used as additives in many other products including cosmetics,

pharmaceuticals, pesticides, printing inks, sealants and adhesives (Glüge et al., 2020).

The diverse application of PFASs together with their high stability have led them to be ubiquitous contaminants in the environment. Even in the case of microbial transformation of PFASs, the perfluorinated residues will persist for a very long time, for this reason, they are referred to as "forever chemicals" (Brunn et al., 2023). PFASs have been detected in all environmental compartments. They are considered both hydrophobic and hydrophilic compounds as they have a polar "head" and a nonpolar "tail" (Christensen et al., 2022). The environmental fate of PFASs can differ according to the chain length, short-chain PFASs (PFCA ≤ 8; PFSA≤6C) are more hydrophilic and water soluble which makes ocean, groundwaters and surface waters their main sinks. The water solubility and relatively low molecular weight of legacy PFASs, makes them more likely to be distributed in the aquatic systems, which are an important transport route for PFASs (Miranda et al., 2022). Long-chain PFASs (PFCA >8; PFSA >6C) are more hydrophobic and proteinophilic (Zarębska and Bajkacz, 2023), and are known to bioaccumulate and biomagnify along the food chain, reaching high concentrations in top predators (Androulakakis et al., 2022; Lewis et al., 2022). The toxicity of PFASs is not yet fully understood, although there is evidence that their exposure impairs the regulation of stress and immune responses, affecting the hormone production (thus they are within the category of endocrine disruptors), causes various oxidative stress reactions (Dickman and Aga, 2022), and alterations in lipid metabolism (Gorrochategui et al., 2014).

The first public concern about PFASs was stated in 1968, when some studies pointed out that these substances were accumulating in the human bloodstream (Taves, 1968). In the earlier 2000s there was strong evidence of their occurrence in humans and widespread distribution in the environment, which encouraged the first regulatory actions on PFASs production (Buck et al., 2011). One of the most significant incidents involved the PFOA contamination resulting from chemical dumping by DuPont factory, a major producer of PFASs. It caused the contamination of drinking water and consequently the exposure to population, and even the death of cattle feeding on a contaminated area (Richter et al., 2018). In 2002, the main producer of PFASs, 3M company, phased out the production of the long-chain compounds PFOS, PFOA, and PFHxS. In 2006, EPA and 8 leading global companies producers of PFASs, including DuPont, agreed to reduce the emissions of PFOA (Buck et al., 2011).

PFOS was included in the Stockholm Convention in 2009, followed by PFOA in 2019 and PFHxS in 2022 (Stockholm Convention, 2022). The Stockholm Convention aims to protect human health and the environment from the effects of persistent organic pollutants (POPs) and controls their emission

and time trends. However, it is not a legislative body. The Water Framework Directive (Directive 2013/39/EU) regulates some PFASs, for example it established an Environmental Quality Standards (EQS) for PFOS and its derivatives at annual average (AA-EQS) of 0.65 ng/L in inland water and 9.1 ng/g in biota, indicating that European surface waters and fish should not exceed these levels. However, PFASs have been recurrently detected in surface waters (Göckener et al., 2023; Podder et al., 2021), soils (Röhler et al., 2021), sediments (Guckert et al., 2022) and biota (Boisvert et al., 2019; Koch et al., 2020; Lopez-Antia et al., 2019). Regulations and the prohibition of use has led to a decrease in the concentrations detected in the environment. Long-term monitoring studies in birds' eggs have reported a decline in PFOS concentrations after its regulation proving the effectiveness of their ban (Colomer-Vidal et al., 2022; Pereira et al., 2021; Wang et al., 2021). However, this decline is not observed for other concerning PFASs as it is a common practice to forbid one chemical and introduce an analogue. For this reason, the EU intends to regulate PFASs as an entire class instead of single-substance regulation. In this direction, Germany, the Netherlands, Denmark, Norway and Sweden are working together on a restriction for the entire substance group of PFASs in the EU (Brunn et al., 2023).

This Thesis focused on a group of 17 PFASs including 4 PFSAs and 13 PFCAs, selected for their widespread occurence in the environment. Figure 1.3 indicates the studied PFASs.

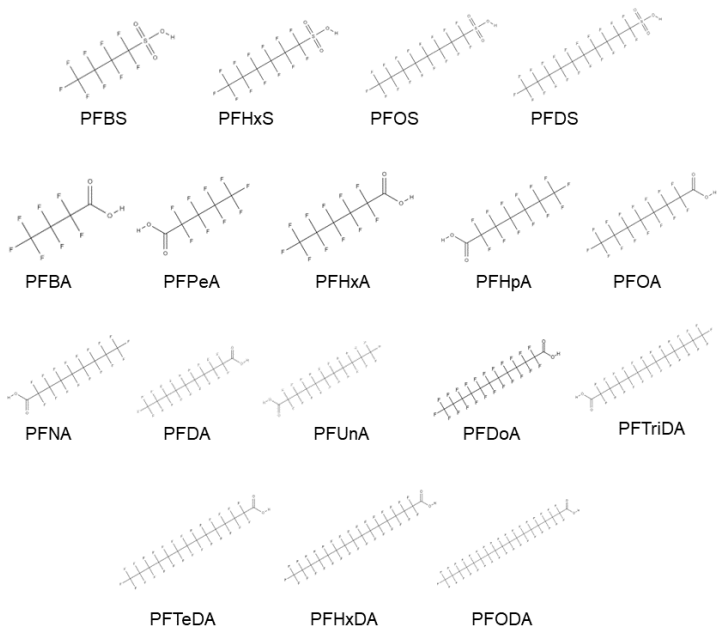

Figure 1.3. Target PFASs studied.

1.4.3. Current use pesticides

The actual agricultural production relies on the use of high volumes of synthetic pesticides to sustain crop yields (EEA, 2023). Pesticides are substances that kill, repel, or control any forms of animal and plant life considered to damage or be a nuisance in agriculture and domestic life. Pesticides include various chemical families, and the most important are (i) herbicides used to destroy or control weeds and other unwanted vegetation; (ii) insecticides used to kill or control insects in agriculture, industry, businesses, and households and (iii) fungicides used to control fungi that are applied on plants or other surfaces where mould or mildew grow and play an important role in protecting crops. Pesticides sales in the EU were 355,175 in 2021, with Spain accounting for 21% of the total sales, followed by France (20%), Germany (14%) and Italy (14%) as the top agricultural producers (Eurostat, 2023). The main pesticides products sold in the EU are fungicides, accounting for 44% of the total sales, followed by herbicides (34%) and insecticides (14%) (Eurostat, 2023).

Current used pesticides exhibit a higher polar nature than legacy pesticides. This polarity is intentionally designed to avoid bioaccumulation, persistence or long-range transport processes compared to legacy compounds (Campanale et al., 2021). Pesticides applied in crops are transferred from agricultural fields to waterbodies representing a threat to freshwater ecosystems. In 2020 at least one or more pesticides were detected at concentrations above the thresholds of concern in 22% of the monitored rivers and lakes across Europe (EEA, 2023). The fate and toxicity of pesticides depend on their structure and mode of action. However, pesticides are active molecules designed to produce harmful effects against organisms, therefore toxic effects on non-target organisms are expected. There are many examples of the toxicity of current pesticides in biodiversity. For instance, one of the most commonly used fungicides, tebuconazole, has been associated with the degradation of soils owing to its persistence and alteration of the microbial community (Han et al., 2021). Also, it is an endocrine disruptor in non-target organisms such as fishes, amphibians and birds (Li et al., 2019; Lopez-Antia et al., 2021). Among the most toxic group of insecticides, there are organophosphorus pesticides (OPPs), derived from phosphoric acid. OPPs are neurotoxins, as they interfere in the synapse of neurons by blocking permanently the enzyme acetylcholinesterase (AChE). These compounds were developed for two main purposes: as war agents during the Second World War and later for insecticide applications (Soltaninejad and Shadnia, 2014). In agriculture, OPPs became very popular as substitutes for banned organochlorine pesticides (OCPs), as they are considered less toxic and less persistent in the environment due to their higher polarity and biodegradability (Walker et al., 2012). However, their persistence in the environment strongly depends on the molecular structure of each compound, and some studies suggest that the persistence of these

pesticides in the environment may have been underestimated. For instance, the half-life of chlorpyrifos was reported to vary from 4 days in sand soils to 1 to 4 months in manured or muck soils, indicating that the persistence of chlorpyrifos depends on the soil and climatic conditions (Ramasubramanian and Paramasivam, 2022). The exposure of OPPs has been related to adverse effects in non-target species, mainly to alterations in the behaviour of organisms due to the interference of the neuronal function, but also to other sublethal effects such as endocrine disruption, cognitive alterations, reduced growth, and growth development in animal species (Iwuozor et al., 2023; Sidhu et al., 2019). OPPs have been found to be highly toxic for pollinators. In Spain, high concentrations of chlorpyrifos and dimethoate were detected in dead honeybees (*Apis mellifera*) during poisoning incidents (Calatayud-Vernich et al., 2016), and residues of OPPs have also been found in bees, pollen, and beeswax from Spanish apiaries (Calatayud-Vernich et al., 2018). Some countries have started to restrict or cancel the use of OPPs because of their impact on human and ecosystem health. For instance, in Europe, chlorpyrifos was banned in 2020 (European Commission, 2020) and malathion is only allowed in greenhouse applications (European Commission, 2023). Nonetheless, many other countries worldwide continue to allow the use OPPs (FAO, 2022).

The European Water Framework has established EQS for some pesticides identified as field priority substances; For instance, the insecticides chlorphenvinfos and chlorpyrifos present an AA-EQS of 100 ng/L and 30 ng/L respectively, the herbicide isoproturon present an AA-EQS set at 300 ng/L in surface waters (Directive 2013/39/EU). New pesticides are continually being synthesised and used to replace the phase-out substances, creating a flow of new products for which their impact in the environment is not known.

In this Thesis, 18 pesticides, including 4 OPPs, were selected due to their widespread application in Spain according to data from the Spanish ministry of agriculture (Figure 1.4).

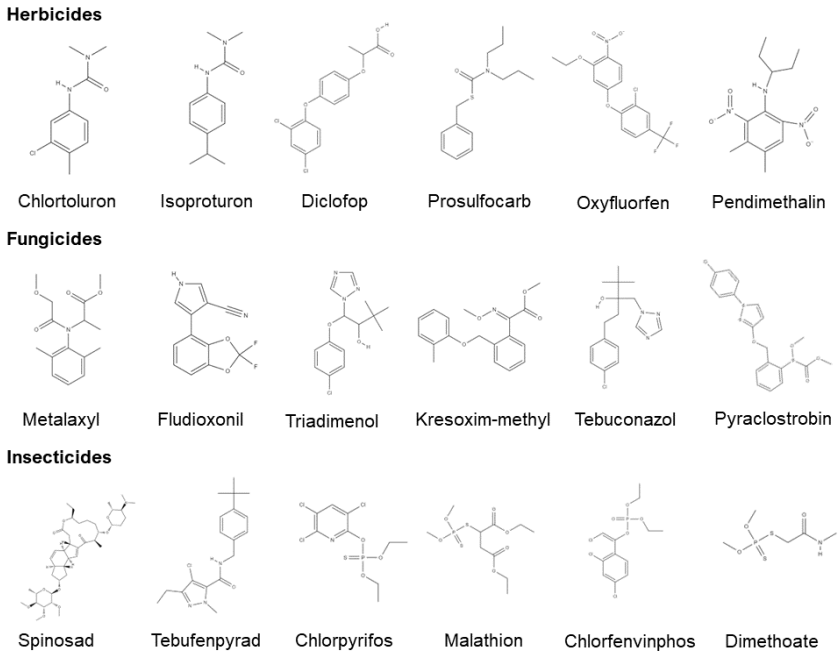

Figure 1.4. Target in-use pesticides studied.

1.4.4. Organophosphate esters (OPEs)

Organophosphate esters (OPEs) are derivatives of phosphoric acid and are divided into halogenated OPEs which are mainly used as flame retardants in textiles, furniture, and electronic products, and non-halogenated OPEs, which are used as antifoaming agents, plasticizers, and additives in hydraulic fluids (Chokwe et al., 2020). The production and use of OPEs in industrial and consumer products increased rapidly after they were used as substitutes of legacy brominated flame retardants. China is the main manufacturer of OPEs, with 363003 annual tonnes produced annually (Huang et al., 2022). OPEs are not chemically bonded to the materials and are easily released, becoming widespread in the environment (Dou and Wang, 2023). They present a wide range of polarities, some of them can readily be absorbed in solid particles and others are highly soluble in water where they can be easily transported by water flows (van der Veen and de Boer, 2012). Residues of OPEs have been detected in numerous environmental compartments as water and sediment (Cristale et al., 2013), and can be sorbed to soils (Cristale et al., 2017). OPEs have been reported at high concentrations in soils impacted by plastic waste, in urban areas and also in agricultural areas at concentration ranges from 0.1 to 10,000 ng/g (Zhang et al., 2022). OPEs are bioaccumulated in biota, and residues have been reported in mussels, fish, mammals and birds from

the Baltic Sea (de Wit et al., 2020) and also in the Mediterranean trophic web (Sala et al., 2022). Moreover, they are concerning compounds as some OPEs are neurotoxic, carcinogenic and endocrine disruptors (van der Veen and de Boer, 2012). Due to their toxicity, most OPEs are regulated under the EU law "Regulation on the Registration, Evaluation, Authorisation and Restriction of Chemicals" (REACH) and their use is being restricted in the EU. Among OPEs, tris(2-chloroethyl) phosphate (TCEP), tris(2-chloro-1-methylethyl) phosphate (TCPP), and tris [2-chloro-1 chloromethyl)ethyl] phosphate (TDCP), have been classified as carcinogenic, mutagenic and toxic to reproduction, and their use is banned in childcare articles (ECHA, 2023a). However, the EU does not include these compounds in any priority list of hazardous substances to be monitored in the environment.

In this Thesis, 2 halogenated OPEs and 4 non-halogenated OPEs have been studied (Figure 1.5).

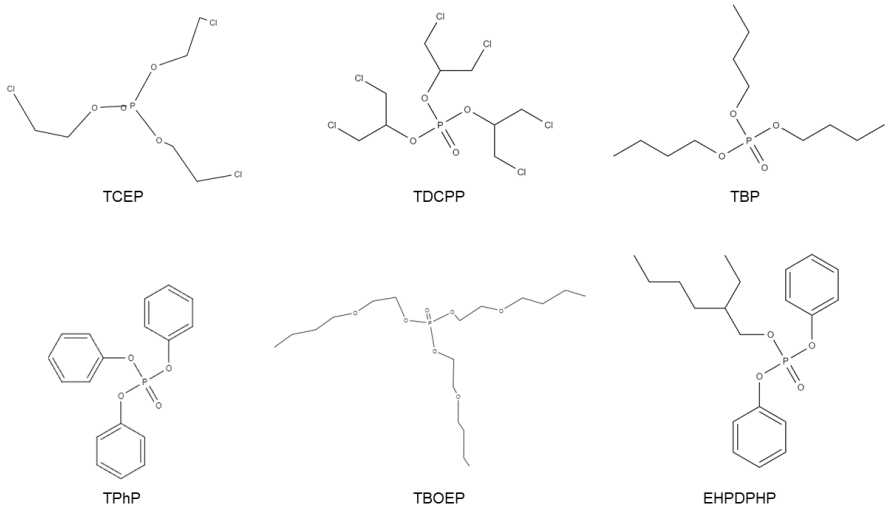

Figure 1.5. Target OPEs studied.

1.4.5. Phthalates and related compounds

If we must point out one material present in everyday life, it is plastic, whose use has become a serious global environmental concern. Plastic pollution, besides the release of this material into the environment, also refers to the release of chemicals known as plasticizers which are additives used to provide specific properties to plastics. Plasticizers, such as phthalates esters (PAEs), are used to make plastics more flexible, transparent and durable. One of the most widely produced PAE is DEHP (Bis(2-ethylhexyl) phthalate)

which is a main additive in the fabrication of polyvinyl chloride (PVC) used in construction materials, pipes, plastic packaging film and sheets, garden hoses, inflatable toys, blood-storage containers, medical tubing, and some children's toys. Other PAE are DnBP (di-n-butyl phthalate), DiBP (di-isobutyl phthalate), DMP (dimethyl phthalate) and BBzP (butyl-benzyl phthalate) used in hundreds of other products such as adhesives, detergents and surfactants, lubricating oils, automotive plastics, impermeable clothes, and personal-care products (e.g. soaps, shampoos, perfumes, and nail polishes) (Sparling, 2016). The production of PAE started in the 1930s and has continued to grow until today. It is estimated that around 6 to 8 million tons of PAEs are consumed each year worldwide, of which European consumption accounts for 1 million tons annually (Net et al., 2015).

PAEs are persistent compounds in the environment, with half lives of more than 100 days for BBzP, around 3 years for DMP and even 2000 years for DEHP (Gao and Wen, 2016). However, in biota phthalates are metabolized to monoesters and excreted in urine and faces (Wang & Qian, 2021). High molecular weight (and lipophilic) compounds are more rapidly transformed to their monoesters (Metcalfe et al., 2022). Phthalates have been described as endocrine disruptors involved in the alteration of reproduction, teratogenic and carcinogenic (Net et al., 2015; Pérez-Albaladejo et al., 2017). In the EU several phthalates, including DEHP, DnBP, DiBP, and BBzP, have been classified as toxic to reproduction (ECHA, 2023b). Consequently, these compounds were banned in plastics to make toys and childcare products, cosmetics, and restricted in food contact materials (Lemke et al., 2021). Furthermore, since July 2020, DEHP, DBP, DIBP, and BBzP have been restricted in a wide range of products, such as children's swimming aids, flooring, coated fabrics and paper, recreational gear, mattresses, footwear, and office supplies (ECHA, 2023b). The European Water Directive lists DEHP as a priority substance in surface water, with an AA-EQS of 13000 ng/L (Directive 2013/39/EU). Other phthalates are not legislated.

Bisphenol A (BPA) is a high production volume chemical used since 1950 in the production of epoxy resins and polycarbonate plastics (PC). PC is a polymer widely used in many construction materials such as water pipes, in the food packaging industry as inner coatings of food and beverage cans, and in many other domestic products including sports equipment, compact discs (CDs), and thermal papers (Hahladakis et al., 2022). BPA is a well-known endocrine disruptor with harmful impacts on the reproductive system, and presents high genotoxicity and neurotoxicity (Chen et al., 2016). BPA has been identified as a concerning compound for its widespread detection in the general population, including in human breast milk (Iribarne-Durán et al., 2022) and in children urine (Rodríguez-Carrillo et al., 2019). Its use has been restricted or prohibited in many consumer products in the EU since March 2018, as in thermal paper since January 2020, and in food contact materials and children's toys (ECHA, 2023c). Despite its progressive replacement by

other bisphenolic compounds such as bisphenol S, there is concern about potential similar toxic effects as they present very similar chemical structures and properties (Chen et al., 2016; Molina-Molina et al., 2019). Despite BPA is a high production volume chemical, there are no EQS in water, soil, or biota in Europe.

Benzophenones are a very broad group of compounds which share a benzophenone molecule as a basic structure and different substituents on the benzene ring (Kim and Choi, 2014). Benzophenones are widely used as UV filters in numerous personal care products such as sunscreen and perfumes, and also as plastic and textile additives as they can delay product aging (Ma et al., 2022). Due to their widespread use, benzophenones are continuously released into the environment, mainly through WWTP discharges but also due to human recreational activities such as bathing (related to the application of sunscreens), resulting in their ubiquity in surface water, where they can be bioaccumulated by organisms and also act as endocrine disruptors (Mao et al., 2019). BP and other bisphenolic compounds have also been detected in white-tailed eagle (*Haliaeetus albicilla*) from Smola, in Norway, suggesting that even in remote areas these compounds can be bioaccumulated in wildlife (González-Rubio et al., 2020; Oró-Nolla et al., 2021).

Nonylphenols (NPs) are intermediate products of the degradation of nonylphenol ethoxylates (NPE) used as surfactants and emulsifiers in various industrial processes, including detergents, wetting and dispersing agents, antistatic agents, demulsifiers and solubilisers (Soares et al., 2008). NPs are also used as antioxidants and plasticizers for the production of plastics (Hermabessiere et al., 2017). 4-Nonylphenol (NP hereafter) is a typical endocrine-disrupting chemical due to its estrogenicity (Pérez-Albaladejo et al., 2017). Several governments, including the EU, have phased out or restricted the use of NP in industrial formulations to a maximum 0.1% (Directive 2003/53/EC). Nonetheless, NP still presents an ubiquitous distribution in the aquatic ecosystem owing to its persistence and its widespread past and present use (Hong et al., 2020). NP is included in the list of priority substances in the surface water directive with an AA-EQS of 300 ng/L (Directive 2013/39/EU).

Plasticizers used in plastic production are ultimately released into the environment. This Thesis includes a selection of representative contaminants whose occurrence in the environment is somehow related to plastic production, including phthalates, alkylphenols, BPA, and benzophenone (Figure 1.6).

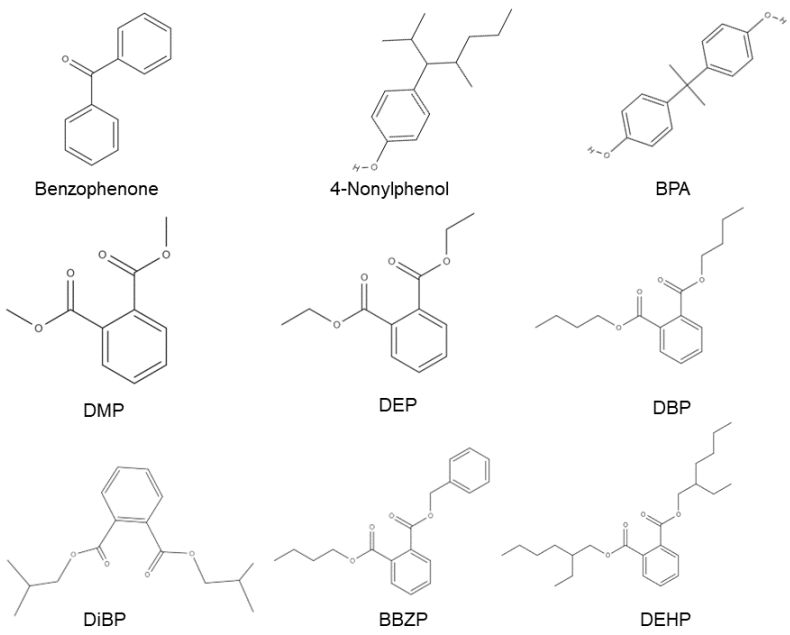

Figure 1.6. Target plasticizers studied.

1.4.6. Polycyclic Aromatic Hydrocarbons (PAHs)

Polycyclic aromatic hydrocarbons (PAHs) are organic pollutants composed of two or more aromatic rings. PAHs are classified according to the number of rings, as low-molecular-weight PAHs (two or three rings; LMW-PAHs) or high-molecular-weight (four or more rings; HMW-PAHs). PAHs are formed by the incomplete combustion of organic matter, including both natural and anthropogenic sources. Natural sources include volcanic eruptions or forest fires. Numerous anthropogenic sources have been identified, related to industrial activities (waste incineration, cement and asphalt manufacturing, chemical and metals production, rubber and tyre manufacturing and burning, power production), vehicle emissions from cars combustion and oil from ships, coal and wood combustion for domestic use, cigarette smoking, and agricultural waste burning and pesticide applications (Patel et al., 2020). PAHs can be also classified according to the process of their formation as pyrogenic or petrogenic sources. Pyrogenic PAHs are HMW PAHs formed due to the incomplete combustion of organic matter and fossil fuels, for example in industrial processes, in the combustion of wood and fossil fuels, wildfires, and volcanic activity. Petrogenic PAHs are LMW PAHs produced spontaneously during crude oil maturation or similar processes, and accordingly, the main sources are oil spills, storage tank leaks, and spills of gasoline and motor oil

(Abdel-Shafy and Mansour, 2016).

PAHs have different volatilities and solubility, and their persistence increases with their molecular weight. LMW-PAHs are soluble in water (1.1 to 31 mg/L at 25ºC) and volatile and are released into water and eventually can be volatilized from water to air, especially when the temperature is high. HMW-PAHs are sorbed to particulate matter in soil or sediment for long periods of time, becoming sinks of PAHs (Alegbeleye et al., 2017). PAHs are also detected in biota samples including in mussels (Bajt et al., 2019), fishes (Ololade et al., 2020), birds (Power et al., 2021) and mammals (Dron et al., 2023). In vertebrates most of the PAHs absorbed are metabolized and excreted, while invertebrates present lower capacity to metabolize PAHs (Lourenço et al., 2021). Due to the metabolization of PAHs, they do not accumulate and biomagnify along the food-web, and thus are not considered POPs. PAHs are well-known activators of the aryl hydrocarbon receptor (AhR), a latent transcription factor with numerous roles in the synthesis of proteins (Yu et al., 2022). PAHs are concerning compounds for their toxicity to organisms as they are carcinogenic, genotoxic, teratogenic and endocrine disruptors for wildlife and humans (Wallace et al., 2020). Some PAHs such as naphthalene, fluoranthene, benzo[a]pyrene, benzo[a]fluoranthene, benzo[k] fluoranthene, benzo[ghi]perylene and indeno[1,2,3-cd]pyrene are included as priority substances in the European Water Framework and EQSs range from 0.17 to 2000 ng/L (Directive 2013/39/EU).

The multiple sources of PAHs make them ubiquitous in the environment. This Thesis has focused on 16 priority PAHs designated by the European Union (EU) and the US EPA due to their known toxicity and widespread distribution in the environment (Figure 1.7).

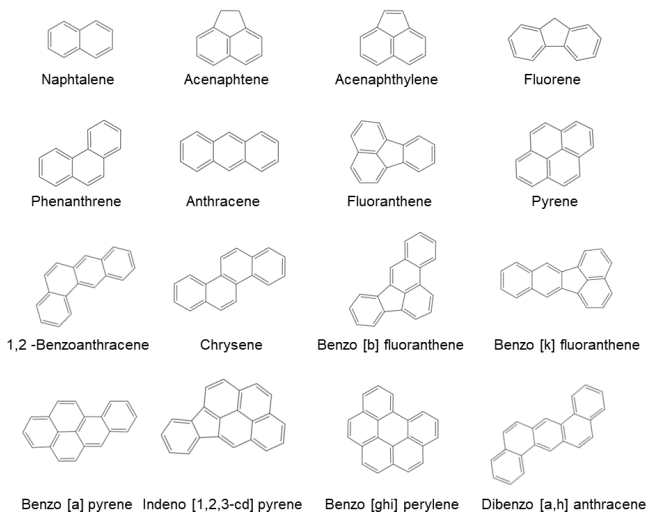

Figure 1.7. Target PAHs studied.

1.4.7. Polychlorinated biphenyls (PCBs)

Polychlorinated biphenyls (PCBs) are a group of organic molecules composed of two benzene rings in which one to ten chlorine atoms can replace hydrogen atoms (Sparling, 2016). There are 209 different PCBs. They are viscous liquids of low volatility with a very high chemical stability.

PCBs were manufactured for the first time in 1929 and used as flame-retardants in transformer fluids. The manufacture of PCBs increased during the Second World War for their use in the war effort by the US government. After the war, the scope of PCBs expanded in numerous applications such as heat transfer fluids, hydraulic fluids, and plasticizers (Erickson and Kaley, 2011). More than 1.3 million tonnes of pure PCBs were produced between 1930 and 1993, resulting in 17 million tonnes of PCBs contaminated materials due to their use and poor management (Breivik et al., 2007). PCBs have entered to the environment through the release of wastes from manufacturing industries, poorly managed disposals, and dumping, becoming widespread in the environment.

PCBs are very persistent and are generally detected in organic-rich matrices, such as soils, sediments or accumulated in biota. PCBs are also biomagnified through the food chain and have been reported at high concentrations in marine top predators such as killer whales (*Orcinus orca*) (Schnitzler et al., 2019) and polar bears (*Ursus maritimus*) (Routti et al., 2019). PCBs bind to the AhR, producing adverse toxic effects to organisms, including endocrine disruption, neurotoxicity, carcinogenicity, and alterations in the reproductive and immune systems (Ngoubeyou et al., 2022).

In 1977 the main producer of PCBs in the USA, Monsanto, stopped their production after ample evidence on their global spread in the environment and toxicity concerns (Markowitz and Rosner, 2018). Other countries banned their production decades after. In Spain, the production of PCBs occurred between 1955 and 1984 with more than 29,000 tonnes produced. The global production of PCBs stopped in 1993, and in 2001 PCBs were included in the Stockholm Convention as POPs (Breivik et al., 2007; Voogt and Brinkman, 1989). Nonetheless, PCBs contamination is not an issue from the past, as nowadays is still widespread in different environment matrices, including biota. The Stockholm convention set a deadline to phase out in-use PCBs by 2025 and ensure the management of PCBs-contaminated materials (>0.005% of PCB content) in 2028, which is estimated to represent the elimination of 1 million tonnes of PCB-containing oils and equipment per year (Melymuk et al., 2022). Today, PCBs are considered legacy contaminants with still high environmental impact.

From the 209 PCBs, this Thesis focuses on seven congeners, the so called "marker PCBs" which are frequently found in environmental samples (Figure 1.8).

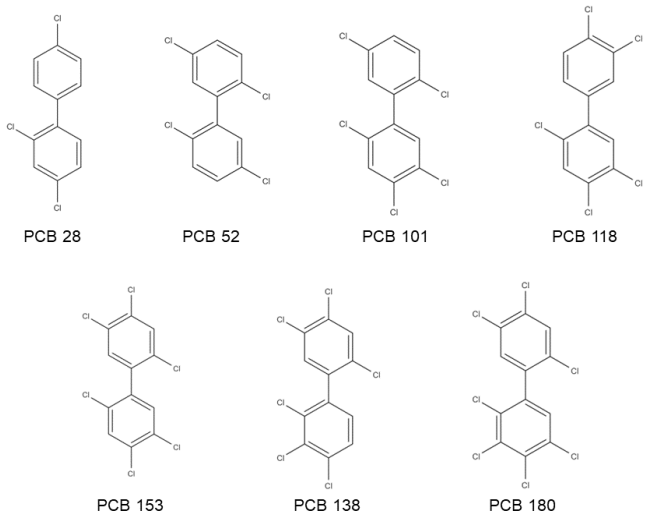

Figure 1.8. Target PCBs studied.

1.4.8. Organochlorine pesticides (OCPs)

Organochlorine pesticides (OCPs) are a large group of molecules that have at least one ring structure with multiple chlorinated atoms and were extensively used as pesticides in the early and mid of the last century (Sparling, 2016). OCPs can be divided into three main types: DDT and its metabolites, hexachlorocyclohexanes (HCHs) and related compounds, and chlorinated cyclodiene insecticides. OCPs were applied worldwide, with an estimated cumulative world production of about 10 million tons from 1945 to 1965 (Tsai, 2014).

Dichlorodiphenyltrichloroethane (DDT) was first synthesised in 1874, but its insecticidal properties were not discovered until 1939 when it became very popular as a vector control disease during the Second World War against malaria and typhus. DDT was widely used in the following years for agricultural and domestic use due to its efficiency as an insecticide and because the low production costs. In fact, nowadays DDT is still applied in some countries to reduce malaria transmission by mosquitoes (Van den Berg et al., 2017). The DDT family of compounds includes DDE, and DDD metabolites, which are formed by the aerobic and anaerobic degradation of the parent compound 4,4'-DDT respectively. The efficiency of DDT led to the synthesis of new organochlorine compounds. In 1942, the insecticidal properties of hexachlorocyclohexanes (HCHs) were acknowledged, being lindane (γ-HCH)

widely applied as seed treatment to control phytophagous and soil-inhabiting insects, and in fruit and vegetable crops, forestry, tobacco and timber (Le Goff and Giraudo, 2019). Shortly, cyclodienes such as aldrin, dieldrin and endosulfan were also discovered and became very popular for spraying applications in agricultural and urban areas for pest and vector disease control. Hexachlorobenzene (HCB) was an organochlorine compound used in industry as a wood-preserving and porosity-control agent, and as a fungicide in seeds for agricultural purposes (Barber et al., 2005). Hexachlorobutadiene (HCBD) is a byproduct of the synthesis of other organochlorine solvents. Its main use is to make rubber compounds, and other uses include scrubbers for removing chlorine-containing contaminants and to produce aluminium and graphite or as a solvent for polymers heat transfer liquid and hydraulic transfer. It was also used for agricultural activities as a vineyard fumigant (Zhang et al., 2019).

The mode of action of OCPs is based on the alteration of the neural function of insects by impairing the sodium chlorine cell flows. However, they are non-selective compounds and the application of these pesticides also affects non-target organisms such as worms, birds, and mammals (Sparling, 2016). OCPs also are known to be endocrine disruptors, capable of interfering with the hormonal function of organisms with implications for their development and reproduction (Mrema et al., 2013). Furthermore, OCPs are persistent and lyophilic compounds, which make them prone to bioaccumulate in the trophic chain and are found at higher concentrations in top predators which are more vulnerable to adverse toxic effects (Sparling, 2016). For example, 4,4'-DDE induced eggshell thinning in several bird species, increasing hatching failure with strong implications in bird populations, especially in raptors (Grove et al., 2009).

In the 1950s it started to grow a general concern for the high mortality rates and reproduction failure of non-target organisms, such as fish and birds (Jarman and Ballschmiter, 2012). In 1962, the publication of "The Silent Spring" by Rachel Carson (Carson, 1962) represented a turning point for the awareness of the harmful implications associated to the use of OCPs treatments for the ecosystem and human health. OCPs started to be phased out in the 1970s, depending on the compounds and countries. The use of OCPs in Spain, including, DDTs, HCHs, and HCB, was totally banned in 1994 (BOE, 1994).

In 2001, OCPs were considered POPs and included in the international agreement of the Stockholm Convention (Stockholm Convention, 2019). Despite its global regulation, OCPs are still widely distributed in the environment, and wildlife is still exposed to their residues. Thus, their presence in the environment has to be monitored. Accordingly, the Directive 2013/39/EU includes several OCPs with established EQS for surface waters, such as DDTs, HCHs, HCB, HCBD, among others.

This Thesis focuses on 14 OCPs: DDTs isomers, HCHs isomers, HCB, HCBD, α-endosulfan and β-endosulfan (Figure 1.9).

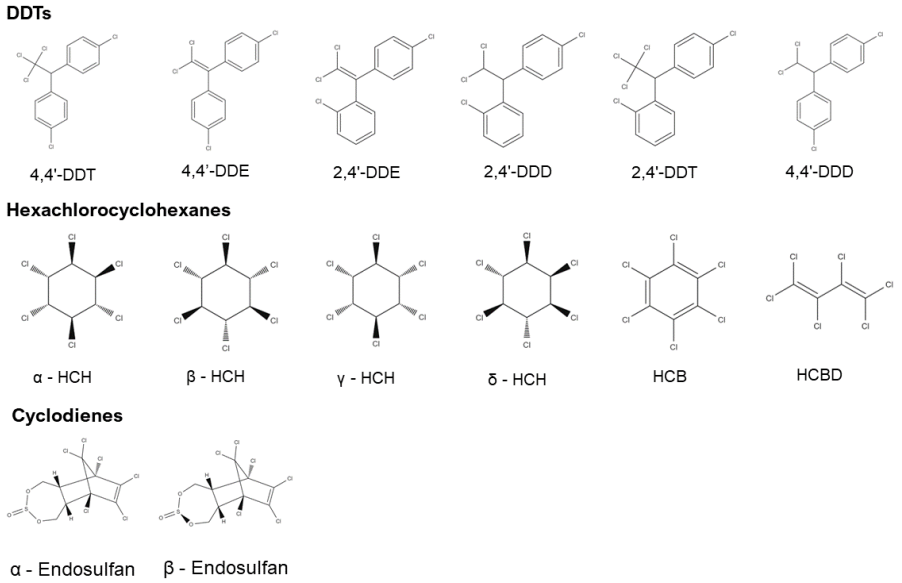

Figure 1.9. Target OCPs studied.

1.5. Monitoring of contaminants in abiotic matrices.

Once contaminants enter the ecosystem they are distributed among different environmental compartments. The fate of organic micropollutants depends on the characteristics of each compartment, as well as the chemical properties of the contaminants themselves. Surface waters, soils, and sediments are monitored to assess their presence, and provide information on contamination patterns, geographical distribution, temporal trends, and impacts. However, not all compounds behave in the same way.

The chemical properties of organic micropollutants can be used to estimate their fate in the environment. The most important chemical properties to consider are their solubility and their polarity, and finally their octanol-water partition coefficient (K_{ow}). The solubility of the chemical substances in water is an important characteristic to estimate their likelihood of detection in the water column and mobility in surface water. It is typically expressed as milligrams per Liter (mg/L) at a specific temperature, usually 25 ºC. The octanol-water partition coefficient (K_{ow}) is a chemical parameter calculated from the equilibrium concentrations of a chemical in a non-polar liquid (octanol) and a polar liquid (water) (eq.1). This parameter is used to determine the level of polarity or hydrophobicity of a chemical compound. K_{ow} is often expressed in logarithmic scale (log K_{ow}) due to the differences in order of magnitude

among compound values. Higher log K_{ow} values indicate that compounds are more likely to bind with organic matter or accumulate in fat tissues, implying a greater tendency to be bioaccumulate in organisms.

$$K_{ow} = \frac{Concentration\ in\ octanol}{Concentration\ in\ water} \qquad \text{eq. 1}$$

The physicochemical properties of the target chemical families are important for determining the compartments in which the target compounds should be monitored. Chemicals may be introduced into the natural ecosystems as a result of multiple anthropogenic activities. Once chemicals are released into the environment, they can be considered contaminants and are distributed through different environmental compartments such as water, soil, sediment, and biota.

1.5.1. Contaminants in freshwater

Surface waters such as rivers and lakes receive organic micropollutants from a wide range of direct or diffuse sources and pathways, and as a result, complex cocktails of contaminants are detected in the µg/L to ng/L range (Adeleye et al., 2022; Morin-Crini et al., 2022; Podder et al., 2021). Polar and soluble compounds are more prone to be found in the water column rather than be adhered to solid particles or sediments, consequently waterbodies are monitored to evaluate their distribution and potential impacts. The hydrophilicity of these polar organic compounds stems largely from the polar functional groups in their molecules, such as those containing oxygen and nitrogen (Christensen et al., 2022). Table 1.1 indicates the physicochemical properties of contaminants studied in surface water, and includes: lifestyle compounds, pharmaceuticals, PFASs, polar pesticides, and OPEs.

Table 1.1. Chemical properties of the target compounds studied in water indicating name, CAS number, formula, molecular weight, water solubility, and Log K_{ow}. Compounds are ordered by chemical family and increasing molecular weight.

	Compound	CAS	Formula	Molecular Weight (g/mol)	Water solubility (mg/L) at 25ºC	Log K_{ow}
Lifestyle compounds	Nicotine	54-11-5	$C_{10}H_{14}N_2$	162.2	1.00E+06	1.17
	Caffeine	58-08-2	$C_8H_{10}N_4O_2$	194.2	2.16E+04	-0.07
Pharmaceuticals	Metformin	657-24-9	$C_4H_{11}N_5$	129.2	1.00E+06	-2.64
	Allopurinol	315-30-0	$C_5H_4N_4O$	136.1	5.69E+02	-0.55
	Paracetamol	103-90-2	$C_8H_9NO_2$	151.2	1.40E+04	0.46
	Levetiracetam	102767-28-2	$C_8H_{14}N_2O_2$	170.2	1.00E+06	-0.49
	Gabapentine	60142-96-3	$C_9H_{17}NO_2$	171.2	2.11E+05	-1.10
	Ibuprofen	15687-27-1	$C_{13}H_{18}O_2$	206.3	2.10E+01	3.97
	Carbamazepine	298-46-4	$C_{15}H_{12}N_2O$	236.3	1.12E+02	2.45
	Sulfamethoxazole	723-46-6	$C_{10}H_{11}N_3O_3S$	253.3	8.69E+02	0.89
	Tramadol	27203-92-5	$C_{16}H_{25}NO_2$	263.4	2.89E+03	2.51
	Atenolol	29122-68-7	$C_{14}H_{22}N_2O_3$	266.3	1.33E+04	0.16
	Venlafaxine	93413-69-5	$C_{17}H_{27}NO_2$	277.4	1.42E+03	3.28
	Pentoxifylline	6493056	$C_{13}H_{18}N_4O_3$	278.3	7.70E+04	0.29
	Diclofenac	15307-86-5	$C_{14}H_{11}Cl_2NO_2$	296.1	1.09E+01	4.51
	Furosemide	54-31-9	$C_{12}H_{11}ClN_2O_5S$	330.7	9.35E+01	2.03
	Trazodone	19794-93-5	$C_{19}H_{22}ClN_5O$	371.9	1.07E+02	3.21
	Quetiapine	111974-69-7	$C_{21}H_{25}N_3O_2S$	383.5	1.58E+03	3.17
	Losartan	114798-26-4	$C_{22}H_{23}ClN_6O$	422.9	9.38E-01	4.01
	Valsartan	137862-53-4	$C_{24}H_{29}N_5O_3$	435.5	2.19E+01	3.65
	Atrovastatin	134523-00-5	$C_{33}H_{35}FN_2O_5$	558.6	1.35E-02	6.36
PFASs	PFBA	375-22-4	$C_4HF_7O_2$	214.0	316	2.14
	PFPA	2706-90-3	$C_5HF_9O_2$	264.1	16.7	2.81
	PFBS	375-73-5	$C_4HF_9O_3S$	300.1	8860	1.82
	PFHxA	307-24-4	$C_6HF_{11}O_2$	314.1	8.53E-01	3.48
	PFHpA	375-85-9	$C_7HF_{13}O_2$	364.1	4.24E-02	4.15
	PFHxS	355-46-4	$C_6HF_{13}O_3S$	400.1	21.7	3.16
	PFOA	335-67-1	$C_8HF_{15}O_2$	414.1	2.00E-03	4.81
	PFNA	375-95-1	$C_9HF_{17}O_2$	464.1	9.94E-05	5.48
	PFOS	1763-23-1	$C_8HF_{17}O_3S$	500.1	5.00E-02	4.49
	PFDA	335-76-2	$C_{10}HF_{19}O_2$	514.1	4.72E-06	6.15
	PFUnA	2058-94-8	$C_{11}HF_{21}O_2$	564.1	5.64E-07	6.82
	PFDS	335-77-3	$C_{10}HF_{21}O_3S$	600.2	1.10E-04	5.83
	PFDoA	307-55-1	$C_{12}HF_{23}O_2$	614.1	6.14E-08	7.49
	PFTriDA	72629-94-8	$C_{13}HF_{25}O_2$	664.1	6.64E-07	8.16
	PFTeDA	376-06-7	$C_{14}HF_{27}O_2$	714.1	7.14E-07	8.83
	PFHxDA	67905-19-5	$C_{16}HF_{31}O_2$	814.1	8.14E-07	10.2
	PFODA	16517-11-6	$C_{18}HF_{35}O_2$	914.1	9.14E-07	11.5

	Compound	CAS	Formula	Molecular Weight (g/mol)	Water solubility (mg/L) at 25ºC	Log K_{ow}
Current use pesticides	Isoproturon	34123-59-6	$C_{12}H_{18}N_2O$	206.3	9.20E+01	2.87
	Chlortoluron	15545-48-9	$C_{10}H_{13}ClN_2O$	212.7	1.36E+02	2.41
	Dimethoate	60-51-5	$C_5H_{12}NO_3PS_2$	229.3	2.33E+04	0.78
	Fludioxonil	131341-86-1	$C_{12}H_6F_2N_2O_2$	248.2	1.80E+00	4.12
	Prosulfocarb	52888-80-9	$C_{14}H_{21}NOS$	251.4	5.69E+00	4.65
	Metalaxyl	57837-19-1	$C_{15}H_{21}NO_4$	279.3	9.25E+03	1.65
	Pendimethalin	40487-42-1	$C_{13}H_{19}N_3O_4$	281.3	3.00E-01	5.19
	Triadimenol	55219-65-3	$C_{14}H_{18}ClN_3O_2$	295.8	1.99E+03	2.90
	Tebuconazol	107534-96-3	$C_{16}H_{22}ClN_3O$	307.8	9.71E+01	3.70
	Kresoxim-methyl	143390-89-0	$C_{18}H_{19}NO_4$	313.3	9.92E-02	3.40
	Diclofop	40843-25-2	$C_{15}H_{12}Cl_2O_4$	327.2	3.84E+00	4.58
	Tebufenpyrad	119168-77-3	$C_{18}H_{24}ClN_3O$	333.9	2.60E+00	4.61
	Chlorpyrifos	2921-88-2	$C_9H_{11}Cl_3NO_3PS$	350.6	1.10E+01	4.96
	Chlorfenvinphos	470-90-6	$C_{12}H_{14}Cl_3O_4P$	359.6	1.98E+01	3.81
	Oxyfluorfen	42874-03-3	$C_{15}H_{11}ClF_3NO_4$	361.7	1.16E-01	4.73
	Pyraclostrobin	175013-18-0	$C_{19}H_{18}ClN_3O_4$	387.8	2.71E-01	3.99
	Isoproturon	34123-59-6	$C_{12}H_{18}N_2O$	206.3	9.20E+01	2.87
	Spinosad	168316-95-8	$C_{83}H_{132}N_2O_20$	1477.9	1.86E+01	5.61
OPEs	Benzophenone	119-61-9	$C_{13}H_{10}O$	182.2	137	3.18
	TBP	126-73-8	$C_{12}H_{27}O_4P$	266.3	280	4.00
	TCEP	115-96-8	$C_6H_{12}Cl_3O_4P$	285.5	7.00	1.44
	TPhP	115-86-6	$C_{18}H_{15}O_4P$	326.3	1.90	4.59

To monitor surface water, the most used method is the grab sampling method or "spot sampling", where water is directly collected by filling a bottle sample (Figure 1.10). This methodology is well established and validated for regulatory and legislation purposes. However, the collected sample is only representative of the time and location at the moment of sampling, as water is continuously changing fluid (Madrid and Zayas, 2007). Typically, 1L of water is collected in an amber glass bottle to avoid light degradation and it is transported refrigerated to the laboratory and stored at 4ºC and analysed shortly after to avoid degradation of the most labile compounds.

Figure 1.10. Sampling of freshwater.

The objective of the extraction procedure is to selectively retain the organic micropollutants present in the samples and concentrate them into an organic solvent. For the analysis of a wide number of compounds, multi-residue methods are employed (Klančar et al., 2018; Sánchez-Avila et al., 2010). The analysis of water is frequently performed by Solid Phase Extraction (SPE) techniques, that allows preconcentrating multiple contaminants in a single step (Amritha et al., 2022; Sabik et al., 2000). These methods consist in passing the water sample through a SPE cartridges that retains the chemical compounds. The SPE cartridges consist of a high-density polypropylene syringe filled by different amounts of sorbent, either silica-based or polymer-based, between two frits (Badawy et al., 2022). The type and amount of sorbent are different according to the target compounds and the total loaded sample. Once the sample is loaded through the SPE cartridges, an elution step is carried out using organic solvents that wash out the chemical compounds. In general, for the analysis of polar compounds such as pharmaceuticals and pesticides, polar solvents such as methanol are preferred for the elution of the target compounds (Badawy et al., 2022). Then the extract is preconcentrated under a flow on N_2 to a final small volume, typically between 0.2 to 1 mL, depending on the expected concentration of target analytes. For the instrumental analysis, liquid chromatography coupled to mass spectrometry (LC-MS/MS) is a commonly used technique for the determination of polar compounds such as pharmaceuticals (Sanusi et al., 2023), pesticides (Gusmaroli et al., 2019), PFASs (Zarębska and Bajkacz, 2023), and OPEs (Wang et al., 2011) in water.

1.5.2. Contaminants in soils and sediments

Soils and sediments are a complex association of mineral particles, organic matter and living organisms. Organic micropollutants with low water solubility and high K_{ow} are prone to become strongly absorbed to soil organic matter (Walker et al., 2012). Because of this, soils and sediments are good matrices to monitor lipophilic and non-polar compounds. Once non-polar compounds are sorbed in soils or sediments they can be retained for a long period and have little tendency to be leached down to groundwater. Table 1.2 indicates the physicochemical properties of those compounds studied in this Thesis that are expected to be retained in soils and sediment compartments and include PAHs, PCBs, OCPs, OPPs, plasticizers, and OPEs.

Table 1.2. Chemical properties of the target compounds studied in soils and sediment indicating name, CAS number, formula, molecular weight, water solubility, and Log K_{ow}. Compounds are ordered by chemical family and molecular weight.

	Compound	CAS	Formula	Molecular Weight (g/mol)	Water solubility (mg/L) at 25ºC	Log Kow
PAHs	Naphthalene	91-20-3	$C_{10}H_8$	128.2	31.0	3.30
	Acenaphthylene	208-96-8	$C_{12}H_8$	152.2	3.93	3.94
	Acenaphthene	83-32-9	$C_{12}H_{10}$	154.2	3.90	3.92
	Fluorene	86-73-7	$C_{13}H_{10}$	166.0	1.69	4.18
	Phenanthrene	85-01-8	$C_{14}H_{10}$	178.2	1.10	4.46
	Anthracene	120-12-7	$C_{14}H_{10}$	178.2	1.29	4.45
	Fluoranthene	206-44-0	$C_{16}H_{10}$	202.3	0.20	5.16
	Pyrene	129-00-0	$C_{16}H_{10}$	202.3	0.14	4.88
	Benzo[a]anthracene	56-55-3	$C_{18}H_{12}$	228.3	9.40E-03	5.76
	Chrysene	218-01-9	$C_{18}H_{12}$	228.3	2.00E-03	5.81
	Benzo[b]fluoranthene	205-99-2	$C_{20}H_{12}$	252.3	1.50E-03	5.78
	Benzo[k]fluoranthene	207-08-9	$C_{20}H_{12}$	252.3	8.00E-04	6.11
	Benzo[a]pyrene	50-32-8	$C_{20}H_{12}$	252.3	1.62E-03	6.13
	Indeno[1,2,3-cd]pyrene	193-39-5	$C_{22}H_{12}$	276.3	1.90E-04	6.70
	Benzo[ghi]perylene	191-24-2	$C_{22}H_{12}$	276.3	2.60E-04	6.63
	Dibenzo[a,h]anthracene	53-70-3	$C_{22}H_{14}$	278.3	6.30E-04	6.50
PCBs	PCB 28	7012-37-5	$C_{12}H_7Cl_3$	257.5	0.34	5.69
	PCB 52	35693-99-3	$C_{12}H_6Cl_4$	292.0	0.09	6.09
	PCB 101	37680-73-2	$C_{12}H_5Cl_5$	326.4	0.01	6.80
	PCB 118	31508-00-6	$C_{12}H_5Cl_5$	326.4	0.01	7.12
	PCB 138	35065-28-2	$C_{12}H_4Cl_6$	360.9	2.36E-03	7.44
	PCB 153	35065-27-1	$C_{12}H_4Cl_6$	360.9	1.28E-03	7.75
	PCB 180	35065-29-3	$C_{12}H_3Cl_7$	395.3	2.84E-04	8.27
OCPs	HCBD	87-68-3	C_4Cl_6	260.8	3.2	4.78
	HCB	118-74-1	C_6Cl_6	284.8	0.3445	5.86
	β-HCH	319-85-7	$C_6H_6Cl_6$	290.8	6.5846	4.26
	γ-HCH (Lindane)	58-89-9	$C_6H_6Cl_6$	290.8	6.5846	3.72
	δ-HCH	319-86-8	$C_6H_6Cl_6$	290.8	6.5846	4.26
	α-HCH	319-84-6	$C_6H_6Cl_6$	290.8	6.5846	4.26
	4,4′-DDE	72-55-9	$C_{14}H_8Cl_4$	318.0	0.065	6.51
	2,4′-DDE	3424-82-6	$C_{14}H_8Cl_4$	318.0	0.04	6.00
	2,4′-DDD	53-19-0	$C_{14}H_{10}Cl_4$	320.0	0.1	5.87
	4,4′-DDD	72-54-8	$C_{14}H_{10}Cl_4$	320.0	0.09	6.02
	2,4′-DDT	789-02-6	$C_{14}H_9Cl_5$	354.5	0.085	6.79
	4,4′-DDT	50-29-3	$C_{14}H_9Cl_5$	354.5	0.0034	6.91
	α-Endosulfan	959-98-8	$C_9H_6Cl_6O_3S$	406.9	0.37	3.83
	β-Endosulfan	19670-15-6	$C_9H_6Cl_6O_3S$	406.9	0.37	3.49

	Compound	CAS	Formula	Molecular Weight (g/mol)	Water solubility (mg/L) at 25ºC	Log Kow
OPPs	Malathion	121-75-5	$C_{10}H_{19}O_6PS_2$	330.4	4.28E+02	2.36
	Chlorpyrifos	2921-88-2	$C_9H_{11}Cl_3NO_3PS$	350.6	1.10E+01	4.96
	Chlorfenvinphos	470-90-6	$C_{12}H_{14}Cl_3O_4P$	359.6	1.98E+01	3.81
Plasticizers	DMP	131-11-3	$C_{10}H_{10}O_4$	194.2	4000	1.60
	NP	25154-52-3	$C_{15}H_{24}O$	220.4	4.90	5.76
	DEP	84-66-2	$C_{12}H_{14}O_4$	222.2	1080	2.42
	BPA	80-05-7	$C_{15}H_{16}O_2$	228.3	120	3.32
	DBP	84-74-2	$C_{16}H_{22}O_4$	278.3	11.2	4.50
	DiBP	84-69-5	$C_{16}H_{22}O_4$	278.3	6.20	4.11
	BBZP	85-68-7	$C_{19}H_{20}O_4$	312.4	2.69	4.73
	DEHP	117-81-7	$C_{24}H_{38}O_4$	390.6	0.27	7.60
OPEs	TCEP	115-96-8	$C_6H_{12}Cl_3O_4P$	285.5	7.00	1.44
	EHDPHP	1241-94-7	$C_{20}H_{27}O_4P$	362.4	1.90	5.73
	TBOEP	78-51-3	$C_{18}H_{39}O_7P$	398.5	1100	3.75
	TDCPP	13674-87-8	$C_9H_{15}Cl_6O_4P$	430.9	7.00	3.65

Approximately 500 g of soil or sediment sample is collected and stored in glass containers or wrapped in aluminium foil for the analysis of organic contaminants. Soils and sediments are heterogeneous matrices and properties such as texture, pH, or composition may affect the sorption of contaminants in the matrix. To obtain a representative sample, several subsamples collected randomly around the sampling area are mixed in a common pool (Hildebrandt et al., 2006). Soils can be collected with different tools depending on the layer of interest, such as stainless shovels or augers (Figure 1.11A). Topsoil samples (0-20 cm) are commonly used for contaminant monitoring, and in most cases the first 4-5 cm are avoided as is the fraction exposed to sunlight or soil erosion that can produce the degradation of contaminants. Vegetation residues, grass, and litter, if present, are removed from the surface before sampling (Orgiazzi et al., 2018). For sediment sampling, usually stainless dredges are used to collect sediments in deep rivers or lakes (Figure 1.11B), in the case of small waterbodies, shovels are also used.

Solid samples usually require a pretreatment before the extraction procedure to obtain a dry and homogenous matrix. Samples can be air/oven dried or freeze-dried to eliminate the total content of water. Once soils and sediments are completely dried, samples are homogenized. Then, it is recommended to sieve the samples to obtain the finest portion of the matrix for the analysis. This fine particle fraction of 125 or 63 µm has the highest content of Total Organic Matter and retain the largest proportion of organic

micropollutants in comparison with larger particles size (Hildebrandt et al., 2006). The extraction of soil and sediments is performed by solid-liquid extraction methods using an organic solvent. The extraction process can be enhanced with a wide range of techniques. For instance, Ultrasonic-Assisted Extraction (UAE) consists in the use of ultrasounds waves to enhance the migration of the compounds from the solid matrix to the liquid phase. It is one of the most used solid-liquid extraction methods as it allows the full extraction within a few minutes, offers high reproducibility, reduction in solvent consumption, simplified manipulation, and a good and selective extraction efficiency (Jinadasa et al., 2023). Other extraction techniques are Soxhlet or Accelerated Solvent Extraction (ASE) but require more sophisticated equipment. Solid-liquid extraction procedures are followed by a clean-up step to obtain a purified extract and avoid matrix effect problems and interferences in the instrumental analysis. The clean-up procedure can be achieved with the application of commercial SPE cartridges filled with florisil, alumina, silica or activated carbon, which are used to retain impurities from the extract, allowing the selective elution of target compounds. As a final step, the resulting aliquots must be preconcentrated to a final volume, typically around 0.1 to 1 mL. Purified concentrated extracts devoid of interferences are needed for the measurement of contaminants with high certainty and accuracy. The determination of non-polar compounds is typically achieved by using Gas Chromatography coupled with tandem Mass Spectrometry (GC-MS/MS) (Barco-Bonilla et al., 2010; Vidal et al., 2010).

Figure 1.11. Sampling of soil (A) and sediment (B).

1.6. Main sources and pathways of contaminants into ecosystems

The main sources of contaminants into natural areas are represented in Figure 1.12. Urban areas are an important source of pollutants that are emitted from activities such as domestic discharges, transportation, construction, combustion, and litter. The contaminants present in urban

environments are washed off during stormwater events, ultimately ending up in surface waters. Stormwater has been identified as a significant pathway for a myriad of micropollutants including PAHs, household and industrial chemicals (Masoner et al., 2019). As an example, cigarette butts, the most common litter in urban areas, are an important source of nicotine in urban waters (Roder Green et al., 2014). Another important pressure associated with urban contamination are roads and associated traffic, which are known to be relevant sources of PAHs (Kicińska and Dmytrowski, 2023) and also other chemical substances leaching from tire wears (McIntyre et al., 2021; Rødland et al., 2023). Consequently, road runoff has also been identified as a major source of micropollutants discharged into rivers and water reservoirs, especially traffic-related compounds such as PAHs and phthalates (Järlskog et al., 2021).

WWTPs effluents are a well-known route of urban chemicals to surface water. Urban waste waters receive a great number of compounds from households (through toilets, showers, sinks, etc.) and other domestic discharges. These waters are treated in WWTPs that are incapable of eliminating many compounds present in influents, and therefore contaminants are released from WWTP effluents to the receiving bodies such as rivers and lakes (Rogowska et al., 2020). WWTPs discharges are considered the main pathway of emerging compounds including pharmaceuticals and lifestyle compounds into surface waters (Lopez-Herguedas et al., 2022).

Agriculture is the main source of pesticides to the environment. Pesticides applied to the soil reach water bodies by surface runoff and by percolation through the soil into the groundwater. Moreover, they can be transported over long distances through volatilization and precipitation (de Souza et al., 2020). Agricultural practices are associated not only with pesticides but also with other substances, such as additives found in pesticide formulations and other organic additives such as plasticizers. Agricultural plastics as mulching films are released into the soil and can be spread to surrounding environments through rainfall and irrigation, and release plasticizers such as phthalates, BPA and OPEs (Cao et al., 2023; Gong et al., 2021).

Another evident cause of contaminants entering the environment is the legal and illegal dumping and landfills, an issue that persists worldwide even in natural areas (Jakiel et al., 2019). All kinds of materials such as food packaging, construction materials, fabrics, etc. are abandoned in natural areas or end up there due to improper waste disposal. The degradation of the abandoned waste materials releases chemical components into soils and water resources. Similarly, the presence of landfills with inadequate management can produce the leaching of contaminants into soils and groundwaters, becoming a source of a wide range of environmental contaminants (Bandala et al., 2021).

Natural sources of contamination must be also considered. Forest fires are a well-known source of PAHs in both terrestrial and aquatic compartments (Campos and Abrantes, 2021). Forestal fires can also contribute to the remobilization of contaminants retained in soils (Isley and Taylor, 2020) and are indirectly related to the release of chemicals in the environment used to extinguish fire such as flame retardants (Campo et al., 2017). Due to the increasingly frequent mega-wildfires, there is growing concern for the occurrence and remobilization of pollutants in fire-affected areas (Chang et al., 2021).

Natural areas are impacted by multiple sources of contaminants even if they are occurring at long distances due to the long-range transportation capacity of contaminants. As a result of the widespread distribution of chemicals in the environment, the earth is now devoid of "pristine" ecosystems (Sigmund et al., 2023). Pollution sources indicated above affect natural areas as IBAs, having an impact on habitats and wildlife. Therefore, it is of outmost importance to determine the sources of pollution, considering the anthropogenic activities affecting these natural sites.

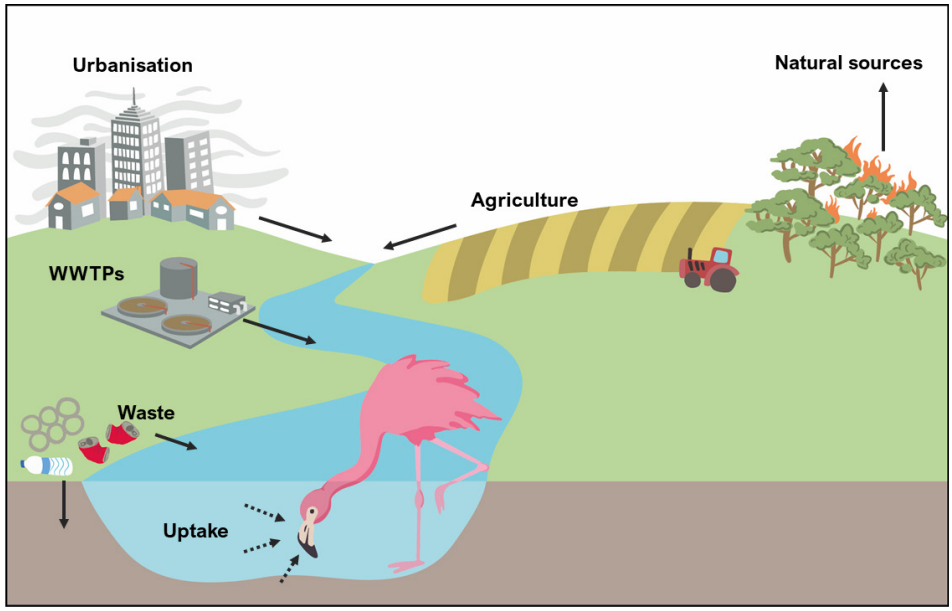

Figure 1.12. Main pathways of organic contaminants to natural areas.

Landscape analysis has emerged as a very useful tool to identify pollution sources in an area, and for the study of the distribution and contamination patterns. The landscape or spatial analysis is based on the geographic information system (GIS), which is a combination of hardware and software programs that can store, manage, manipulate, and visualize geographic data. Geographical data is based on the provision of information encoded

with latitude and longitude coordinates. These geolocations are associated with multiple attributes, for example vectorial layers provide categorical information such as land-use cover (surface occupied by agriculture, artificial, forestal, water etc.), types of soil or vegetation, hydrography, designation of protected areas, etc. Also, geographical data can be linked to quantitative datasets, providing information about the altitude, temperature, precipitation, or population density. These parameters can be integrated with other type of information, such as contaminant concentrations determined in different locations. The use of GIS is spread in environmental fields that require the management of spatial information, for instance in agriculture, urban planning, and many other studies such as wildlife population and movements. GIS is also a useful tool for spatial ecotoxicology that enables identifying hot spots of contamination, using georeferenced monitoring data to derive quantitative exposure-response relationships and assess complex exposures with realism (Eccles et al., 2019).

There are multitude of available spatial databases where data can be downloaded to perform spatial analysis. A relevant source of landscape information is the Corine Land Cover from Copernicus database. It is the primary spatial data source on land covers used by the Environmental European Agency (EEA), and it is widely used for environmental modelling and land cover/use change in the European context (Copernicus, 2023). Corine Land Cover provides a vectorial layer with qualitative information of land cover categories (e.g., agricultural area, artificial surface, forest and semi-natural areas, wetlands, and waterbodies). This qualitative information can be transformed in quantitative data by calculating the percentage of surface occupied in an area. Other relevant layers are available from governmental databases, such as the protected surface under Natura 2000 framework (https://www.eea.europa.eu/data-and-maps), location of WWTP discharges available from the European Commission (www.uwwtd.eu), or the location of industrial sites and urban buildings from the Spanish government database (www.cnig.es). Also, other features such as the presence and typology of roads can be obtained from open-source databases as OpenStreetMap (www.openstreermap.org).

The integration of different spatial information layers and the monitoring dataset results of an expanded database (Figure 1.13). This combined information is utilized in spatial analysis to evaluate the distribution of contaminants and enhance subsequent data analysis. The expanded dataset, which includes both contaminant monitoring and spatial information, enables the assessment of relationships between contaminant occurrence and spatial variables through multivariate analysis techniques such as Principal Component Analysis (PCA), clustering analysis, or regression models. This allows the identification of relevant spatial characteristics related to sources and distribution patterns of contaminants. This information can then be used

for decision support regarding legislative actions, remediation, or evaluation of impacts.

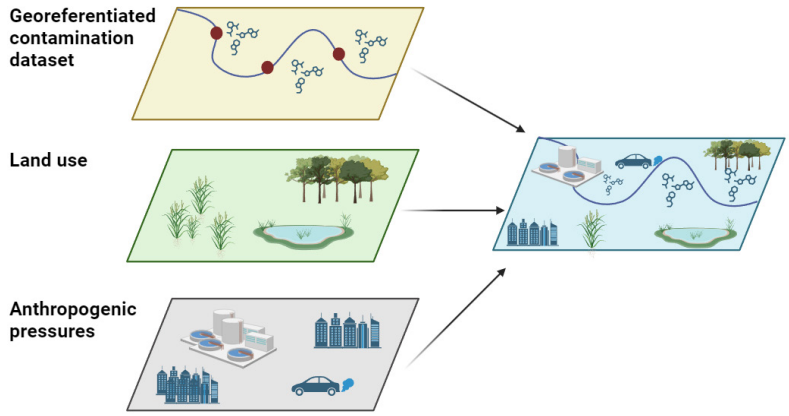

Figure 1.13. Combination of data layers used for spatial analysis.

1.7. Environmental Risk Assessment

The presence of organic micropollutants in the environment does not necessary imply an adverse toxic effect on organisms. Environmental Risk Assessment (ERA) is a process used to predict the likelihood of a chemical substance to produce an adverse impact to the environment (USEPA, 1998). ERA approach is based on the risk characterization of environmental contaminants, which estimates the probability of a specific toxic effect to occur in a wide range of exposures or doses (KEMI, 2020). The characterization of contaminants risk is based on two main factors: the intrinsic hazard of the chemical substances and the environmental exposure to contaminants. A general overview of the process of risk characterization is shown in Figure 1.14.

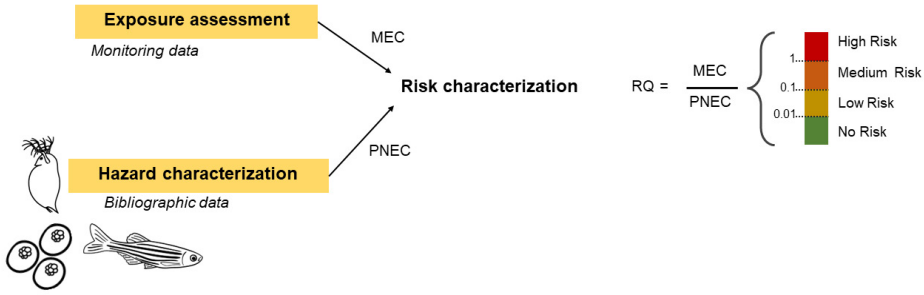

Figure 1.14. Environmental Risk Assessment process based on monitoring data.

The hazard characterization aims to obtain a value to classify the substance regarding its intrinsic hazardous properties for the environment and to determine a non-effect concentration below which toxic adverse effects are not expected. The Predicted Non-Effect Concentration (PNEC) is defined as the highest concentration of a contaminant in the environment at which no adverse effects on the organisms are expected. There are several databases that provide ecotoxicological information of chemical substances, examples of relevant databases are: ECOTOX database from EPA (USEPA, 2023), NORMAN Ecotoxicology Database (NORMAN, 2022) or PubChem from the National Institutes of Health (NIH, 2023). Toxicological information is based on experimental studies performed with laboratory models. For its practicality and ethical reasons, most of the toxicological testing is performed with aquatic organisms such as algae, daphnids or fishes. Especially, in the case of pharmaceuticals, environmental toxicity studies are commonly performed with aquatic organisms as are the group expected to be directly affected by environmental concentration of pharmaceuticals present in the aquatic ecosystems (Vestel et al., 2016). The measured effect in aquatic species can be also extrapolated to other environmental compartments such as sediment or soil (KEMI, 2020). Experimental studies are not available for all chemical substances, in this case toxicological information can be estimated using quantitative structure-activity relationship (QSAR) models, which are mathematical algorithms that predict the physicochemical, biological, and environmental fate properties of compounds based on their chemical structures (ECHA, 2016). This approach has raised interest in the recent years as it has the potential to reduce the cost and number of animals used in laboratory. For risk assessment within the EU the PNEC values are calculated by applying an assessment factor (AF) to toxicological reference values such as EC_{10} (Effect Concentration that affects the 10% of the population) or NOEC (No-Observed-Effect Concentration) for the most sensitive tested organisms (KEMI, 2020).

The extent of exposure of the target contaminants to the environment can be predicted using exposure models to obtain the Predicted Environmental Concentrations (PEC) or can be evaluated using Measured Environmental Concentrations (MEC) based on the monitoring data of contaminants in the environment.

Finally, the risk characterization is based on the calculation of risk quotients (RQs) as the ratio between the MEC (or PEC in case of monitoring data not available) and the PNEC values. The resulting RQs provides the probability of the contaminants to produce an adverse effect to the exposed organisms, when the MEC are higher than the PNEC values it is indicative of high-risk concentrations, as toxic adverse effects to exposed organism are expected (Figure 1.14). Although the risk is calculated based on aquatic organisms, the affection on these organisms may have repercussions on the trophic web,

leading to habitat degradation and ultimately affecting higher trophic levels such as birds' populations.

1.8. Biomonitoring of contaminants in birds

Birds are recognized as valuable sentinel species for the biomonitoring of contaminants, as they are widely distributed, are long-lived animals and are found in a large range of habitats and trophic positions (Gómez-Ramírez et al., 2014). Figure 1.15 shows a scheme of biomonitoring strategies based on birds. Sampling of birds is often based on active monitoring efforts when the collection of samples requires the manipulation of the live bird for example to obtain blood or plasma, picking up feathers or preen oil, or without manipulation collecting feathers, eggs, or pellets in active nest. On the other hand, passive or non-invasive monitoring is based on the collection of samples from carcasses or in non-active nests to collect desert eggs, feathers, faeces, or pellets. Passive monitoring is gaining more attention in recent years as it presents more ethical and practical advantages due to the lack of animal disturbance (Espín et al., 2021). When sampling biological matrices it is important to collect mandatory information about the bird, including specie, sex, age, and body condition and pathological issues or cause of death (Espín et al., 2021). Also, other contextual data regarding their ecological traits such as diet, habitat or reproductive performance is of interest for the correct interpretation of the contaminant results (Ratajc et al., 2023). Also, different bird's tissues can be used to perform chemical analysis such as feathers, preen oil, eggs, blood, and internal organs, as shown in Figure 1.15. However, not all tissues are suitable to analyse all chemical contaminants, and a few considerations must be made before establishing biomonitoring programmes based on birds, considering the distribution of the different species, the sampling effort, and the type of contaminants to be monitored.

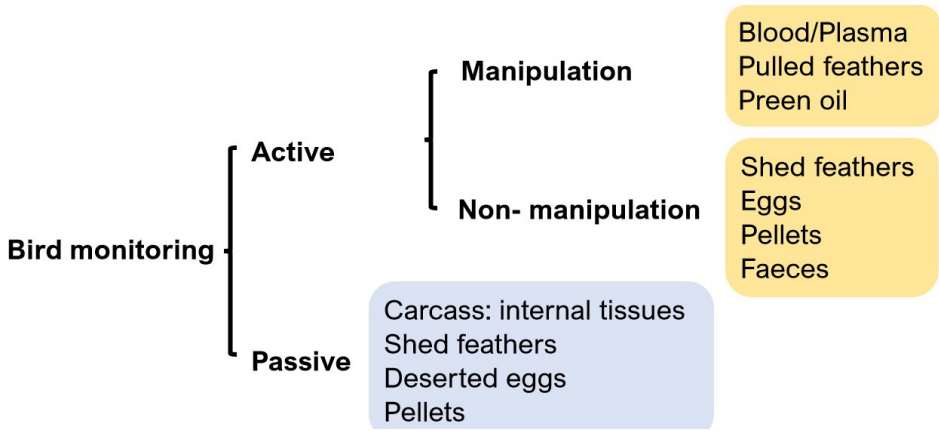

Figure 1.15. Examples of samples obtained from birds by performing active and passive monitoring.

Blood is a common tissue used in active monitoring programmes. Once micropollutants are absorbed into the organism they are transported and distributed by the bloodstream to other internal tissues. The fate and transport of micropollutants in the body depends on their chemical structure and properties. Polar compounds (low K_{ow}) tend to be dissolved in blood, while lipophilic compounds (high K_{ow}) tend to associate with fat, lipoproteins and membranes of blood cells (Walker et al., 2012). The half-lives of contaminants in blood are typically shorter than in other matrices; because of this the analysis of blood provides a measurement of the recent exposure to compounds (Espín et al., 2016). It must be considered that physiological alterations in birds' bodies, for example during the breeding season, can produce remobilization of compounds stored in fat tissues to the bloodstream (Thorstensen et al., 2021). Contaminants can be measured in whole blood samples or in fractions of blood such as plasma, serum or blood cells depending on the contaminants of interest. In general, the analysis of whole blood prevents potential loss of compounds in the cellular fraction and a smaller volume is needed for the analysis, which is interesting considering that the volume of blood extracted from birds cannot exceed 1% of the birds body weight to ensure the bird well-being (Espín et al., 2016; Fair and Jones, 2010). Blood must be collected by veterinarians or trained and authorised personnel. Samples are collected with hypodermic needles and syringes, with optimum gauge size according to the mass of the bird, from brachial, tarsus or jugular vein (Figure 1.16) (Espin et al., 2014) and placed in tubes with anticoagulant such as EDTA or heparin if recommended. Samples must transport refrigerated (4ºC) and then stored at -21ºC until chemical analysis.

Figure 1.16. Extraction of blood from tarsal vein.

Table 1.3 indicates studies using blood to monitor several chemical families. Bird's blood have been used to determine the exposure of birds to emerging compounds such as pharmaceuticals (Bean et al., 2018; Blanco et al., 2023), pesticides (Rodrigues et al., 2023), and PFASs (Custer et al., 2019; Thorstensen et al., 2021). The analysis of these families of contaminants is commonly performed using a liquid-liquid extraction, which can be accelerated by vortex and ultrasonication, followed by a clean-up for the purification of the samples performed with SPE methods. As blood is a matrix with low content of lipids, the clean-up procedure is not always necessary. The determination of the

analytes is achieved by LC methods. Other contaminants studied in avian blood are PCBs (da Silva et al., 2023; Goutner et al., 2011; Thorstensen et al., 2021), OCPs (Abbasi et al., 2017; da Silva et al., 2023; Goutner et al., 2011) and PAHs (Jodice et al., 2023; Morin-crini et al., 2020). For these chemical families, the clean-up procedure consists of SPE cartridges typically alumina, silica, or florisil, and the determination of analytes is achieved by GC analysis.

Table 1.3. Birds' biomonitoring studies using blood to determine the chemical families under study. Indicating species, location, extraction and cleanup procedures, type of analysis, and concentration ranges. Concentrations expressed in ng/g w.w. or ng/mL if indicated. UAE stands for Ultrasonic-Assisted Extraction.

Contaminant	Species and location	Extraction and Clean up	Analysis	Concentration range	Ref.
Pharmaceuticals (Plasma)	Osprey (*Pandion haliaetus*) USA	UAE liquid-liquid and SPE	LC/MS-MS	Acetaminophen 2.85 to 3.95 ng/mL Diclofenac: LOD - 3.73 ng/mL	(Bean et al., 2018)
Pharmaceuticals	White stork (*Ciconia ciconia*) Spain	UAE liquid-liquid	HPLC-TOF-MS	Acetaminophen: 9.45 ng/mL Marbofloxacin: 7.21 ng/mL	(Blanco et al., 2023)
Current use pesticides and personal products	Grey partridges (Perdix perdix)France	UAE liquid-liquid	LC-MS/MS	0.010 - 71.38	(Rodrigues et al., 2023)
PFASs (Plasma)	Tree swallow (*Tachycineta bicolor*) USA	SPE weak anion exchange cartridges	LC-MS/MS	0.5 - 6.91 ng/mL	(Custer et al., 2019)
PFASs	Herring gull (*Larus argentatus*) Common eider (*Somateria mollissima*) Norway	Liquid-liquid extraction	UPLC-QTOF	Herring gull: <LOD - 31.3 Common eider <LOD - 45.2	(Thorstensen et al., 2021)
PCBs	Herring gull (*Larus argentatus*) Common eider (*Somateria mollissima*) Norway	Liquid-liquid extraction and clean up with silica column	GC/MS-MS	Herring gull: <LOD - 65.1 Common eider: 92.6 - 543	(Thorstensen et al., 2021)
PCBs	Cinereous vulture (*Aegypius monachus*) Griffon vulture (*Gyps fulvus*) Greece	UAE Liquid-liquid and clean up with silica, alumina and Na2SO4	GC-MSD	Cinereus vulture: 2.86 - 21.2 Griffon vulture: 3.58 - 20.4	(Goutner et al., 2011)
PCBs	Trindade petrel (*Pterodroma arminjoniana*) Brazil	Liquid-liquid vortex assisted, clean up with acidified silica	GC/MS-MS	7.28 - 55.1	(da Silva et al., 2023)
DDTs	Trindade petrel (*Pterodroma arminjoniana*) Brazil	Liquid-liquid vortex assisted, clean up with silica column	GC/MS-MS	2.62 - 17.4	(da Silva et al., 2023)

Contaminant	Species and location	Extraction and Clean up	Analysis	Concentration range	Ref.
OCPs	Cinereous vulture (*Aegypius monachus*) Griffon vulture (*Gyps fulvus*) Greece	UAE Liquid-liquid and clean up with silica, alumina and Na2SO4	GC-MSD	Cinereus vulture: 5.05 - 27.65 Griffon vulture: 9.42 - 45.27	(Goutner et al., 2011)
OCPs	Black kite (*Milvus migrans*) Spotted owlet (*Athene brama*) Pakistan	Soxhlet, clean up with silica	GC-MS/MS	Black kite: 0.08 - 16.68 Spotted owlet: LOD - 13.3	(Abbasi et al., 2017)
PAHs	Red Kite (*Milvus milvus*) France	Liquid-liquid vortex assisted	GC/MS-MS	LOD- 8.5 ng/mL	(Morin-crini et al., 2020)
PAHs	Brown pelicans (*Pelecanus occidentalis*) Mexico	Liquid-liquid vortex assisted	GC/MS-MS	42.4 - 326.6	(Jodice et al., 2023)

Liver is by far the most commonly analysed internal tissue for contaminant monitoring (Espín et al., 2016). After the distribution of compounds through the bloodstream one of the first organs to receive blood after the uptake of nutrients (and contaminants) in the alimentary tract is liver whose function is to detoxify blood. Consequently, the liver becomes the initial recipient of contaminants from the bloodstream. Given the high adipose nature of liver, persistent and lipophilic compounds, like POPs, tend to be bioaccumulated in this tissue at a higher rate than its elimination. Because of this, the contaminant concentrations in liver are a key indicator of bioaccumulation and long-term exposure (Espín et al., 2016). It must be considered that organisms accumulate persistent contaminants through time, as a result, older individuals tend to present higher concentrations than younger ones. The obtention of liver from protected bird species is limited to the collection of dead individuals in the field or rehabilitations centres, though it is worth mentioning that passive monitoring using liver samples requires a lower level of expertise and permits than the collection of other matrices that involve the manipulation of live birds. Internal tissue samples are obtained by practising necropsies of the dead birds (Figure 1.17). Necropsies should be carried out by trained personnel following protocols to avoid both potential exposure to zoonotic diseases and

Figure 1.17. Necropsy of a bird of prey to collect liver sample.

chemical contamination of the sample (Espín et al., 2021). If the necropsy cannot be performed on the fresh carcass, it can be frozen at -21°C, and thawed before necropsy. Liver samples should be folded in aluminium foil and stored in freezers until chemical analysis.

There are numerous studies on livers exemplifying the usefulness of this matrix to stablish monitoring programmes with dead birds. Table 1.4 indicates the methods used to analyse the contaminants in liver samples. Livers are freeze dried and homogenized with a mortar and a pestle before chemical analysis. Several studies have performed biomonitoring studies using livers from dead top- predators and scavengers to determine the levels of emerging compounds such as BPA and benzophenone (Oró-Nolla et al., 2021), pharmaceuticals (Badry et al., 2022; Herrero-villar et al., 2020), OPEs (Verreault et al., 2018), polar pesticides and PFASs (Badry et al., 2022). The determination of these compounds is achieved by solid-liquid extraction followed by a clean-up with SPE cartridges, although some procedures only consist of the filtration and dilution of the sample without purification step. The determination of the compounds is performed by LC or GC methods depending on the compounds to be analysed. Most studies are directed to monitor OCPs (Acampora et al., 2017; Badry et al., 2022; Espín et al., 2010; Roque et al., 2022), PCBs (Acampora et al., 2017; Badry et al., 2022) and PAHs (Acampora et al., 2017; Morin-crini et al., 2020) as the methods used to analyse this group of compounds are better stablished than those for emerging compounds. As a result of this, little information is available in the open bibliography to determine the presence and impact of pharmaceuticals,v pesticides, or plasticizers in birds.

Table 1.4. Birds' biomonitoring studies using liver to determine the chemical families under study. Indicating species, location, extraction and cleanup procedures, type of analysis, and concentration ranges. Concentrations expressed in ng/g w.w. ASE stands for Accelerated Solvent Extraction.

Contaminant	Species and location	Extraction and Clean up	Analysis	Concentration range	Ref.
BPA and benzophenones	White-Tailed Eagle (*Haliaeetus albicilla*) Norway	Solid-liquid	UPLC-MS/MS	BPA: 3.36 - 33.8 Bzp: 2.07 - 7.94	(Oró-Nolla et al., 2021)
Pharmaceuticals	Griffon vulture (*Gyps fulvus*) Spain	Solid-liquid UAE syringe-filtered	LC-ESI-MS LC-QTOF-MS	0.02 - 4.91	(Herrero-villar et al., 2020)
Pharmaceuticals	White-Tailed Eagle (*Haliaeetus albicilla*) Germany	ASE, clean up with SPE mix (Oasis HLB, Strata X-CW, X-AW, Isolute ENV+)	LC-/ GC-HR-MS	1.76 - 49.9	(Badry et al., 2022)

Contaminant	Species and location	Extraction and Clean up	Analysis	Concentration range	Ref.
Current use pesticides	White-Tailed Eagle (*Haliaeetus albicilla*) Germany	ASE, clean up with SPE mix (Oasis HLB, Strata X-CW, X-AW, Isolute ENV+)	LC-/ GC-HR-MS	1.04 - 53.7	(Badry et al., 2022)
PFASs	White-Tailed Eagle (*Haliaeetus albicilla*) Germany	ASE, clean up with SPE mix (Oasis HLB, Strata X-CW, X-AW, Isolute ENV+)	LC-/ GC-HR-MS	0.05 - 773	(Badry et al., 2022)
OPEs	Glaucous gull (*Larus hyperboreus*) Canada	Solid-liquid ASE, clean up silica gel	LC-/ GC-HR-MS	0.18 - 82.1	(Verreault et al., 2018)
DDTs	White-Tailed Eagle (*Haliaeetus albicilla*) Germany	ASE and clean up with florisil	LC-/ GC-HR-MS	0.79 - 222	(Badry et al., 2022)
OCPs	Common tern (*Sterna hirundo*) Ireland	Solid-liquid, clean up with silica gel	GC/MS	4.84 - 38.1	(Acampora et al., 2017)
OCPs	Barn Owl (*Tyto alba*) Portugal	ASE and clean up with florisil	GC/MS	22 - 448	(Roque et al., 2022)
OCPs	Razorbills (*Alca torda*) Spain	ASE and clean up with florisil	GC ECD	495 - 14696	(Espín et al., 2010)
PCBs	Common tern (*Sterna hirundo*) Ireland	Solid-liquid, clean up with silica gel	GC/MS	11 - 104	(Acampora et al., 2017)
PCBs	White-Tailed Eagle (*Haliaeetus albicilla*) Germany	ASE and clean up with florisil	LC-/ GC-HR-MS	0.29 - 502	(Badry et al., 2022)
PAHs	Common tern (*Sterna hirundo*) Ireland	Solid-liquid, clean up with silica gel	GC/MS	4.49 - 78.8	(Acampora et al., 2017)
PAHs	Red Kite (*Milvus milvus*) France	Solid-liquid vortex assisted	GC/MS-MS	LOD- 130	(Morincrini et al., 2020)

1.9. References

Abbasi, N.A., Arukwe, A., Jaspers, V.L.B., Eulaers, I., Mennilo, E., Ibor, O.R., Frantz, A., Covaci, A., Malik, R.N., 2017. Oxidative stress responses in relationship to persistent organic pollutant levels in feathers and blood of two predatory bird species from Pakistan. Sci. Total Environ. 580, 26–33. https://doi.org/https://doi.org/10.1016/j.scitotenv.2016.11.197

Abbasi, N.A., Shahid, S.U., Majid, M., Tahir, A., 2022. Chapter 1 - Introduction to environmental micropollutants, in: Hashmi, M.Z., Wang, S., Ahmed, Z. (Eds.), Environmental Micropollutants, Advances in Pollution Research. Elsevier, pp. 1–12. https://doi.org/https://doi.org/10.1016/B978-0-323-90555-8.00010-6

Abdel-Shafy, H.I., Mansour, M.S.M., 2016. A review on polycyclic aromatic hydrocarbons: Source, environmental impact, effect on human health and remediation. Egypt. J. Pet. 25, 107–123. https://doi.org/https://doi.org/10.1016/j.ejpe.2015.03.011

Acampora, H., White, P., Lyashevska, O., O'Connor, I., 2017. Presence of persistent organic pollutants in a breeding common tern (*Sterna hirundo*) population in Ireland. Environ. Sci. Pollut. Res. 24, 13025–13035. https://doi.org/10.1007/s11356-017-8931-7

Adeleye, A.S., Xue, J., Zhao, Y., Taylor, A.A., Zenobio, J.E., Sun, Y., Han, Z., Salawu, O.A., Zhu, Y., 2022. Abundance, fate, and effects of pharmaceuticals and personal care products in aquatic environments. J. Hazard. Mater. 424, 127284. https://doi.org/10.1016/j.jhazmat.2021.127284

Alegbeleye, O.O., Opeolu, B.O., Jackson, V.A., 2017. Polycyclic aromatic hydrocarbons: a critical review of environmental occurrence and bioremediation. Environ. Manage. 60, 758–783.

Amritha, P.S., Vinod, V., Harathi, P.B., 2022. A critical review on extraction and analytical methods of phthalates in water and beverages. J. Chromatogr. A 1675, 463175. https://doi.org/https://doi.org/10.1016/j.chroma.2022.463175

Ancora, S., Bianchi, N., Leonzio, C., Renzoni, A., 2008. Heavy metals in flamingos (*Phoenicopterus ruber*) from Italian wetlands: The problem of ingestion of lead shot. Environ. Res. 107, 229–236. https://doi.org/10.1016/j.envres.2008.02.004

Androulakakis, A., Alygizakis, N., Gkotsis, G., Nika, M.C., Nikolopoulou, V., Bizani, E., Chadwick, E., Cincinelli, A., Claßen, D., Danielsson, S., Dekker, R.W.R.J., Duke, G., Glowacka, N., Jansman, H.A.H., Krone, O., Martellini, T., Movalli, P., Persson, S., Roos, A., O'Rourke, E., Siebert, U., Treu, G., van den Brink, N.W., Walker, L.A., Deaville, R., Slobodnik, J., Thomaidis, N.S., 2022. Determination of 56 per- and polyfluoroalkyl substances in top predators and their prey from Northern Europe by LC-MS/MS. Chemosphere 287, 131775. https://doi.org/10.1016/J.CHEMOSPHERE.2021.131775

Aulsebrook, L.C., Bertram, M.G., Martin, J.M., Aulsebrook, A.E., Brodin, T., Evans, J.P., Hall, M.D., O'Bryan, M.K., Pask, A.J., Tyler, C.R., Wong, B.B.M., 2020. Reproduction in a polluted world: implications for wildlife. Reproduction 160, R13–R23. https://doi.org/10.1530/REP-20-0154

Badawy, M.E.I., El-Nouby, M.A.M., Kimani, P.K., Lim, L.W., Rabea, E.I., 2022. A review of the modern principles and applications of solid-phase extraction techniques in chromatographic analysis. Anal. Sci. 38, 1457–1487. https://doi.org/10.1007/s44211-022-00190-8

Badry, A., Treu, G., Gkotsis, G., Nika, M.-C., Alygizakis, N., Thomaidis, N.S., Voigt, C.C., Krone, O., 2022. Ecological and spatial variations of legacy and emerging contaminants in white-tailed sea eagles from Germany: Implications for prioritisation and future risk management. Environ. Int. 158, 106934. https://doi.org/https://doi.org/10.1016/j.envint.2021.106934

Bajt, O., Ramšak, A., Milun, V., Andral, B., Romanelli, G., Scarpato, A., Mitrić, M., Kupusović, T., Kljajić, Z., Angelidis, M., Çullaj, A., Galgani, F., 2019. Assessing chemical contamination in the coastal waters of the Adriatic Sea using active mussel biomonitoring with Mytilus galloprovincialis. Mar. Pollut. Bull. 141, 283–298. https://doi.org/https://doi.org/10.1016/j.marpolbul.2019.02.007

Bandala, E.R., Liu, A., Wijesiri, B., Zeidman, A.B., Goonetilleke, A., 2021. Emerging materials and technologies for landfill leachate treatment: A critical review. Environ. Pollut. 291, 118133. https://doi.org/https://doi.org/10.1016/j.envpol.2021.118133

Barber, J.L., Sweetman, A.J., van Wijk, D., Jones, K.C., 2005. Hexachlorobenzene in the global environment: Emissions, levels, distribution, trends and processes. Sci. Total Environ. 349, 1–44. https://doi.org/https://doi.org/10.1016/j.scitotenv.2005.03.014

Barco-Bonilla, N., Romero-González, R., Plaza-Bolaños, P., Garrido Frenich, A., Martínez Vidal, J.L., 2010. Analysis and study of the distribution of polar and non-polar pesticides in wastewater effluents from modern and conventional treatments. J. Chromatogr. A 1217, 7817–7825. https://doi.org/https://doi.org/10.1016/j.chroma.2010.10.011

Bean, T.G., Rattner, B.A., Lazarus, R.S., Day, D.D., Burket, S.R., Brooks, B.W., Haddad, S.P., Bowerman, W.W., 2018. Pharmaceuticals in water , fi sh and osprey nestlings in Delaware River. Environ. Pollut. 232, 533–545. https://doi.org/10.1016/j.envpol.2017.09.083

BirdLife International, 2023. Data Zone [WWW Document]. URL http://www.birdlife.org/datazone (accessed 7.11.23).

BirdLife International, 2022. State of the World's Birds 2022: Insights and solutions for the biodiversity crisis. Cambridge, UK.

BirdLife International, 2006. Monitoring Important Bird Areas: a global framework.

Blanco, G., Gómez-Ramírez, P., Espín, S., Sánchez-Virosta, P., Frías, Ó., García-Fernández, A.J., 2023. Domestic Waste and Wastewaters as Potential Sources of Pharmaceuticals in Nestling White Storks (*Ciconia ciconia*). Antibiotics 12. https://doi.org/10.3390/antibiotics12030520

BOE, 1994. Orden de 4 de febrero de 1994 por la que se prohíbe la comercialización y utilización de plaguicidas de uso ambiental que contienen determinados ingredientes activos peligrosos.

Boisvert, G., Sonne, C., Rigét, F.F., Dietz, R., Letcher, R.J., 2019. Bioaccumulation and biomagnification of perfluoroalkyl acids and precursors in East Greenland polar bears and their ringed seal prey. Environ. Pollut. 252, 1335–1343. https://doi.org/https://doi.org/10.1016/j.envpol.2019.06.035

Bouzas-Monroy, A., Wilkinson, J.L., Melling, M., Boxall, A.B.A., 2022. Assessment of the Potential Ecotoxicological Effects of Pharmaceuticals in the World's Rivers. Environ. Toxicol. Chem. 41, 2008–2020. https://doi.org/https://doi.org/10.1002/etc.5355

Breivik, K., Sweetman, A., Pacyna, J.M., Jones, K.C., 2007. Towards a global historical emission inventory for selected PCB congeners—a mass balance approach: 3. An update. Sci. Total Environ. 377, 296–307. https://doi.org/https://doi.org/10.1016/j.scitotenv.2007.02.026

Brunn, H., Arnold, G., Körner, W., Rippen, G., Steinhäuser, K.G., Valentin, I., 2023. PFAS: forever chemicals—persistent, bioaccumulative and mobile. Reviewing the status and the need for their phase out and remediation of contaminated sites. Environ. Sci. Eur. 35, 20. https://doi.org/10.1186/s12302-023-00721-8

Buck, R.C., Franklin, J., Berger, U., Conder, J.M., Cousins, I.T., de Voogt, P., Jensen, A.A., Kannan, K., Mabury, S.A., van Leeuwen, S.P.J., 2011. Perfluoroalkyl and polyfluoroalkyl substances in the environment: Terminology, classification, and origins. Integr. Environ. Assess. Manag. 7, 513–541. https://doi.org/https://doi.org/10.1002/ieam.258

Burns, F., Eaton, M.A., Burfield, I.J., Klvaňová, A., Šilarová, E., Staneva, A., Gregory, R.D., 2021. Abundance decline in the avifauna of the European Union reveals cross-continental similarities in biodiversity change. Ecol. Evol. 11, 16647–16660. https://doi.org/https://doi.org/10.1002/ece3.8282

Butchart, S.H.M., Clarke, M., Smith, R.J., Sykes, R.E., Scharlemann, J.P.W., Harfoot, M., Buchanan, G.M., Angulo, A., Balmford, A., Bertzky, B., Brooks, T.M., Carpenter, K.E., Comeros-Raynal, M.T., Cornell, J., Ficetola, G.F., Fishpool, L.D.C., Fuller, R.A., Geldmann, J., Harwell, H., Hilton-Taylor, C., Hoffmann, M., Joolia, A., Joppa, L., Kingston, N., May, I., Milam, A., Polidoro, B., Ralph, G., Richman, N., Rondinini, C., Segan, D.B., Skolnik, B., Spalding, M.D., Stuart, S.N., Symes, A., Taylor, J., Visconti, P., Watson, J.E.M., Wood, L., Burgess, N.D., 2015. Shortfalls and Solutions for Meeting National and Global Conservation Area Targets. Conserv. Lett. 8, 329–337. https://doi.org/https://doi.org/10.1111/conl.12158

Butchart, S.H.M., Scharlemann, J.P.W., Evans, M.I., Quader, S., Aricò, S., Arinaitwe, J., Balman, M., Bennun, L.A., Bertzky, B., Besançon, C., Boucher, T.M., Brooks, T.M., Burfield, I.J., Burgess, N.D., Chan, S., Clay, R.P., Crosby, M.J., Davidson, N.C., de Silva, N., Devenish, C., Dutson, G.C.L., Fernández, D.F.D., Fishpool, L.D.C., Fitzgerald, C.,

Foster, M., Heath, M.F., Hockings, M., Hoffmann, M., Knox, D., Larsen, F.W., Lamoreux, J.F., Loucks, C., May, I., Millett, J., Molloy, D., Morling, P., Parr, M., Ricketts, T.H., Seddon, N., Skolnik, B., Stuart, S.N., Upgren, A., Woodley, S., 2012. Protecting important sites for biodiversity contributes to meeting global conservation targets. PLoS One 7. https://doi.org/10.1371/journal.pone.0032529

Calatayud-Vernich, P., Calatayud, F., Simó, E., Picó, Y., 2018. Pesticide residues in honey bees, pollen and beeswax: Assessing beehive exposure. Environ. Pollut. 241, 106–114. https://doi.org/https://doi.org/10.1016/j.envpol.2018.05.062

Calatayud-Vernich, P., Calatayud, F., Simó, E., Suarez-Varela, M.M., Picó, Y., 2016. Influence of pesticide use in fruit orchards during blooming on honeybee mortality in 4 experimental apiaries. Sci. Total Environ. 541, 33–41. https://doi.org/https://doi.org/10.1016/j.scitotenv.2015.08.131

Campanale, C., Massarelli, C., Losacco, D., Bisaccia, D., Triozzi, M., Uricchio, V.F., 2021. The monitoring of pesticides in water matrices and the analytical criticalities: A review. TrAC Trends Anal. Chem. 144, 116423. https://doi.org/https://doi.org/10.1016/j.trac.2021.116423

Campo, J., Lorenzo, M., Cammeraat, E.L.H., Picó, Y., Andreu, V., 2017. Emerging contaminants related to the occurrence of forest fires in the Spanish Mediterranean. Sci. Total Environ. 603–604, 330–339. https://doi.org/https://doi.org/10.1016/j.scitotenv.2017.06.005

Campos, I., Abrantes, N., 2021. Forest fires as drivers of contamination of polycyclic aromatic hydrocarbons to the terrestrial and aquatic ecosystems. Curr. Opin. Environ. Sci. Heal. 24, 100293. https://doi.org/https://doi.org/10.1016/j.coesh.2021.100293

Cao, X., Liang, Y., Jiang, J., Mo, A., He, D., 2023. Organic additives in agricultural plastics and their impacts on soil ecosystems: Compared with conventional and biodegradable plastics. TrAC Trends Anal. Chem. 166, 117212. https://doi.org/https://doi.org/10.1016/j.trac.2023.117212

Cardinale, B.J., Duffy, J.E., Gonzalez, A., Hooper, D.U., Perrings, C., Venail, P., Narwani, A., Mace, G.M., Tilman, D., Wardle, D.A., 2012. Biodiversity loss and its impact on humanity. Nature 486, 59–67.

Carson, R., 1962. Silent Spring. Houghton Mifflin, Boston.

Chang, D., Richardot, W.H., Miller, E.L., Dodder, N.G., Sedlak, M.D., Hoh, E., Sutton, R., 2021. Framework for nontargeted investigation of contaminants released by wildfires into stormwater runoff: Case study in the northern San Francisco Bay area. Integr. Environ. Assess. Manag. 17, 1179–1193. https://doi.org/https://doi.org/10.1002/ieam.4461

Chen, D., Kannan, K., Tan, H., Zheng, Z., Feng, Y.-L., Wu, Y., Widelka, M., 2016. Bisphenol Analogues Other Than BPA: Environmental Occurrence, Human Exposure, and Toxicity—A Review. Environ. Sci. Technol. 50, 5438–5453. https://doi.org/10.1021/acs.est.5b05387

Chokwe, T.B., Abafe, O.A., Mbelu, S.P., Okonkwo, J.O., Sibali, L.L., 2020. A review of sources, fate, levels, toxicity, exposure and transformations of organophosphorus flame-retardants and plasticizers in the environment. Emerg. Contam. 6, 345–366. https://doi.org/https://doi.org/10.1016/j.emcon.2020.08.004

Christensen, E.R., Wang, Y., Huo, J., Li, A., 2022. Properties and fate and transport of persistent and mobile polar organic water pollutants: A review. J. Environ. Chem. Eng. 10, 107201. https://doi.org/https://doi.org/10.1016/j.jece.2022.107201

Colomer-Vidal, P., Bertolero, A., Alcaraz, C., Garreta-Lara, E., Santos, F.J., Lacorte, S., 2022. Distribution and ten-year temporal trends (2009–2018) of perfluoroalkyl substances in gull eggs from Spanish breeding colonies. Environ. Pollut. 293, 118555. https://doi.org/10.1016/J.ENVPOL.2021.118555

Copernicus, 2023. Copernicus Global Land Service [WWW Document]. URL https://land.copernicus.eu/

Cristale, J., Álvarez-Martín, A., Rodríguez-Cruz, S., Sánchez-Martín, M.J., Lacorte, S., 2017. Sorption and desorption of organophosphate esters with different hydrophobicity by soils. Environ. Sci. Pollut. Res. 24, 27870–27878. https://doi.org/10.1007/s11356-017-0360-0

Cristale, J., Vázquez, A.G., Barata, C., Lacorte, S., 2013. Priority and emerging fl ame retardants in rivers : Occurrence in water and sediment , Daphnia magna toxicity and risk assessment. Environ. Int. 59, 232–243. https://doi.org/10.1016/j.envint.2013.06.011

Custer, C.M., Custer, T.W., Delaney, R., Dummer, P.M., Schultz, S., Karouna-Renier, N., 2019. Perfluoroalkyl Contaminant Exposure and Effects in Tree Swallows Nesting at Clarks Marsh, Oscoda, Michigan, USA. Arch. Environ. Contam. Toxicol. 77, 1–13. https://doi.org/10.1007/s00244-019-00620-1

Custer, T.W., Custer, C.M., Dummer, P.M., Goldberg, D., Franson, J.C., Erickson, R.A., 2017. Organic contamination in tree swallow (*Tachycineta bicolor*) nestlings at United States and binational Great Lakes Areas of Concern. Environ. Toxicol. Chem. 36, 735–748. https://doi.org/https://doi.org/10.1002/etc.3598

da Silva, D.A.M., Colabuono, F.I., Taniguchi, S., Petry, M.V., Montone, R.C., 2023. Persistent organic pollutant patterns in seabirds from marine protected areas in the tropical Atlantic Ocean. Mar. Pollut. Bull. 186, 114461. https://doi.org/https://doi.org/10.1016/j.marpolbul.2022.114461

Dasgupta, P., 2021. The economics of biodiversity: the Dasgupta review. HM Treasury, London.

de Souza, R.M., Seibert, D., Quesada, H.B., de Jesus Bassetti, F., Fagundes-Klen, M.R., Bergamasco, R., 2020. Occurrence, impacts and general aspects of pesticides in surface water: A review. Process Saf. Environ. Prot. 135, 22–37. https://doi.org/https://doi.org/10.1016/j.psep.2019.12.035

de Wit, C.A., Bossi, R., Dietz, R., Dreyer, A., Faxneld, S., Garbus, S.E., Hellström, P., Koschorreck, J., Lohmann, N., Roos, A., Sellström, U., Sonne, C., Treu, G., Vorkamp, K., Yuan, B., Eulaers, I., 2020. Organohalogen compounds of emerging concern in Baltic Sea biota: Levels, biomagnification potential and comparisons with legacy contaminants. Environ. Int. 144, 106037. https://doi.org/https://doi.org/10.1016/j.envint.2020.106037

Dickman, R.A., Aga, D.S., 2022. A review of recent studies on toxicity, sequestration, and degradation of per- and polyfluoroalkyl substances (PFAS). J. Hazard. Mater. 436, 129120. https://doi.org/https://doi.org/10.1016/j.jhazmat.2022.129120

Directive 2003/53/EC of The European Parliment And Of The Council of 18 June 2003 amending for the 26th time Council Directive 76/769/EEC relating to restrictions on the marketing and use of certain dangerous substances and preparations, 2003.

Directive 2009/147/EC of the European Parliament and of the Council of 30 November 2009 on the conservation of wild birds., 2009.

Directive 2013/39/EU of the European Parliament and of the Council of 12 August 2013 amending Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy, 2013.

Donald, P.F., Fishpool, L.D.C., Ajagbe, A., Bennun, L.A., Bunting, G., Burfield, I.J., Butchart, S.H.M., Capellan, S., Crosby, M.J., Dias, M.P., Diaz, D., Evans, M.I., Grimmett, R., Heath, M., Jones, V.R., Lascelles, B.G., Merriman, J.C., O'brien, M., Ramírez, I., Waliczky, Z., Wege, D.C., 2019. Important Bird and Biodiversity Areas (IBAs): the development and characteristics of a global inventory of key sites for biodiversity. Bird Conserv. Int. 29, 177–198. https://doi.org/10.1017/S0959270918000102

Dou, M., Wang, L., 2023. A review on organophosphate esters: Physiochemical properties, applications, and toxicities as well as occurrence and human exposure in dust environment. J. Environ. Manage. 325, 116601. https://doi.org/https://doi.org/10.1016/j.jenvman.2022.116601

Dron, J., Wafo, E., Boissery, P., Dhermain, F., Bouchoucha, M., Chamaret, P., Lafitte, D., 2023. Occurrence and distribution of PAHs in stranded dolphin tissues from the Northwestern Mediterranean. Mar. Pollut. Bull. 191, 114898. https://doi.org/https://doi.org/10.1016/j.marpolbul.2023.114898

Ebele, A.J., Abou-Elwafa Abdallah, M., Harrad, S., 2017. Pharmaceuticals and personal care products (PPCPs) in the freshwater aquatic environment. Emerg. Contam. 3, 1–16. https://doi.org/10.1016/J.EMCON.2016.12.004

Eccles, K.M., Pauli, B.D., Chan, H.M., 2019. The Use of Geographic Information Systems for Spatial Ecological Risk Assessments: An Example from the Athabasca Oil Sands Area in Canada. Environ. Toxicol. Chem. 38, 2797–2810. https://doi.org/https://doi.org/10.1002/etc.4577

ECHA, 2023a. Regulatory strategy for flame retardants. Finland. https://doi.org/10.2823/854233

ECHA, 2023b. Phthalates [WWW Document]. URL https://echa.europa.eu/hot-topics/phthalates

ECHA, 2023c. Bisphenols [WWW Document]. URL https://echa.europa.eu/en/hot-topics/bisphenols

ECHA, 2016. How to use and report (Q)SARs. https://doi.org/10.2823/81818

EEA, 2023. How pesticides impact human health and ecosystems in Europe [WWW Document]. https://doi.org/doi: 10.2800/760240

Erickson, M.D., Kaley, R.G., 2011. Applications of polychlorinated biphenyls. Environ. Sci. Pollut. Res. 18, 135–151. https://doi.org/10.1007/s11356-010-0392-1

Espín, S., Andevski, J., Duke, G., Eulaers, I., Gómez-Ramírez, P., Hallgrimsson, G.T., Helander, B., Herzke, D., Jaspers, V.L.B., Krone, O., Lourenço, R., María-Mojica, P., Martínez-López, E., Mateo, R., Movalli, P., Sánchez-Virosta, P., Shore, R.F., Sonne, C., van den Brink, N.W., van Hattum, B., Vrezec, A., Wernham, C., García-Fernández, A.J., 2021. A schematic sampling protocol for contaminant monitoring in raptors. Ambio 50, 95–100. https://doi.org/10.1007/s13280-020-01341-9

Espín, S., García-Fernández, A.J., Herzke, D., Shore, R.F., van Hattum, B., Martínez-López, E., Coeurdassier, M., Eulaers, I., Fritsch, C., Gómez-Ramírez, P., Jaspers, V.L.B., Krone, O., Duke, G., Helander, B., Mateo, R., Movalli, P., Sonne, C., van den Brink, N.W., 2016. Tracking pan-continental trends in environmental contamination using sentinel raptors—what types of samples should we use? Ecotoxicology 25, 777–801. https://doi.org/10.1007/s10646-016-1636-8

Espin, S., García Fernández, A.J., Herzke, D., Shore, R.F., van Hattum, B., Martinez-López, E., Coeurdassier, M., Eulaers, I., Fritsch, C., Gómez-Ramírez, P., Jaspers, V.L.B., Krone, O., Duke, G., Helander, B., Mateo, R., Movalli, P., Sonne, C., van den Brink, N.W., EURAPMON, 2014. Sampling and contaminant monitoring protocol for raptors 34.

Espín, S., Martínez-López, E., Gómez-Ramírez, P., María-Mojica, P., García-Fernández, A.J., 2010. Assessment of organochlorine pesticide exposure in a wintering population of razorbills (*Alca torda*) from the southwestern Mediterranean. Chemosphere 80, 1190–1198. https://doi.org/https://doi.org/10.1016/j.chemosphere.2010.06.015

European Commission, 2023. Active substance: Malathion [WWW Document]. URL https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/start/screen/active-substances/details/273 (accessed 4.22.23).

European Commission, 2022. Commission Implementing Decision (EU)2022/1307 of 22 July 2022 establishing a watch list of substances for Union-wide monitoring in the field of water policy pursuant to Directive 2008/105/EC of the European Parliament and of the Council. Official Journal of the European Union.

European Commission, 2021. EU Biodiversity Strategy for 2030: Bringing nature back into our lives.

European Commission, 2020. Commission implementing regulation (EU) 2020/18 of 10 January 2020 concerning the non-renewal of the approval of the active substance chlorpyrifos, in accordance with Regulation (EC) No 1107/2009. Off. J. Eur. Union L 7/14.

European Commission, Joint Research Centre, Napierska, D., Sanseverino, I., Loos, R., Marinov, D., Lettieri, T., 2018. Review of the 1st Watch List under the Water Framework Directive and recommendations for the 2nd Watch List. Publications Office. https://doi.org/doi/10.2760/614367

Eurostat, 2023. EU sales of pesticides rebounded in 2021 [WWW Document]. URL https://ec.europa.eu/eurostat/

Fair, J.M., Jones, J., 2010. Guidelines to the use of wild birds in research. Ornithological council.

FAO, 2022. Pesticides and environmental incidents: Rotterdam Convention on the prior informed consent procedure for certain hazardous chemicals and pesticides in international trade. Rome, Italy. https://doi.org/https://doi.org/10.4060/cb6761en

Fernández-Vizcaíno, E., Ortiz-Santaliestra, M.E., Fernández-Tizón, M., Mateo, R., Camarero, P.R., Mougeot, F., 2022. Bird exposure to fungicides through the consumption of treated seeds: A study of wild red-legged partridges in central Spain. Environ. Pollut. 292, 118335. https://doi.org/https://doi.org/10.1016/j.envpol.2021.118335

Fraixedas, S., Lindén, A., Piha, M., Cabeza, M., Gregory, R., Lehikoinen, A., 2020. A state-of-the-art review on birds as indicators of biodiversity: Advances, challenges, and future directions. Ecol. Indic. 118, 106728. https://doi.org/https://doi.org/10.1016/j.ecolind.2020.106728

Gao, D.-W., Wen, Z.-D., 2016. Phthalate esters in the environment: A critical review of their occurrence, biodegradation, and removal during wastewater treatment processes. Sci. Total Environ. 541, 986–1001. https://doi.org/https://doi.org/10.1016/j.scitotenv.2015.09.148

Geldmann, J., Manica, A., Burgess, N.D., Coad, L., Balmford, A., 2019. A global-level assessment of the effectiveness of protected areas at resisting anthropogenic pressures. Proc. Natl. Acad. Sci. 116, 23209–23215. https://doi.org/10.1073/pnas.1908221116

Glüge, J., Scheringer, M., Cousins, I.T., DeWitt, J.C., Goldenman, G., Herzke, D., Lohmann, R., Ng, C.A., Trier, X., Wang, Z., 2020. An overview of the uses of per-and polyfluoroalkyl substances (PFAS). Environ. Sci. Process. Impacts 22, 2345–2373.

Göckener, B., Fliedner, A., Weinfurtner, K., Rüdel, H., Badry, A., Koschorreck, J., 2023. Tracking down unknown PFAS pollution – The direct TOP assay in spatial monitoring of surface waters in Germany. Sci. Total Environ. 898, 165425. https://doi.org/https://doi.org/10.1016/j.scitotenv.2023.165425

Gómez-Canela, C., Pueyo, V., Barata, C., Lacorte, S., Marcé, R.M., 2019. Development of predicted environmental concentrations to prioritize the occurrence of pharmaceuticals in rivers from Catalonia. Sci. Total Environ. 666, 57–67. https://doi.org/10.1016/j.scitotenv.2019.02.078

Gómez-Ramírez, P., Shore, R.F., van den Brink, N.W., van Hattum, B., Bustnes, J.O., Duke, G., Fritsch, C., García-Fernández, A.J., Helander, B.O., Jaspers, V., Krone, O., Martínez-López, E., Mateo, R., Movalli, P., Sonne, C., 2014. An overview of existing raptor contaminant monitoring activities in Europe. Environ. Int. 67, 12–21. https://doi.org/10.1016/j.envint.2014.02.004

Gong, X., Zhang, W., Zhang, S., Wang, Y., Zhang, X., Lu, Y., Sun, H., Wang, L., 2021. Organophosphite Antioxidants in Mulch Films Are Important Sources of Organophosphate Pollutants in Farmlands. Environ. Sci. \& Technol. 55, 7398–7406. https://doi.org/10.1021/acs.est.0c08741

González-Rubio, S., Vike-Jonas, K., Gonzalez, S. V., Ballesteros-Gómez, A., Sonne, C., Dietz, R., Boertmann, D., Rasmussen, L.M., Jaspers, V.L.B., Asimakopoulos, A.G., 2020. Bioaccumulation potential of bisphenols and benzophenone UV filters: A multiresidue approach in raptor tissues. Sci. Total Environ. 741, 140330. https://doi.org/10.1016/j.scitotenv.2020.140330

Gorrochategui, E., Pérez-Albaladejo, E., Casas, J., Lacorte, S., Porte, C., 2014. Perfluorinated chemicals: Differential toxicity, inhibition of aromatase activity and alteration of cellular lipids in human placental cells. Toxicol. Appl. Pharmacol. 277, 124–130. https://doi.org/https://doi.org/10.1016/j.taap.2014.03.012

Goutner, V., Skartsi, T., Konstantinou, I.K., Sakellarides, T.M., Albanis, T.A., Vasilakis, D., Elorriaga, J., Poirazidis, K., 2011. Organochlorine residues in blood of cinereous vultures and Eurasian griffon vultures in a northeastern Mediterranean area of nature conservation. Environ. Monit. Assess. 183, 259–271. https://doi.org/10.1007/s10661-011-1919-8

Gregory, R.D., van Strien, A., 2010. Wild bird indicators: using composite population trends of birds as measures of environmental health. Ornithol. Sci. 9, 3–22.

Grove, R.A., Henny, C.J., Kaiser, J.L., 2009. Osprey: Worldwide Sentinel Species for Assessing and Monitoring Environmental Contamination in Rivers, Lakes, Reservoirs, and Estuaries. J. Toxicol. Environ. Heal. Part B 12, 25–44. https://doi.org/10.1080/10937400802545078

Guckert, M., Scheurer, M., Schaffer, M., Reemtsma, T., Nödler, K., 2022. Combining target analysis with sum parameters—a comprehensive approach to determine sediment contamination with PFAS and further fluorinated substances. Environ. Sci. Pollut. Res. 29, 85802–85814. https://doi.org/10.1007/s11356-022-21588-x

Guitart, R., Clavero, R., Mateo, R., Máñez, M., 2005. Levels of Persistent Organochlorine Residues in Eggs of Greater Flamingos from the Guadalquivir Marshes (Doñana), Spain. J. Environ. Sci. Heal. Part B 40, 753–760. https://doi.org/10.1080/03601230500189394

Gusmaroli, L., Buttiglieri, G., Petrovic, M., 2019. The EU watch list compounds in the ebro delta region: Assessment of sources, river transport, and seasonal variations. Environ. Pollut. 253, 606–615. https://doi.org/10.1016/j.envpol.2019.07.052

Hahladakis, J.N., Iacovidou, E., Gerassimidou, S., 2022. An overview of the occurrence, fate, and human risks of the bisphenol-A present in plastic materials, components, and products. Integr. Environ. Assess. Manag. 19, 45–62. https://doi.org/https://doi.org/10.1002/ieam.4611

Han, L., Kong, X., Xu, M., Nie, J., 2021. Repeated exposure to fungicide tebuconazole alters the degradation characteristics, soil microbial community and functional profiles. Environ. Pollut. 287, 117660. https://doi.org/https://doi.org/10.1016/j.envpol.2021.117660

Harfoot, M.B.J., Johnston, A., Balmford, A., Burgess, N.D., Butchart, S.H.M., Dias, M.P., Hazin, C., Hilton-Taylor, C., Hoffmann, M., Isaac, N.J.B., Iversen, L.L., Outhwaite, C.L., Visconti, P., Geldmann, J., 2021. Using the IUCN Red List to map threats to terrestrial vertebrates at global scale. Nat. Ecol. Evol. 5, 1510–1519. https://doi.org/10.1038/s41559-021-01542-9

Heath, M.F., Evans, M.I., Hoccom, D.G., Payne, A.J., Peet, N.B., 2000. Important Bird Areas in Europe: priority sites for conservation. Volume 2: Southern Europe. BirdLife International, Cambridge, UK.

Hermabessiere, L., Dehaut, A., Paul-Pont, I., Lacroix, C., Jezequel, R., Soudant, P., Duflos, G., 2017. Occurrence and effects of plastic additives on marine environments and organisms: A review. Chemosphere 182, 781–793. https://doi.org/https://doi.org/10.1016/j.chemosphere.2017.05.096

Herrero-Villar, M., Delepoulle, É., Suárez-Regalado, L., Solano-Manrique, C., Juan-Sallés, C., Iglesias-Lebrija, J.J., Camarero, P.R., González, F., Álvarez, E., Mateo, R., 2021. First diclofenac intoxication in a wild avian scavenger in Europe. Sci. Total Environ. 782, 146890. https://doi.org/https://doi.org/10.1016/j.scitotenv.2021.146890

Herrero-Villar, M., Taggart, M.A., Mateo, R., 2023. Medicated livestock carcasses and landfill sites: Sources of highly toxic veterinary pharmaceuticals and caffeine for avian scavengers. J. Hazard. Mater. 459, 132195. https://doi.org/https://doi.org/10.1016/j.jhazmat.2023.132195

Herrero-villar, M., Velarde, R., Camarero, P.R., Taggart, M.A., Fonseca, C., Marco, I., Mateo, R., 2020. NSAIDs detected in Iberian avian scavengers and carrion after diclofenac registration for veterinary use in Spain. Environ. Pollut. 115157. https://doi.org/10.1016/j.envpol.2020.115157

Hildebrandt, A., Lacorte, S., Barceló, D., 2006. Sampling of water, soil and sediment to trace organic pollutants at a river-basin scale. Anal. Bioanal. Chem. 386, 1075–1088. https://doi.org/10.1007/s00216-006-0486-2

Hogue, A.S., Breon, K., 2022. The greatest threats to species. Conserv. Sci. Pract. 4, e12670.

Hong, Y., Feng, C., Yan, Z., Wang, Y., Liu, D., Liao, W., Bai, Y., 2020. Nonylphenol occurrence, distribution, toxicity and analytical methods in freshwater. Environ. Chem. Lett. 18, 2095–2106. https://doi.org/10.1007/s10311-020-01060-3

Huang, J., Ye, L., Fang, M., Su, G., 2022. Industrial Production of Organophosphate Flame Retardants (OPFRs): Big Knowledge Gaps Need to Be Filled? Bull. Environ. Contam. Toxicol. 108, 809–818. https://doi.org/10.1007/s00128-021-03454-7

Huertas, D., Grimalt, J.O., Jover, L., Sanpera, C., Ruiz, X., 2016. Organochlorine compounds in Purple Heron eggs (*Ardea purpurea*) nesting in sites located around a chlor-alkali plant (Ebro River). Sci. Total Environ. 540, 211–220. https://doi.org/10.1016/j.scitotenv.2015.07.037

Infante, O., Fuente, U. y Atienza, J.C., 2011. Las Áreas Importantes para la Conservación de las Aves en España. SEO/BirdLife.

IPBES, 2019. Global assessment report of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Service. IPBES secretaria, Bonn, German.

Iribarne-Durán, L.M., Serrano, L., Peinado, F.M., Peña-Caballero, M., Hurtado, J.A., Vela-Soria, F., Fernández, M.F., Freire, C., Artacho-Cordón, F., Olea, N., 2022. Biomonitoring bisphenols, parabens, and benzophenones in breast milk from a human milk bank in Southern Spain. Sci. Total Environ. 830, 154737. https://doi.org/https://doi.org/10.1016/j.scitotenv.2022.154737

Isley, C.F., Taylor, M.P., 2020. Atmospheric remobilization of natural and anthropogenic contaminants during wildfires. Environ. Pollut. 267, 115400. https://doi.org/https://doi.org/10.1016/j.envpol.2020.115400

IUCN, 2023. The IUCN Red List of Threatened Species [WWW Document]. https://doi.org/ISSN 2307-8235

Iwuozor, K.O., Emenike, E.C., Gbadamosi, F.A., Ighalo, J.O., Umenweke, G.C., Iwuchukwu, F.U., Nwakire, C.O., Igwegbe, C.A., 2023. Adsorption of organophosphate pesticides from aqueous solution: a review of recent advances. Int. J. Environ. Sci. Technol. 20, 5845–5894.

Jakiel, M., Bernatek-Jakiel, A., Gajda, A., Filiks, M., Pufelska, M., 2019. Spatial and temporal distribution of illegal dumping sites in the nature protected area: the Ojców National Park, Poland. J. Environ. Plan. Manag. 62, 286–305. https://doi.org/10.1080/09640568.2017.1412941

Järlskog, I., Strömvall, A.-M., Magnusson, K., Galfi, H., Björklund, K., Polukarova, M., Garção, R., Markiewicz, A., Aronsson, M., Gustafsson, M., Norin, M., Blom, L., Andersson-Sköld, Y., 2021. Traffic-related microplastic particles, metals, and organic pollutants in an urban area under reconstruction. Sci. Total Environ. 774, 145503. https://doi.org/https://doi.org/10.1016/j.scitotenv.2021.145503

Jarman, W.M., Ballschmiter, K., 2012. From coal to DDT: the history of the development of the pesticide DDT from synthetic dyes till Silent Spring. Endeavour 36, 131–142. https://doi.org/https://doi.org/10.1016/j.endeavour.2012.10.003

Jaureguiberry, P., Titeux, N., Wiemers, M., Bowler, D.E., Coscieme, L., Golden, A.S., Guerra, C.A., Jacob, U., Takahashi, Y., Settele, J., 2022. The direct drivers of recent global anthropogenic biodiversity loss. Sci. Adv. 8, eabm9982.

Jinadasa, B.K.K.K., Moreda-Piñeiro, A., Fowler, S.W., 2023. Ultrasound-Assisted Extraction in Analytical Applications for Fish and Aquatic Living Resources, a Review. Food Rev. Int. 39, 2684–2707. https://doi.org/10.1080/87559129.2021.1967378

Jodice, P.G.R., Lamb, J.S., Satgé, Y.G., Perkins, C., 2023. Spatial and individual factors mediate the tissue burden of polycyclic aromatic hydrocarbons in adult and chick brown pelicans in the northern Gulf of Mexico. Front. Ecol. Evol. 11. https://doi.org/10.3389/fevo.2023.1185659

Joppa, L.N., Pfaff, A., 2009. High and Far: Biases in the Location of Protected Areas. PLoS One 4, 1–6. https://doi.org/10.1371/journal.pone.0008273

KEMI, 2020. Guidance on national chemicals control for other countries. Hazard and risk assessment of chemicals - an introduction. Stockholm, Sweden.

Kicińska, A., Dmytrowski, P., 2023. Anthropogenic impact on soils of protected areas—example of PAHs. Sci. Rep. 13, 1524. https://doi.org/10.1038/s41598-023-28726-6

Kim, S., Choi, K., 2014. Occurrences, toxicities, and ecological risks of benzophenone-3, a common component of organic sunscreen products: A mini-review. Environ. Int. 70, 143–157. https://doi.org/10.1016/j.envint.2014.05.015

Klančar, A., Trontelj, J., Roškar, R., 2018. Development of a Multi-Residue Method for Monitoring 44 Pharmaceuticals in Slovene Surface Water by SPE-LC-MS/MS. Water, Air, Soil Pollut. 229, 192. https://doi.org/10.1007/s11270-018-3845-7

Koch, A., Jonsson, M., Yeung, L.W.Y., Kärrman, A., Ahrens, L., Ekblad, A., Wang, T., 2020. Per- and Polyfluoroalkyl-Contaminated Freshwater Impacts Adjacent Riparian Food Webs. Environ. Sci. Technol. 54, 11951–11960. https://doi.org/10.1021/acs.est.0c01640

Kukkala, A.S., Santangeli, A., Butchart, S.H.M., Maiorano, L., Ramirez, I., Burfield, I.J., Moilanen, A., 2016. Coverage of vertebrate species distributions by Important Bird and Biodiversity Areas and Special Protection Areas in the European Union. Biol. Conserv. 202, 1–9. https://doi.org/https://doi.org/10.1016/j.biocon.2016.08.010

Le Goff, G., Giraudo, M., 2019. Effects of Pesticides on the Environment and Insecticide Resistance, in: Picimbon, J.-F. (Ed.), Olfactory Concepts of Insect Control - Alternative to Insecticides: Volume 1. Springer International Publishing, Cham, pp. 51–78. https://doi.org/10.1007/978-3-030-05060-3_3

Lemke, N., Murawski, A., Lange, R., Weber, T., Apel, P., Dębiak, M., Koch, H.M., Kolossa-Gehring, M., 2021. Substitutes mimic the exposure behaviour of REACH regulated phthalates – A review of the German HBM system on the example of plasticizers. Int. J. Hyg. Environ. Health 236, 113780. https://doi.org/https://doi.org/10.1016/j.ijheh.2021.113780

Lewis, A.J., Yun, X., Spooner, D.E., Kurz, M.J., McKenzie, E.R., Sales, C.M., 2022. Exposure pathways and bioaccumulation of per- and polyfluoroalkyl substances in freshwater aquatic ecosystems: Key considerations. Sci. Total Environ. 822, 153561. https://doi.org/https://doi.org/10.1016/j.scitotenv.2022.153561

Li, S., Sun, Q., Wu, Q., Gui, W., Zhu, G., Schlenk, D., 2019. Endocrine disrupting effects of tebuconazole on different life stages of zebrafish (*Danio rerio*). Environ. Pollut. 249, 1049–1059. https://doi.org/https://doi.org/10.1016/j.envpol.2019.03.067

Lopez-Antia, A., Groffen, T., Lasters, R., AbdElgawad, H., Sun, J., Asard, H., Bervoets, L., Eens, M., 2019. Perfluoroalkyl acids (PFAAs) concentrations and oxidative status in two generations of great tits inhabiting a contamination hotspot. Environ. Sci. Technol. 53, 1617–1626.

Lopez-Antia, A., Ortiz-Santaliestra, M.E., Mougeot, F., Camarero, P.R., Mateo, R., 2021. Birds feeding on tebuconazole treated seeds have reduced breeding output. Environ. Pollut. 271, 116292. https://doi.org/10.1016/j.envpol.2020.116292

Lopez-Herguedas, N., González-Gaya, B., Castelblanco-Boyacá, N., Rico, A., Etxebarria, N., Olivares, M., Prieto, A., Zuloaga, O., 2022. Characterization of the contamination fingerprint of wastewater treatment plant effluents in the Henares River Basin (central Spain) based on target and suspect screening analysis. Sci. Total Environ. 806. https://doi.org/10.1016/j.scitotenv.2021.151262

Lourenço, R.A., Taniguchi, S., da Silva, J., Gallotta, F.D.C., Bícego, M.C., 2021. Polycyclic aromatic hydrocarbons in marine mammals: A review and synthesis. Mar. Pollut. Bull. 171, 112699. https://doi.org/https://doi.org/10.1016/j.marpolbul.2021.112699

Ma, J., Qin, C., Waigi, M.G., Gao, Y., Hu, X., Mosa, A., Ling, W., 2022. Functional group substitutions influence the binding of benzophenone-type UV filters with DNA. Chemosphere 299, 134490. https://doi.org/https://doi.org/10.1016/j.chemosphere.2022.134490

Madrid, Y., Zayas, Z.P., 2007. Water sampling: Traditional methods and new approaches in water sampling strategy. TrAC Trends Anal. Chem. 26, 293–299. https://doi.org/https://doi.org/10.1016/j.trac.2007.01.002

Mao, F., He, Y., Gin, K.Y.-H., 2019. Occurrence and fate of benzophenone-type UV filters in aquatic environments: a review. Environ. Sci. Water Res. Technol. 5, 209–223.

Markowitz, G., Rosner, D., 2018. Monsanto, PCBs, and the creation of a "world-wide ecological problem." J. Public Health Policy 39, 463–540. https://doi.org/10.1057/s41271-018-0146-8

Masoner, J.R., Kolpin, D.W., Cozzarelli, I.M., Barber, L.B., Burden, D.S., Foreman, W.T., Forshay, K.J., Furlong, E.T., Groves, J.F., Hladik, M.L., Hopton, M.E., Jaeschke, J.B., Keefe, S.H., Krabbenhoft, D.P., Lowrance, R., Romanok, K.M., Rus, D.L., Selbig, W.R., Williams, B.H., Bradley, P.M., 2019. Urban Stormwater: An Overlooked Pathway of Extensive Mixed Contaminants to Surface and Groundwaters in the United States. Environ. Sci. Technol. 53, 10070–10081. https://doi.org/10.1021/acs.est.9b02867

McIntyre, J.K., Prat, J., Cameron, J., Wetzel, J., Mudrock, E., Peter, K.T., Tian, Z., Mackenzie, C., Lundin, J., Stark, J.D., King, K., Davis, J.W., Kolodziej, E.P., Scholz, N.L., 2021. Treading Water: Tire Wear Particle Leachate Recreates an Urban Runoff Mortality Syndrome in Coho but Not Chum Salmon. Environ. Sci. Technol. 55, 11767–11774. https://doi.org/10.1021/acs.est.1c03569

Melymuk, L., Blumenthal, J., Sáňka, O., Shu-Yin, A., Singla, V., Šebková, K., Pullen Fedinick, K., Diamond, M.L., 2022. Persistent Problem: Global Challenges to Managing PCBs. Environ. Sci. Technol. 56, 9029–9040. https://doi.org/10.1021/acs.est.2c01204

Metcalfe, C.D., Bayen, S., Desrosiers, M., Muñoz, G., Sauvé, S., Yargeau, V., 2022. An introduction to the sources, fate, occurrence and effects of endocrine disrupting chemicals released into the environment. Environ. Res. 207, 112658. https://doi.org/https://doi.org/10.1016/j.envres.2021.112658

Miranda, D. de A., Peaslee, G.F., Zachritz, A.M., Lamberti, G.A., 2022. A worldwide evaluation of trophic magnification of per- and polyfluoroalkyl substances in aquatic ecosystems. Integr. Environ. Assess. Manag. 18, 1500–1512. https://doi.org/https://doi.org/10.1002/ieam.4579

Molina-Molina, J.M., Jiménez-Díaz, I., Fernández, M.F., Rodriguez-Carrillo, A., Peinado, F.M., Mustieles, V., Barouki, R., Piccoli, C., Olea, N., Freire, C., 2019. Determination of bisphenol A and bisphenol S concentrations and assessment of estrogen- and anti-androgen-like activities in thermal paper receipts from Brazil, France, and Spain. Environ. Res. 170, 406–415. https://doi.org/https://doi.org/10.1016/j.envres.2018.12.046

Morin-Crini, N., Lichtfouse, E., Liu, G., Balaram, V., Ribeiro, A.R.L., Lu, Z., Stock, F., Carmona, E., Teixeira, M.R., Picos-Corrales, L.A., Moreno-Piraján, J.C., Giraldo, L., Li, C., Pandey, A., Hocquet, D., Torri, G., Crini, G., 2022. Worldwide cases of water pollution by emerging contaminants: a review. Environ. Chem. Lett. 20, 2311–2338. https://doi.org/10.1007/s10311-022-01447-4

Morin-crini, N., Scheifler, R., Amiot, C., Riols, R., 2020. Determination of polycyclic aromatic hydrocarbon (PAH) contents in micro-volumes of the whole blood and liver of Red Kite by a simplified GC-MS / MS method. Int. J. Environ. Anal. Chem. 00, 1–10. https://doi.org/10.1080/03067319.2020.1726899

Mrema, E.J., Rubino, F.M., Brambilla, G., Moretto, A., Tsatsakis, A.M., Colosio, C., 2013. Persistent organochlorinated pesticides and mechanisms of their toxicity. Toxicology 307, 74–88. https://doi.org/https://doi.org/10.1016/j.tox.2012.11.015

Naidu, R., Biswas, B., Willett, I.R., Cribb, J., Kumar Singh, B., Paul Nathanail, C., Coulon, F., Semple, K.T., Jones, K.C., Barclay, A., Aitken, R.J., 2021. Chemical pollution: A growing peril and potential catastrophic risk to humanity. Environ. Int. 156, 106616. https://doi.org/https://doi.org/10.1016/j.envint.2021.106616

Net, S., Sempéré, R., Delmont, A., Paluselli, A., Ouddane, B., 2015. Occurrence, Fate, Behavior and Ecotoxicological State of Phthalates in Different Environmental Matrices. Environ. Sci. Technol. 49, 4019–4035. https://doi.org/10.1021/es505233b

Ngoubeyou, P.S.K., Wolkersdorfer, C., Ndibewu, P.P., Augustyn, W., 2022. Toxicity of polychlorinated biphenyls in aquatic environments – A review. Aquat. Toxicol. 251, 106284. https://doi.org/https://doi.org/10.1016/j.aquatox.2022.106284

NIH, 2023. PubChem [WWW Document]. URL https://pubchem.ncbi.nlm.nih.gov/

NORMAN, 2022. NORMAN Ecotoxicology Database - Lowest PNECs [WWW Document]. URL https://www.norman-network.com/nds/ecotox/lowestPnecsIndex.php (accessed 3.16.22).

Oaks, J.L., Gilbert, M., Virani, M.Z., Watson, R.T., Meteyer, C.U., Rideout, B.A., Shivaprasad, H.L., Ahmed, S., Chaudhry, M.J.I., Arshad, M., Mahmood, S., Ali, A., Khan, A.A., 2004. Diclofenac residues as the cause of vulture population decline in Pakistan. Nature 427, 630–633. https://doi.org/10.1038/nature02317

Ololade, I.A., Oladoja, N.A., Ololade, O.O., Saliu, T.D., Alabi, A.B., Obadawo, S.B., Anifowose, M.M., 2020. Bioaccumulation and toxic potencies of polycyclic aromatic hydrocarbons in freshwater biota from the Ogbese River, Nigeria. Environ. Monit. Assess. 193, 8. https://doi.org/10.1007/s10661-020-08759-4

Orgiazzi, A., Ballabio, C., Panagos, P., Jones, A., Fernández-Ugalde, O., 2018. LUCAS Soil, the largest expandable soil dataset for Europe: a review. Eur. J. Soil Sci. 69, 140–153. https://doi.org/https://doi.org/10.1111/ejss.12499

Oró-Nolla, B., Lacorte, S., Vike-Jonas, K., Gonzalez, S. V, Nygård, T., Asimakopoulos, A.G., Jaspers, V.L.B., 2021. Occurrence of Bisphenols and Benzophenone UV Filters in White-Tailed Eagles (*Haliaeetus albicilla*) from Smøla, Norway. Toxics 9. https://doi.org/10.3390/toxics9020034

Pascual, L.-L., Luigi, M., Alessandra, F., Emilio, B., Luigi, B., 2011. Hotspots of species richness, threat and endemism for terrestrial vertebrates in SW Europe. Acta Oecologica 37, 399–412. https://doi.org/https://doi.org/10.1016/j.actao.2011.05.004

Patel, A.B., Shaikh, S., Jain, K.R., Desai, C., Madamwar, D., 2020. Polycyclic Aromatic Hydrocarbons: Sources, Toxicity, and Remediation Approaches. Front. Microbiol. 11. https://doi.org/10.3389/fmicb.2020.562813

Patel, M., Kumar, R., Kishor, K., Mlsna, T., Pittman, C.U.J., Mohan, D., 2019. Pharmaceuticals of Emerging Concern in Aquatic Systems: Chemistry, Occurrence, Effects, and Removal Methods. Chem. Rev. 119, 3510–3673. https://doi.org/10.1021/acs.chemrev.8b00299

Pavón-Jordán, D., Abdou, W., Azafzaf, H., Balaž, M., Bino, T., Borg, J.J., Božič, L., Butchart, S.H.M., Clausen, P., Sniauksta, L., Dakki, M., Devos, K., Domsa, C., Encarnaçao, V., Etayeb, K., Faragó, S., Fox, A.D., Frost, T., Gaudard, C., Georgiev, V., Goratze, I., Hornman, M., Keller, V., Kostiushyn, V., Langendoen, T., Ławicki, Ł., Ieronymidou, C., Lewis, L.J., Lorentsen, S.H., Luigujoe, L., Meissner, W., Mikuska, T., Molina, B., Musil, P., Musilova, Z., Nagy, S., Natykanets, V., Nilsson, L., Paquet, J.Y., Portolou, D., Ridzon, J., Santangeli, A., Sayoud, S., Šćiban, M., Stipniece, A., Teufelbauer, N., Topić, G., Uzunova, D., Vizi, A., Wahl, J., Yavuz, K.E., Zenatello, M., Lehikoinen, A., 2020. Positive impacts of important bird and biodiversity areas on wintering waterbirds under changing temperatures throughout Europe and North Africa. Biol. Conserv. 246, 108549. https://doi.org/10.1016/J.BIOCON.2020.108549

Pereira, M.G., Lacorte, S., Walker, L.A., Shore, R.F., 2021. Contrasting long term temporal trends in perfluoroalkyl substances (PFAS) in eggs of the northern gannet (*Morus bassanus*) from two UK colonies. Sci. Total Environ. 754, 141900. https://doi.org/10.1016/j.scitotenv.2020.141900

Pereira, M.G., Lawlor, A., Bertolero, A., Díez, S., Shore, R.F., Lacorte, S., 2019. Temporal and Spatial Distribution of Mercury in Gulls Eggs from the Iberian Peninsula. Arch. Environ. Contam. Toxicol. 76, 394–404. https://doi.org/10.1007/s00244-018-0584-0

Pereira, M.G., Walker, L.A., Wright, J., Best, J., Shore, R.F., 2009. Concentrations of polycyclic aromatic hydrocarbons (PAHs) in the eggs of predatory birds in Britain. Environ. Sci. Technol. 43, 9010–9015. https://doi.org/10.1021/es901805e

Pérez-Albaladejo, E., Fernandes, D., Lacorte, S., Porte, C., 2017. Comparative toxicity, oxidative stress and endocrine disruption potential of plasticizers in JEG-3 human placental cells. Toxicol. Vitr. 38, 41–48. https://doi.org/10.1016/j.tiv.2016.11.003

Podder, A., Sadmani, A.H.M.A., Reinhart, D., Chang, N. Bin, Goel, R., 2021. Per and poly-fluoroalkyl substances (PFAS) as a contaminant of emerging concern in surface water: A transboundary review of their occurrences and toxicity effects. J. Hazard. Mater. 419, 126361. https://doi.org/10.1016/j.jhazmat.2021.126361

Power, A., White, P., McHugh, B., Berrow, S., McKeown, A., Crowley, D., Newton, S., McGovern, E., Murphy, S., O'Connor, I., 2021. Polycyclic aromatic hydrocarbons (PAHs) in seabird eggs in Ireland. Mar. Pollut. Bull. 170, 112636. https://doi.org/https://doi.org/10.1016/j.marpolbul.2021.112636

Ramasubramanian, T., Paramasivam, M., 2022. Soil persistence and environmental risk assessment of chlorpyrifos under different organic manuring in the tropical sugarcane ecosystem. Int. J. Environ. Anal. Chem. 102, 7754–7766. https://doi.org/10.1080/03067319.2020.1838493

Ratajc, U., Lourenço, R., Espín, S., Virosta, P.S., Birrer, S., Studler, D., Wernham, C., Vrezec, A., 2023. The importance of population contextual data for large-scale biomonitoring using an apex predator: The Tawny Owl (*Strix aluco*). Sci. Total Environ. 860, 160530. https://doi.org/https://doi.org/10.1016/j.scitotenv.2022.160530

Richter, L., Cordner, A., Brown, P., 2018. Non-stick science: Sixty years of research and (in)action on fluorinated compounds. Soc. Stud. Sci. 48, 691–714. https://doi.org/10.1177/0306312718799960

Rigal, S., Dakos, V., Alonso, H., Auniņš, A., Benkő, Z., Brotons, L., Chodkiewicz, T., Chylarecki, P., de Carli, E., Del Moral, J.C., Domşa, C., Escandell, V., Fontaine, B., Foppen, R., Gregory, R., Harris, S., Herrando, S., Husby, M., Ieronymidou, C., Jiguet, F., Kennedy, J., Klvaňová, A., Kmecl, P., Kuczyński, L., Kurlavičius, P., Kålås, J.A., Lehikoinen, A., Lindström, Å., Lorrillière, R., Moshøj, C., Nellis, R., Noble, D., Eskildsen, D.P., Paquet, J.-Y., Pélissié, M., Pladevall, C., Portolou, D., Reif, J., Schmid, H., Seaman, B., Szabo, Z.D., Szép, T., Florenzano, G.T., Teufelbauer, N., Trautmann, S., van Turnhout, C., Vermouzek, Z., Vikstrøm, T., Vořílek, P., Weiserbs, A., Devictor, V., 2023. Farmland practices are driving bird population decline across Europe. Proc. Natl. Acad. Sci. U. S. A. 120, e2216573120. https://doi.org/10.1073/pnas.2216573120

Roder Green, A.L., Putschew, A., Nehls, T., 2014. Littered cigarette butts as a source of nicotine in urban waters. J. Hydrol. 519, 3466–3474. https://doi.org/10.1016/J.JHYDROL.2014.05.046

Rødland, E.S., Heier, L.S., Lind, O.C., Meland, S., 2023. High levels of tire wear particles in soils along low traffic roads. Sci. Total Environ. 903, 166470. https://doi.org/https://doi.org/10.1016/j.scitotenv.2023.166470

Rodrigues, A., Gaffard, A., Moreau, J., Monceau, K., Delhomme, O., Millet, M., 2023. Analytical development for the assessment of pesticide contaminations in blood and plasma of wild birds: The case of grey partridges (*Perdix perdix*). J. Chromatogr. A 1687, 463681. https://doi.org/https://doi.org/10.1016/j.chroma.2022.463681

Rodríguez-Carrillo, A., Mustieles, V., Pérez-Lobato, R., Molina-Molina, J.M., Reina-Pérez, I., Vela-Soria, F., Rubio, S., Olea, N., Fernández, M.F., 2019. Bisphenol A and cognitive function in school-age boys: Is BPA predominantly related to behavior? Neurotoxicology 74, 162–171. https://doi.org/https://doi.org/10.1016/j.neuro.2019.06.006

Rogowska, J., Cieszynska-Semenowicz, M., Ratajczyk, W., Wolska, L., 2020. Micropollutants in treated wastewater. Ambio 49, 487–503. https://doi.org/10.1007/s13280-019-01219-5

Röhler, K., Haluska, A.A., Susset, B., Liu, B., Grathwohl, P., 2021. Long-term behavior of PFAS in contaminated agricultural soils in Germany. J. Contam. Hydrol. 241, 103812. https://doi.org/https://doi.org/10.1016/j.jconhyd.2021.103812

Roque, I., Lourenço, R., Marques, A., Martínez-López, E., Espín, S., Gómez-Ramirez, P., García-Fernández, A.J., Roulin, A., Rabaça, J.E., 2022. A First Record of Organochlorine Pesticides in Barn Owls (*Tyto alba*) from Portugal: Assessing

Trends from Variation in Feather and Liver Concentrations. Bull. Environ. Contam. Toxicol. 109, 436–442. https://doi.org/10.1007/s00128-022-03576-6

Routti, H., Atwood, T.C., Bechshoft, T., Boltunov, A., Ciesielski, T.M., Desforges, J.-P., Dietz, R., Gabrielsen, G.W., Jenssen, B.M., Letcher, R.J., McKinney, M.A., Morris, A.D., Rigét, F.F., Sonne, C., Styrishave, B., Tartu, S., 2019. State of knowledge on current exposure, fate and potential health effects of contaminants in polar bears from the circumpolar Arctic. Sci. Total Environ. 664, 1063–1083. https://doi.org/https://doi.org/10.1016/j.scitotenv.2019.02.030

Sabik, H., Jeannot, R., Rondeau, B., 2000. Multiresidue methods using solid-phase extraction techniques for monitoring priority pesticides, including triazines and degradation products, in ground and surface waters. J. Chromatogr. A 885, 217–236. https://doi.org/https://doi.org/10.1016/S0021-9673(99)01084-5

Sala, B., Giménez, J., Fernández-Arribas, J., Bravo, C., Lloret-Lloret, E., Esteban, A., Bellido, J.M., Coll, M., Eljarrat, E., 2022. Organophosphate ester plasticizers in edible fish from the Mediterranean Sea: Marine pollution and human exposure. Environ. Pollut. 292, 118377. https://doi.org/https://doi.org/10.1016/j.envpol.2021.118377

Sánchez-Avila, J., Meyer, J., Lacorte, S., 2010. Spatial distribution and sources of perfluorochemicals in the NW Mediterranean coastal waters (Catalonia, Spain). Environ. Pollut. 158, 2833–2840. https://doi.org/10.1016/j.envpol.2010.06.022

Sanusi, I.O., Olutona, G.O., Wawata, I.G., Onohuean, H., 2023. Occurrence, environmental impact and fate of pharmaceuticals in groundwater and surface water: a critical review. Environ. Sci. Pollut. Res. https://doi.org/10.1007/s11356-023-28802-4

Schnitzler, J.G., Reckendorf, A., Pinzone, M., Autenrieth, M., Tiedemann, R., Covaci, A., Malarvannan, G., Ruser, A., Das, K., Siebert, U., 2019. Supporting evidence for PCB pollution threatening global killer whale population. Aquat. Toxicol. 206, 102–104. https://doi.org/https://doi.org/10.1016/j.aquatox.2018.11.008

Sebastiano, M., Jouanneau, W., Blévin, P., Angelier, F., Parenteau, C., Pallud, M., Ribout, C., Gernigon, J., Lemesle, J.C., Robin, F., Pardon, P., Budzinski, H., Labadie, P., Chastel, O., 2023. Physiological effects of PFAS exposure in seabird chicks: A multi-species study of thyroid hormone triiodothyronine, body condition and telomere length in South Western France. Sci. Total Environ. 901, 165920. https://doi.org/https://doi.org/10.1016/j.scitotenv.2023.165920

Sharp, S., Sardiña, P., Metzeling, L., McKenzie, R., Leahy, P., Menkhorst, P., Hinwood, A., 2021. Per- and Polyfluoroalkyl Substances in Ducks and the Relationship with Concentrations in Water, Sediment, and Soil. Environ. Toxicol. Chem. 40, 846–858. https://doi.org/https://doi.org/10.1002/etc.4818

Sheehan, D.K., Gregory, R.D., Eaton, M.A., Bubb, P.J., Chenery, A.M., 2010. The wild bird index–guidance for national and regional use. UNEP-WCMC, Cambridge 25.

Sidhu, G.K., Singh, S., Kumar, V., Dhanjal, D.S., Datta, S., Singh, J., 2019. Toxicity, monitoring and biodegradation of organophosphate pesticides: a review. Crit. Rev. Environ. Sci. Technol. 49, 1135–1187.

Sigmund, G., Ågerstrand, M., Antonelli, A., Backhaus, T., Brodin, T., Diamond, M.L., Erdelen, W.R., Evers, D.C., Hofmann, T., Hueffer, T., Lai, A., Torres, J.P.M., Mueller, L., Perrigo, A.L., Rillig, M.C., Schaeffer, A., Scheringer, M., Schirmer, K., Tlili, A., Soehl, A., Triebskorn, R., Vlahos, P., vom Berg, C., Wang, Z., Groh, K.J., 2023. Addressing chemical pollution in biodiversity research. Glob. Chang. Biol. 29, 3240–3255. https://doi.org/https://doi.org/10.1111/gcb.16689

Sigmund, G., Ågerstrand, M., Brodin, T., Diamond, M.L., Erdelen, W.R., Evers, D.C., Lai, A., Rillig, M.C., Schäffer, A., Soehl, A., Torres, J.P.M., Wang, Z., Groh, K.J., 2022. Broaden chemicals scope in biodiversity targets. Science (80-.). 376, 1280. https://doi.org/10.1126/science.add3070

Smits, J.E.G., Fernie, K.J., 2013. Avian wildlife as sentinels of ecosystem health. Comp. Immunol. Microbiol. Infect. Dis. 36, 333–342. https://doi.org/10.1016/j.cimid.2012.11.007

Soares, A., Guieysse, B., Jefferson, B., Cartmell, E., Lester, J.N., 2008. Nonylphenol in the environment: A critical review on occurrence, fate, toxicity and treatment in wastewaters. Environ. Int. 34, 1033–1049. https://doi.org/https://doi.org/10.1016/j.envint.2008.01.004

Soltaninejad, K., Shadnia, S., 2014. History of the use and epidemiology of organophosphorus poisoning. Basic Clin. Toxicol. organophosphorus Compd. 25–43.

Sparling, D.W., 2016. Ecotoxicology essentials: environmental contaminants and their biological effects on animals and plants. Academic Press.

Stockholm Convention, 2022. Chemicals proposed for listing under the Convention [WWW Document]. URL http://www.pops.int/TheConvention/ThePOPs/ChemicaWlsProposedforListing

Stockholm Convention, 2019. All POPs listed in the Stockholm Convention [WWW Document]. URL http://chm.pops.int/TheConvention (accessed 6.20.23).

Szabo, D., Lavers, J.L., Shimeta, J., Green, M.P., Mulder, R.A., Clarke, B.O., 2021. Correlations between Per - and Poly fl uoroalkyl Substances and Body Morphometrics in Fledgling Shearwaters Impacted by Plastic Consumption from a Remote Paci fi c Island 40, 799–810. https://doi.org/10.1002/etc.4924

Taves, D.R., 1968. Evidence that there are two forms of fluoride in human serum. Nature 217, 1050–1051.

Thorstensen, H., Ruus, A., Helberg, M., Bæk, K., Enge, E.K., Borgå, K., 2021. Common Eider and Herring Gull as Contaminant Indicators of Different Ecological Niches of an Urban Fjord System. Integr. Environ. Assess. Manag. 17, 422–433. https://doi.org/https://doi.org/10.1002/ieam.4340

Tomza-Marciniak, A., Pilarczyk, B., Witczak, A., Rząd, I., Pilarczyk, R., 2019. PCB residues in the tissues of sea ducks wintering on the south coast of the Baltic Sea, Poland. Environ. Sci. Pollut. Res. 26, 11300–11313. https://doi.org/10.1007/s11356-019-04586-4

Tsai, W.-T., 2014. Organochlorine Insecticides, in: Wexler, P. (Ed.), Encyclopedia of Toxicology (Third Edition). Academic Press, Oxford, pp. 711–713. https://doi.org/https://doi.org/10.1016/B978-0-12-386454-3.00172-X

USEPA, 2023. ECOTOX Knowledgebase [WWW Document]. URL https://cfpub.epa.gov/ecotox/

USEPA, 2022. Fact Sheet: Fifth Contaminant Candidate List (CCL 5).

USEPA, 1998. Guidelines for ecological risk assessment. Risk Assessment Forum, US Environmental Protection Agency, Washigton,DC, USA.

Van den Berg, H., Manuweera, G., Konradsen, F., 2017. Global trends in the production and use of DDT for control of malaria and other vector-borne diseases. Malar. J. 16, 1–8.

van der Veen, I., de Boer, J., 2012. Phosphorus flame retardants: Properties, production, environmental occurrence, toxicity and analysis. Chemosphere 88, 1119–1153. https://doi.org/https://doi.org/10.1016/j.chemosphere.2012.03.067

Verreault, J., Letcher, R.J., Gentes, M.L., Braune, B.M., 2018. Unusually high Deca-BDE concentrations and new flame retardants in a Canadian Arctic top predator, the glaucous gull. Sci. Total Environ. 639, 977–987. https://doi.org/10.1016/j.scitotenv.2018.05.222

Vestel, J., Caldwell, D.J., Constantine, L., D'Aco, V.J., Davidson, T., Dolan, D.G., Millard, S.P., Murray-Smith, R., Parke, N.J., Ryan, J.J., Straub, J.O., Wilson, P., 2016. Use of acute and chronic ecotoxicity data in environmental risk assessment of pharmaceuticals. Environ. Toxicol. Chem. 35, 1201–1212. https://doi.org/https://doi.org/10.1002/etc.3260

Vidal, J. luis martínez, Sánchez, J.A.P., Plaza-bolaños, P., Frenich, A.G., Romero-González, R., 2010. Use of Pressurized Liquid Extraction for the Simultaneous Analysis of 28 Polar and 94 Non-polar Pesticides in Agricultural Soils by GC/QqQ-MS/MS and UPLC/QqQ-MS/MS. J. AOAC Int. 93, 1715–1731. https://doi.org/10.1093/jaoac/93.6.1715

Voogt, P. de, Brinkman, U.T., 1989. Production, properties and usage of polychlorinated biphenyls. Halogenated biphenyls, terphenyls, naphthalenes, dibenzodioxins Relat. Prod. 3–45.

Vrezec, A., Duke, G., Kovács, A., Saurola, P., Wernham, C., Burfield, I., Movalli, P., Bertoncelj, I., 2014. Overview of raptor monitoring activities in Europe. Acrocephalus 33, 145–157. https://doi.org/10.2478/v10100-012-0003-y

Waliczky, Z., Fishpool, L.D.C., Butchart, S.H.M., Thomas, D., Heath, M.F., Hazin, C., Donald, P.F., Kowalska, A., Dias, M.P., Allinson, T.S.M., 2019. Important Bird and Biodiversity Areas (IBAs): their impact on conservation policy, advocacy and action. Bird Conserv. Int. 29, 199–215. https://doi.org/10.1017/S0959270918000175

Walker, C.H., Sibly, R.M., Hopkin, S.P., Peakall, D.B., 2012. Principles of Ecotoxicology, Fourth edi. ed. CRC Press, Boca Raton.

Wallace, S.J., de Solla, S.R., Head, J.A., Hodson, P. V., Parrott, J.L., Thomas, P.J., Berthiaume, A., Langlois, V.S., 2020. Polycyclic aromatic compounds (PACs) in the Canadian environment: Exposure and effects on wildlife. Environ. Pollut. 265, 114863. https://doi.org/10.1016/j.envpol.2020.114863

Wang, W., Lee, J., Oh, J.-K., Lee, S.-J., Choi, S.-D., Oh, J.-E., 2021. Per- and polyfluoroalkyl substances and their alternatives in black-tailed gull (*Larus crassirostris*) eggs from South Korea islands during 2012–2018. J. Hazard. Mater. 411, 125036. https://doi.org/https://doi.org/10.1016/j.jhazmat.2020.125036

Wang, X., Liu, J., Yin, Y., 2011. Development of an ultra-high-performance liquid chromatography–tandem mass spectrometry method for high throughput determination of organophosphorus flame retardants in environmental water. J. Chromatogr. A 1218, 6705–6711. https://doi.org/https://doi.org/10.1016/j.chroma.2011.07.067

Wang, Y., Qian, H., 2021. Phthalates and Their Impacts on Human Health. Healthc. (Basel, Switzerland) 9. https://doi.org/10.3390/healthcare9050603

Wang, Z., Walker, G.W., Muir, D.C.G., Nagatani-Yoshida, K., 2020. Toward a Global Understanding of Chemical Pollution: A First Comprehensive Analysis of National and Regional Chemical Inventories. Environ. Sci. Technol. 54, 2575–2584. https://doi.org/10.1021/acs.est.9b06379

Wei, L., Huang, Q., Qiu, Y., Zhao, J., Rantakokko, P., Gao, H., Huang, F., Bignert, A., Bergman, Å., 2023. Legacy persistent organic pollutants (POPs) in eggs of night herons and poultries from the upper Yangtze Basin, Southwest China. Environ. Sci. Pollut. Res. 30, 93744–93759. https://doi.org/10.1007/s11356-023-28974-z

Wenny, D.G., Devault, T.L., Johnson, M.D., Kelly, D., Sekercioglu, C.H., Tomback, D.F., Whelan, C.J., 2011. The Need to Quantify Ecosystem Services Provided By Birds. Auk 128, 1–14. https://doi.org/10.1525/auk.2011.10248

Whelan, C.J., Şekercioğlu, Ç.H., Wenny, D.G., 2015. Why birds matter: from economic ornithology to ecosystem services. J. Ornithol. 156, 227–238. https://doi.org/10.1007/s10336-015-1229-y

WWF, 2022. Living Planet Report 2022 -Building a nature positive society. WWF, Gland, Switzerland.

Yu, Y., Jin, H., Lu, Q., 2022. Effect of polycyclic aromatic hydrocarbons on immunity. J. Transl. Autoimmun. 5, 100177. https://doi.org/https://doi.org/10.1016/j.jtauto.2022.100177

Zapata, P., Ballesteros-Cano, R., Colomer, P., Bertolero, A., Viana, P., Lacorte, S., Santos, F.J., 2018. Presence and impact of Stockholm Convention POPs in gull eggs from Spanish and Portuguese natural and national parks. Sci. Total Environ. 633, 704–715. https://doi.org/10.1016/j.scitotenv.2018.03.081

Zarębska, M., Bajkacz, S., 2023. Poly– and perfluoroalkyl substances (PFAS) - recent advances in the aquatic environment analysis. TrAC Trends Anal. Chem. 163, 117062. https://doi.org/https://doi.org/10.1016/j.trac.2023.117062

Zhang, H., Shen, Y., Liu, W., He, Z., Fu, J., Cai, Z., Jiang, G., 2019. A review of sources, environmental occurrences and human exposure risks of hexachlorobutadiene and its association with some other chlorinated organics. Environ. Pollut. 253, 831–840. https://doi.org/https://doi.org/10.1016/j.envpol.2019.07.090

Zhang, Q., Wang, Y., Zhang, C., Yao, Y., Wang, L., Sun, H., 2022. A review of organophosphate esters in soil: Implications for the potential source, transfer, and transformation mechanism. Environ. Res. 204, 112122. https://doi.org/https://doi.org/10.1016/j.envres.2021.112122

2. OBJECTIVES AND THESIS STRUCTURE

The main objective of this Thesis is to determine the presence and impact of chemical pollution in natural areas of ecological significance for bird and biodiversity conservation and to assess the exposure of contaminants to several bird species. To achieve this purpose, a representative selection of organic micropollutants, including emerging and legacy compounds, were studied to determine their presence in water, soil, sediment, and evaluate their occurrence and potential impact in birds.

The specific objectives of this Thesis are:

1. To develop sampling and analytical methods to determine the presence of lifestyle compounds, pharmaceuticals, in-use pesticides, OPEs and PFASs in water, and OCPs, PCBs, PAHs, plasticizers and OPEs in soils and sediments.
2. To evaluate the contamination status of 140 Important Bird and Biodiversity Areas (IBAs) and to identify the distribution pattern of contaminants and potential sources of contamination.
3. To perform an Environmental Risk Assessment to identify the most impacted areas by chemical pollution and the most concerning compounds.
4. To assess the feasibility of biomonitoring schemes based on birds as sentinel species to monitor environmental contaminants.
5. To determine the exposure and impact of contaminants on waterbird and raptor species.

To fulfil the objectives of the Thesis, it is divided in the following two parts:

Part I: Contaminants in Important Birds and Biodiversity Areas

The first part of the Thesis comprises four chapters based on the analysis of environmental contaminants in water, soils, and sediments from IBAs. The four chapters focus on objectives 1, 2 and 3 of the Thesis. The sampling procedure and analytical methods are established to determine the presence of 59 organic micropollutants in water samples and 52 organic micropollutants in soils and sediment samples from 140 IBAs from Spain. The chapters assess the contamination status of the selected IBAs, analysing the sources and evaluate the distribution of pollutants through GIS. Also, an Environmental Risk Assessment is performed in all matrices to identify the most polluted sites and the concerning compounds potentially impacting these natural areas.

Chapter I: Pilot monitoring scheme of water pollutants in Important Bird and Biodiversity Areas.

Chapter II: Water pollution threats in Important Bird and Biodiversity areas from Spain.

Chapter III: Impact of organic contaminants in soils from Important Bird and Biodiversity Areas.

Chapter IV: Assessing sediments contamination status in Important Bird and Biodiversity areas.

Part II: Biomonitoring contaminants in birds

The second part of the Thesis includes three chapters focusing on the significance of birds as sentinel species to monitor environmental contaminants. Chapter V focuses on objective number 4 providing a review of constraints identified to be important to address to successfully establish a pan-European monitoring scheme based on raptor samples. Chapters VI and VII fulfil objective 5 of the Thesis. All target compounds analysed in water, soils, and sediments from IBAs are analysed in birds' samples, to provide an insight of the implication of the contamination of natural areas to wildlife inhabiting these sites. The exposure to contaminants is assessed in blood from flamingos' chicks, showing the first evidence on the exposure of organic micropollutants in the Ebro delta flamingo's breeding colony. Finally, the exposure to contaminants is determined in the livers of five species of road-killed nocturnal raptors from Portugal. The study assesses the different patterns of exposure among species related to their habitat and trophic position.

Chapter IV: A review of constraints and solutions for collecting raptor samples and contextual data for a European Raptor Biomonitoring Facility.

Chapter VI: Legacy and emerging contaminants in flamingos' chicks' blood from the Ebro Delta Natural Park.

Chapter VII: Assessing contamination profiles in livers from road-killed owls.

The Thesis follows with a general discussion on the main findings and the final conclusions. Overall, this Thesis provides evidence of the importance of monitoring contaminants in natural areas and proposes the use of birds as sentinel species to address the threat of chemical pollution in biodiversity.

3. RESULTS

PART I: CONTAMINANTS IN IMPORTANT BIRD AND BIODIVERSITY AREAS

CHAPTER I

PILOT MONITORING SCHEME OF WATERS POLLUTANTS IN IMPORTANT BIRD

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ABSTRACT

In this study we have established a monitoring scheme to determine the presence and distribution of widely used pharmaceuticals, pesticides, organophosphate esters (OPEs) and perfluoroalkyl substances (PFASs) in water bodies from Important Bird and Biodiversity Areas (IBAs) from Spain. The monitoring scheme included the georeferenced sampling of rocky mountain, Atlantic forest, riparian forest, Mediterranean forest, agricultural, inland aquatic and coastal aquatic IBAs, with the aim to evaluate the impact of widely used chemicals in those aquatic resources. Water samples were extracted using a generic solid-phase extraction protocol and analysed by 3 analytical methods based on liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). Quality parameters such as compound recovery, intra and inter-day variation, linearity and limits of detection were calculated in order to validate the methods. In addition, the ionization conditions and the optimization of the most appropriate transitions permitted unequivocal identification. Once the sampling and analytical procedure was set-up, 59 target compounds were monitored in 63 samples. Pharmaceutical, followed by pesticides, OPEs and PFASs were widespread along all IBAs studied at concentrations from 0.5 to 41083 ng/L. Overall, study highlights the need to monitor the presence of contaminants in areas of high ecological interest to contribute to pollution control and mitigation towards protection of biodiversity.

1. INTRODUCTION

Environmental pollution of surface waters is a worldwide threat to freshwaters ecosystems leading to habitat degradation and biodiversity loss (Dudgeon, 2019). The impact of pollution on biodiversity can be critical for the preservation of natural values in vulnerable areas. Important Bird and Biodiversity Areas (IBAs) are sites of ecological interest and of importance for the conservation of biodiversity, identified by BirdLife International under scientific criteria, specifically for bird populations (Donald et al., 2019). However, IBAs are affected by anthropogenic activities such expansion and intensification of agriculture and livestock rearing, urban settlements, wastewater treatment plants (WWTP) discharges, road networks, and also related to direct human intrusion as tourism, picnic areas and hiking (Hausmann et al., 2019). Directly or indirectly, these activities affect water quality of these areas and pose these ecosystems at risk.

Waters from IBAs are categorized as headwater streams, springs, ditches, flushes, small lakes, and ponds. Some of them are within the category of small water bodies. They are vital for freshwater biodiversity, but the presence of contaminants albeit at generally low concentrations pose these fragile ecosystems at risk (Albert et al., 2021). However, the evaluation of the contamination status in hotspots of biodiversity as IBAs is often overlooked because of the difficulty to implement monitoring programmes that provide data on the sources and distribution of micropollutants in poorly accessible areas. This contrasts with the multitude of studies on wastewater effluents or waters affected by urban and industrial discharges, as reflected in recent reviews (e.g. Rathi et al., 2021). Monitoring schemes of IBAs require a particular sampling effort, logistics for sample transport and coordination with the analytical laboratory to process the samples quickly to minimize degradation of contaminants. For these valuable samples, it is crucial to use multiresidue methods to assess multiple polar contaminants in water as different chemical families with specific modes of action can provide a broader vision on in-use chemicals impacting IBAs. Liquid chromatography coupled to tandem mass spectrometry techniques (LC-MS/MS) serve for this purpose as allows routine water monitoring at trace level (Petrie et al., 2016), as the concentration of contaminants from IBAs is expected to be low.

The main objective of this pilot study is to develop and implement a sampling and analytical procedure to determine 59 organic contaminants in waters from IBAs. A generic extraction was performed followed by 3 LC-MS/MS methods to determine pharmaceuticals, pesticides, organophosphate esters (OPEs), benzophenone, and perfluoroalkyl substances (PFASs). In this study, the effectiveness of the proposed field and analytical methodology was evaluated in 21 IBAs as a first step to establish a thorough water

monitoring scheme. Studied compounds were selected because they are high consumption chemicals and are considered mobile, ubiquitous, and persistent emerging contaminants (Barbosa et al., 2016; Gómez-Canela et al., 2019). The presence of organic micropollutants in IBA sites from Spain nor over the world has not been reported previously, despite contamination is a pressure than can affect the well-being of these ecosystems.

2. MATERIALS AND METHODS

2.1. Chemicals and materials

This study comprises the analysis of 59 compounds, being 21 pharmaceuticals, 17 pesticides, 3 OPEs, benzophenone and 17 PFASs. Standards of 98-99% purity were purchased from Sigma-Aldrich (St. Louis, USA), Dr. Ehrenstorfer (Germany), and Wellington laboratories (New Zeland), except for pregabalin and paracetamol that were purchased from European Pharmacopeia Reference Standard. The list of contaminants analysed is indicated in Table S1 of Supplementary Information (SI). Stock standards solutions were prepared at a concentration of 1000 ng/µL and working solutions at 1 - 100 ng/µL all in methanol. The Internal Standard (IS) used were acetaminophen-methyl-d_3, lidocaine-diethyl-d_{10}, isoproturon-d_6, and triphenyl phosphate-d_{15} from Sigma-Aldrich (St. Louis, MO USA), sulfamethoxazole-d_4, and carbamazepine-d_2 from Santa Cruz Biotechnology (Sta. Cruz, CA, USA), MPFOA and MPFOS from Wellington (New Zeland). A mixture containing all internal standards was prepared at 1 ng/ µL in methanol.

Methanol (MeOH) and HPLC water (LiChrosolv grade) were supplied by Merck (Darmstadt, Germany), ammonium formate (NH_4HCO_2), ammonium acetate ($NH_4CH_3CO_2$) and formic acid (HCOOH) from Sigma-Aldrich, and acetonitrile (ACN) from Fisher Scientific Chemical (Bridgewater, USA).

2.2. Sampling

In 2019 a total of 21 IBAs representative from different Spanish habitats were sampled: rocky mountain, Atlantic forest, riparian forest, Mediterranean forest, agricultural, inland aquatic and coastal aquatic (Table 1). The sampling area was geolocalized and potential sources of pollution were determined in ArcGIS 10.1 (ESRI) using spatial data obtained from CNIG (www.centrodedescargas.cnig.es/, land cover information). The same day three water samples were collected within each IBA during the period April-August 2019. One Liter of freshwater was collected using a telescope beaker scoop from the shore of streams, rivers, canals, lakes, or ponds inside each IBA. Samples were dosed in amber glass bottles and kept cold for transportation and analysed within 1-3 days after collection to avoid degradation of contaminants.

Table 1. Habitat type, IBA codes and name, and province of the 21 selected areas throughout Spain.

Habitat	IBA Code	IBA Name	Province
Rocky Mountain	ES014	Babia-Somiedo	Asturias, León
Rocky Mountain	ES420	Montaña Central de León	León
Rocky Mountain	ES020	Picos de Europa	Cantabria, León, Asturias
Atlantic forest	ES015	Sierras de Gistreo y Coto	León
Atlantic forest	ES016	Sierras Centrales de la Cordillera Cantabrica	Asturias, León
Atlantic forest	ES022	Sierras de Peñalabra y del Cordel	Cantabria, Burgos
Riparian forest	ES042	Rio Pisuerga en Dueñas	Palencia, Valladolid
Riparian forest	ES072	Carrizales y Sotos de Aranjuez	Toledo
Riparian forest	ES053	Cañon del Duraton	Segovia
Mediterranean forest	ES071	El Pardo-Viñuelas	Madrid
Mediterranean forest	ES227	Sierras al Sur de Jaen	Jaén, Granada
Mediterranean forest	ES235	Sierra Morena de Córdoba	Cordoba
Agricultural	ES058	Tordesillas - Mota del Marques	Valladolid
Agricultural	ES202	Llanos de Oropesa	Toledo
Agricultural	ES008	A Limia	Orense
Inland aquatic	ES188	Alto Tajo y Tajuña	Guadalajara, Cuenca
Inland aquatic	ES023	Embalse del Ebro	Cantabria, Burgos
Inland aquatic	ES192	El Hito	Cuenca
Coastal	ES035	Urdaibai - Matxitxako	Vizcaya
Coastal	ES140	Delta del Llobregat	Barcelona
Coastal	ES148	Delta de l'Ebre	Tarragona

2.3. SPE extraction and analysis

A single generic solid-phase extraction (SPE) protocol was used to preconcentrate all target compounds considering the dissolved and particulate fractions, to have the total concentration of contaminants in water. 200 mL of unfiltered water were spiked with 50 ng of the surrogate standards. HLB SPE cartridges (6 cc, 200 mg, Waters, Milford, USA) were conditioned with 12 mL of methanol and 6 mL of milliQ water and samples were loaded at a flow of 1 mL/min. After preconcentration, SPE cartridges were dried for 25 min and the elution was performed with 15 mL of methanol and 6 mL of acetone. The extracts were evaporated to approximately 0.5 mL in a TurboVap® LV (Uppsala, Sweden) under N_2 stream at 25ºC, and transferred to a 1.5 mL chromatographic vial using 1 mL methanol as washing solvent. Finally, samples were evaporated to dryness with a ReactiVap®, and reconstituted with 100 µL of methanol and 100 µL of water. Samples were

stored at -20ºC until analysis. Samples containing solid particles were filtered with 13 mm nylon filters of 0.22 µm pore diameter (Clarify, Phenomenex, USA).

Liquid chromatography coupled to a triple quadrupole mass analyser (LC-MS/MS) with an electrospray ion source (Waters, Milford, USA) was used in 3 methods with different conditions to determine (i) pharmaceuticals, (ii) pesticides, OPEs and benzophenone, and (iii) PFASs. In all cases, a BEH C18 analytical column (100 mm x 2.1 mm, particle size 1.7 µm) (Acquity, Waters, Milford, USA) was deployed. The flow rate was set at 200 µL/min and the sample injection volume was 10 µL. Method optimization was performed by flow injection analysis of individual standard solutions at 1 ng/µL, where the cone voltage was optimized from 10 to 130 V to select the molecular ion with the highest intensity and then the collision energy was optimized in a range from 10 to 50 eV to select the most intense and selective transitions. In all cases, acquisition was performed in selected reaction monitoring (SRM) mode using two transitions from the precursor ion to the product ion to identify each compound. Identification criteria included the retention time and 2 transitions, one used for quantification and the other for confirmation. Internal standard quantification was performed using acetaminophen-methyl-d_3, sulfamethoxazole-d_4, lidocaine-diethyl-d_{10} and, carbamazepine-d_2 for pharmaceuticals, isoproturon-d_6 and triphenyl phosphate-d_{15} for pesticides and OPEs, and MPFOS and MPFOA for PFASs. The system and data management were processed using MassLynx v4.1 software package.

2.3.1. Analysis of Pharmaceuticals

Mobile phase composition consisted of binary mixtures with acetonitrile with 0.1% formic acid (A) and water with 0.1% formic acid (B). Gradient elution started at 10% A and 90% B, increasing to 80% A in 19.4 min and to 100% of A in 2.6 min, held for 1 min and returned to initial conditions in 3 min. All compounds were measured under positive electrospray ionization (ESI+), except for furosemide that was detected in negative electrospray ionization (ESI-).

2.3.2. Analysis of Pesticides and OPEs

Mobile phase composition consisted of binary mixtures with methanol with 0.1% formic acid and 5 mM ammonium formate (A) and water with 0.1% formic acid and 5 mM ammonium formate (B). Gradient elution started at 5% A and 95% B, increasing to 55% A in 7 min, to 95% in 7 min and to 100% of A in 1 min, held for 2 min and returned to initial conditions in 1 min. Most compounds were measured under ESI+, except for fludioxonyl and diclofop that were detected in ESI-.

2.3.3. Analysis of Perfluorinated Compounds

The analysis was adapted from the method previously described by (Sánchez-Avila et al., 2010). Mobile phase composition consisted of binary mixtures methanol and acetonitrile (80:20) with 10 mM of ammonium acetate in (A) and water with 10 mM of ammonium acetate (B). Gradient elution started at 50% A and B, increasing to 100% A in 3 minutes, and held for 7 minutes, and returned to initial conditions in 5 minutes. All compounds were measured under ESI-.

2.4. Quality Control/Quality Analysis

For all compounds studied, the three methods developed were assessed for accuracy, linearity, sensitivity, selectivity, and extraction efficiency. Intra and inter-day accuracy were determined by injecting a 0.05 ng/µL standard during 5 consecutive injections and in 5 different days, respectively. Linearity was studied over a concentration range of 0.001 to 0.6 ng/µL, with surrogate standards kept at a constant concentration of 0.05 ng/µL. Calibration standards were prepared in methanol: water (1:1). Instrumental limits of detection (IDL) were calculated as the amount of analyte that gives a signal to noise ratio of 3 (S/N=3) and method detection limits (MDL) as the concentration that gave a S/N=3 using spiked MilliQ water, considering the extraction and analytical procedure. Extraction efficiency was determined by spiking water at a concentration of 100 ng/L and performing the analysis in quadruplicate. The MDL was used as criteria for quantification as the concentration in waters from IBAs are expected to be low. Samples values below MDL were given a value of zero in a way that the concentrations were not overestimated during statistical analysis.

3. RESULTS AND DISCUSSION

3.1. Method performance and validation

In this study, we have optimized a single extraction method and analysis by 3 LC-MS/MS methods to determine pharmaceuticals, pesticides, OPEs and PFASs. Based on FIA analysis, SRM transitions were selected considering the peak abundance of the parent ion (normally the molecular ion) and the specific fragment ions for each compound. The cone voltage and the capillary energy were also optimized, and the conditions selected provided the highest peak abundance. The optimal acquisition parameters are displayed in Table S2 for pharmaceuticals, Table S3 for pesticides, OPEs, and benzophenone and Table S4 for PFASs. The MRM transitions allowed unequivocal identification with high compound specificity and sensitivity.

For all 3 chromatographic methods used for the different chemical families, the same C_{18} column was used with different mobile phases and gradient conditions to maximize compound detectability. Gradient elution was optimized (results not shown) to avoid elution at the front, as most compounds have high polarity. Good chromatographic resolution was obtained for all compounds and no coelutions were observed. Figure S1 to S3 shows the chromatograms obtained from the 3 methods developed, showing that peaks were well resolved at their specific MRM transition.

Quality parameters including inter-day and intra-day precision, linearity, sensitivity, and extraction efficiency are also indicated in Table S2-S4. Good correlation was obtained (r^2=0.99) over a concentration range of 0.001-0.6 ng/µL using internal standard quantification. The inter-day precision of the method at 0.05 ng/µL was between 5 to 29% for pharmaceuticals, from 5 to 23% for pesticides, except for fludioxonyl, diclofop and pendimethalin with values >29%, 12 to 23% for OPEs and benzophenone, and from 1 to 22% for PFASs, except for PFTriDA and PFODA with 55 and 41% variation. Recovery studies were performed in milliQ water as waters from IBA sites are considered pristine. Recoveries were performed at 100 ng/L level as this value is recommended as threshold concentration to be monitored by the Water Framework Directive (Directive 39/2013/EU). Recovery of pharmaceuticals were between the acceptable range of 60 to 120% (Table S2), although metformin (50%), allopurinol (21%), gabapentine (34%), atrovastatin (56%) had the lowest recoveries due to degradation in water while for nicotine, atenolol, paracetamol, caffeine or valsartan, high recoveries were observed (>136%) due to the low response of the IS used. The recoveries for pesticides, OPEs and benzophenone (Table S3) were also adequate for most compounds, despite of the low values for the less stable compounds as tebufenpyrad (19%), oxyfluorfen (58%), tebuconazol (48%), kresoxim-methyl (47%) and fludioxonyl (56%). PFASs were recovered with good extraction yields except for long-chained PFHxDA (28%) and PFODA (17%) (Table S4). MDL for pharmaceuticals were from 0.05 to 17 ng/L, for pesticides from 0.10 to 8.36 ng/L, for OPEs between 0.05 and 0.53 ng/L, and for PFASs between 0.05 and 0.55 ng/L. These low MDL enabled trace determination of target contaminants in water.

3.2. Occurrence of contaminants in IBAs

The proposed sampling and analytical procedure were used to evaluate the occurrence of micropollutants in 63 samples from 21 IBAs along the Spanish territory. A total of 49 out from 59 analysed compounds were detected in all samples, indicative that IBAs are impacted by pollution. Regarding detection frequency, pharmaceuticals and OPEs were more ubiquitous than pesticides and PFASs. According to median concentrations the detection of the different

chemical families followed the order: pharmaceuticals > OPEs > Pesticides > PFASs (Figure 1). The most frequently detected compounds were caffeine present in 79% of the analysed samples with a median concentration of 46.5 ng/L, TCEP (57% detection frequency, median 3.29 ng/L), valsartan (57% detection frequency, median 330 ng/L), TBP (56% detection frequency, median 10.9 ng/L) and metformin (43% detection frequency, median 11.7 ng/L). Fourteen compounds including pharmaceuticals and pesticides were detected in 20-35% of the samples, 16 compounds including pesticides and PFASs were detected between 10 and 20% of the samples and 14 compounds were detected sporadically. Compounds never detected were quetiapine, clorphenvinfos, spinosad, pendimethalin, PFUnA, PFoDA, PFTriDA, PFTeDA, PFHxDA, and PFODA.

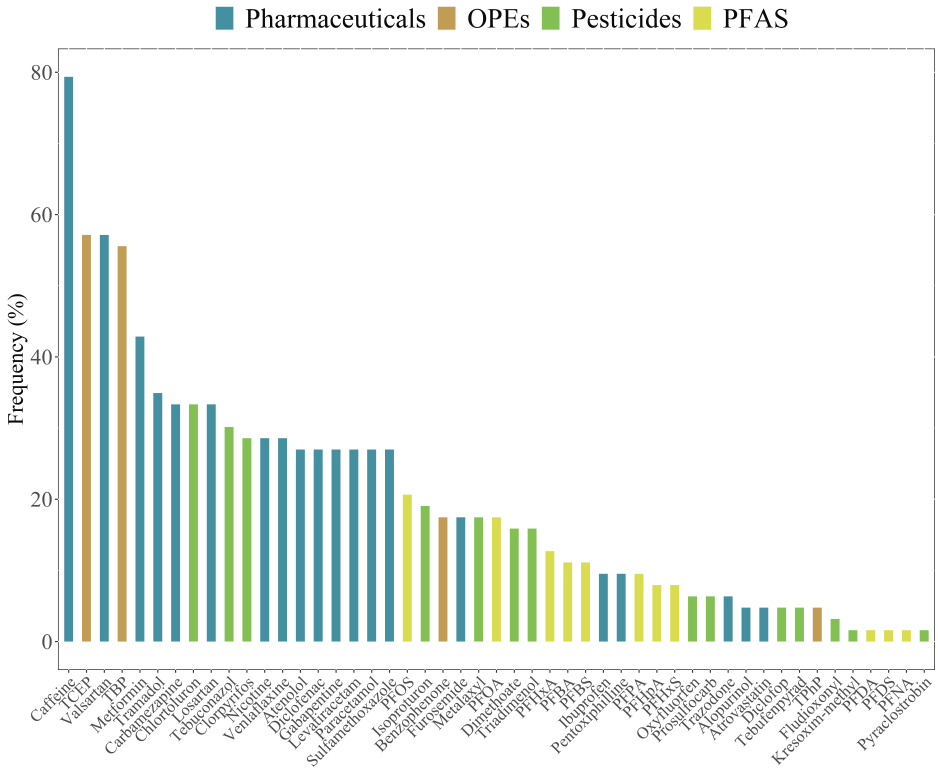


Figure 1. Frequency of detection of contaminants detected in the samples (n=63).

Considering the different categories of IBAs, the number of compounds detected were higher in coastal aquatic and riparian forest than agricultural, inland aquatic and Mediterranean Forest, while rocky mountain and Atlantic forest were the IBAs with the smallest number of compounds detected (Figure 2). Regarding the total concentrations, agricultural, coastal aquatic,

and riparian forest were the IBAs with the highest levels, followed by inland aquatic, while Mediterranean forest, Atlantic forest and rocky mountain were the IBAs with the lowest contamination levels. The IBAs located close to urban settlements or agricultural areas are more exposed to contaminants, considering both detection frequency and concentrations detected.

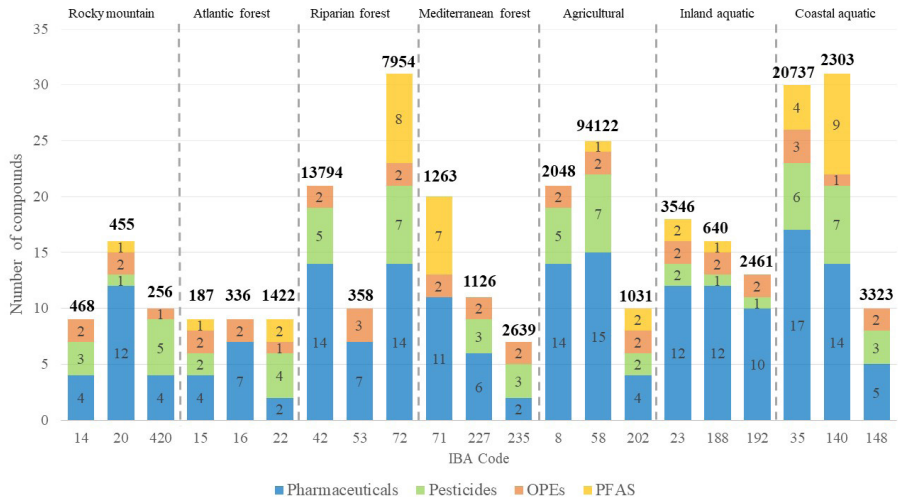


Figure 2. Number of detected compounds in selected IBAs grouped by chemical families: pharmaceuticals (n = 21), pesticides (n = 17), OPEs (n = 3), benzophenone and PFASs (n = 17). In bold, the total concentration of contaminants (ng/L) in each IBA.

The concentration of each contaminant is indicated in Figure 3. The contamination profile dominated by pharmaceuticals was observed in all IBAs with concentrations ranging from 1 to 41083 ng/L and a median concentration of 105 ng/L. The most frequently detected compound was caffeine, with a concentration ranging from 2 to 41083 ng/L. Besides being used as pharmaceutical, caffeine is strongly related to its presence in coffee or other beverages (Peteffi et al., 2018). It is released by WWTP effluents and it is a useful marker for anthropogenic impact in freshwater (Borrull et al., 2021). Caffeine was detected at high concentrations levels at IBA ES058 (further details in Table 1), a sample collected from a small water body next to a town drain. The lack of water treatment and the small volume of water are determinant factors that explain the high concentrations found in the area. This IBA is an area without any form of legal protection (BirdLife International, 2023). Similar caffeine concentrations were reported in previous studies in surface waters (Li et al., 2020; Paíga et al., 2019). Ibuprofen, atrovastatin and tramadol were detected at high concentrations between 58 to 5592 ng/L, 45 to 433 ng/L and 1 to 848 ng/L respectively. Ibuprofen is a common pharmaceutical detected at relevant concentrations levels in surface waters due to its high consumption (Camacho-Muñoz et al., 2010). The presence

of pharmaceuticals such as atorvastatin, for the treatment of cholesterol, or tramadol an opioid analgesic, are associated to consumption patterns, water stability, and continuous release from WWTP effluents due to incomplete removal (Kasprzyk-Hordern et al., 2009). In general compounds frequently detected (Figure 1) corresponded with the ones with high consumption among population, as valsartan (30 t/year) and paracetamol (1500 t/year) in Spain (Ortiz de García et al., 2013). Metformin, used against type 2 diabetes, is also among the most consumed drugs worldwide (Gómez-Canela et al., 2019), and is excreted unaltered in the urine, thus favouring its detection in the surface water (Godoy et al., 2018). In the IBAs studied, metformin was detected in 43% of the samples at concentrations from 1 to 106 ng/L. The European Medicine Agency indicates that pharmaceuticals present at concentrations > 10 ng/L should be assessed for risk in the aquatic environment (EMA, 2006); 12% of the samples surpassed this limit. The compounds that presented the highest number of values above 10 ng/L were caffeine (79%) and valsartan (57%), corresponding also to the two most ubiquitous compounds detected. The impact of pharmaceuticals in waters has been recently reviewed and authors highlight that because they are bioactive molecules, they can interfere in the regulation and expression of genes and immune responses, impair hatching and development in adult organisms and affect the neuroendocrine and cardiovascular system (González-González et al., 2022). Therefore, the ubiquitous presence of pharmaceuticals in IBA water can have important ecological relevance and need to be surveyed to minimize potential adverse effects on wildlife.

∑Pesticides were detected in 60% of the samples, ranging from 0.5 to 2640 ng/L, with a median concentration of 7.50 ng/L. In 14% of the samples, five or more different pesticides were detected, and those samples were situated close to agricultural areas. The number of detected pesticides depends on crop types and environmental conditions as periods of heavy rains (Moreno-González et al., 2013). Our results correspond to the period spring-summer when pesticides are mostly applied. In this preliminary study, the most frequently detected pesticides were the herbicide chlortoluron (33% detection frequency) at concentrations ranging from 0.5 to 91 ng/L, followed by the systemic fungicide tebuconazole (30% detection frequency) and the organophosphorus pesticide chlorpyrifos (29%). Chlorpyrifos has been detected as an ubiquitous insecticide in surface waters from Spain (Claver et al., 2006; Moreno-González et al., 2013; Pascual Aguilar et al., 2017). The highest occurrence of pesticides was detected at IBA ES072, containing 7 pesticides. Despite being classified as riparian forest habitat, the 60% of the area is occupied by agricultural land-use. This is an indicative that the generic classification of habitat types may not be useful to identify sites potentially affected by pesticides.

∑OPEs were detected in 76% of the samples. TBP and TCEP were detected in 56% and 57% of the samples at a concentration from 1 to 91.5 ng/L. TPhP was detected only in three samples (33.5-99 ng/L). These compounds are used as flame retardants in several consumer and construction goods, but also as additives in plastics, foams, paints, furniture and electronic materials (Du et al., 2019), and their presence in surface waters is mostly associated to wastewater discharges (Wang et al., 2020), but also via plastic trash and industrial waste (Hou et al., 2016; Kim and Choi, 2014). The ubiquity of OPEs in Spanish surface waters have been reported in previous studies, with comparable concentrations range as found in Nalón, Arga and Besós river (Cristale et al., 2013) and Ebro, Llobregat, Júcar and Guadalquivir for TCEP (Gorga et al., 2015). Benzophenone was detected in eleven samples (17%) at concentrations from 47.5 to 1040 ng/L. This compound is widely used in cosmetics but also as plastic additive, the highest concentrations were found in IBAs ES058 (agricultural) and ES035 (coastal). Both areas are influenced by urban proximity and water presented waste residues when sampling.

∑PFASs were present in less than 21% of the samples (Figure 1), and 11 out of the 17 target perfluorinated compounds were detected at levels ranging from 5.2 to 1257 ng/L. PFASs are a diverse group of chemicals used in a high number of industrial and consumer products like surfactants, agrochemicals, fire-fighting foams, oil-resistant packaging for food products, and water and stain proofing agents. The most ubiquitous compound was PFOS (21% detection frequency) at concentrations from 7.9 to 1257 ng/L, followed by PFOA (17% detection frequency) from 21.6 to 73.5 ng/L. Other compounds detected in a sporadic way were PFHxA (13% detection frequency), PFBA (11% detection frequency) and PFBS (11% detection frequency). In general, the contamination profile of PFASs in European surface waters is dominated by PFOA and PFOS, associated to tourism, urban runoff, and wastewaters (Podder et al., 2021). PFOS was found at high concentrations in IBA ES022 (1257 ng/L) that corresponds to Atlantic forest and is a pristine area except for PFOS, the main pressure of the site is tourism infrastructures, particularly for skiing activities (BirdLife International, 2021a). PFOS was also the main contaminant in IBA ES023 (317 ng/L), an inland aquatic IBA where the sample was collected next to WWTP discharge, and high concentrations of caffeine were also detected, indicating that it is an area influenced by anthropogenic pressures (BirdLife International, 2021b). PFASs are highly accumulative and have a high potential for trophic transfer. They have been detected in insects larvae and other invertebrates (Fernández-Sanjuan et al., 2010), amphibians (Abercrombie et al., 2019), mussels, clams and fish (Miranda et al., 2021) and in top predators at alarming levels (Androulakakis et al., 2022), thus reinforcing the need to control their occurrence in IBA sites.

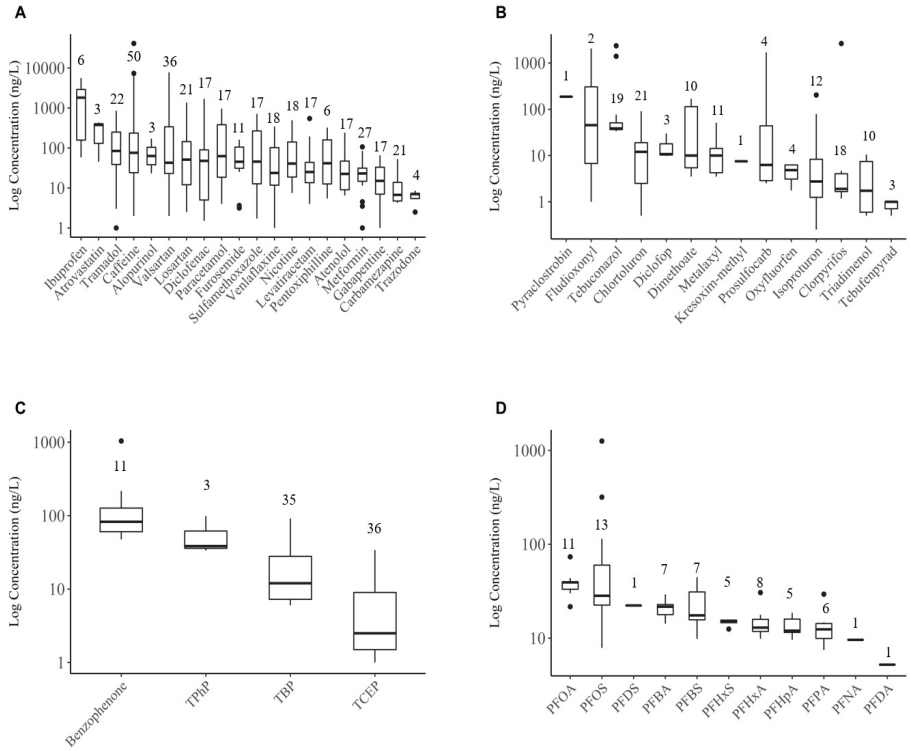


Figure 3. Boxplot indicating the 25%, 75% quartile and median concentrations (ng/L) of pharmaceuticals (A), pesticides (B), flame retardants and benzophenone (C) and perfluorinated compounds (D) detected in 21 different IBAs, ordered from the highest to lowest median concentration. Numbers on top of each compound indicate the number of positive samples out of 63 analysed.

3.3. Contaminants as a critical pressure in IBAs or other protected areas

This study evidences the lack of information regarding the presence of organic contaminants in IBAs or Natural or National parks, despite being areas of high ecological interest, natural beauty, and hotspots of biodiversity. These areas generally hold some degree of protection, but nonetheless are affected by anthropogenic activities. Table 2 summarizes main contaminants detected in headwaters or waters from natural reserves. Pharmaceutical contamination, mainly analgesics, were observed in the protected estuarine waters of the Ebro delta attributed to runoff (Čelić et al., 2019). Pharmaceuticals, along with life-style compounds and pesticides were detected in the upper Tagus River (Spain) and their presence was related to land use (Arenas-Sánchez et al., 2019; Rico et al., 2019). In the Colorado Plateau National Park (USA), pharmaceuticals and personal-care compounds, bisphenol A, caffeine and OPEs were detected at a mean frequency of 10–20%, with concentrations up to 5360 ng/L and refer to WWTP effluents as main

contamination sources (Weissinger et al., 2018). Pesticides were ubiquitous in Auvézère and Aixette headwaters (France) associated to their use in nearby agricultural areas (Guibal *et al.*, 2018). A suite of 44 commonly used pesticides, out of 94 analyzed, were detected in lentic small water bodies from northern Germany at concentrations that produce toxic effects in algae, macrophytes and invertebrates, thus affecting the ecosystem services of these valuable landscapes (Ulrich et al., 2022). In fact, pesticides have been identified as a main threat of aquatic resources (Lorenz et al., 2017). Even isolated habitats as mountain freshwater ecosystems act as a reservoir of organic contaminants, although little is known on the toxicological effects on biodiversity patterns (Schmeller et al., 2018). OPEs and PFASs were detected at concentrations up to 330 ng/L in surface waters from the Valencia Albufera Natural Park, often exceeding EQS, and authors point to need to identify pollution sources to minimize the environmental impact of those contaminants (Lorenzo et al., 2019). A recent study undertaken in a biodiversity spot in Clinch River watershed (Virginia, USA) indicates that chronic and pervasive contamination produces a decline in mussel populations and this affect ecosystem equilibrium (Cope et al., 2021). Trace concentrations of wastewater-associated contaminants impaired structural degradation of German streams with significant impact on invertebrate populations (Stalter et al., 2013). Without measures to address water quality and habitat restoration, biodiversity loss and decline in populations will continue, and hence, this study highlights the need to implement thorough monitoring programs in IBAs to protect these vulnerable ecosystems.

Table 2. Type and range concentrations of contaminants detected in waters from areas holding some type of protection (compounds ordered alphabetically).

Study Area	Protection status	Pharmaceuticals	Pesticides	OPEs	PFASs	Reference
Ebro delta (NE Spain)	Reference site	2.1–6.3 ng/L Carbamazepine Citalopram Desloratadine Gemfibrozil Phenazone Venlaflaxine				Čelić et al., (2019)
Tagus River (NW Spain)	Headwaters far from direct pollution sources (samples 1–5)	0.2–50 ng/L Acetaminophen Atenolol Azithromycin Caffeine Carbamazepine Citalopram Diclofenac Erytromycin Estrone Gemfibrocil Ibuprofen Omeprazole Paraxantine Sulfamethoxazole Trimethorprim Valsartan Venlaflaxine				Arenas-Sánchez et al., (2019)
Colorado Plateau National Park (USA)	Surface waters	11–464 ng/L Caffeine Gabapentin Hydrochlorazide Lamotrigine Metformin Methylparaben Naproxen Sulfamethoxazole Triclosan DEET	11.2–116 ng/L 2,4-D Hexazione Metalaxyl Metholachlor			Weissinger et al., (2018)
Tagus River (NW Spain)	Headwaters far from direct pollution sources (samples 1–5)		0.03–15.3 ng/L Carbendazim Chlortoluton Dimethoate Diuron Imidacloprid Metribuzine Simazine Tebuconazole Terbuthrin Terbutylazine			Rico et al., (2019)
Auvézère and Aixette (France)	Only upstream waters		5–110 ng/L Atrazine Chlortoluron Diuron Desethylatrazine Imidacloprid Isoproturon Metolachlor Simazine (Others at trace levels)			Guibal et al., (2018)

Study Area	Protection status	Pharmaceuticals	Pesticides	OPEs	PFASs	Reference
Lowland catchment (Northern Germany)	Lentic small water bodies		120–4830 ng/L 44 pesticides Azxystrobin Bixafen Boscalid Metolachlor Tebuconazole Terbuthylazine (Plus others and degradation products)			Ulrich et al., (2022)
Albufera Valencia (Spain)	Surface water			<LOD-330 ng/L 6 OPEs	<LOD-47.8 ng/L 10 PFASs	Lorenzo et al., (2019)

4. CONCLUSIONS

The aim of this pilot study was to propose a sampling and analytical methodology to assess the presence of micropollutants in surface water from areas of high ecological interest, such as IBAs. Regarding the analytical protocol, we propose a single extraction procedure followed by 3 LC-MS/MS methods to determine 59 water contaminants at trace levels in an efficient and sensitive way. The developed procedure was applied as pilot study to determine the contamination patterns in 63 water samples from 21 IBAs in Spain. The preliminary results indicated the presence of 49 target compounds in the studied areas, being pharmaceuticals, the most widespread compounds followed by OPEs, pesticides and PFASs. It is remarkable that caffeine, used as tracer of urban pollution, was detected in 79% of the samples and provided indication of human intrusion. Implementation of the monitoring strategy permitted to evaluate the quality of IBA waters, and it was evidenced that in-use chemicals were frequently detected and, in some cases, at high concentrations. IBA sites are meant to be refugees for wildlife, and thus efforts are needed to protect these areas against chemical contamination and to promote biodiversity conservations actions. This study serves to implement a future large-scale monitoring to evaluate the contamination status of a representative number of IBAs from Spain with the overarching aim to assess the sources of pollution, evaluate their impact and contribute to pollution control in vulnerable areas such as IBAs.

REFERENCES

Abercrombie, S.A., de Perre, C., Choi, Y.J., Tornabene, B.J., Sepúlveda, M.S., Lee, L.S., Hoverman, J.T., 2019. Larval amphibians rapidly bioaccumulate poly- and perfluoroalkyl substances. Ecotoxicol. Environ. Saf. 178, 137–145. https://doi.org/10.1016/J.ECOENV.2019.04.022

Albert, J.S., Destouni, G., Duke-Sylvester, S.M., Magurran, A.E., Oberdorff, T., Reis, R.E., Winemiller, K.O., Ripple, W.J., 2021. Scientists' warning to humanity on the freshwater biodiversity crisis. Ambio 50, 85–94. https://doi.org/10.1007/s13280-020-01318-8

Androulakakis, A., Alygizakis, N., Gkotsis, G., Nika, M.C., Nikolopoulou, V., Bizani, E., Chadwick, E., Cincinelli, A., Claßen, D., Danielsson, S., Dekker, R.W.R.J., Duke, G., Glowacka, N., Jansman, H.A.H., Krone, O., Martellini, T., Movalli, P., Persson, S., Roos, A., O'Rourke, E., Siebert, U., Treu, G., van den Brink, N.W., Walker, L.A., Deaville, R., Slobodnik, J., Thomaidis, N.S., 2022. Determination of 56 per- and polyfluoroalkyl substances in top predators and their prey from Northern Europe by LC-MS/MS. Chemosphere 287, 131775. https://doi.org/10.1016/J.CHEMOSPHERE.2021.131775

Arenas-Sánchez, A., Rico, A., Rivas-Tabares, D., Blanco, A., Garcia-Doncel, P., Romero-Salas, A., Nozal, L., Vighi, M., 2019. Identification of contaminants of concern in the upper Tagus river basin (central Spain). Part 2: Spatio-temporal analysis and ecological risk assessment. Sci. Total Environ. 667, 222–233. https://doi.org/10.1016/J.SCITOTENV.2019.02.286

Barbosa, M.O., Moreira, N.F.F., Ribeiro, A.R., Pereira, M.F.R., 2016. Occurrence and removal of organic micropollutants : An overview of the watch list of EU Decision 2015 / 495 94. https://doi.org/10.1016/j.watres.2016.02.047

BirdLife International, 2023. Important Bird Areas factsheet: Tordesillas-Mota del Marqués [WWW Document]. URL http://www.birdlife.org (accessed 8.30.23).

BirdLife International, 2021a. Important Bird Areas factsheet: Peña Labra and Cordel mountain ranges [WWW Document]. URL http://datazone.birdlife.org/ (accessed 11.8.21).

BirdLife International, 2021b. Important Bird Areas factsheet: River Ebro dam [WWW Document]. URL http://datazone.birdlife.org/ (accessed 12.12.21).

Borrull, J., Colom, A., Fabregas, J., Borrull, F., Pocurull, E., 2021. Presence, behaviour and removal of selected organic micropollutants through drinking water treatment. Chemosphere 276, 130023. https://doi.org/10.1016/j.chemosphere.2021.130023

Camacho-Muñoz, D., Martín, J., Santos, J.L., Aparicio, I., Alonso, E., 2010. Occurrence, temporal evolution and risk assessment of pharmaceutically active compounds in Doñana Park (Spain). J. Hazard. Mater. 183, 602–608. https://doi.org/10.1016/j.jhazmat.2010.07.067

Čelić, M., Gros, M., Farré, M., Barceló, D., Petrović, M., 2019. Pharmaceuticals as chemical markers of wastewater contamination in the vulnerable area of the Ebro Delta (Spain). Sci. Total Environ. 652, 952–963. https://doi.org/10.1016/J.SCITOTENV.2018.10.290

Claver, A., Ormad, P., Rodríguez, L., Ovelleiro, J.L., 2006. Study of the presence of pesticides in surface waters in the Ebro river basin (Spain). Chemosphere 64, 1437–1443. https://doi.org/10.1016/j.chemosphere.2006.02.034

Cope, W.G., Bergeron, C.M., Archambault, J.M., Jones, J.W., Beaty, B., Lazaro, P.R., Shea, D., Callihan, J.L., Rogers, J.J., 2021. Understanding the influence of multiple pollutant stressors on the decline of freshwater mussels in a biodiversity hotspot. Sci. Total Environ. 773, 144757. https://doi.org/10.1016/J.SCITOTENV.2020.144757

Cristale, J., Vázquez, A.G., Barata, C., Lacorte, S., 2013. Priority and emerging fl ame retardants in rivers : Occurrence in water and sediment, *Daphnia magna* toxicity and risk assessment. Environ. Int. 59, 232–243. https://doi.org/10.1016/j.envint.2013.06.011

Donald, P.F., Fishpool, L.D.C., Ajagbe, A., Bennun, L.A., Bunting, G., Burfield, I.J., Butchart, S.H.M., Capellan, S., Crosby, M.J., Dias, M.P., Diaz, D., Evans, M.I., Grimmett, R., Heath, M., Jones, V.R., Lascelles, B.G., Merriman, J.C., O'brien, M., Ramírez, I., Waliczky, Z., Wege, D.C., 2019. Important Bird and Biodiversity Areas (IBAs): the development and characteristics of a global inventory of key sites for biodiversity. Bird Conserv. Int. 29, 177–198. https://doi.org/10.1017/S0959270918000102

Du, J., Li, H., Xu, S., Zhou, Q., Jin, M., Tang, J., 2019. A review of organophosphorus flame retardants (OPFRs): occurrence, bioaccumulation, toxicity, and organism exposure. Environ. Sci. Pollut. Res. 26, 22126–22136. https://doi.org/10.1007/s11356-019-05669-y

Dudgeon, D., 2019. Multiple threats imperil freshwater biodiversity in the Anthropocene. Curr. Biol. 29, R960–R967. https://doi.org/10.1016/J.CUB.2019.08.002

EMA, 2006. Guideline on the environmental risk assessment of medicinal products for human use.

Fernández-Sanjuan, M., Meyer, J., Damásio, J., Faria, M., Barata, C., Lacorte, S., 2010. Screening of perfluorinated chemicals (PFCs) in various aquatic organisms. Anal. Bioanal. Chem. 398, 1447–1456. https://doi.org/10.1007/s00216-010-4024-x

Godoy, A.A., Domingues, I., Arsénia Nogueira, A.J., Kummrow, F., 2018. Ecotoxicological effects, water quality standards and risk assessment for the anti-diabetic metformin. Environ. Pollut. 243, 534–542. https://doi.org/10.1016/j.envpol.2018.09.031

Gómez-Canela, C., Pueyo, V., Barata, C., Lacorte, S., Marcé, R.M., 2019. Development of predicted environmental concentrations to prioritize the occurrence of pharmaceuticals in rivers from Catalonia. Sci. Total Environ. 666, 57–67. https://doi.org/10.1016/j.scitotenv.2019.02.078

González-González, R.B., Sharma, P., Singh, S.P., Américo-Pinheiro, J.H.P., Parra-Saldívar, R., Bilal, M., Iqbal, H.M.N., 2022. Persistence, environmental hazards, and mitigation of pharmaceutically active residual contaminants from water matrices. Sci. Total Environ. 821. https://doi.org/10.1016/j.scitotenv.2022.153329

Gorga, M., Insa, S., Petrovic, M., Barceló, D., 2015. Occurrence and spatial distribution of EDCs and related compounds in waters and sediments of Iberian rivers. Sci. Total Environ. 503–504, 69–86. https://doi.org/10.1016/j.scitotenv.2014.06.037

Guibal, R., Lissalde, S., Leblanc, J., Cleries, K., Charriau, A., Poulier, G., Mazzella, N., Rebillard, J.-P., Brizard, Y., Guibaud, G., 2018. Two sampling strategies for an overview of pesticide contamination in an agriculture-extensive headwater stream. Environ. Sci. Pollut. Res. 25, 14280–14293. https://doi.org/10.1007/s11356-017-9883-7

Hausmann, A., Toivonen, T., Fink, C., Heikinheimo, V., Tenkanen, H., Butchart, S.H.M., Brooks, T.M., Di Minin, E., 2019. Assessing global popularity and threats to Important Bird and Biodiversity Areas using social media data. Sci. Total Environ. 683, 617–623. https://doi.org/10.1016/j.scitotenv.2019.05.268

Hou, R., Xu, Y., Wang, Z., 2016. Review of OPFRs in animals and humans : Absorption , bioaccumulation , metabolism , and internal exposure research. Chemosphere 153, 78–90. https://doi.org/10.1016/j.chemosphere.2016.03.003

Kasprzyk-Hordern, B., Dinsdale, R.M., Guwy, A.J., 2009. The removal of pharmaceuticals, personal care products, endocrine disruptors and illicit drugs during wastewater treatment and its impact on the quality of receiving waters. Water Res. 43, 363–380. https://doi.org/10.1016/j.watres.2008.10.047

Kim, S., Choi, K., 2014. Occurrences, toxicities, and ecological risks of benzophenone-3, a common component of organic sunscreen products: A mini-review. Environ. Int. 70, 143–157. https://doi.org/10.1016/j.envint.2014.05.015

Li, S., Wen, J., He, B., Wang, J., Hu, X., Liu, J., 2020. Occurrence of caffeine in the freshwater environment: Implications for ecopharmacovigilance. Environ. Pollut. 263, 114371. https://doi.org/10.1016/j.envpol.2020.114371

Lorenz, S., Rasmussen, J.J., Süß, A., Kalettka, T., Golla, B., Horney, P., Stähler, M., Hommel, B., Schäfer, R.B., 2017. Specifics and challenges of assessing exposure and effects of pesticides in small water bodies. Hydrobiologia 793, 213–224. https://doi.org/10.1007/s10750-016-2973-6

Lorenzo, M., Campo, J., Morales Suárez-Varela, M., Picó, Y., 2019. Occurrence, distribution and behavior of emerging persistent organic pollutants (POPs) in a Mediterranean wetland protected area. Sci. Total Environ. 646, 1009–1020. https://doi.org/10.1016/j.scitotenv.2018.07.304

Miranda, D.A., Benskin, J.P., Awad, R., Lepoint, G., Leonel, J., Hatje, V., 2021. Bioaccumulation of Per- and polyfluoroalkyl substances (PFASs) in a tropical estuarine food web. Sci. Total Environ. 754, 142146. https://doi.org/10.1016/J.SCITOTENV.2020.142146

Moreno-González, R., Campillo, J.A., García, V., León, V.M., 2013. Seasonal input of regulated and emerging organic pollutants through surface watercourses to a Mediterranean coastal lagoon. Chemosphere 92, 247–257. https://doi.org/10.1016/j.chemosphere.2012.12.022

Ortiz de García, S., Pinto Pinto, G., García Encina, P., Irusta Mata, R., 2013. Consumption and occurrence of pharmaceutical and personal care products in the aquatic environment in Spain. Sci. Total Environ. 444, 451–465. https://doi.org/10.1016/j.scitotenv.2012.11.057

Paíga, P., Ramos, S., Jorge, S., Silva, J.G., Delerue-Matos, C., 2019. Monitoring survey of caffeine in surface waters (Lis River) and wastewaters located at Leiria Town in Portugal. Environ. Sci. Pollut. Res. 26, 33440–33450. https://doi.org/10.1007/s11356-019-06168-w

Pascual Aguilar, J.A., Andreu, V., Campo, J., Picó, Y., Masiá, A., 2017. Pesticide occurrence in the waters of Júcar River, Spain from different farming landscapes. Sci. Total Environ. 607–608, 752–760. https://doi.org/10.1016/j.scitotenv.2017.06.176

Peteffi, G.P., Fleck, J.D., Kael, I.M., Girardi, V., Bündchen, R., Krajeski, D.M., Demoliner, M., Silva, F.P., da Rosa, D.C., Antunes, M.V., Linden, R., 2018. Caffeine levels as a predictor of Human mastadenovirus presence in surface waters—a case study in the Sinos River basin—Brazil. Environ. Sci. Pollut. Res. 25, 15774–15784. https://doi.org/10.1007/s11356-018-1649-3

Petrie, B., Youdan, J., Barden, R., Kasprzyk-Hordern, B., 2016. Multi-residue analysis of 90 emerging contaminants in liquid and solid environmental matrices by ultra-high-performance liquid chromatography tandem mass spectrometry. J. Chromatogr. A 1431, 64–78. https://doi.org/10.1016/J.CHROMA.2015.12.036

Podder, A., Sadmani, A.H.M.A., Reinhart, D., Chang, N. Bin, Goel, R., 2021. Per and poly-fluoroalkyl substances (PFAS) as a contaminant of emerging concern in surface water: A transboundary review of their occurrences and toxicity effects. J. Hazard. Mater. 419, 126361. https://doi.org/10.1016/j.jhazmat.2021.126361

Rathi, B.S., Kumar, P.S., Vo, D.V.N., 2021. Critical review on hazardous pollutants in water environment: Occurrence, monitoring, fate, removal technologies and risk assessment. Sci. Total Environ. 797, 149134. https://doi.org/10.1016/J.SCITOTENV.2021.149134

Rico, A., Arenas-Sánchez, A., Alonso-Alonso, C., López-Heras, I., Nozal, L., Rivas-Tabares, D., Vighi, M., 2019. Identification of contaminants of concern in the upper Tagus river basin (central Spain). Part 1: Screening, quantitative analysis and comparison of sampling methods. Sci. Total Environ. 666, 1058–1070. https://doi.org/10.1016/J.SCITOTENV.2019.02.250

Sánchez-Avila, J., Meyer, J., Lacorte, S., 2010. Spatial distribution and sources of perfluorochemicals in the NW Mediterranean coastal waters (Catalonia, Spain). Environ. Pollut. 158, 2833–2840. https://doi.org/10.1016/j.envpol.2010.06.022

Schmeller, D.S., Loyau, A., Bao, K., Brack, W., Chatzinotas, A., De Vleeschouwer, F., Friesen, J., Gandois, L., Hansson, S. V., Haver, M., Le Roux, G., Shen, J., Teisserenc, R., Vredenburg, V.T., 2018. People, pollution and pathogens – Global change impacts in mountain freshwater ecosystems. Sci. Total Environ. 622–623, 756–763. https://doi.org/10.1016/J.SCITOTENV.2017.12.006

Stalter, D., Magdeburg, A., Quednow, K., Botzat, A., Oehlmann, J., 2013. Do Contaminants Originating from State-of-the-Art Treated Wastewater Impact the Ecological Quality of Surface Waters? PLoS One 8, 1–10. https://doi.org/10.1371/journal.pone.0060616

Ulrich, U., Lorenz, S., Hörmann, G., Stähler, M., Neubauer, L., Fohrer, N., 2022. Multiple pesticides in lentic small water bodies: Exposure, ecotoxicological risk, and contamination origin. Sci. Total Environ. 816. https://doi.org/10.1016/j.scitotenv.2021.151504

Wang, X., Zhu, Q., Yan, X., Wang, Y., Liao, C., Jiang, G., 2020. A review of organophosphate flame retardants and plasticizers in the environment: Analysis, occurrence and risk assessment. Sci. Total Environ. 731, 139071. https://doi.org/https://doi.org/10.1016/j.scitotenv.2020.139071

Weissinger, R.H., Blackwell, B.R., Keteles, K., Battaglin, W.A., Bradley, P.M., 2018. Bioactive contaminants of emerging concern in National Park waters of the northern Colorado Plateau, USA. Sci. Total Environ. 636, 910–918. https://doi.org/10.1016/j.scitotenv.2018.04.332

SUPPLEMENTARY INFORMATION

Table S1. Compounds studied and standard supplier.

Compound	Supplier
Caffeine	Sigma-Aldrich (St. Louis, USA)
Nicotine	Sigma-Aldrich (St. Louis, USA)
Valsartan	Sigma-Aldrich (St. Louis, USA)
Carbamazepine	Sigma-Aldrich (St. Louis, USA)
Tramadol	Sigma-Aldrich (St. Louis, USA)
Venlafaxine	Sigma-Aldrich (St. Louis, USA)
Sulfamethoxazole	Sigma-Aldrich (St. Louis, USA)
Losartan	Sigma-Aldrich (St. Louis, USA)
Metformin	Sigma-Aldrich (St. Louis, USA)
Paracetamol	European Pharmacopea Reference Standard
Furosemide	Sigma-Aldrich (St. Louis, USA)
Levetiracetam	Sigma-Aldrich (St. Louis, USA)
Diclofenac	Sigma-Aldrich (St. Louis, USA)
Gabapentin	Sigma-Aldrich (St. Louis, USA)
Atenolol	Sigma-Aldrich (St. Louis, USA)
Pentoxyphilline	Sigma-Aldrich (St. Louis, USA)
Trazodone	Sigma-Aldrich (St. Louis, USA)
Alopurinol	Sigma-Aldrich (St. Louis, USA)
Ibuprofen	Sigma-Aldrich (St. Louis, USA)
Atrovastatin	Sigma-Aldrich (St. Louis, USA)
Quetiapine	Sigma-Aldrich (St. Louis, USA)
Chlortoluron	Sigma-Aldrich (St. Louis, USA)
Clorpyrifos	Dr. Ehrenstorfer
Isoproturon	Sigma-Aldrich (St. Louis, USA)
Dimethoate	Sigma-Aldrich (St. Louis, USA)
Tebuconazol	Sigma-Aldrich (St. Louis, USA)
Metalaxyl	Dr. Ehrenstorfer
Triadimenol	Dr. Ehrenstorfer
Prosulfocarb	Sigma-Aldrich (St. Louis, USA)
Oxyfluorfen	Sigma-Aldrich (St. Louis, USA)
Diclofop	Sigma-Aldrich (St. Louis, USA)
Pendimethalin	Sigma-Aldrich (St. Louis, USA)
Pyraclostrobin	Dr. Ehrenstorfer
Fludioxonyl	Dr. Ehrenstorfer
Tebufenpyrad	Dr. Ehrenstorfer

Compound	Supplier
Spinosad	Dr. Ehrenstorfer
Kresoxim-methyl	Sigma-Aldrich (St. Louis, USA)
Chlorfenvinphos	Sigma-Aldrich (St. Louis, USA)
Tributyl phosphate (TBP)	Sigma-Aldrich (St. Louis, USA)
Triphenyl phosphate (TPhP)	Sigma-Aldrich (St. Louis, USA)
Benzophenone	Sigma-Aldrich (St. Louis, USA)
Tris (2- chloroethyl) phosphate (TCEP)	Sigma-Aldrich (St. Louis, USA)
Perfluoropentanoic acid (PFPA)	Wellington Laboratories
Perfluorobutanoic acid (PFBA)	Wellington Laboratories
Perfluorobutane sulfonate (PFBS)	Wellington Laboratories
Perfluorohexanoic acid (PFHxA)	Wellington Laboratories
Perfluoroheptanoic acid (PFHpA)	Wellington Laboratories
Perfluorohexane sulfonate (PFHxS)	Wellington Laboratories
Perfluorooctanoic acid (PFOA)	Wellington Laboratories
Perfluorononanoic acid (PFNA)	Wellington Laboratories
Perfluorooctane sulfonate (PFOS)	Wellington Laboratories
Perfluorodecanoic acid (PFDA)	Wellington Laboratories
Perfluoroundecanoic acid (PFUnA)	Wellington Laboratories
Perfluorodecane sulfonate (PFDS)	Wellington Laboratories
Perfluorododecanoic acid (PFDoA)	Wellington Laboratories
Perfluorotridecanoic acid (PFTriDA)	Wellington Laboratories
Perfluorotetradecanoic acid (PFTeDA)	Wellington Laboratories
Perfluorohexadecanoic acid (PFHxDA)	Wellington Laboratories
Perfluorooctadecanoic acid (PFODA)	Wellington Laboratories
Internal Standards	
Estrone-d_2	Sigma-Aldrich (St. Louis, USA)
Acetaminophen-methyl-d_3	Sigma-Aldrich (St. Louis, USA)
Lidocaine-diethyl-d_{10}	Sigma-Aldrich (St. Louis, USA)
Carbamazepine-d_2	Sigma-Aldrich (St. Louis, USA)
Sulfamethoxazole-d_4	Sigma-Aldrich (St. Louis, USA)
Isoproturon-d_6	Sigma-Aldrich (St. Louis, USA)
Triphenyl phosphate-d_{15} (TPhP-d_{15})	Sigma-Aldrich (St. Louis, USA)
M-PFOA	Wellington Laboratories
M-PFOS	Wellington Laboratories

Table S2. LC-MS/MS conditions and quality parameters for the analysis of pharmaceuticals (ordered by retention time: R.T.), indicating quantification (Q) and confirmation (q) transitions, Collision Energy (C.E.), the response Factor (F) of the calibration curve, Instrument Detection Limit (IDL), Method Detection Limit (MDL), intra-day precision (%, n=5), inter-day precision (%, n=5) and percentage recovery with standard deviation (%R±SD). Coefficient of determination (R^2) was 0.99 for all compounds over a concentration range of 0.001-0.6 ng/µL.

Compound	R.T (min)	Precursor ion $[M+H]^+$	Cone (V)	Q transition	C.E. (eV)	q transition	C.E. (eV)	F	IDL (ng)	MDL (ng/L)	Intra-day precision	Inter-day precision	% R±SD
Metformin	1.37	130	25	130>60	15	130>71	20	18.08	0.08	0.41	5	12	50±3
Nicotine	1.62	163	30	163>130	20	163>117	25	1.794	0.12	1.94	6	9	150±33
Allopurinol	1.66	137	70	137>110	20	137>54	20	0.262	0.27	3.19	16	6	21±18
Atenolol	2.22	267	10	267>190	25	267>145	25	0.110	0.20	4.11	3	16	141±32
Paracetamol	2.51	152	32	152>110	18	152>93	22	0.510	0.15	1.77	6	7	136±27
Levetiracetam	2.58	171	20	171>126	13	171>154	5	0.500	0.14	2.37	12	21	68±14
Caffeine	3.18	195	24	195>138	20	195>110	22	1.035	0.44	0.86	4	7	162±37
Gabapentin	3.32	172	22	172>154	12	172>137	14	6.151	0.11	1.19	2	19	34±7
Pentoxifylline	5.35	279	30	279>181	15	279>138	30	2.444	0.02	0.31	3	5	119±12
Tramadol	5.95	264	20	264>264	0	264>58	15	1.597	0.45	1.66	1	11	120±24
Sulfamethoxazole	6.7	254	36	254>156	13	254>92	26	0.543	0.55	0.78	11	17	115±23
Venlafaxine	7.52	278	20	278>260	10	278>58	15	2.309	0.07	0.39	3	23	135±29
Trazodone	8.06	372	7	372>148	34	372>176	18	2.800	0.05	0.09	12	12	61±12
Quetiapine	8.39	384	5	384>221	40	384>253	30	1.347	0.21	0.22	9	17	60±12
Carbamazepine	9.18	237	32	237>194	20	237>174	34	12.29	0.03	0.12	5	10	86±17
Furosemide	9.42	329 $[M+H]^-$	36	329>285	16	329>205	22	0.192	1.12	3.06	19	22	65±13
Losartan	10.19	423	25	423>405	15	423>207	15	2.986	0.30	0.38	12	16	126±27
Valsartan	12.65	436	10	436>418	12	436>235	18	0.525	0.19	0.98	10	20	148±31
Atrovastatin	14.57	559	50	559>440	18	559>250	36	0.269	0.32	2.05	9	28	56±11
Diclofenac	14.66	296	26	296>215	18	296>250	10	2.048	0.36	0.24	18	28	108±22
Ibuprofen	15.06	161	44	161>119	14	161>105	14	0.267	22.2	17.1	20	29	62±34
Acetaminophen-d_3	2.51	155	34	155>111	16	155>65	22						
Lidocaine -d_{10}	5.03	245	30	245>245	0	245>96	15						
Sulfamethoxazole-d_4	6.7	258	35	258>160	15	258>96	20						
Carbamazepine-d_2	9.14	240	30	240>240	0	240>196	35						

Table S3. LC-MS/MS conditions and quality parameters for the analysis of pesticides and OPEs (*) (ordered by retention time: R.T.), indicating quantification (Q) and confirmation (q) transitions used, Collision Energy (C.E.), the response Factor (F) of the calibration curve, Instrument Detection Limit (IDL), Method Detection Limit (MDL), intra-day precision (%, n=5), inter-day precision (%, n=5) and percentage recovery with standard deviation (%R±SD). Coefficient of determination (R^2) was 0.99 for all compounds over a concentration range of 0.001-0.6 ng/µL.

Compound	R.T. (min)	Precursor ion $[M+H]^+$	Cone (V)	Q transition	C.E. (eV)	q transition	C.E. (eV)	F	IDL (ng)	MDL (ng/L)	Intra-day precision	Inter-day precision	% R±SD
Dimethoate	7.49	230	16	230 > 199	20	230 > 125	10	0.316	0.06	0.50	2	6	95±8
TCEP	9.81	287	29	287>99	15	287>63	15	0.105	0.36	0.53	13	12	79±6
Chlortoluron	10.65	350	22	350 > 198	30	350 > 97	20	0.671	0.17	0.31	3	9	116±9
Isoproturon	11.06	207	28	207 > 72	-	-	-	0.657	0.04	0.29	7	6	107±14
Metalaxyl	11.09	280	26	280 > 220	20	280 > 192	10	0.054	0.11	0.57	4	2	94±1
Benzophenone *	12.16	183	25	183>105	20	183>77	20	0.100	0.23	0.22	8	16	101±34
Fludioxonyl	12.19	247 $[M-H]^-$	50	247 > 180	30	247 > 126	30	0.014	0.25	0.76	36	53	48±14
Triadimenol	12.75	296	20	296 > 70	-	-	-	0.329	0.34	0.87	17	16	114±4
Kresoxim-methyl	13.49	314	14	314 > 116	60	314 > 89	15	0.027	0.26	0.39	16	9	47±7
Tebuconazol	13.66	308	24	308 > 70	40	308 > 125	20	0.217	0.10	0.10	6	10	48±14
TPhP*	13.79	327	47	327>215	25	327>152	25	1.572	0.13	0.05	6	14	60±11
Chlorfenvinphos	13.8	213	22	213 > 72	20	213 > 46	20	2.009	0.07	0.16	18	18	92±15
Pyraclostrobin	13.87	388	20	388 > 194	20	388 > 163	10	0.601	0.03	0.11	5	23	79±28
Diclofop	14.18	325 $[M-H]^-$	25	325 > 325	10	325 > 253	10	0.004	1.89	8.36	10	30	96±12
Spinosad	14.28	733	40	733 > 142	60	733 > 98	30	0.481	0.59	0.29	4	9	92±6
TBP*	14.54	267	22	267>155	10	267>99	10	1.151	0.14	0.15	4	23	68±26
Prosulfocarb	14.69	252	22	252 > 91	60	252 > 65	20	3.715	0.09	0.13	7	17	113±42
Oxyfluorfen	14.89	362	28	362 > 237	20	362 > 316	20	0.038	0.96	0.28	19	23	58±8
Tebufenpyrad	14.96	334	38	334 > 145	40	334 > 117	30	0.330	0.16	0.22	4	7	19±10
Chlorpyrifos	15.38	361	30	361>155	15	361>127	15	0.456	0.16	0.54	8	13	98±32
Pendimethalin	15.46	282	15	282 > 212	20	282 > 194	10	0.313	0.32	0.32	5	29	87±30
Isoproturon-d_6	11.03	213	29	213>78	20	213>52	20						
TPhP-d_{15}*	13.7	342	40	342>161	40	342 > 82	20						

Table S4. LC-MS/MS conditions and quality parameters for the analysis of PFASs (ordered by retention time: R.T.), indicating quantification (Q) and confirmation (q) transitions used, Collision Energy (C.E.), the response Factor (F) of the calibration curve, Instrument Detection Limit (IDL), Method Detection Limit (MDL), intra-day precision (%, n=5), inter-day precision (%, n=5) and percentage recovery with standard deviation (%R±SD). Coefficient of determination (R^2) was 0.99 for all compounds over a concentration range of 0.001-0.6 ng/µL.

Compound	R.T. (min)	Precursor ion $[M + H]^+$	Cone (V)	Q transition	C.E. (eV)	q transition	C.E. (eV)	F	IDL (ng)	MDL (ng/L)	Intra-day precision	Inter-day precision	% R±SD
PFBA	1.71	213	20	213>169	10	-	-	0.228	0.10	0.08	7	6	106±0.5
PFPA	2.37	263	25	263>219	10	-	-	0.131	0.08	0.10	9	17	89±3
PFBS	2.67	299	45	299>80	23	299>99	30	0.182	0.05	0.10	11	9	105±2
PFHxA	3.83	313	16	313>269	10	313>119	24	0.172	0.08	0.10	4	10	111±2
PFHpA	5.91	363	20	363>319	10	363>3169	14	0.262	0.06	0.20	7	12	117±1
PFHxS	6.25	399	55	399>80	32	399>90	31	0.218	0.03	0.05	4	5	105±1
PFOA	7.09	413	18	413>369	11	413>169	13	0.214	0.06	0.05	2	6	104±1
PFNA	7.91	463	25	463>419	11	463>169	23	0.171	0.05	0.15	3	8	131±1
PFOS	8.03	499	65	499>99	35	499>80	35	0.228	0.06	0.10	2	5	108±1
PFDA	8.6	513	14	513>469	9	513>169	11	0.238	0.05	1.35	12	15	115±2
PFUnA	9.06	563	29	563>519	10	563>269	12	0.145	0.20	0.15	1	1	135±2
PFDS	9.1	599	80	599>80	41	599>99	40	0.199	0.08	0.05	8	6	74±4
PFDoA	9.52	613	20	613>569	15	613>319	16	0.170	0.08	0.10	3	7	90±4
PFTriDA	9.9	663	31	663>619	17	663>169	24	0.118	0.14	0.15	0	55	86±4
PFTeDA	10.19	713	29	713>669	10	713>319	18	0.209	0.07	0.10	11	12	65±1
PFHxDA	10.68	813	35	813>769	10	813>169	45	0.151	0.18	0.35	18	22	28±3
PFODA	11.11	913	45	913>869	12	913>169	48	0.115	0.37	0.55	29	41	17±3
M-PFOA	7.09	417	21	417>372	11	417>172	12						
M-PFOS	8.07	503	70	503>80	36	503>99	36						

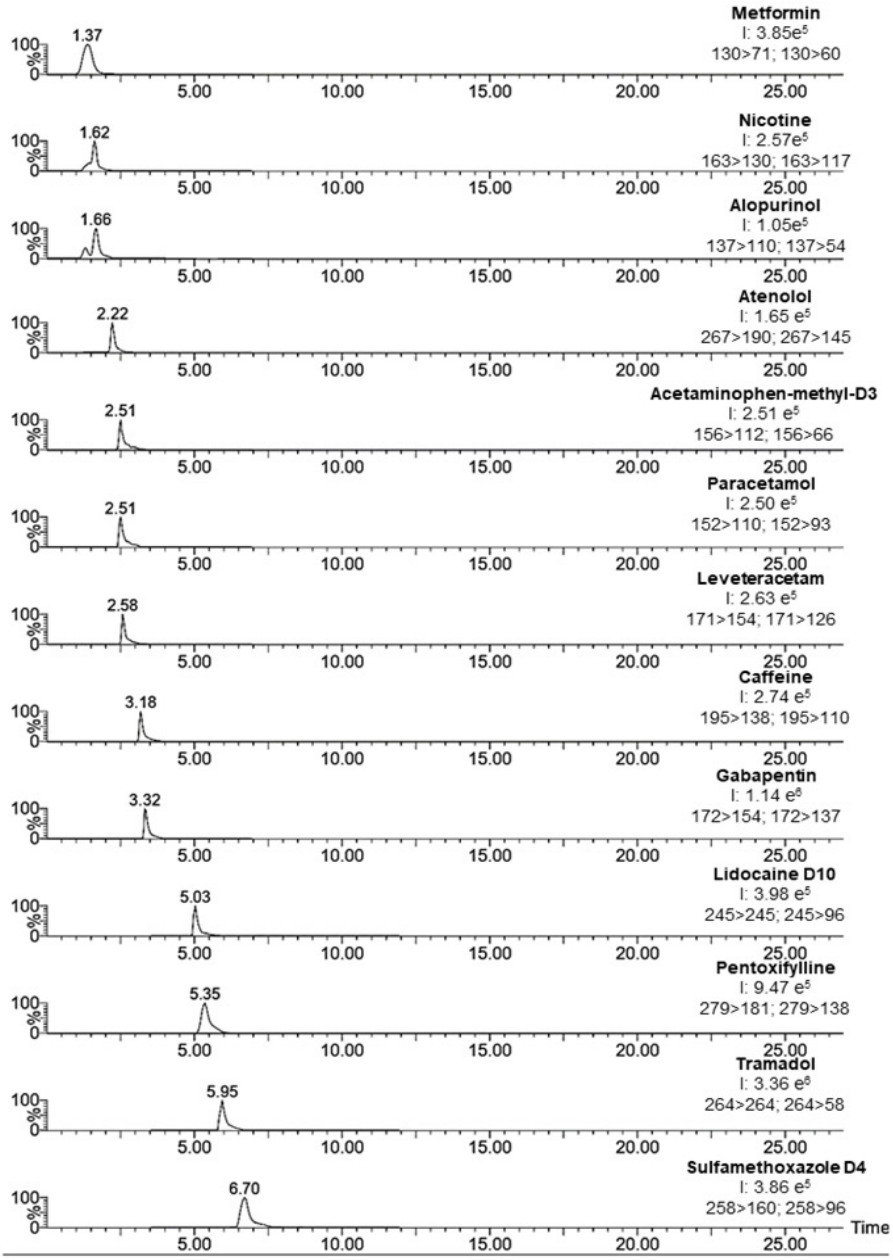


Figure S1. UPLC-MS/MS chromatogram of a pharmaceuticals standard at 0.6 ng/µL.

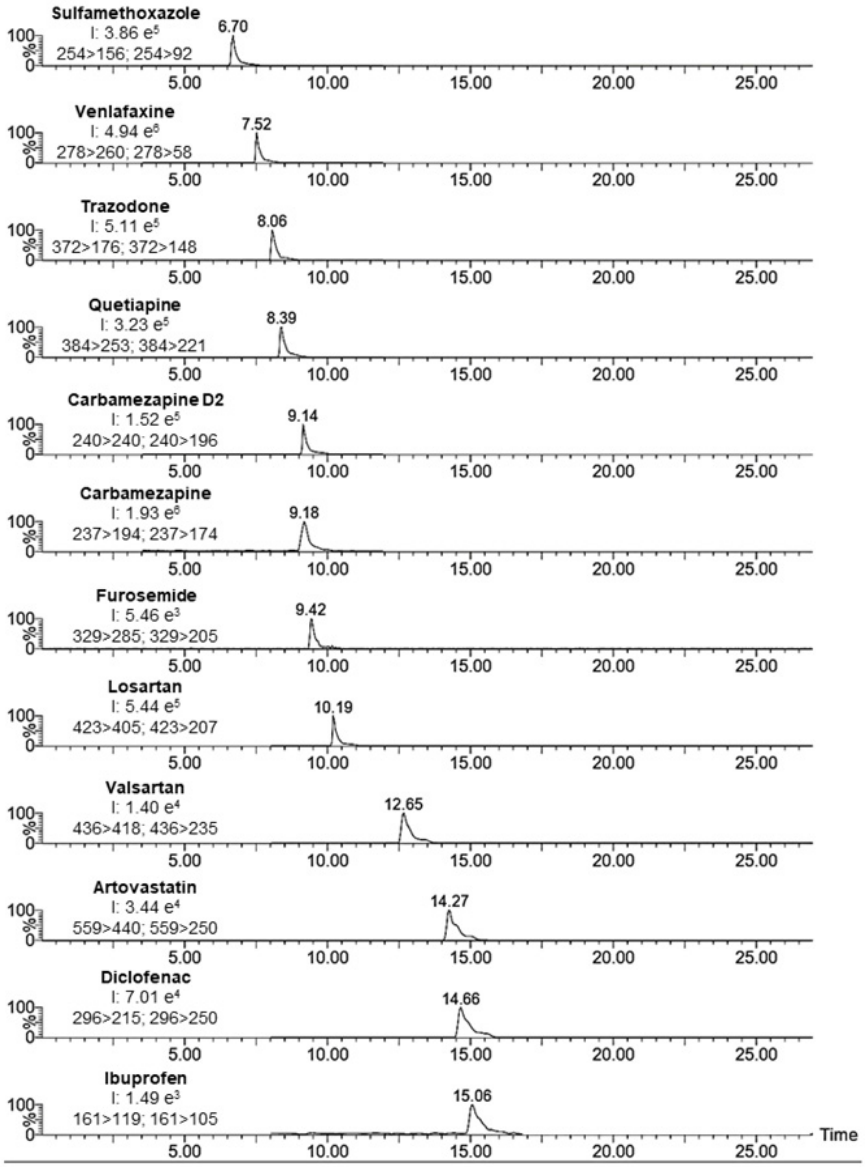


Figure S1. UPLC-MS/MS chromatogram of a pharmaceutical's standard at 0.6 ng/μL

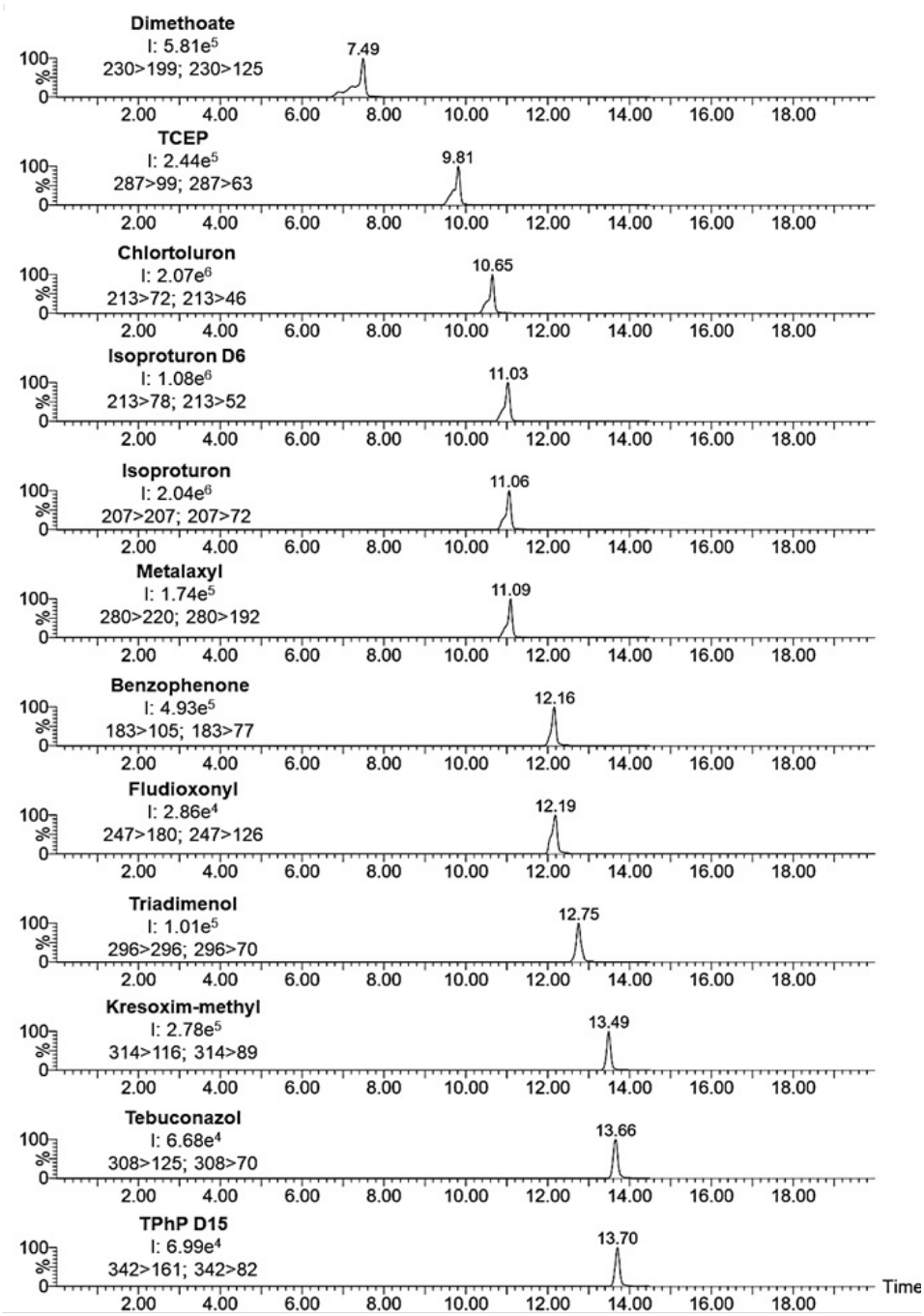


Figure S2. UPLC-MS/MS chromatogram of a pesticide and organophosphoshate ester standard at 0.6 ng/µL.

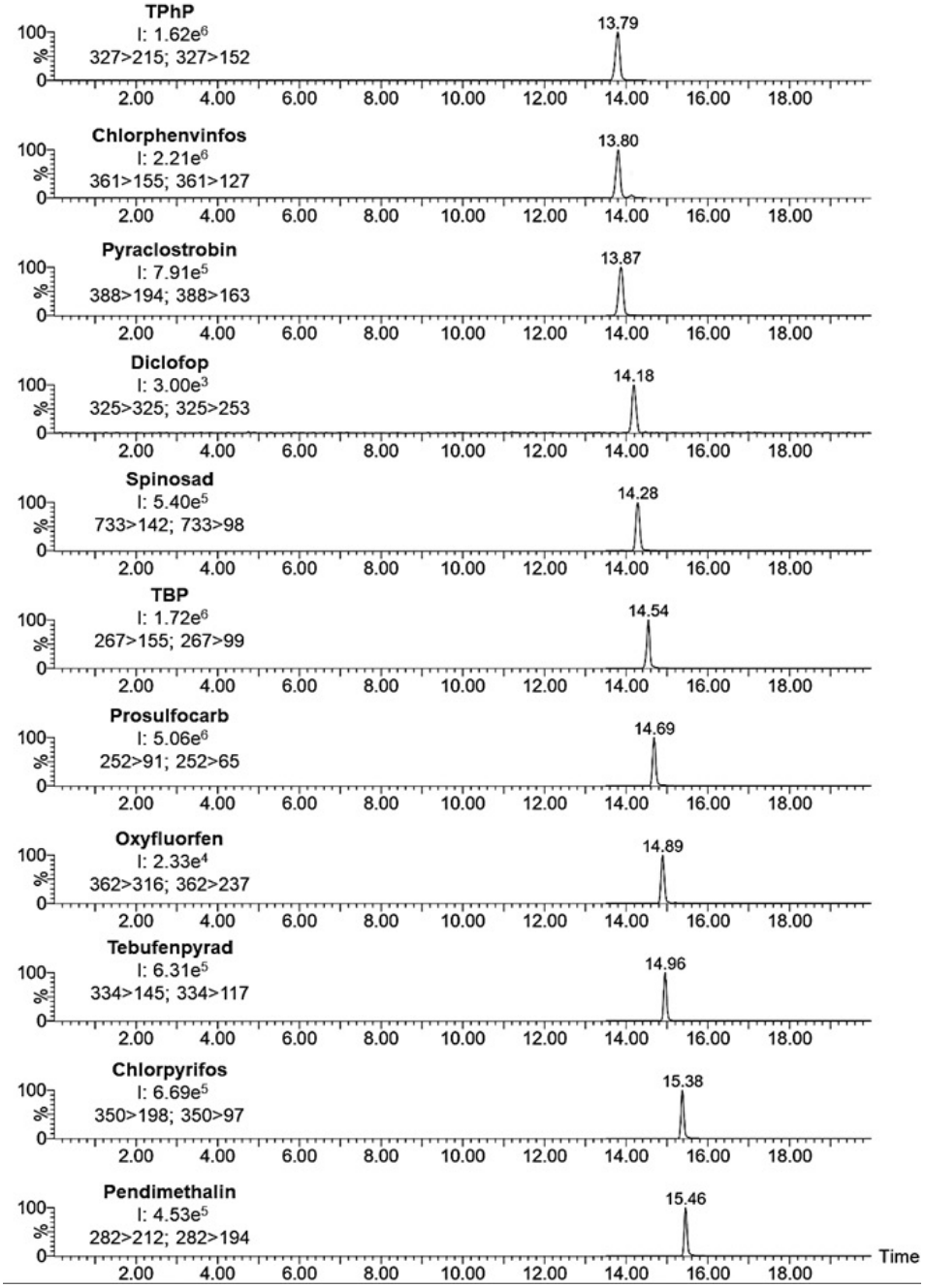


Figure S2. UPLC-MS/MS chromatogram of a pesticide and organophosphoshate ester standard at 0.6 ng/µL.

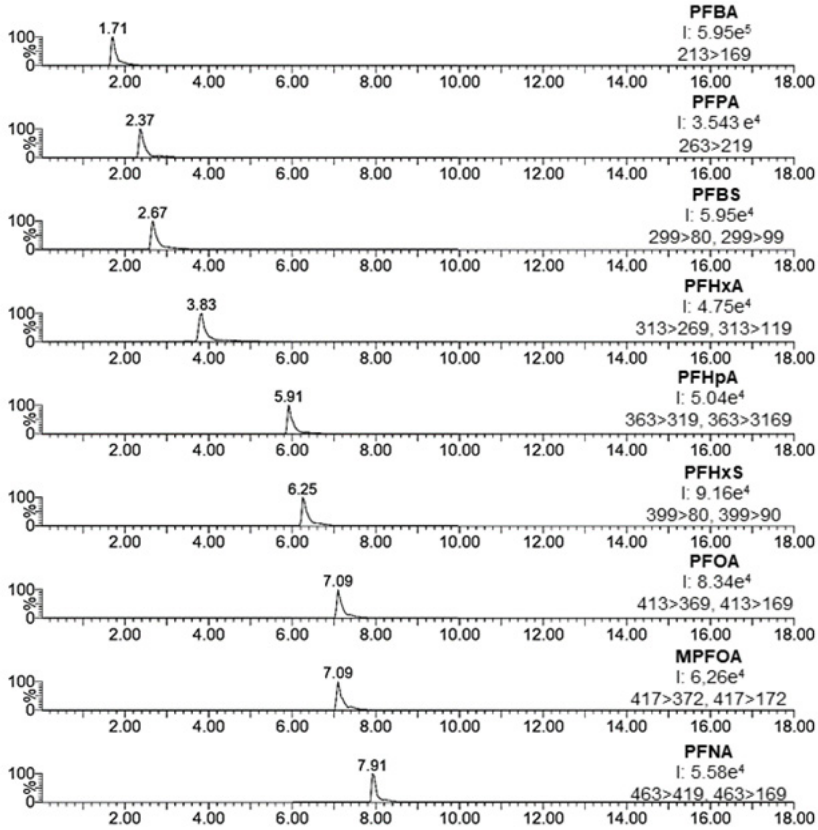
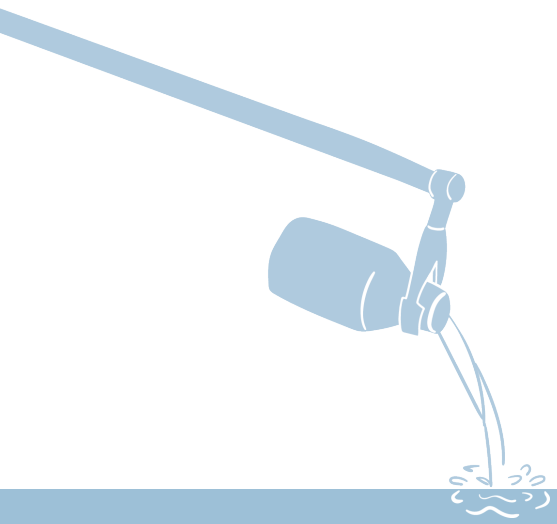


Figure S3. UPLC-MS/MS chromatogram of a perfluoroalkyl substances standard at 0.6 ng/µL.

Figure S3. UPLC-MS/MS chromatogram of a perfluoroalkyl substances standard at 0.6 ng/μL



CHAPTER II

WATER POLLUTION THREATS IN IMPORTANT BIRD AND BIODIVERSITY AREAS FROM SPAIN

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ABSTRACT

Chemical pollution is still an underestimated threat to surface waters from natural areas. This study has analysed the presence and distribution of 59 organic micropollutants (OMPs) including pharmaceuticals, lifestyle compounds, pesticides, organophosphate esters (OPEs), benzophenone and perfluoroalkyl substances (PFASs) in 411 water samples from 140 Important Bird and Biodiversity Areas (IBAs) from Spain, to evaluate the impact of these pollutants in sites of environmental relevance. Lifestyle compounds, pharmaceuticals and OPEs were the most ubiquitous chemical families, while pesticides and PFASs showed a detection frequency below 25% of the samples. The mean concentrations detected ranged from 0.1 to 301 ng/L. According to spatial data, agricultural surface has been identified as the most important source of all OMPs in natural areas. Lifestyle compounds and PFASs have been related to the presence of artificial surface and wastewater treatment plants (WWTPs) discharges, which were also an important source of pharmaceuticals to surface waters. Fifteen out of 59 OMPs have been found at levels posing a high risk for the aquatic IBAs ecosystems, being the insecticide chlorpyrifos, the antidepressant venlafaxine and perfluorooctanesulfonic acid (PFOS) the most concerning compounds. This is the first study to quantify water pollution in IBAs and evidence that OMPs are an emerging threat to freshwater ecosystems that are essential for biodiversity conservation.

INTRODUCTION

A great number of European surface water bodies do not achieve good quality status due to chemical pollution (Posthuma et al., 2019a). Water pollution is a worldwide environmental problem that has increased in the last decades as a result of population growth and can pose the ecosystems at risk (Arenas-Sánchez et al., 2019). It has been long recognized that surface waters are a common endpoint for organic micropollutants (OMPs) released from anthropogenic activities including wastewater treatment plants (WWTP) discharges, urban runoff, industry and agriculture. The intensification of these activities has caused the widespread distribution of emerging contaminants such as pharmaceuticals, polar pesticides, organophosphate esters (OPEs), perfluoro alkyl substances (PFASs) and other chemicals in rivers and estuaries (Luo et al., 2014; Sousa et al., 2018). However, there is still scarce information about their occurrence and distribution in natural areas and the potential risk that those chemicals can represent for biodiversity conservation, especially regarding the assessment of complex chemical mixtures which raise concerns from a toxicological point of view (Altenburger et al., 2013). The presence of OMPs in surface water has a negative impact on living organisms and may directly lead to habitat degradation and loss of ecosystem services (Posthuma, Brack, et al., 2019). For this reason, water pollution is an important risk to consider for the management of key areas for biodiversity.

Important Bird and Biodiversity Areas (IBAs) are sites designated and managed by the conservationist non-profit organization BirdLife International, with the aim to conserve and protect areas of interest for the preservation of long-term bird populations (Donald et al., 2019). The IBAs network is identified using scientific criteria based on current knowledge as the most important places for conserving biodiversity (Butchart et al., 2012). The programme has already mapped over 13,000 sites of international significance for birds, making it the world's largest network of biodiversity importance (Waliczky et al., 2019). Even though they are not an official figure of protection, the IBAs inventory is the most up-to-date and precise benchmark used as a reference in the European Union in the designation of the Special Protection Areas (SPA) under the Birds Directive 2009/147/EC as part of the Natura 2000 network. In Europe and north Africa, IBAs play a crucial role in the conservation of waterbirds (Pavón-Jordán et al., 2020), and maintaining these areas could be key for ensuring the survival of species likely to be affected by global change, as well as for reducing biodiversity loss (Butchart et al., 2012). Although an important number of IBAs have a protected status, a great number of them suffer high anthropogenic pressures that may hamper their conservation, such as tourism, pesticide use and deforestation, among many others (Buchanan et al., 2009; Hausmann et al., 2019; Rattner & Ackerson, 2008). All those pressures are directly or indirectly linked to chemical pollution. BirdLife

International has identified 277 IBAs from 48 countries worldwide under very high pressure that need immediate action, known as "IBAs in Danger". Spain is in the top 10 countries with the highest number of IBAs in unfavourable conditions, and pollution has been identified as the major threat (BirdLife International, 2023).

Our motivation was to assess the occurrence of chemical pollution in waters from IBAs for the following reasons: (i) they are areas of high ecological interest which need conservation measures; (ii) they are often influenced by the different agricultural, urban and recreational activities carried out within their boundaries; (iii) they are regions of landscape beauty where mitigation of pollution can have high societal and environmental benefits; and (iv) they cover large areas of the territory with heterogeneity of the landscape.

The aim of this study was to assess the water pollution status in 140 Spanish IBAs. For this purpose, we determined the presence and distribution of 59 OMPs in surface waters from 3 sites within each IBA. Spatial data was used to characterize the anthropogenic pressures (such as WWTP discharges, urban areas, agriculture, roads, etc.) of each IBA to unravel the sources of pollution of the different chemical families studied. The risk was assessed using Predicted Non-Effect Concentrations (PNEC) values to identify the most concerning compounds detected in freshwater and the most impacted IBAs. Overall, this study is intended to enhance the management of IBAs and other natural areas worldwide against chemical pollution. To the best of our knowledge, this is the first study to evaluate the threat of water pollution in IBAs.

2. MATERIALS AND METHOD

2.1. Sample collection

In Spain, 469 IBAs have been identified covering more than 23,000,000 ha, comprising seven habitat types as agricultural, Atlantic forest, Mediterranean forest, riparian forest, rocky mountain, inland aquatic and coastal. In the present study, a representative subset of 140 IBAs from the seven categories was chosen (Figure S1, Table S1). Within the 140 IBAs selected, 94% contain or overlap with surfaces from Natura 2000 protected areas.

To have a good representativeness of the study area, three different sampling locations within each IBA were selected following a gradient of anthropogenic pollution, labelled as (i) high impact: close to discharges from WWTPs, tourist centres, main roads, urban settlements, industrial areas, landfills, intensive agricultural areas; (ii) medium impact: 500 m away from point 1; (iii) non-evident impact: more pristine areas, and when possible, close to water sources (springs, upstream waters). Because some sampling points

were dry, a total of 411 samples were finally analysed. Freshwater samples were collected following the procedure described by Dulsat-Masvidal et al., 2022 and sampling sites were geolocated. All samples were collected from 2019 to 2020.

2.2. Target compounds

A total of 59 target OMPs from 5 main chemical families were studied: 19 pharmaceuticals, 2 lifestyle compounds (nicotine and caffeine), 17 pesticides, 3 OPEs, benzophenone and 17 PFASs (Table S2). Caffeine and nicotine were selected for their widespread consumption and occurrence in freshwater (Li et al., 2020a). Pharmaceuticals were selected according to their Predicted Environmental Concentrations (PEC) in Spain, including compounds which are highly consumed by the general population or with low degradability (Gómez-Canela et al., 2019). The most used pesticides in agriculture were selected according to the Ministry of Agriculture in Spain (https://www.mapa.gob.es). Selected OPEs and benzophenone are high production volume chemicals (ECHA, 2022) and widely present in surface waters (Cristale et al., 2013). PFASs studied included those with the highest mobility and persistence in the environment.

2.3. Chemical analysis

Water samples were analysed using the procedure described by Dulsat-Masvidal et al., 2022 where quality control results including recoveries, limits of detection, repetitivity and reproducibility of the method and mass spectrometric conditions are reported in detail. Briefly, 200 mL of unfiltered water samples were spiked with 50 ng of a mixture of labelled surrogate standards (acetaminophen-methyl-d_3, lidocaine-diethyl-d_{10}, isoproturon-d_6, triphenyl phosphate-d_{15}, sulfamethoxazole-d_4, carbamazepine-d_2, MPFOA and MPFOS) and extracted by solid-phase extraction (SPE) using Oasis HLB 200 mg, 6 cc cartridges (Waters, Milford, USA). After preconcentration, SPE cartridges were dried under vacuum and eluted with 15 mL of methanol and 6 mL of acetone. The extracts were evaporated to 0.5 mL in a TurboVap® LV (Caliper Lifesciences, Uppsala, Sweden) under N_2 stream at 25ºC, and transferred to a 1.5 mL chromatographic vial using 1 mL methanol as a washing solvent. Finally, samples were evaporated to dryness with a ReactiVap® (Pierce, Gemini lab, Apeldoorn, The Netherlands) and reconstituted with 100 µL of methanol and 100 µL of water. Samples were stored at -20ºC until analysis. Samples were analysed by liquid chromatography coupled to a triple quadrupole mass analyser (LC-MS/MS) with an electrospray ion source (Waters, Milford, USA). Three analytical methods with different conditions were used to determine: (i) pharmaceuticals, (ii) pesticides, OPEs and benzophenone and (iii) PFASs.

In all cases, an Acquity BEH C18 analytical column (100 mm x 2.1 mm, 1.7 µm particle size) (Waters, Milford, USA) was deployed. Concentrations are reported in ng/L.

2.4. Data and spatial analysis

Data below the Method Detection Limits (MDL) were given a value of 0 to avoid overestimating the concentrations or risks in case contaminants were not detected. The frequency of detection was calculated as the coefficient between the number of samples with concentrations of target compounds above MDL and the total number of samples (n=411) multiplied by 100. Data was log-transformed (x+1). Normality of the data was tested by performing normality and density plots. Since not all variables achieved normality despite log transformation, nonparametric statistical tests were performed. Kruskal Wallis followed by Dunn's test were used to assess differences between groups (chemical families, habitats, IBAs or sampling points within each IBA). Spearman's correlations were used to assess the association between chemical concentrations and each IBA's total extension, altitude, and percentage of protected surfaces. The protected surface data were obtained from the Natura 2000 dataset (https://www.eea.europa.eu/data-and-maps). The percentage of protection was calculated by considering the surface of Natura 2000 protection figures as Sites of Community Importance (SCI), Special Areas of Conservation (SAC) and SPA and the total extension of each IBA. The significance level was set at $p<0.05$.

To assess the main sources of pollution, we built five models with the total concentration of each family of contaminants (∑pharmaceuticals, ∑lifestyle compounds, ∑pesticides, ∑OPEs and ∑PFASs) as the dependent variable, and different combinations of spatial data considering a buffer area as explanatory variables. A preliminary multiscaling approach was done with 0.25, 0.5, 1, 5 and 10 km buffer area around each sampling point to obtain representative spatial data information. A buffer area of 0.25 and 0.5 km were not representative of the anthropogenic pressures close to the sampled area, while buffer areas of 5 and 10 km were too large compared to our sampled area. Finally, 1 km buffer area was considered the optimum scale. As the dependent variable was censored at zero, we performed a Tobit model analysis using the censReg function from the censReg package (Henningsen, 2022). The spatial data considered included the number of WWTP discharges, density of buildings, altitude, density of roads, and Corine land use information reclassified in major land-use classes: agricultural surface, artificial surface (including urban and industrial areas), and wetland surface. WWTP discharges data was obtained from the European Commission Urban wastewater database (https://uwwtd.eu/). Buildings and altitude (a digital elevation model) were obtained from the

Spanish Geographic National Institute (https://www.cnig.es). Roads were obtained from OpenStreetMap (https://www.openstreetmap.org). Land use data were obtained from Corine Land Cover 2018 (https://land.copernicus.eu/). Spatial data were obtained combining all layers in a single database (further details in Table S1). Multicollinearity of covariates was assessed by Variance Inflation Factors (VIF) (VIF <5, James et al., 2013). All covariates were scaled and centred. Forest surface was excluded as was strongly correlated with agricultural surface. The top models were selected following the Log-likelihood values and Likelihood ratio test.

Spatial and statistical analysis was performed using open-sources software QGIS (version 3.18.2) and R studio (R version 4.0.3). All figures were elaborated using ggplot2 package from R studio software and QGIS.

2.5. Environmental risk of chemicals mixtures

The potential adverse effects of the target OMPs in the aquatic ecosystems were assessed by performing a Tier I Ecological Risk Assessment (ERA), following the European guidelines (European Commission, 2003)**.** Individual Risk Quotients (RQs) for each compound were calculated as the ratio between the Measured Environmental Concentration (MEC) and the lowest Predicted Non-Effect Concentration (PNEC) in freshwater from the Norman network database (https://www.norman-network.com) (Table S2). MEC values are referred to the median concentration (n=3) of the target compounds in each IBA. Norman lowest PNECs are based on experimental eco-toxicity data of aquatic organisms (algae, daphnid or fish) or Quantitative Structure-Activity Relationship (QSAR) predictions in case of lack of empirical data. The environmental risk of the detected micropollutants at IBAs was determined according to RQ value (Li et al., 2020b), where:

RQ ≥ 1 indicates a high risk for aquatic ecosystems.

0.1 ≤ RQ <1 indicates a medium risk for aquatic ecosystems.

0.01 ≤ RQ <0.1 indicates a low risk for aquatic ecosystems.

RQ < 0.01 indicates an insignificant risk for aquatic ecosystems.

The IBAs with high risk levels of individual compounds (RQ≥ 1) were classified in groups according to the main sources of OMPs identified: (A) high agricultural surface (>20%), presence of artificial surface and WWTP discharges; (B) agricultural surface and WWTP discharges; (C) agricultural and artificial surfaces; (D) mainly agricultural surface, and (E) mainly artificial surface.

The chemical mixtures risk for each IBA was calculated by summing up the RQs for every target compound (i) in each site (j) (eq. 1). The calculation

of ∑RQs disregards different modes of action from the different chemicals, therefore, is based on a "Concentration Addition" approach, which is used as first-tier for environmental risk assessment (Backhaus & Faust, 2012; Kienzler et al., 2019). Considering that our ∑RQs spanned from 0 to 1327, 4 levels were stablished: no risk (0-1), low risk (1-10), medium (10-100) and high risk (100-1500).

$$RQ_{j,i} = \sum_{i=1}^{n} \frac{MEC_i}{PNEC_i} \quad \text{(eq. 1)}$$

The compounds of the highest concern for all studied IBAs were identified by calculating $RQ_{f,i}$ (eq.2), which is used to make a distinction between the pollutants that supposed a frequent risk and those producing a risk in a limited number of samples (Figuière et al., 2022):

$$RQ_{f,i} = \sum_{i=1}^{n} \frac{MEC_{average,i}}{PNEC_i} \times \frac{Number\ of\ samples\ where\ MEC_i > PNEC_i}{Total\ number\ of\ IBAs\ (n=140)} \quad \text{(eq. 2)}$$

3. RESULTS AND DISCUSSION

3.1. Occurrence of micropollutants in IBA's water

The occurrence of the analysed OMPs in IBA's surface waters is detailed in Table 1. From the 59 target compounds, 54 were detected in at least one of the 411 analysed water samples. Pharmaceuticals and lifestyle compounds were the most ubiquitous chemical families, detected in 84% and 76% of the samples, respectively, at concentrations ranging from 0.5 to 41,083 ng/L. Their widespread presence is attributed to their pseudo-persistent behaviour as are continuously released into the environment despite their polar nature and the relatively short half-lifetime in the water (Ebele et al., 2017). Caffeine was the most ubiquitous compound, present in 73% of the samples at concentrations ranging from 0.9 to 41,083 ng/L, and nicotine in 23% of the samples at levels from 2.5 to 567 ng/L. The concentrations of caffeine reported in the present study are similar to the levels detected in freshwaters worldwide (11 to 144,179 ng/L) (Adeleye et al., 2022). The anti-hypertensive valsartan was found in 43% of the samples at levels ranging from 2.0 to 22,473 ng/L. Concentrations of anti-hypertensives and analgesics are globally reported at the µg/L - mg/L range in freshwaters particularly in developed countries due to the high consumption volumes and low removal in WWTPs (Adeleye et al., 2022). In the present study, the anticonvulsant carbamazepine was present in 38% of the samples (1.0 to 698 ng/L), the opiod agonist pain reliever tramadol was detected in 32% of samples (3.0 to 1,714 ng/L) and the antidepressant venlafaxine presented an occurrence of 31% (2.1 to 1,142 ng/L). Other pharmaceuticals found at relevant mean concentrations but with an occurrence lower than 30%, were paracetamol (2.0 to 9,611 ng/L), ibuprofen (27.5 to 5,592 ng/L), and diclofenac (0.5 to 1,684 ng/L), and the rest of pharmaceuticals detected are indicated in Table 1.

Table 1. Compounds detected ordered by chemical family and number of occurrences (N) out of 411 samples analyzed and frequency of detection (%, in parenthesis), mean ± SE (Standard Error), minimum and maximum concentrations and method detection limit (MDL) of detected compounds, expressed in ng/L.

	N (%)	Mean ± SE	Min	Max	MDL (ng/L)
Lifestyle compounds					
Caffeine	301 (73)	296 ± 107	0.9	41083	0.86
Nicotine	93 (23)	24.5 ± 3.4	2.5	567	1.94
Pharmaceuticals					
Valsartan	178 (43)	301 ± 80	2.0	22473	0.98
Carbamazepine	157 (38)	8.5 ± 2.1	1.0	698	0.12
Tramadol	133 (32)	62.1 ± 9.6	3.0	1714	1.66
Venlafaxine	126 (31)	31.7 ± 5.7	2.1	1142	0.39
Sulfamethoxazole	110 (27)	32.2 ± 9.7	1.2	3045	0.78
Losartan	98 (24)	34.5 ± 7.8	1.6	2146	0.38
Metformin	97 (24)	9.0± 1.4	1.0	386	0.41
Paracetamol	97 (24)	93.2 ± 34	2.0	9611	1.77
Furosemide	88 (21)	18.6 ± 4.9	3.1	1327	3.06
Levetiracetam	82 (20)	16.4 ± 4.3	2.5	1348	2.37
Diclofenac	71 (17)	35.2 ± 8.1	0.5	1684	0.24
Gabapentin	62 (15)	5.8 ± 1.1	2.6	273	1.19
Atenolol	59 (14)	20.9 ± 6.9	5.3	1729	4.11
Pentoxyfylline	40 (10)	28.4 ± 12	1.0	4670	0.31
Trazodone	35 (9)	1.0 ± 0.3	0.5	45	0.09
Allopurinol	25 (6)	10.8 ± 3.7	4.5	897	3.19
Ibuprofen	15 (4)	64.4 ± 25	27	5592	17.1
Atorvastatin	12 (3)	3.7 ± 1.6	20	433	2.05
Quetiapine	2 (0.5)	0.2 ± 0.1	31	33	0.22
Pesticides					
Chlortoluron	100 (24)	44.5 ± 13	0.5	4841	0.31
Chlorpyrifos	99 (24)	13.8 ± 6.9	1.7	2639	0.54
Isoproturon	92 (22)	8.0 ± 2.7	0.3	913	0.29
Dimethoate	79 (19)	176 ± 107	0.5	31546	0.50
Tebuconazol	71 (17)	40.6 ± 15	28	5162	0.10
Metalaxyl	60 (14)	11.7 ± 3.1	3.0	730	0.57
Triadimenol	45 (11)	2.2 ± 0.6	1.0	150	0.87
Prosulfocarb	36 (9)	167 ± 132	2.5	53126	0.13
Oxyfluorfen	25 (6)	0.9 ± 0.4	0.3	132	0.28
Diclofop	17 (4)	129 ± 123	9.8	49981	8.36
Pendimethalin	14 (3)	1.2 ± 0.5	6.5	116	0.32
Pyraclostrobin	10 (2)	1.4 ± 0.6	1.0	149	0.11
Fludioxonil	8 (2)	30 ± 20	1.0	7817	0.76

Tebufenpyrad	7 (2)	0.8 ± 0.7	0.5	289	0.22
Spinosad	5 (1)	0.3 ± 0.2	4.5	66	0.29
Kresoxim-methyl	4 (1)	2.3 ± 1.6	7.5	507	0.39
Chlorfenvinfos	2 (0.5)	0.1 ± 0.0	6.2	19	0.16
OPEs and benzophenone					
TCEP	140 (34)	18.2 ± 3.9	2.2	1085	0.53
TBP	130 (32)	20.4 ± 3.8	1.5	901	0.15
Benzophenone	113 (27)	278± 49	0.5	10266	0.22
TPhP	73 (18)	7.3 ± 1.7	0.5	341	0.05
Perfluorinated compounds					
PFOA	68 (16)	8.2 ± 1.1	22	246	0.05
PFOS	53 (13)	101 ± 80	7.9	32607	0.10
PFBA	49 (12)	3.2 ± 0.7	12	268	0.08
PFHxA	40 (10)	2.5 ± 0.5	9.0	82	0.10
PFPA	38 (9)	2.6 ± 0.5	6.7	107	0.10
PFHpA	29 (7)	1.40 ± 0.3	9.6	46	0.20
PFBS	26 (6)	1.50 ± 0.4	9.0	78	0.10
PFHxS	21 (5)	1.60 ± 0.4	12	78	0.05
PFNA	4 (1)	0.10 ± 0.00	6.7	11	0.15
PFDA	4 (1)	0.10 ± 0.1	5.2	17	1.35
PFDS	1 (0.2)	-	22	22	0.05
PFHxDA	1 (0.2)	-	16	16	0.35

OPEs and benzophenone were also ubiquitous, being detected in 18-34% of the samples. The most prevalent OPE was tris(2-chloroethyl) phosphate (TCEP) present at concentrations from 2.2 to 1,085 ng/L, followed by tributyl phosphate (TBP) from 1.5 to 901 ng/L and triphenylphosphate (TPhP) from 0.5 to 341 ng/L. Cristale et al., 2013 reported slightly lower concentrations for TCEP (1.5 to 330 ng/L), TPhP (0.6 to 35 ng/L) and TBP (2.2 to 370 ng/L) in several rivers from Spain. Benzophenone was detected in 27% of the samples at higher concentrations levels than OPEs, ranging from 0.5 to 10,266 ng/L.

Pesticides showed a detection frequency below 25%. The most predominant were the herbicide chlortoluron found in 24% of the samples (0.5 to 4,841 ng/L) and the organophosphorus insecticide chlorpyrifos detected in 24% of the samples (1.7 to 2,639 ng/L), followed by the herbicide isoproturon present in 22% of the samples (0.3 to 913 ng/L). Chlorpyrifos is one of the most prevalent insecticides in surface waters in Spain detected in 22% of the 14600 data values at concentrations ranging from 5 to 96,000 ng/L (Rico et al., 2021). The pesticides detected at the highest concentrations it the present study were dimethoate (19%) ranging from 0.5 to 31,546 ng/L, prosulfocarb (9%) from 2.5 to 53,126 ng/L and diclofop (4%) from 9.8 to 49,981 ng/L. All

the mentioned pesticides have GUS (Groundwater Ubiquity Score) indexes between 1.8 and 2.8, meaning that they have a moderate leaching capacity (PPDB, 2022). The exception is chlorpyrifos, which has a low aqueous solubility and a GUS index of 0.58, indicating it can be adsorbed onto particulate matter and be moderately persistent in soil.

Finally, PFASs were the least detected compounds and at lower concentrations levels than the rest of OMPs. The most prevalent compounds were PFOA detected in 16% of the samples at 22 to 246 ng/L and PFOS in 13% of the samples at concentrations ranging from 7.9 to 32,607 ng/L. Our results are consistent with previous monitoring studies which found PFOS and PFOA to be the most dominant PFASs in surface waters from the Danube river (Ng et al., 2022). Other relevant PFASs were PFBA detected in 12% of the samples (12.3 to 268 ng/L) and PFHxA detected in 10% (9 to 82 ng/L). PFASs detected at less than 10% of the samples are indicated in Table 1. Non-detected compounds were long-chain carboxylic acids (PFDoA, PFTriDA, PFTeDA, PFODA and PFUnA).

3.2. Distribution of micropollutants in IBAs

IBAs are a heterogenic group of natural areas with different geographical characteristics that influence the distribution of OMPs, for instance they are placed at different altitude, comprise different habitats, have different extension, and even legal protection status. As shown in Figure 1, the sum of the concentrations of the five chemical families presented a positive correlation among them, indicating that they co-occur and have similar sources and pattern distributions among the studied IBAs (Figure 1, Figure S2). The total amount of chemicals was negatively correlated with altitude, which implies that those IBAs located at high altitudes had the lowest concentrations of contaminants (Figure 1). This is expected, since upper water bodies are usually closer to their river source, therefore the water course has received fewer anthropogenic pressures. This agrees with the lower concentration levels for all chemical groups ($p<0.05$) in Atlantic forest and rocky mountain habitats, which correspond to the IBAs located at the highest altitudes (Table S1). It must be pointed out that IBAs at high altitude also correspond to the ones with the greatest terrestrial habitat extension, which presented slightly lower concentrations of pesticides, OPEs and PFASs (Figure 1). In contrast, IBAs in low altitudes are more likely to be impacted by water pollution, as described in coastal surface waters typically having a higher degree of urbanization and therefore are more polluted (Biel-Maeso et al., 2018; Munaron et al., 2012).

EU countries are required to manage Natura 2000 sites to maintain or improve the conservation status of species and habitats listed in the Birds

Directive (2009/147/EC) and Habitats Directive (92/43/EC). Figure 2 shows the Natura 2000 framework and the specific Directives and corresponding protection figures. The Bird directive addresses the conservation of wild bird

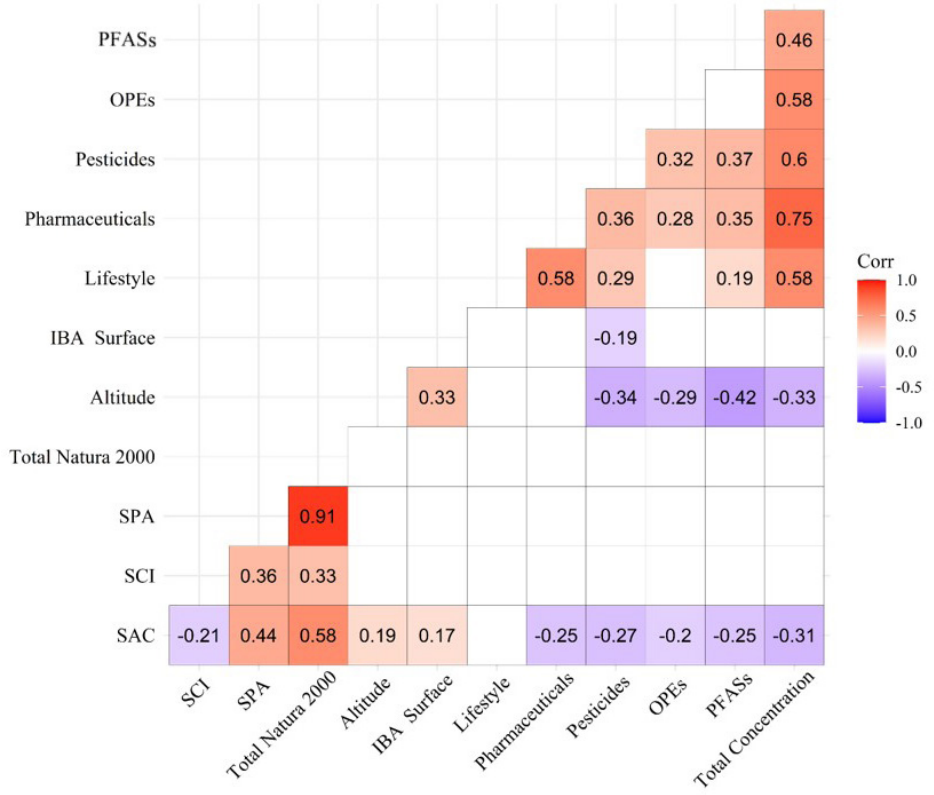


Figure 1. Spearman correlation matrix for the explanatory variables and the concentration of chemical groups. Only significant results (p- value<0.05) are shown.

species through the designation of bird sanctuaries under the designation of SPA. Despite IBAs are used as a reference to designate SPA, their protection status often does not cover the total IBA's surface (Table S2). The Habitat Directive promotes the conservation of natural habitats, wild fauna, and flora to maintain biodiversity. To do that, member states propose Sites of Community Importance (SCI), which once the EU accepts, the member states must protect the designated habitats as Special Areas of Conservation (SAC). The underlying goal of the designation of protected areas (PAs) is to halt biodiversity loss in Europe. The EU biodiversity strategy for 2030 has the ambitious goal of restoring biodiversity by 2030, implementing more than 100 actions by 2030, including the legal protection of a minimum of 30% of the EU's land area (target 1). Although this legal protection does not directly address any form of pollution impact, its designation may help reduce the release of micropollutants in some areas by regulating some anthropogenic

activities that are known to be sources of OMPs in IBAs, such as tourism, urbanism, or industrial and agricultural practices. As shown in Figure 1, the percentage of surface protected by Natura 2000 was not related to a decrease in the concentration of OMPs. Yet, IBAs with the greatest surface designated as SAC under the habitat Directive presented a significantly lower concentration of pharmaceuticals, pesticides, OPEs, and PFASs. Therefore, increasing the protection land cover may help reduce OMPs; however, this reduction is not reflected in the total Natura 2000 network protection status (Figure 1).

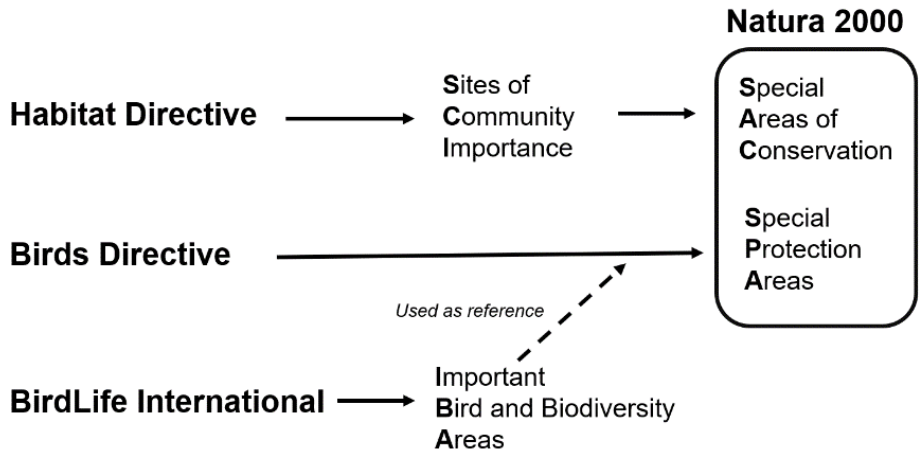


Figure 2. Natura 2000 framework related European Directives and protection figures that have been used to designate the level of protection of IBAs and the impact of contaminants.

3.3. Sources of micropollutants in IBAs

A second aim of this study was to identify the potential sources of contaminants in IBAs waterbodies. No significant differences ($p>0.05$) were observed between the sampling points 1, 2, and 3 within each IBA considering the individual compounds or either considering the sum of the chemical families. This indicates that even the most pristine sites within the IBA's boundaries are affected by water pollution. Among the different explanatory variables tested, the best performing models to explain the sources of the five chemical groups included: agricultural surface, artificial surface and WWTP discharges (Figure 3).

The total concentration of pharmaceuticals was found to be higher in those IBAs with the greatest agricultural surface ($\beta=0.85$, p-value <0.001) and a higher number of WWTPs ($\beta=0.804$, p-value <0.001). Lifestyle compounds (nicotine and caffeine) were also positively correlated with agricultural surface ($\beta=0.66$, p-value <0.001) and the number of WWTPs ($\beta=0.44$, p-value <0.001), in addition to artificial surface ($\beta=0.49$, p-value <0.001). The primary route of pharmaceuticals and personal care products into the environment

is human consumption and subsequent discharge via WWTP effluents into receiving waters, and the use of veterinary drugs in cattle (Adeleye et al., 2022). Also, pharmaceutical manufacturing sites have been found to be an underestimated source of water contamination, even in countries with high environmental standards (Anliker et al., 2020). The pharmaceuticals analysed in the present study have been detected in WWTP effluents from Spain, suggesting they are an important source of these contaminants to river systems (Gómez-Canela et al., 2021). Pharmaceuticals and lifestyle compounds can be also related to agronomic practices since they can be present in WWTPs sewage sludge applied to the fields as fertilizer to improve soil productivity in agriculture (Ivanová et al., 2018). In Spain, 72% of the organic solids derived from sewage treatment processes are used in agriculture (Collivignarelli et al., 2019). An additional contamination source is through irrigation or by applying reclaimed water directly into the fields, which is a widely used practice especially in those areas with water deficits as a way to efficiently manage water resources (Pedrero et al., 2010). However, the water quality and safety of this practice is highly dependent on the treatment and efficiency of each WWTPs to remove OMPs (Singh, 2021).

The concentrations and widespread presence of lifestyle compounds (nicotine and caffeine) were positively related to artificial surface. Stimulants are contained in tea, coffee, soft drinks, energy drinks, and chocolate products (Quadra et al., 2020). Because caffeine is partially metabolized by humans, it is present at high concentrations in raw wastewaters and incompletely eliminated during wastewater treatment, resulting in caffeine residues in the final effluents (Adeleye et al., 2022). Its presence is used as an excellent indicator of domestic wastewater pollution (Camacho-Muñoz et al., 2012) and urban runoff (Warner et al., 2019). On the other hand, nicotine is widely present in urban areas due to cigarette butts, which in contact with water release a significant amount of nicotine, and thus become a useful indicator of urban run-off (Roder Green et al., 2014).

The main source of pesticides in surface waters was the agricultural land-use (β=1.785, p-value <0.001) (Figure 3). It is well established the relationship of pesticides (herbicides, insecticides, fungicides) in agriculture and aquatic pollution (Chow et al., 2020). Wolfram et al. (2021) identified intensive agricultural regions from Spain to have pesticide concentrations at risk levels for the aquatic ecosystems. In these areas, intensive irrigation is commonly used due to the semi-arid climate with recurring droughts and strong seasonal rainfall variety (Eurostat, 2018). This practice has been identified to be a significant factor in increasing pesticide runoff to surface waters, as pesticides are more likely to reach surface waters due to high drainage potentials, increased water to land interface area, through discharge channels, and agricultural intensification (Wolfram et al., 2019).

OPEs and benzophenone have also been found to be positively related to the agricultural surface (β=0.647, p-value <0.001); the estimates were positive for artificial surface and number of WWTP, but they were not significant (Figure 3). OPEs are used as flame retardants and plasticizers in textiles, electronic goods and many other consumer products (Greaves & Letcher, 2017). OPEs reach the aquatic environment through WWTP discharges, run-off, and atmospheric deposition, and the concentration and profile of OPEs in surface waters are highly dependent on local emissions and dilution factors (Pantelaki & Voutsa, 2019). Similar to other OMPs, the occurrence of OPEs in agricultural areas has been found to be related to the use of sewage sludge as fertilizer and the use of reclaimed water for irrigation (Eggen et al., 2013). Some studies have also pointed to the use of plastic mulch film in agriculture as a source of OPEs in the fields due to the oxidation of organophosphite antioxidants, auxiliary antioxidants used in plastic polymers that contain TCEP and TPhP (Gong et al., 2021).

The total concentration of PFASs has been positively associated with agricultural surface (β=1.155, p-value <0.001), artificial surface (β=0.816, p-value <0.001) and number of WWTPs (β=0.923, p-value <0.001) (Figure 3). Similarly, the major sources of PFASs to the aquatic environment include industrial runoff, WWTPs, landfills, atmospheric deposition, and aqueous film-forming foams (AFFFs) storage and use in both training exercises and actual fire emergencies (Sims et al., 2022). PFASs have been also detected in streams from agricultural regions in US, whose presence has been attributed to the application of WWTP biosolids and reclaimed water to the fields (Kolpin et al., 2021).

Summarizing, agricultural areas have been related to an increase in concentrations of all OMPs families. It must be highlighted that agricultural land use is the most prevalent anthropogenic pressure present in IBAs, as 89 out of the 140 areas have more than 20% of its total surface occupied by agricultural use. Therefore, agricultural practices have a higher impact in the release of OMPs than artificial surface and WWTPs, which present a lower prevalence in natural areas, especially in the most protected ones. In a recent study, Wolfram et al., 2023 also found a positive relationship between agricultural surface and increased environmental risk concentrations of pesticides in protected areas from Germany.

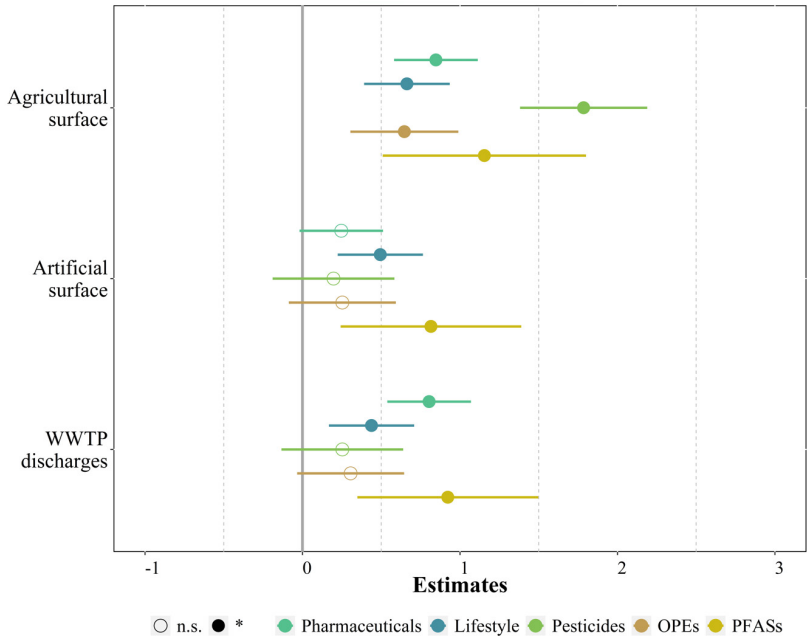
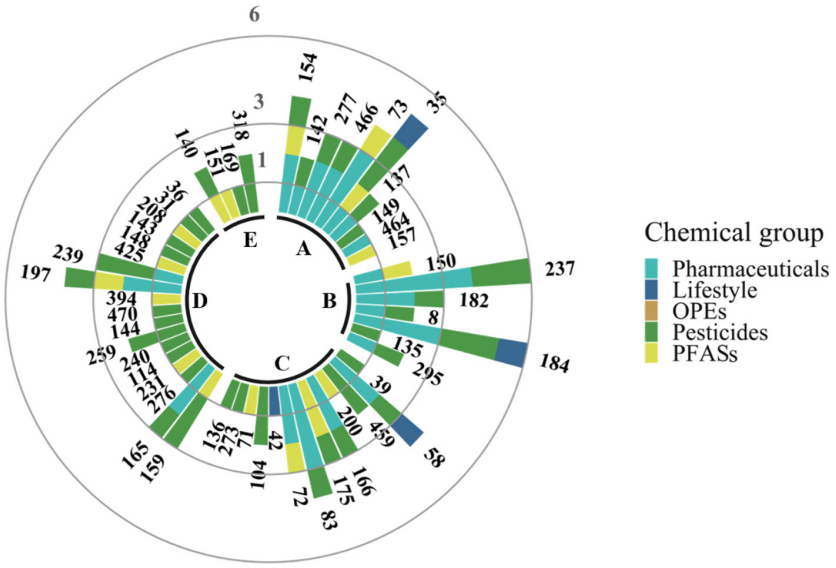


Figure 3. Estimates for the effects of spatial data on micropollutants concentrations. Full dots are indicative of significant differences ($p<0.05$).

3.4. Geographical distribution and environmental risk of chemicals in IBAs

The environmental RQs for the target compounds in each IBA are indicated in Table S3. Out of the 140 IBAs, only 50 did not present any target compound at concentrations considered to be of concern for the aquatic ecosystems (RQ<0.01). Following, 38 IBAs presented medium risk values of compounds (0.01<RQ<1) and 52 IBAs had high risk concentrations for aquatic ecosystems (RQ>1). Figure 4 shows those IBAs with RQs >1, classified according to the main pressures identified in the area (indicated in section 2.5). As discussed above, different pollution sources lead to releases of OMP, and therefore, to high probabilities of toxicological risk for the aquatic ecosystems. Those IBAs with the highest number of pharmaceuticals with RQ>1 corresponded to those which presents (A) agricultural surfaces, artificial, and WWTPs or (B) agricultural surface and WWTPs. IBAs where the main pressure identified were (C) agricultural and artificial surface or (D) only agricultural surface presented the greatest proportion of pesticides at high-risk concentration. Finally, in IBAs where the (E) artificial surface was the only pressure, the RQ>1 corresponded to pesticides and PFASs.



(>20%) and presence of artificial surface and WWTP discharges; B: Agricultural pressure and WWTPs; C: Agricultural and artificial surfaces; D: Agricultural surface; E: Mainly artificial surface. The numbers indicate IBA codes.

OMPs in surface waters from IBAs are present as mixtures of compounds. Although little information is available about the potential interactions between different chemical groups in the environment, it is well established that chemical cocktails have a greater impact to the environment than individual compounds (Altenburger et al., 2013, Posthuma et al., 2019a). Therefore, IBAs with high ∑RQs, meaning concentrations far higher than the toxicological levels, are expected to be the most impacted and with a higher risk of biodiversity loss. The total risk per IBA is mapped in Figure 5, classified in four levels from no risk ($\sum RQ_{j,i}$ <1) to high risk ($\sum RQ_{j,i}$ >100). Using this approach, nine IBAs presented a high risk $\sum RQ_{j,i}$ mainly caused by the presence of pesticides and PFASs (Table S3). The top three most impacted areas IBAs were 237 Campiña de Carmona (Sevilla) with a $\sum RQ_{j,i}$ of 1327, mostly driven by the organophosphate insecticide dimethoate detected at mean concentrations of 30,590 ng/L. IBA 175 - Guadalentín salt marshes (Murcia) and IBA 157-Turia canyon and Los Serranos (Valencia, Cuenca, and Teruel) presented a high $RQ_{j,i}$ because of the high concentrations of PFOS, detected at a mean concentration of 352 ng/L and 292 ng/L, respectively.

Considering the worst-case scenario with the lowest freshwaters PNEC

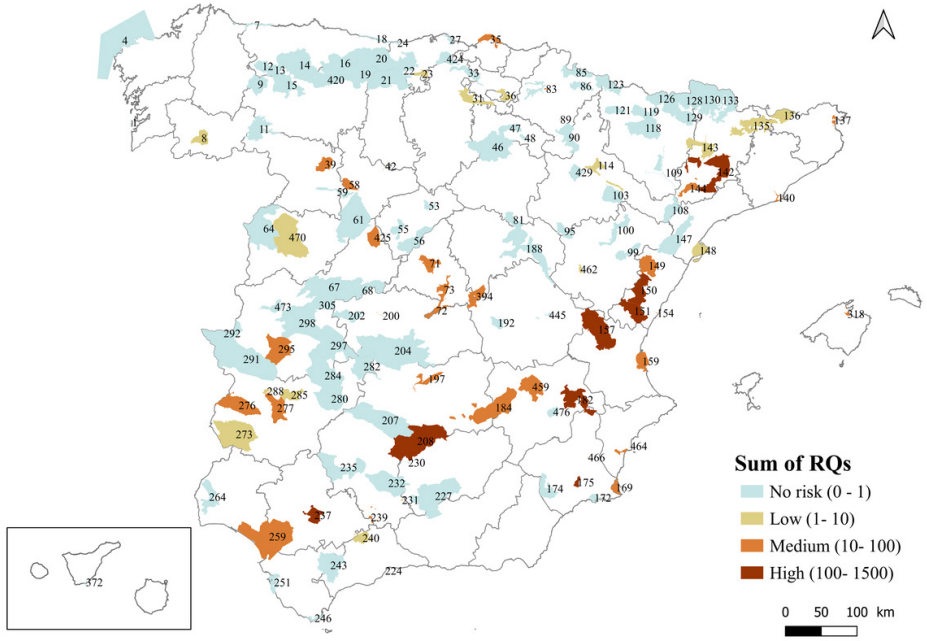


Figure 5. Total sum of risk quotients (∑RQs) in the selected Important Bird and Biodiversity Areas. Numbers indicates BirdLife International IBA's codes (Table S1).

(NORMAN, 2022), 15 out of the 59 target compounds presented values of high risk considering all analysed IBAs, according to $RQ_{f,i}$ value, as indicated in Table 2. This list represents the most concerning compounds for freshwater ecosystems. The most hazardous compound is chlorpyrifos, an organophosphorus insecticide widely used in agricultural applications and domestic pest control, and one of the most toxic compounds due to its neurotoxicity and genotoxicity (Ubaid ur Rahman et al., 2021) and high ecotoxicity for aquatic organisms (Huang et al., 2020). Because of its high persistence and risk for human and environmental health, the use of this insecticide has been banned in Europe (European Commision, 2020) and it has been proposed to be listed in Annex A (for elimination) of the Stockholm Convention (Stockholm Convention, 2022). Chlorpyrifos has been detected at 25% of the IBAs at high-risk concentrations for freshwater ecosystems and the highest concentrations were found in agricultural areas (r=0.2, p=0.04). Also, 8 samples from 6 different IBAs exceeded the Maximum Allowable Concentration - Environmental Quality Standard (MAC-EQS) threshold of 100 ng/L established by European directive (Directive 2013/39/EU). The results are in accordance with previous studies that have identified this insecticide at levels of concern in freshwaters from the Iberian peninsula (Ccanccapa et al., 2016; Rico et al., 2021). Altogether, chlorpyrifos can be considered as a threat for the conservation of biodiversity values in IBAs.

Following, the antidepressant venlafaxine was also detected at risk concentrations in 13% of the IBAs. Venlafaxine has been identified as one of the top ten most frequently detected compounds in surface waters from Europe and Africa (Fekadu et al., 2019). Our results are consistent with previous studies that have identified venlafaxine as a concerning compound in surface waters from Spain (Čelić et al., 2021; Fernández-Rubio et al., 2019). Because of its high bioacumulative properties, residues of venlafaxine accumulates in aquatic organisms such as mussels and fish (Madikizela & Ncube, 2022). The danger of bioaccumulation enhances the risk of exposure to top consumers such as aquatic birds.

The third most concerning compound found was PFOS, detected at high risk concentrations in 12% of the IBAs. Despite not being one of the most ubiquitous compounds, it has a low PNEC value of 0.65 ng/L. It is also listed in the European Water Framework (Directive 2013/39/EU), with a MAC-EQS of 36000 ng/L. None of the samples from the present study exceeded this threshold. PFOS has been found to be widespread in biota due to its bioaccumulation potential, and its presence has been reported in several bird species, such as gulls and flamingos breeding in the Ebro Delta, one of the most important IBAs in Spain for waterbird conservation (Colomer-Vidal et al., 2022; Dulsat-Masvidal et al., 2023), exemplifying the impact of OMPs exposure on biodiversity and birds.

Overall, the water monitoring scheme procedure based on the analysis of in-use chemicals allowed us to determine the IBAs having the highest risk due to chemical pollutants and permitted to prioritize main hazardous compounds and their contamination sources. This methodology can be applied for identifying the pollution status in IBAs, in protected areas or in high conservation areas which are home on many wild species.

Table 2. Prioritization of the most concerning target compounds ($RQ_{f,i}$ >0), and percentage (%) of IBAs (N=140) with degree of environmental risk, ordered by concerning level according to % of high risk RQs (RQ>1).

Compound	Group	PNEC (ng/L)[1]	% No Risk	% Low Risk (0.01< RQ < 0.1)	% Medium Risk (0.1<RQ<1)	% High Risk (RQ>1)	RQf,i
Chlorpyrifos	Pesticide	0.46	75	0	0	25	34.223
Venlafaxine	Pharmaceutical	38	71	1	15	13	0.0766
PFOS	PFASs	0.65	88	0	0	12	20.418
Diclofenac	Pharmaceutical	50	87	1	4	8	0.0226
Dimethoate	Pesticide	70	81	6	9	4	0.1439
Caffeine	Pharmaceutical	1200	32	51	14	3	0.0047
Atorvastatin	Pharmaceutical	10	97	0	0	3	0.0036
Tebuconazol	Pesticide	240	84	1	13	3	0.0027
Prosulfocarb	Pesticide	500	94	1	5	1	0.0009
Ibuprofen	Pharmaceutical	1000	97	1	1	1	0.0009
Chlortoluron	Pesticide	600	82	10	6	1	0.0008
Carbamazepine	Pharmaceutical	50	59	12	28	1	0.0007
Trazodone	Pharmaceutical	16	95	1	2	1	0.0005
PFOA	PFASs	180	86	0	14	1	0.0003
Furosemide	Pharmaceutical	710	94	5	1	1	0.0001

[1]Lowest PNEC values in freshwater (NORMAN, 2022)

4. CONCLUSIONS

This is the first study to quantify water pollution as a threat to IBAs. OMPs have been found to be widespread in natural freshwater ecosystems key for biodiversity conservation. Lifestyle compounds and pharmaceuticals were the most ubiquitous pollutants, present in 76 and 84% of the analysed samples, respectively. Chlorpyrifos and PFOS, together with the antidepressant venlafaxine presented an occurrence below 25% of the total samples but have been found to be of high concern for the aquatic ecosystems. Our results evidenced a strong influence of the land use inside and outside the boundaries of IBAs to explain the sources and distribution of pollutants. Agricultural practices are one of the main anthropogenic pressures in natural areas and have been identified as a source of OMPs in surface waters. WWTP discharges have been related to the release of pharmaceuticals, lifestyle compounds and PFASs. Artificial surface close to water bodies has been identified as a source of lifestyle compounds and PFASs from urban run-off. Our findings indicate that IBAs located in low altitudes are the most vulnerable to be affected by water pollution. Also, those with a smaller surface have a higher input of pesticides; however, this relation was not found for other chemical groups.

Finally, we have identified 52 IBAs out of 140 studied with levels of OMPs that could pose the aquatic ecosystems at risk and therefore, their conservation values. This study serves to delineate the importance of water monitoring studies within IBAs and other natural areas as a first approach to contribute to pollution management actions and minimize the impact of pollutants on biodiversity.

ENVIRONMENTAL IMPLICATION

The presence of organic contaminants in Important Bird and Biodiversity Areas (IBAs) can have huge implications in the conservation of waterbirds and in the maintenance of global biodiversity. IBAs are used as a reference benchmark in the European Union in the designation of the Special Protection Areas (SPA) under the Birds Directive (2009/147/EC) and have become part of the Natura 2000 network. Knowledge on pollution status of water resources, sources of contaminants and risks will allow a better management and conservation actions of these vulnerable areas, key to ensure planet's biodiversity.

REFERENCES

Adeleye, A.S., Xue, J., Zhao, Y., Taylor, A.A., Zenobio, J.E., Sun, Y., Han, Z., Salawu, O.A., Zhu, Y., 2022. Abundance, fate, and effects of pharmaceuticals and personal care products in aquatic environments. J. Hazard. Mater. 424, 127284. https://doi.org/10.1016/j.jhazmat.2021.127284

Altenburger, R., Backhaus, T., Boedeker, W., Faust, M., Scholze, M., 2013. Simplifying complexity: Mixture toxicity assessment in the last 20 years. Environ. Toxicol. Chem. 32, 1685–1687. https://doi.org/10.1002/etc.2294

Anliker, S., Loos, M., Comte, R., Ruff, M., Fenner, K., Singer, H., 2020. Assessing Emissions from Pharmaceutical Manufacturing Based on Temporal High-Resolution Mass Spectrometry Data. Environ. Sci. Technol. 54, 4110–4120. https://doi.org/10.1021/acs.est.9b07085

Arenas-Sánchez, A., Rico, A., Rivas-Tabares, D., Blanco, A., Garcia-Doncel, P., Romero-Salas, A., Nozal, L., Vighi, M., 2019. Identification of contaminants of concern in the upper Tagus river basin (central Spain). Part 2: Spatio-temporal analysis and ecological risk assessment. Sci. Total Environ. 667, 222–233. https://doi.org/10.1016/J.SCITOTENV.2019.02.286

Backhaus, T., Faust, M., 2012. Predictive environmental risk assessment of chemical mixtures: A conceptual framework. Environ. Sci. Technol. 46, 2564–2573. https://doi.org/10.1021/es2034125

Biel-Maeso, M., Baena-Nogueras, R.M., Corada-Fernández, C., Lara-Martín, P.A., 2018. Occurrence, distribution and environmental risk of pharmaceutically active compounds (PhACs) in coastal and ocean waters from the Gulf of Cadiz (SW Spain). Sci. Total Environ. 612, 649–659. https://doi.org/10.1016/J.SCITOTENV.2017.08.279

BirdLife International, 2023. Data Zone [WWW Document]. URL http://www.birdlife.org/datazone (accessed 7.11.23).

Buchanan, G.M., Donald, P.F., Fishpool, L.D.C., Arinaitwe, J.A., Balman, M., Mayaux, P., 2009. An assessment of land cover and threats in Important Bird Areas in Africa. Bird Conserv. Int. 19, 49–61. https://doi.org/10.1017/S0959270908007697

Butchart, S.H.M., Scharlemann, J.P.W., Evans, M.I., Quader, S., Aricò, S., Arinaitwe, J., Balman, M., Bennun, L.A., Bertzky, B., Besançon, C., Boucher, T.M., Brooks, T.M., Burfield, I.J., Burgess, N.D., Chan, S., Clay, R.P., Crosby, M.J., Davidson, N.C., de Silva, N., Devenish, C., Dutson, G.C.L., Fernández, D.F.D., Fishpool, L.D.C., Fitzgerald, C.,

Foster, M., Heath, M.F., Hockings, M., Hoffmann, M., Knox, D., Larsen, F.W., Lamoreux, J.F., Loucks, C., May, I., Millett, J., Molloy, D., Morling, P., Parr, M., Ricketts, T.H., Seddon, N., Skolnik, B., Stuart, S.N., Upgren, A., Woodley, S., 2012. Protecting important sites for biodiversity contributes to meeting global conservation targets. PLoS One 7. https://doi.org/10.1371/journal.pone.0032529

Camacho-Muñoz, D., Martín, J., Santos, J.L., Aparicio, I., Alonso, E., 2012. Effectiveness of conventional and low-cost wastewater treatments in the removal of pharmaceutically active compounds. Water. Air. Soil Pollut. 223, 2611–2621. https://doi.org/10.1007/s11270-011-1053-9

Ccanccapa, A., Masiá, A., Navarro-Ortega, A., Picó, Y., Barceló, D., 2016. Pesticides in the Ebro River basin: Occurrence and risk assessment. Environ. Pollut. 211, 414–424. https://doi.org/10.1016/j.envpol.2015.12.059

Čelić, M., Jaén-Gil, A., Briceño-Guevara, S., Rodríguez-Mozaz, S., Gros, M., Petrović, M., 2021. Extended suspect screening to identify contaminants of emerging concern in riverine and coastal ecosystems and assessment of environmental risks. J. Hazard. Mater. 404. https://doi.org/10.1016/j.jhazmat.2020.124102

Chow, R., Scheidegger, R., Doppler, T., Dietzel, A., Fenicia, F., Stamm, C., 2020. A review of long-term pesticide monitoring studies to assess surface water quality trends. Water Res. X 9, 100064. https://doi.org/10.1016/j.wroa.2020.100064

Collivignarelli, M.C., Abbà, A., Frattarola, A., Miino, M.C., Padovani, S., Katsoyiannis, I., Torretta, V., 2019. Legislation for the reuse of biosolids on agricultural land in Europe: Overview. Sustain. 11. https://doi.org/10.3390/su11216015

Colomer-Vidal, P., Bertolero, A., Alcaraz, C., Garreta-Lara, E., Santos, F.J., Lacorte, S., 2022. Distribution and ten-year temporal trends (2009–2018) of perfluoroalkyl substances in gull eggs from Spanish breeding colonies. Environ. Pollut. 293, 118555. https://doi.org/10.1016/J.ENVPOL.2021.118555

Council Directive 92/43/EEC, 1992. Council Directive 92/43/EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora.

Cristale, J., Vázquez, A.G., Barata, C., Lacorte, S., 2013. Priority and emerging fl ame retardants in rivers : Occurrence in water and sediment , Daphnia magna toxicity and risk assessment. Environ. Int. 59, 232–243. https://doi.org/10.1016/j.envint.2013.06.011

Daughton, C.G., 2004. Non-regulated water contaminants: emerging research. Environ. Impact Assess. Rev. 24, 711–732.

Directive 2009/147/EC of the European Parliament and of the Council of 30 November 2009 on the conservation of wild birds., 2009.

Directive 2013/39/EU of the European Parliament and of the Council of 12 August 2013 amending Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy, 2013.

Donald, P.F., Fishpool, L.D.C., Ajagbe, A., Bennun, L.A., Bunting, G., Burfield, I.J., Butchart, S.H.M., Capellan, S., Crosby, M.J., Dias, M.P., Diaz, D., Evans, M.I., Grimmett, R., Heath, M., Jones, V.R., Lascelles, B.G., Merriman, J.C., O'brien, M., Ramírez, I., Waliczky, Z., Wege, D.C., 2019. Important Bird and Biodiversity Areas (IBAs): the development and characteristics of a global inventory of key sites for biodiversity. Bird Conserv. Int. 29, 177–198. https://doi.org/10.1017/S0959270918000102

Dulsat-Masvidal, M., Bertolero, A., Mateo, R., Lacorte, S., 2023. Legacy and emerging contaminants in flamingos' chicks' blood from the Ebro Delta Natural Park. Chemosphere 312, 18–26. https://doi.org/10.1016/j.chemosphere.2022.137205

Dulsat-Masvidal, M., Ciudad, C., Infante, O., Mateo, R., Lacorte, S., 2022. Pilot monitoring scheme of water pollutants in Important Bird and Biodiversity Areas. Ecotoxicol. Environ. Saf. 237, 113562. https://doi.org/10.1016/j.ecoenv.2022.113562

Ebele, A.J., Abou-Elwafa Abdallah, M., Harrad, S., 2017. Pharmaceuticals and personal care products (PPCPs) in the freshwater aquatic environment. Emerg. Contam. 3, 1–16. https://doi.org/10.1016/J.EMCON.2016.12.004

ECHA, 2022. European Chemicals Agency [WWW Document]. URL https://echa.europa.eu/ (accessed 2.8.22).

Eggen, T., Heimstad, E.S., Stuanes, A.O., Norli, H.R., 2013. Uptake and translocation of organophosphates and other

emerging contaminants in food and forage crops. Environ. Sci. Pollut. Res. 20, 4520–4531. https://doi.org/10.1007/s11356-012-1363-5

European Commision, 2020. Commission Implementing Regulation (EU) 2020/18 of 10 January 2020. Brussels, Belgium.

European Commission, 2003. Technical Guidance Document on Risk Assessment.

Eurostat, 2018. Agricultural census in Spain.

Fekadu, S., Alemayehu, E., Dewil, R., Van der Bruggen, B., 2019. Pharmaceuticals in freshwater aquatic environments: A comparison of the African and European challenge. Sci. Total Environ. 654, 324–337. https://doi.org/10.1016/j.scitotenv.2018.11.072

Fernández-Rubio, J., Rodríguez-Gil, J.L., Postigo, C., Mastroianni, N., López de Alda, M., Barceló, D., Valcárcel, Y., 2019. Psychoactive pharmaceuticals and illicit drugs in coastal waters of North-Western Spain: Environmental exposure and risk assessment. Chemosphere 224, 379–389. https://doi.org/10.1016/j.chemosphere.2019.02.041

Figuière, R., Waara, S., Ahrens, L., Golovko, O., 2022. Risk-based screening for prioritisation of organic micropollutants in Swedish freshwater. J. Hazard. Mater. 429, 128302. https://doi.org/10.1016/j.jhazmat.2022.128302

Gómez-Canela, C., Edo, S., Rodríguez, N., Gotor, G., Lacorte, S., 2021. Comprehensive characterization of 76 pharmaceuticals and metabolites in wastewater by lc-ms/ms. Chemosensors 9, 1–19. https://doi.org/10.3390/chemosensors9100273

Gómez-Canela, C., Pueyo, V., Barata, C., Lacorte, S., Marcé, R.M., 2019. Development of predicted environmental concentrations to prioritize the occurrence of pharmaceuticals in rivers from Catalonia. Sci. Total Environ. 666, 57–67. https://doi.org/10.1016/j.scitotenv.2019.02.078

Gong, X., Zhang, W., Zhang, S., Wang, Y., Zhang, X., Lu, Y., Sun, H., Wang, L., 2021. Organophosphite Antioxidants in Mulch Films Are Important Sources of Organophosphate Pollutants in Farmlands. Environ. Sci. \& Technol. 55, 7398–7406. https://doi.org/10.1021/acs.est.0c08741

Greaves, A.K., Letcher, R.J., 2017. A review of organophosphate esters in the environment from biological effects to distribution and fate. Bull. Environ. Contam. Toxicol. 98, 2–7. https://doi.org/https://doi.org/10.1007/s00128-016-1898-0

Hausmann, A., Toivonen, T., Fink, C., Heikinheimo, V., Tenkanen, H., Butchart, S.H.M., Brooks, T.M., Di Minin, E., 2019. Assessing global popularity and threats to Important Bird and Biodiversity Areas using social media data. Sci. Total Environ. 683, 617–623. https://doi.org/10.1016/j.scitotenv.2019.05.268

Henningsen, A., 2022. censReg: Censored Regression (Tobit) Models.

Huang, X., Cui, H., Duan, W., 2020. Ecotoxicity of chlorpyrifos to aquatic organisms: A review. Ecotoxicol. Environ. Saf. 200, 110731. https://doi.org/https://doi.org/10.1016/j.ecoenv.2020.110731

Ivanová, L., Mackuľak, T., Grabic, R., Golovko, O., Koba, O., Staňová, A.V., Szabová, P., Grenčíková, A., Bodík, I., 2018. Pharmaceuticals and illicit drugs – A new threat to the application of sewage sludge in agriculture. Sci. Total Environ. 634, 606–615. https://doi.org/10.1016/j.scitotenv.2018.04.001

James, G., Witten, D., Hastie, T., Tibshirani, R., 2013. An introduction to statistical learning. Springer.

Kienzler, A., Bopp, S., Halder, M., Embry, M., Worth, A., 2019. Application of new statistical distribution approaches for environmental mixture risk assessment: A case study. Sci. Total Environ. 693, 133510. https://doi.org/10.1016/j.scitotenv.2019.07.316

Kolpin, D.W., Hubbard, L.E., Cwiertny, D.M., Meppelink, S.M., Thompson, D.A., Gray, J.L., 2021. A Comprehensive Statewide Spatiotemporal Stream Assessment of Per- and Polyfluoroalkyl Substances (PFAS) in an Agricultural Region of the United States. Environ. Sci. Technol. Lett. 8, 981–988. https://doi.org/10.1021/acs.estlett.1c00750

Li, S., Wen, J., He, B., Wang, J., Hu, X., Liu, J., 2020. Occurrence of caffeine in the freshwater environment: Implications for ecopharmacovigilance. Environ. Pollut. 263, 114371. https://doi.org/10.1016/j.envpol.2020.114371

Li, Y., Zhang, L., Ding, J., Liu, X., 2020. Prioritization of pharmaceuticals in water environment in China based on environmental criteria and risk analysis of top-priority pharmaceuticals. J. Environ. Manage. 253, 109732. https://doi.org/10.1016/j.jenvman.2019.109732

Luo, Y., Guo, W., Ngo, H.H., Nghiem, L.D., Hai, F.I., Zhang, J., Liang, S., Wang, X.C., 2014. A review on the occurrence of micropollutants in the aquatic environment and their fate and removal during wastewater treatment. Sci. Total Environ. 473–474, 619–641. https://doi.org/10.1016/j.scitotenv.2013.12.065

Madikizela, L.M., Ncube, S., 2022. Health effects and risks associated with the occurrence of pharmaceuticals and their metabolites in marine organisms and seafood. Sci. Total Environ. 837, 155780. https://doi.org/10.1016/j.scitotenv.2022.155780

Munaron, D., Tapie, N., Budzinski, H., Andral, B., Gonzalez, J.L., 2012. Pharmaceuticals, alkylphenols and pesticides in Mediterranean coastal waters: Results from a pilot survey using passive samplers. Estuar. Coast. Shelf Sci. 114, 82–92. https://doi.org/10.1016/J.ECSS.2011.09.009

Ng, K., Alygizakis, N., Androulakakis, A., Galani, A., Aalizadeh, R., Thomaidis, N.S., Slobodnik, J., 2022. Target and suspect screening of 4777 per- and polyfluoroalkyl substances (PFAS) in river water, wastewater, groundwater and biota samples in the Danube River Basin. J. Hazard. Mater. 436, 129276. https://doi.org/https://doi.org/10.1016/j.jhazmat.2022.129276

NORMAN, 2022. NORMAN Ecotoxicology Database - Lowest PNECs [WWW Document]. URL https://www.norman-network.com/nds/ecotox/lowestPnecsIndex.php (accessed 3.16.22).

Pantelaki, I., Voutsa, D., 2019. Organophosphate flame retardants (OPFRs): A review on analytical methods and occurrence in wastewater and aquatic environment. Sci. Total Environ. 649, 247–263. https://doi.org/https://doi.org/10.1016/j.scitotenv.2018.08.286

Pavón-Jordán, D., Abdou, W., Azafzaf, H., Balaž, M., Bino, T., Borg, J.J., Božič, L., Butchart, S.H.M., Clausen, P., Sniauksta, L., Dakki, M., Devos, K., Domsa, C., Encarnaçao, V., Etayeb, K., Faragó, S., Fox, A.D., Frost, T., Gaudard, C., Georgiev, V., Goratze, I., Hornman, M., Keller, V., Kostiushyn, V., Langendoen, T., Ławicki, Ł., Ieronymidou, C., Lewis, L.J., Lorentsen, S.H., Luigujoe, L., Meissner, W., Mikuska, T., Molina, B., Musil, P., Musilova, Z., Nagy, S., Natykanets, V., Nilsson, L., Paquet, J.Y., Portolou, D., Ridzon, J., Santangeli, A., Sayoud, S., Šćiban, M., Stipniece, A., Teufelbauer, N., Topić, G., Uzunova, D., Vizi, A., Wahl, J., Yavuz, K.E., Zenatello, M., Lehikoinen, A., 2020. Positive impacts of important bird and biodiversity areas on wintering waterbirds under changing temperatures throughout Europe and North Africa. Biol. Conserv. 246, 108549. https://doi.org/10.1016/J.BIOCON.2020.108549

Pedrero, F., Kalavrouziotis, I., Alarcón, J.J., Koukoulakis, P., Asano, T., 2010. Use of treated municipal wastewater in irrigated agriculture-Review of some practices in Spain and Greece. Agric. Water Manag. 97, 1233–1241. https://doi.org/10.1016/j.agwat.2010.03.003

Posthuma, L., Altenburger, R., Backhaus, T., Kortenkamp, A., Müller, C., Focks, A., de Zwart, D., Brack, W., 2019a. Improved component-based methods for mixture risk assessment are key to characterize complex chemical pollution in surface waters. Environ. Sci. Eur. 31. https://doi.org/10.1186/s12302-019-0246-5

Posthuma, L., Brack, W., van Gils, J., Focks, A., Müller, C., de Zwart, D., Birk, S., 2019b. Mixtures of chemicals are important drivers of impacts on ecological status in European surface waters. Environ. Sci. Eur. 31. https://doi.org/10.1186/s12302-019-0247-4

PPDB, 2022. Databases-Background and support information [WWW Document]. URL http://sitem.herts.ac.uk/aeru/ppdb/en/support_docs.htm (accessed 3.10.22).

Quadra, G.R., Paranaíba, J.R., Vilas-Boas, J., Roland, F., Amado, A.M., Barros, N., Dias, R.J.P., Cardoso, S.J., 2020. A global trend of caffeine consumption over time and related-environmental impacts. Environ. Pollut. 256, 113343. https://doi.org/https://doi.org/10.1016/j.envpol.2019.113343

Rattner, B.A., Ackerson, B.K., 2008. Potential environmental contaminant risks to avian species at important bird areas in the Northeastern United States. Integr. Environ. Assess. Manag. 4, 344–357. https://doi.org/10.1897/IEAM_2007-091.1

Rico, A., Dafouz, R., Vighi, M., Rodríguez-Gil, J.L., Daam, M.A., 2021. Use of Postregistration Monitoring Data to Evaluate the Ecotoxicological Risks of Pesticides to Surface Waters: A Case Study with Chlorpyrifos in the Iberian Peninsula. Environ. Toxicol. Chem. 40, 500–512. https://doi.org/10.1002/etc.4927

Roder Green, A.L., Putschew, A., Nehls, T., 2014. Littered cigarette butts as a source of nicotine in urban waters. J. Hydrol. 519, 3466–3474. https://doi.org/10.1016/J.JHYDROL.2014.05.046

Schwarzenbach, R., Anderson, M.G., Mcdonnell, J., Ximing, C., Cline, S. a, Balance, W.W., Rockstrom, J., Daily, G.C., Ehrlich, P.R., Reidy, C. a, Dynesius, M., Revenga, C., Dams, L., Reichel, E., Global, M.A., Green, P., Salisbury, J., Lammers, R.B., Kanae, S., Oki, T., Hoekstra, a Y., Eichert, P., Abbaspour, K.C., Zehnder, a B., Kitoh, A., Hosaka, M., Dunne, K. a, Vecchia, a V, Valeo, C., Heal, K., Science, I., Bengtsson, M., Agata, Y., Kim, H., Science, E., 2006. The Challenge of Micropollutants. Sci. Technol. 313, 1072–1077.

Silva, B.F. da, Jelic, A., López-Serna, R., Mozeto, A.A., Petrovic, M., Barceló, D., 2011. Occurrence and distribution of pharmaceuticals in surface water, suspended solids and sediments of the Ebro river basin, Spain. Chemosphere 85, 1331–1339. https://doi.org/10.1016/j.chemosphere.2011.07.051

Sims, J.L., Stroski, K.M., Kim, S., Killeen, G., Ehalt, R., Simcik, M.F., Brooks, B.W., 2022. Global occurrence and probabilistic environmental health hazard assessment of per- and polyfluoroalkyl substances (PFASs) in groundwater and surface waters. Sci. Total Environ. 816, 151535. https://doi.org/https://doi.org/10.1016/j.scitotenv.2021.151535

Singh, A., 2021. A review of wastewater irrigation: Environmental implications. Resour. Conserv. Recycl. 168, 105454. https://doi.org/10.1016/j.resconrec.2021.105454

Sousa, J.C.G., Ribeiro, A.R., Barbosa, M.O., Pereira, M.F.R., Silva, A.M.T., 2018. A review on environmental monitoring of water organic pollutants identified by EU guidelines. J. Hazard. Mater. 344, 146–162. https://doi.org/10.1016/j.jhazmat.2017.09.058

Stockholm Convention, 2022. Chemicals proposed for listing under the Convention [WWW Document]. URL http://www.pops.int/TheConvention/ThePOPs/ChemicalsProposedforListing

Ubaid ur Rahman, H., Asghar, W., Nazir, W., Sandhu, M.A., Ahmed, A., Khalid, N., 2021. A comprehensive review on chlorpyrifos toxicity with special reference to endocrine disruption: Evidence of mechanisms, exposures and mitigation strategies. Sci. Total Environ. 755. https://doi.org/10.1016/j.scitotenv.2020.142649

Waliczky, Z., Fishpool, L.D.C., Butchart, S.H.M., Thomas, D., Heath, M.F., Hazin, C., Donald, P.F., Kowalska, A., Dias, M.P., Allinson, T.S.M., 2019. Important Bird and Biodiversity Areas (IBAs): their impact on conservation policy, advocacy and action. Bird Conserv. Int. 29, 199–215. https://doi.org/10.1017/S0959270918000175

Warner, W., Licha, T., Nödler, K., 2019. Qualitative and quantitative use of micropollutants as source and process indicators. A review. Sci. Total Environ. 686, 75–89. https://doi.org/10.1016/j.scitotenv.2019.05.385

Wolfram, J., Bub, S., Petschick, L.L., Schemmer, A., Stehle, S., Schulz, R., 2023. Pesticide occurrence in protected surface waters in nature conservation areas of Germany. Sci. Total Environ. 858, 160074. https://doi.org/https://doi.org/10.1016/j.scitotenv.2022.160074

Wolfram, J., Stehle, S., Bub, S., Petschick, L.L., Schulz, R., 2021. Water quality and ecological risks in European surface waters – Monitoring improves while water quality decreases. Environ. Int. 152, 106479. https://doi.org/10.1016/j.envint.2021.106479

Wolfram, J., Stehle, S., Bub, S., Petschick, L.L., Schulz, R., 2019. Insecticide Risk in US Surface Waters: Drivers and Spatiotemporal Modeling. Environ. Sci. Technol. 53, 12071–12080. https://doi.org/10.1021/acs.est.9b04285

SUPPLEMENTARY INFORMATION

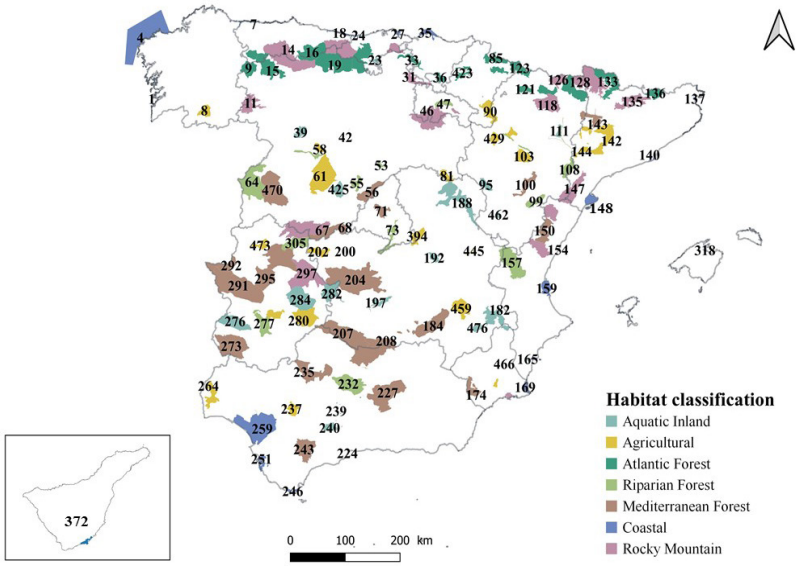


Figure S1. Map of the selected Important Bird and Biodiversity Areas from Spain. Numbers indicate IBA code.

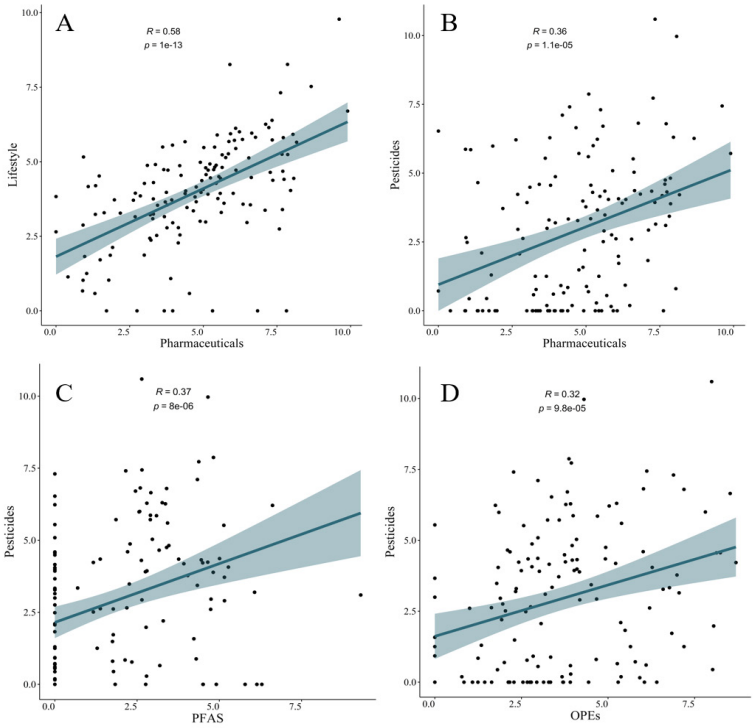


Figure S2. Spearman correlations between the concentrations of chemical families.

Table S1. Information of IBAs name, habitat, sampling point, spatial data, protection status, and the ∑pharmaceuticals, ∑lifestyle compounds, ∑pesticides, ∑OPEs and benzophenone and ∑PFASs. Table S1 is attached as electronic supplementary material due to its large size. Please scan the QR code below to display the table.

Table S2. Target compounds, indicating CAS number and the Lowest PNEC values (ng/L) from Norman Database.

Compound	CAS	Lowest Fresh Water PNEC (ng/L)	Source
Lifestyle compounds			
Nicotine	54-11-5	5450	Verified by NORMAN
Caffeine	58-08-2	1200	Verified by NORMAN
Pharmaceuticals			
Nervious System			
Paracetamol	103-90-2	134000	Verified by NORMAN
Tramadol	27203-92-5	8650	Verified by NORMAN
Carbamazepine	298-46-4	50	Verified by NORMAN
Gabapentine	60142-96-3	10000	Verified by NORMAN
Levetiracetam	102767-28-2	38000	Verified by NORMAN
Venlaflaxine	93413-69-5	38	Verified by NORMAN
Trazodone	19794-93-5	16	Verified by NORMAN
Quetiapine	111974-69-7	140	Verified by NORMAN
Cardiovascular System			
Pentoxifylline	6493056	6020	Predicted by QSAR and experimentally-based values
Losartan	114798-26-4	78000	Verified by NORMAN
Valsartan	137862-53-4	560000	Verified by NORMAN
Atrovastatin	134523-00-5	10	Verified by NORMAN
Atenolol	29122-68-7	150000	Verified by NORMAN
Muscular-Alimentary-Antiinfective			
Metformin	657-24-9	156000	Verified by NORMAN
Diclofenac	15307-86-5	50	Verified by NORMAN
Sulfamethoxazole	723-46-6	600	Verified by NORMAN
Furosemide	54-31-9	710	Verified by NORMAN
Ibuprofen	15687-27-1	1000	Predicted by QSAR and experimentally-based values
Allopurinol	315-30-0	20600	Verified by NORMAN

Compound	CAS	Lowest Fresh Water PNEC (ng/L)	Source
Pesticides			
Insecticide			
Dimethoate	60-51-5	70	Verified by NORMAN
Tebufenpyrad	119168-77-3	82	Predicted by QSAR and experimentally-based values
Chlorpyrifos	2921-88-2	0.46	Verified by NORMAN
Chlorfenvinphos	470-90-6	100	Verified by NORMAN
Spinosad	168316-95-8	24	Verified by NORMAN
Herbicide			
Chlortoluron	15545-48-9	600	Verified by NORMAN
Isoproturon	34123-59-6	640	Verified by NORMAN
Diclofop	40843-25-2	460	Predicted by QSAR and experimentally-based values
Prosulfocarb	52888-80-9	500	Verified by NORMAN
Pendimethalin	40487-42-1	300	Verified by NORMAN
Oxyfluorfen	42874-03-3	94	Predicted by QSAR and experimentally-based values
Fungicide			
Tebuconazol	107534-96-3	240	Verified by NORMAN
Metalaxyl	*57837-19-1*	20000	Verified by NORMAN
Triadimenol	55219-65-3	3200	Verified by NORMAN
Fludioxonil	131341-86-1	100	Verified by NORMAN
Kresoxim-methyl	143390-89-0	630	Verified by NORMAN
Pyraclostrobin	175013-18-0	200	Verified by NORMAN
Flame retardants and benzophenone			
Tributyl phosphate (TBP)	126-73-8	66000	Verified by NORMAN
Tris (2- chloroethyl) phosphate (TCEP)	115-96-8	4000	Verified by NORMAN
Triphenyl phosphate (TPhP)	115-86-6	740	Verified by NORMAN
Benzophenone	119-61-9	5400	Verified by NORMAN
Perfluoroalkyl substances			
Perfluoropentanoic acid (PFPA)	2706-90-3	3910	Verified by NORMAN
Perfluorobutanoic acid (PFBA)	375-22-4	27800	Verified by NORMAN
Perfluorobutane sulfonate (PFBS)	375-73-5	4080	Verified by NORMAN
Perfluorohexanoic acid (PFHxA)	307-24-4	140000	Verified by NORMAN
Perfluoroheptanoic acid (PFHpA)	375-85-9	500	Verified by NORMAN
Perfluorohexane sulfonate (PFHxS)	355-46-4	870	Verified by NORMAN
Perfluorooctanoic acid (PFOA)	335-67-1	180	Verified by NORMAN

Compound	CAS	Lowest Fresh Water PNEC (ng/L)	Source
Perfluorononanoic acid (PFNA)	375-95-1	1000	Verified by NORMAN
Perfluorooctane sulfonate (PFOS)	1763-23-1	0.65	Verified by NORMAN
Perfluorodecanoic acid (PFDA)	335-76-2	170	Predicted by QSAR and experimentally-based values
Perfluoroundecanoic acid (PFUnA)	2058-94-8	130	Verified by NORMAN
Perfluorodecane sulfonate (PFDS)	335-77-3	160	Predicted by QSAR and experimentally-based values
Perfluorododecanoic acid (PFDoA)	307-55-1	110	Predicted by QSAR and experimentally-based values
Perfluorotridecanoic acid (PFTriDA)	72629-94-8	100	Verified by NORMAN
Perfluorotetradecanoic acid (PFTeDA)	376-06-7	83	Verified by NORMAN
Perfluorohexadecanoic acid (PFHxDA)	67905-19-5	78	Verified by NORMAN
Perfluorooctadecanoic acid (PFODA)	16517-11-6	69	Verified by NORMAN

Table S3. RQ values for each sample and target compounds. Table S3 is attached as electronic supplementary material due to its large size. Please scan the QR code below to display the table.

CHAPTER III

IMPACT OF ORGANIC CONTAMINANTS IN SOILS FROM IMPORTANT BIRD AND BIODIVERSITY AREAS

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ABSTRACT

Soils act as sinks for many organic contaminants, posing a threat to biodiversity and essential ecosystem services. In this study, we assessed the contamination status of soils in 140 Important Bird and Biodiversity Areas (IBAs) in Spain. Fifty-two organic contaminants including organochlorine pesticides (OCPs), organophosphorus pesticides (OPPs), polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), and plasticizers or plastic related such as phthalates, bisphenol A, nonylphenol, and organophosphate esters (OPEs) were analysed by gas chromatography coupled to tandem mass spectrometry (GC-MS/MS). The mean soil concentration ranged from 1.41 and 917 ng/g and plasticizer and PAHs were detected at the highest concentrations while OCPs were the most frequently detected. Hierarchical Clustering on Principal Components (HCPC) and land use data associated PCBs with artificial land, phthalates with industrial sites and incineration plants and PAHs with burned areas, and in a lesser extent pesticides with agricultural activities. A tier I Environmental Risk Assessment (ERA) was performed to identify the most impacted natural areas and the most concerning compounds. Out of the 140 IBAs, 95 presented at least one compound at high-risk concentrations (RQ>1) for soil organisms. The OPPs chlorpyrifos and malathion, together with the PAH benzo[b]fluoranthene, were detected at high-risk concentrations. Overall, this study highlights the widespread presence of organic contaminants in areas of high natural value and the importance of implementing monitoring studies to identify potential contaminated sites that require conservation and remediation actions for the protection of biodiversity.

1. INTRODUCTION

Soils are non-renewal resources and represent a compartment of global biodiversity, which is crucial for providing ecosystem services such as agricultural and biomass production, supply of raw materials, filtration of contaminants, regulation of water, and nutrient cycling (Ferreira et al., 2022). However, in recent decades, there has been an increasing concern regarding the rapid degradation of soils, which have the potential to negatively impact landscapes and ecosystems. Soils are globally threatened mostly by human activities, such as unsustainable practices in agriculture and forestry, industry emissions, waste discharges, and soil sealing through urbanization and infrastructures (European Comission, 2020). Soil contamination, either from natural or anthropogenic sources, can represent a serious impact on biodiversity and ecosystem functions (Liu et al., 2023). In the EU most soils are considered unhealthy as 2.8 million sites are known to be contaminated (European Comission, 2020). Soil contamination can be local, where there is a clear source of contamination affecting a limited area, or diffuse, which is much harder to manage as there is not a directly apparent source of pollution and it can affect a very large area (FAO, 2015).

Soils are sinks of legacy contaminants such as organochlorine pesticides (OCPs), widely used in the past in domestic and agricultural applications, and polychlorinated biphenyls (PCBs) used for industrial purposes. Despite important efforts have been done to restrict, ban, and eliminate the production and use of these compounds through international treaties (Stockholm Convention, 2019), residues are present in the global environment, even in remote areas with low human footprint due to their persistency and long-range atmospheric transport (Kim et al., 2021; Mishra et al., 2022). After the ban on OCPs, organophosphorus pesticides (OPPs) such as chlorpyrifos, malathion and chlorfenvinphos have been extensively used in agriculture, and although they are more easily degraded, they trigger toxicological adverse effects. Another important soil contaminants are Polycyclic Aromatic Hydrocarbons (PAHs), which are formed by the combustion of organic matter such as natural sources through wildfires, volcanic activity, or geological formation of fossil fuels, or from anthropogenic sources such as industrial emissions, traffic, and heating systems for households (gas, wood or coal burning) (Rengarajan et al., 2015). They impact soils as they have mutagenic and carcinogenic properties (Shukla et al., 2022). Finally, high-volume production chemicals such as phthalates, organophosphate esters (OPEs), bisphenol A and nonylphenol are ubiquitous in the environment (Wang et al., 2020) and have raised concern due to their capacity to bioaccumulate and biomagnify along the trophic chains and affect humans and wildlife (Greaves and Letcher, 2017; Y. Zhang et al., 2022).

The contamination of soils has been studied mostly in urban and agricultural areas and there are few monitoring studies on soils from natural areas (Aichner et al., 2013). Filling this gap of knowledge is of outmost importance, as natural areas are crucial for the conservation of global biodiversity and can play an important role in the retention of contaminants. Furthermore, in the actual context of climate change, the re-emission of persistent compounds from soil is more likely to occur, and in fact, this phenomenon has been already reported in European soils (Degrendele et al., 2016; Ren et al., 2019). To assess the health of soils, many countries have implemented long-term surveys focused on metals and organic matter, but lack of harmonized soil monitoring system for organic contaminants, and the real extent of soil contamination, especially for emerging contaminants, is still unknown (FAO, 2015).

Important Bird and Biodiversity Areas (IBAs) are sites designated by the non-profit organisation BirdLife International to preserve natural areas with high value for birds and biodiversity conservation (Donald et al., 2019). In recent studies, freshwaters from IBAs have been found to be impacted by organic micropollutants, evidencing the underlying pressure of contaminants in these natural areas (Dulsat-Masvidal et al., 2023). In the present study we performed a monitoring survey to determine 52 organic contaminants including persistent (PAHs, OCPs, PCBs) and emerging (OPEs, OPPs, phthalate esters (PAEs), bisphenol A (BPA), nonylphenol (NP)) in soils from 140 IBAs in Spain with the aim to assess the pollution patterns in these natural areas and identify the distribution and potential sources of the studied contaminants and the risk they may pose to soil conservation.

2. MATERIALS AND METHODS

2.1. Chemicals and reagents

A total of 52 persistent compounds were investigated in the present study, including 16 PAHs, 14 OCPs, 3 OPPs, 7 marker PCBs congeners, 4 OPEs, 6 PAEs, BPA and 4-Nonylphenol (NP). Surrogate standards consisted of deuterated PAHs solution mix containing naphthalene-d_8, acenaphthene-d_{10}, phenanthrene-d_{10}, chrysene-d_{12} and perylene-d_{12}, purchased from Sigma-Aldrich (Darmstadt, Germany and St. Louis, MO, USA). These deuterated PAHs elute along the chromatogram and were used to quantify compounds present in each retention time-window. Further details of the analyte standards are provided in Table S1. Calibration standards were prepared in hexane. Solvents used were hexane from Merck (Darmstadt, Germany) and dichloromethane from Carlo Erba Reagents (Sabadell, Spain).

2.2. Sample collection

Soil samples were collected in Spain between 2019 and 2020 from 140 IBAs representative of 7 ecosystems: agricultural, Atlantic forest, Mediterranean forest, riparian forest, rocky mountain, inland aquatic, and coastal habitats (Figure S1, Table S2). Soil was sampled using a auger or a scoop and avoiding the first 4-5 cm upper soil, as the top surface is affected by sunlight, erosion and deterioration which could produce the degradation of some contaminants. Each sample consisted of 0.8 and 1 kg of soil composed of a minimum of 8 subsamples collected 10 m apart to enhance representativeness. We georeferenced the sampling points and provided an observational land description (e.g. presence of trash). Samples were placed in glass containers and sent refrigerated to the laboratory, where they were dried at 40°C until constant weight. Although this procedure might affect the extraction efficiency of the more volatile PAHs (Narizzano et al., 2013), an important additional factor affecting the volatilization of volatile contaminants is the movement of air over the sample controlled by the use of a fan powered air circulation (Beriro et al., 2014). In our case, and considering that most contaminants analysed have low vapour pressures, we used a natural convection oven drying (J.P. Selecta, Abrera, Barcelona). Such low cost and more expedient technique balanced potential vaporisation losses (Beriro et al., 2014). Soils were finally sieved through 500 and 125 µm stainless steel mesh and the latter fraction was analysed. Samples were preserved at 4ºC in amber glass vials until chemical analysis.

2.3. Sample treatment and chemical analysis

One g of sample was weighed in 60 mL glass centrifuge tubes and spiked with 50 ng of the internal standard solution. As extraction solvent, 30 mL of hexane:dichloromethane (1:1) were added, following a previous procedure for the analysis of contaminants in dust (Velázquez-Gómez et al. 2018). Samples were vortexed (1 min) and ultra-sonicated (10 min), and this procedure was repeated 3 times without changing the solvent, and finally centrifuged (10 min, 1560 rcf, 20ºC). The supernatant was collected and transferred to 40 mL amber vial and concentrated to 2 mL using a gentle N_2 flow at 20ºC using a TurboVap® LV (Caliper Lifesciences, Uppsala, Sweden). Clean-up was performed with 5 g Bond Elut Florisil cartridges (Agilent Technologies, Santa Clara, CA, USA). Cartridges were conditioned with 30 mL hexane: dichloromethane (1:1) dropwise, and samples were loaded and eluted with 30 mL of the same solvent mix. The extract was evaporated again using the TurboVap® LV to near dryness and transferred to chromatographic vials to a final volume of 0.5 mL in hexane. If some solid particles appeared during the final preconcentration step, extracts were filtered through 0.2 µm x 13 mm nylon filters (Clarify, Phenomenex, Torrance, USA).

Samples were analysed by gas chromatography coupled to a triple quadrupole (QqQ) mass spectrometer (Agilent 7890A chromatograph and 7000A MS from Agilent Technologies, Santa Clara, CA, USA) with electron ionisation (EI) at 70 eV. Separation of target compounds was achieved using a HP-5MS Agilent column (30 m x 0.25 mm internal diameter, 0.25 µm film thickness). The initial temperature was set at 70ºC and kept for 1 min, then increased to 175ºC in 4 min, from 175°C to 235°C in 20 min, and to 305ºC in 8 min. The slow gradient elution that lasted 60 min allowed the multiresidue analysis to resolve the 52 contaminants belonging to the different chemical families. Compounds were identified by retention time and by the specific MS/MS transition (Velázquez-Gómez et al., 2018), so that all of them could be determined. The injection sequence included the analysis of a standard at 0.05 ng/µL every 15 samples that was used as injection standard. If the response varied within 30% of the theoretical (initial) response, a cleaning procedure of the column, change of liner or ultimately cleaning of the ion source was undertaken. The data was processed by Mass Hunter Quantitative software. Internal standard quantification was performed and Table S3 indicates the compounds eluting in each time-window and the deuterated PAH standards used for quantification. Concentrations are given as ng/g dry weight (dw).

2.4. Quality control/quality analysis

The analytical method was assessed for precision, accuracy, linearity, sensitivity, selectivity, and extraction efficiency. Inter-day precision was determined by injecting a 0.05 ng/µL standard in 5 different days. Linearity was studied over a concentration range of 0.001–0.8 ng/µL. To evaluate the extraction efficiency (recoveries), a pristine and acetone washed freeze-dried soil was spiked with 50 ng/g of a mixture of 52 contaminants (n = 5) and analysed by the described analytical procedure. Unspiked soil was also extracted to guarantee the absence of initial contaminant contribution. Instrumental limits of detection (IDL) were calculated as the amount of analyte that gives a signal-to-noise ratio of 3 (S/N = 3) using the 0.001 ng/µL standard. Method detection limits (MDL) were calculated as the concentration that give a S/N = 3 using the 50 ng/g spiked soil, except for phthalates and OPEs that had a strong blank contribution and then the MDL were calculated as 3 times the standard deviation of the blank contribution (n = 10). Quality parameters including recoveries and MDL of the target compounds are indicated in Table S3. All target analytes were recovered within the 70 - 130%, except for PCB118 that had a recovery of 135% and dibenzo[a,h]anthracene, benzo[ghi] perylene, DMP, DBP, BBZP, TDCPP, EHDPHP were recovered in less than 50%. The method had a good precision and the MDL were within the 0.02 to 13.8 ng/g dw except for phthalates and OPEs that much higher levels were obtained due to the blank contribution. Samples values below MDL were given a value of zero to avoid overestimation in statistical analysis.

2.5. Data analysis

To assess the geographical distribution of the detected compounds we considered spatial data around each sampling point. We estimated a dominant influence of contaminants sources within a 10 km buffer radius, based on previous contaminant deposition studies (Hu et al., 2021; Rossini et al., 2005). We collected spatial information including Corine land-use grouped in major classes (agricultural, artificial, and natural surface), number of industrial sites, incineration plants, density of roads considering the total length of roads in 10 km area, and surface burned areas in the period of 5 years before sampling. Spatial information was expressed as percentage. For each sampling point, the Total Organic Carbon (TOC) was obtained from the Topsoil Soil Organic Carbon (LUCAS) (de Brogniez et al., 2015). The spatial information used here is detailed in Table S4.

The spatial data was obtained from the following databases: Corine Land Cover 2018 (https://land.copernicus.eu/), Spanish Ministry for Ecological Transition and the Demographic Challenge services (https://www.miteco.gob.es), European Forest Fire Information System (https://effis.jrc.ec.europa.eu/), European Soil Data Centre (https://esdac.jrc.ec.europa.eu/).

To characterize the contamination profile of each IBA, we performed a Hierarchical Clustering on Principal Component (HCPC) analysis with log x+1 concentrations of compounds detected in more than 10% of soils and considering the spatial information of each sampling area around 10 km of the sampling point. The Kaiser–Meyer–Olkin (KMO) was used to assess the suitability of Principal Component Analysis (PCA), where KMO>0.5 indicates variables enough interdependent for PCA (Dziuban et al., 1979).

Spatial analysis and maps were performed in open-source software QGIS (version 3.18.2). Statistical analyses were performed in R studio (R version 4.0.3). FactoMineR package was used for HCPC analysis and figures were elaborated using ggplot2 package.

2.6. Environmental risk assessment

A Tier I Environmental Risk Assessment (ERA) was performed for the detected compounds in soils. Risk Quotients (RQs) were assessed as the ratio between the Measured Environmental Concentrations (MEC) for each compound *(i)* and the Predicted No-Effect Concentration (PNEC) (eq. 1).

$$RQ_i = \frac{MEC_i}{PNEC_i} \quad \text{(eq. 1)}$$

There is limited ecotoxicological data in soil organisms, compared to freshwater organisms and obtaining the toxicity data is not straightforward. To perform a unified estimation of RQs for all compounds, all values were

extrapolated using the equilibrium partitioning method following the European Technical Guidance on Risk Assessment (European Commission, 2003) considering the organic carbon-water partition coefficient (K_{oc}) for each compound obtained from EPI Suite (EPA, 2012), the weight fraction of organic carbon in soil (f_{oc}) and the bulk density of wet suspended matter (RHO_{susp}) obtained from the European Technical Guidance on Risk Assessment (European Commission, 2003) (eq. 2). The lowest PNEC values in freshwater were obtained from NORMAN database (NORMAN, 2022). The calculation and full list of PNEC values for soil is detailed in Table S5.

$$PNEC_{comp.} = \frac{K_{oc} \cdot f_{oc}}{RHO_{susp}} \cdot PNEC_{water} \cdot 1000 \quad \text{(eq. 2)}$$

The prioritisation of the most concerning compounds among IBAs was performed calculating the $RQ_{f,i}$ described by Zhou et al., (2019), where f corresponds to the frequency of MECs exceeding PNEC (RQ>1) (eq. 3):

$$RQ_{f,i} = \sum_{i=1}^{n} \frac{MEC_i}{PNEC_i} \times \frac{Number\ of\ samples\ where\ MEC_i > PNEC_i}{Total\ number\ of\ IBAs\ (n=140)} \quad \text{(eq.3)}$$

3. RESULTS AND DISCUSSION

3.1. Levels and occurrence of contaminants in soils

Fifty target compounds out of 52 analysed were detected in soils from Spanish IBAs (Table 1). According to the detection frequency, the chemical families detected followed the order: OCPs > PAHs > plasticizers > PCBs > OPPs > OPEs. The mean concentration of target compounds followed the order: plasticizers > PAHs > OPPs > OCPs > OPEs > PCBs. Figure 1 maps the distribution of the most relevant chemical families classified in percentiles according to their concentrations.

Table 1. Detected target compounds in soils (n = 140) ordered by detection frequency within each chemical family, number of occurrences (N) and detection frequency (%), mean ± S.E. (Standard Error), minimum and maximum concentrations, expressed in ng/g dw. Endosulfans not detected.

Compound	N (%)	Mean ± S.E.	Min.	Max.
4,4'-DDE	107 (76)	8.6 ± 3.22	0.07	358
4,4'-DDD	39 (28)	1.56 ± 0.57	0.06	56.2
4,4'-DDT	33 (24)	9.28 ± 3.28	2.67	308
2,4'-DDD	18 (13)	0.12 ± 0.04	0.13	2.39
2,4'-DDT	12 (9)	0.62 ± 0.26	1.24	27.7
2,4'-DDE	12 (9)	0.09 ± 0.03	0.09	3.01
β-HCH	40 (29)	0.09 ± 0.03	0.02	2.46

Compound	N (%)	Mean ± S.E.	Min.	Max.
γ-HCH	27 (19)	3.44 ± 2.96	0.02	410
α-HCH	8 (6)	1.02 ± 0.68	2.43	90.7
δ-HCH	1 (0.7)	-	0.51	0.51
HCB	18 (13)	0.28 ± 0.18	0.07	24.2
HCBD	7 (5)	0.32 ± 0.23	0.27	31.1
∑OCPs	**122 (87)**	**25.4 ± 7.65**	**0.03**	**626**
Pyrene (HMW)	85 (61)	37.4 ± 19	0.81	2427
Benzo[b]fluoranthene (HMW)	75 (54)	60.3 ± 46.1	1.04	6462
Benzo[k]fluoranthene (HMW)	74 (53)	53.1 ± 40.6	1.52	5687
Benzo[ghi]perylene (HMW)	63 (45)	23.9 ± 13.7	2.57	1909
Fluoranthene (HMW)	59 (42)	40.9 ± 25.5	4.17	3561
Benzo[a]anthracene (HMW)	56 (40)	23.1 ± 15.4	2.82	2153
Phenanthrene (LMW)	48 (34)	8.75 ± 4.78	1.25	663
Indeno[1,2,3-cd] pyrene (HMW)	38 (27)	31.3 ± 20.7	7.16	2889
Chrysene (HMW)	37 (26)	12.0 ± 6.6	3.92	917
Acenaphthene (LMW)	37 (26)	3.33 ± 0.6	4.92	52.4
Benzo[a]pyrene (HMW)	25 (18)	31.4 ± 21.7	13.85	3027
Anthracene (LMW)	19 (14)	6.8 ± 5.5	0.62	766
Fluorene (LMW)	16 (11)	1.16 ± 0.56	0.69	57.2
Naphthalene (LMW)	9 (6)	5.13 ± 2.92	1.4	386
Dibenzo[a,h]anthracene (HMW)	9 (6)	3.34 ± 2.13	6.19	259
Acenaphthylene (LMW)	7 (5)	0.33 ± 0.14	1.45	14.1
∑PAHs	**96 (69)**	**342 ± 220**	**1.4**	**30816**
NP	29 (21)	1155 ± 100	1247	3488
DEP	16 (11)	181 ± 56.7	534	5475
DiBP	16 (11)	100 ± 35.8	175.8	2921
DEHP	11 (8)	149 ± 49.2	1223	4073
DMP	10 (7)	0.86 ± 0.32	5.5	32.3
BPA	7 (5)	3.58 ± 1.47	37.1	148
DBP	6 (4)	19.2 ± 8.42	200	771
BBZP	3 (2)	0.76 ± 0.44	25.1	41.4
∑Plasticizers	**70 (50)**	**917 ± 118**	**5.5**	**7026**
PCB 153	54 (39)	0.46 ± 0.15	0.08	18.4
PCB 101	30 (21)	0.06 ± 0.02	0.03	1.86
PCB 138	28 (20)	0.43 ± 0.19	0.07	24.7
PCB 180	25 (18)	0.44 ± 0.18	0.11	22.7
PCB 52	12 (9)	0.01 ± 0	0.02	0.38
PCB 28	2 (0.7)	0.01 ± 0	0.42	0.5
PCB 118	2 (0.7)	-	0.1	0.15
∑PCBs	**61 (44)**	**1.41 ± 0.54**	**0.02**	**67.8**

Compound	N (%)	Mean ± S.E.	Min.	Max.
Chlorpyrifos	38 (27)	162 ± 27.7	125	1587
Malathion	17 (12)	0.9 ± 0.38	1.03	48.3
Chlorfenvinphos	1 (0.7)	-	79.3	79.3
∑OPPs	**51 (36)**	**163 ± 27.7**	**1.03**	**1587**
TBOEP	4 (3)	7.03 ± 5.91	42.6	824
EHDPHP	2 (0.7)	2.3 ± 1.68	114	207
TDCPP	1 (0.7)	-	27.6	27.6
TDCEP	1 (0.7)	-	5.84	5.84
∑OPEs	**7 (5)**	**9.56 ± 6.32**	**5.84**	**851**

∑OCPs were the most frequently detected compounds in soils, found in 87% of the IBAs at concentrations ranging from 0.03 to 626 ng/g (Table 1). ∑DDTs were detected in 79% of the samples and contributed to 80% of ∑OCPs. Despite the high ubiquity, the 50th percentile of ∑DDTs was 0.5 ng/g indicating that most samples presented low concentrations (Figure 1). The highest levels of ∑DDTs were found at IBAs 064 (Rio Huebra - Arribes del Duero, Salamanca), 144 (Cogul – Alfes, Lleida) and 001 (Islas Cies, Pontevedra) (Figure 1), that had concentrations from 483 to 626 ng/g dw. Among DDTs isomers, the metabolite 4,4'-DDE was detected in 76% of the samples at levels from 0.07 to 358 ng/g, followed by 4,4'-DDD present in 28% at concentrations from 0.06 to 56.2 ng/g, and 4,4'-DDT detected in 24% of the samples at levels from 2.67 to 308 ng/g. In the environment, DDT is aerobically degraded to DDE or anaerobically degraded to DDD (Qu et al., 2019). Only 6 samples (IBAs 197, 424, 36, 200, 73, 59, see Figure S1 and Table SI 2 for details of those IBAs) showed higher concentrations of DDD than DDE, indicating a higher prevalence of anaerobic degradation of DDT. Despite being a long-banned pesticide, DDTs and its metabolites are still one of the most widespread insecticide residues in European agricultural soils, and 4,4'-DDE is the most prevalent compound (Silva et al., 2019). ∑HCHs were present at 39% of the samples, but their concentrations were generally found at trace levels. The 90th percentile concentration for ∑HCHs was 0.8 ng/g, indicating that nearly all samples had concentrations below this level and indicate a low-level historical pollution in IBAs from Spain (Figure 1). Technical HCHs, consisted in 90% of γ-HCH isomer, also known as lindane, used as insecticide. Although β-HCH was detected in 29% of the samples at concentrations ranging from 0.02 to 2.46 ng/g, γ-HCH was present in 19% of the samples but at much higher concentrations (from 0.02 to 410 ng/g), and α-HCH present only in 6% of the samples at levels from 2.43 to 90.7 ng/g. IBA 119 (Oturia – Cancias, Huesca, north east Spain, Figure 1) was the most impacted by HCHs as with ∑HCHs of 417 ng/g following the pattern: γ-HCH at 410 ng/g, α-HCH at 4.58 ng/g, β-HCH at 2.34 ng/g and δ-HCH at 0.51 ng/g. IBA 119 is located in Sardas and Bailín-

Sabiñánigo (Aragón, Spain), a landfill used by the Inquinosa company which produced 160000 tons of HCHs waste between 1975 and 1988 (Gómez-Lavín et al., 2018). Our results indicate that the IBA close to this area is still strongly polluted by the historic production of lindane.

∑PAHs were detected in 69% of the IBAs at concentrations from 1.4 to 30816 ng/g. As it is shown in Figure 1, the 50th percentile was of 24.4 ng/g, indicating that half of the sampling locations presented concentrations below this value. This median value is higher than the other chemicals groups, meaning that overall, PAHs were present at relative high concentrations (Figure 1). Northern Spanish IBAs were more impacted than southern IBAs, with concentrations within the 75th (369 ng/g) and 90th (30816 ng/g) percentiles. The maximum concentrations of PAHs were found in IBA 424 (Soba - Castro Valnera - Ordunte Burgos, Cantabria and Vizcaya, north west Spain) which presented a total concentration of 30816 ng/g, almost 20 times higher than other sites, as IBA 072 (Carrizales y Sotos de Aranjuez, Toledo, central Spain) and IBA 035 (Urdaibai – Matxitxako, Vizcaya, a UNESCO World Heritage in the north east Spain) which presented ∑PAHs of 1745 and 1738 ng/g, respectively. Overall, pyrene was the most frequently detected PAH, present in 61% of the IBAs at levels from 0.81 to 2427 ng/g, followed by benzo[b]fluoranthene present in 54% and from 1.04 to 6462 ng/g and benzo[k]fluoranthene detected in 53% at concentrations ranging from 1.52 to 5687 ng/g. High-molecular-weight PAHs (HMW, 4 or more rings) were more prevalent than low-molecular-weight PAHs (LMW, 2 or 3 rings) (Table 1). HMW are mainly originated from pyrogenic sources (fossil fuel, coal, or biomass combustions) while LMW have a petrogenic origin (petroleum and its refining). The highest prevalence of HMW PAHs in front of LMW is indicative of PAHs originated from pyrogenic sources as vehicular emissions or forest fires. Aichner et al. (2013) also reported a higher prevalence of HMW than LMW PAHs in German forest soils, with ∑PAHs ranging from 105 to 14889 ng/g, slightly higher than the concentrations detected in the present study.

∑Plasticizers were detected in 50% of the IBAs and were the chemical group found at the highest mean concentrations (917 ± 118 ng/g), ranging from 5.50 to 7026 ng/g (Table 1). Because of blank contribution and the consequent high MDL, the detection frequency was low. The 50th percentile was of 9 ng/g, while the 75th percentile concentration was 2658 ng/g, and the 90th percentile concentration reached 7026 ng/g, indicating a high variability in concentrations. Plasticizers had a heterogeneous distribution among IBAs (Figure 1). The maximum concentration was found in IBA 230 (Embalse de Marmolejo - La Ropera, Jaen, central-south Spain), where the sample was collected in a path next to a WWTP discharge, and a great amount of plastic waste and wipes were observed and documented during sampling. The main plastic-related compound was NP, detected at mean concentrations of 1155 ng/g, and ranging from 1247 to 3488 ng/g. The half-life of NP in soils

is relatively short, between 1.4 and 16.7 days (Rivier et al., 2019), indicating that it does not persist in soils for extended periods of time. However, its presence in 21% of the soils can be attributed to their constant release from many sources, including pesticide formulations, irrigation with reclaimed water, use of sewage sludge as fertilizer, and breakdown of ethoxylated alkylphenols (Kim et al., 2019). Among PAEs, diethyl phthalate (DEP), di-iso-butyl phthalate (DiBP) and bis(2-ethylhexyl) phthalate (DEHP) were detected in 8-11% of the samples at mean concentrations ranging from 100 to 181 ng/g dw, but reaching up to 4073 ng/g. PAEs are released into the environment as a result of their use as plasticizers in a significant number of industrial and consumer plastic-based products. For instance, DEHP is used as plasticizer in the synthesis of polyvinyl chloride (PVC), and DiBP is used to provide flexibility and durability to plastic, while DEP is mainly used as additive in personal care products (nail polish, shampoos soaps, dyes), pharmaceuticals, and as solvent binders. PAEs are not chemically bonded to the plastics polymer and therefore migrate into the environment during the manufacturing, usage and disposal of plastic materials (Prasad, 2021). Once PAEs are deposited in soils they can persist for a wide range of periods depending on their chain length. DEHP half-life in soil has been reported from 4.6 to 301 days, while the shorter chain DEP has a half-life in soil from 2 to 16 days and is decomposed more rapidly (Li et al., 2023).

∑PCBs were the chemical group detected at the lowest concentrations, despite being present in 44% of the samples, with concentrations ranging from 0.02 to 67.8 ng/g, which are in the same order of magnitude as measurements reported in European background soils (0.21 to 21 ng/g) (Schuster et al., 2011) and Canadian remote mountains (0.24 to 24 ng/g) (Abdul Hussain et al., 2019). Out of 61 IBAs with PCBs, 90% presented values below 1.5 ng/g (Figure 1). The maximum concentrations of PCBs were detected in IBA 295 (Llanos entre Cáceres y Trujillo - Aldea del Cano, Caceres, central west Spain) which is an area with a high urban and agricultural pressure (BirdLife International, 2023). Urban areas are a source of PCBs in surface soils due to atmospheric deposition, especially in the case of heavier PCBs (Yadav et al., 2017). High-chlorinated PCBs (101, 138, 153 and 180) showed a higher prevalence than low-chlorinated PCBs (28, 52), a profile related to the composition of the commonly used technical PCBs mixtures (Clorphen A60 and Aroclors 1254 and 1268), containing congeners 138, 153, and 180 as the most abundant constituents (Aichner et al., 2013). In fact, PCB 153 was the most prevalent compound detected in 39% of the soils at levels from 0.08 to 18.4 ng/g.

∑OPPs were detected in 36% of soils at levels from 1.03 to 1587 ng/g, indicative of local pollution of these compounds rather than a diffuse distribution (Figure 1). Chlorpyrifos was the most frequently detected OPPs, present in 27% of the samples at concentrations ranging from 125 to 1587 ng/g. Due to its effectiveness against fruit and vine pests, chlorpyrifos has

been one of the most widely used pesticides in southern Europe, until its ban in 2020. The agricultural soils from IBA 204 (Montes de Toledo – Cabañeros, Toledo and Ciudad Real, central south Spain) and IBA 137 (Aiguamolls de l'Emporda, Girona, north east Spain) were the most impacted. Previous studies in Spain indicated that chlorpyrifos was an ubiquitous insecticide in the environment (García et al., 2022; Rico et al., 2021). Other OPPs were malathion, detected in 12% of the samples at levels from 1.03 to 48.3 ng/g, and chlorfenvinphos detected once at 79.3 ng/g.

∑OPEs were only detected in 5% of the samples at levels from 5.84 to 851 ng/g. The low frequency of detection is explained in part due to blank contribution and the high MDL for these compounds (Table S3). TBOEP was present in 4 IBAs at concentrations from 42.6 to 824 ng/g, EHDPHP twice (114 to 207 ng/g) and TDCPP and TDCEP only in one sample (Table 1). The maximum concentrations were found in IBA 036 (Montes de Izki y de Vitoria, Alava, Burgos, north Spain) in a recreational area, containing TBOEP at 824 ng/g and TDCPP at 28 ng/g. It is worth to highlight that 6 out of the 7 IBAs where OPEs were detected showed litter around the sampling points, according to visual observation when sampling. OPEs are not chemically bound to products and can be easily released from abandoned waste materials to the environment (Wang et al., 2021). OPEs in soil have been reported at high concentrations (500 to 75000 ng/g dw) in areas contaminated by plastic and e-waste (Zapata et al., 2023), and in soils from urban cities containing 24.9 to 27900 ng/g dw (Yadav et al., 2018). Considering that IBAs are natural sites and not pollution hotspots, the low detection frequency of OPEs is expected.

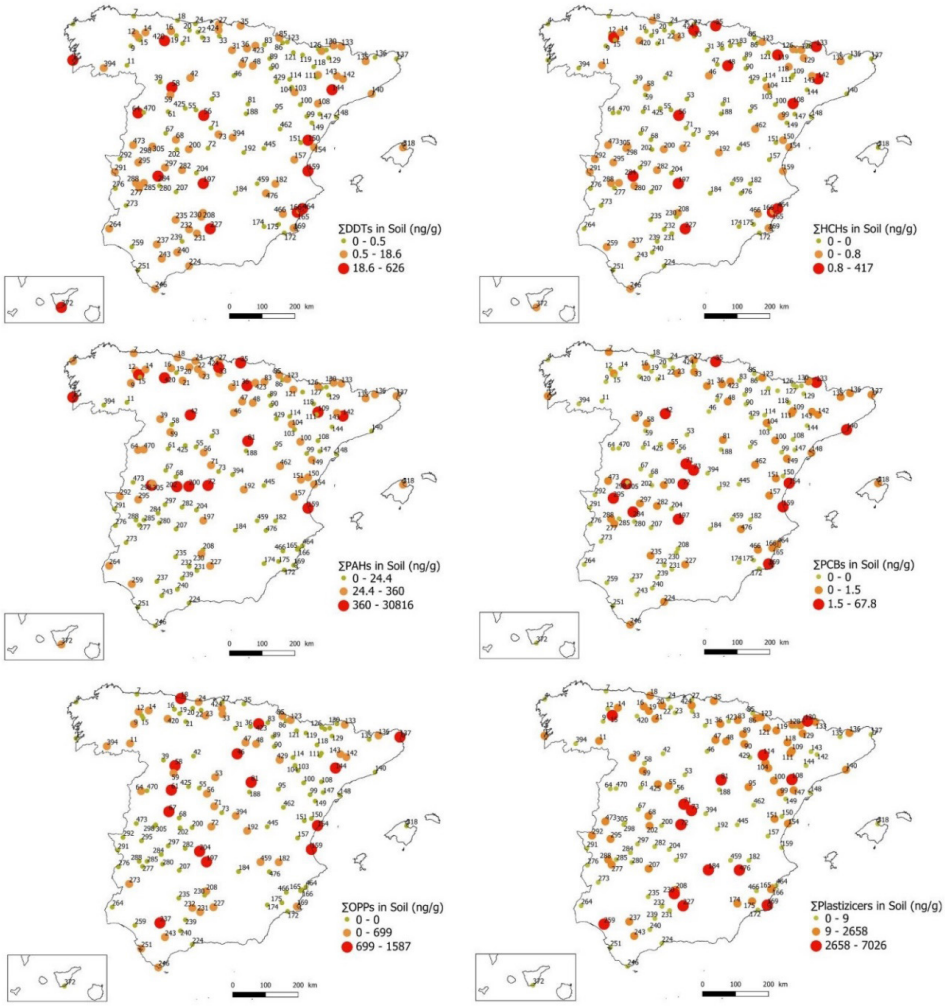


Figure 1. Spatial distribution of the most relevant chemical families detected in IBAs soils, classified in percentiles: minimum to 50%, 50% to 90% and 90% to maximum for each chemical group. Numbers in the maps indicate IBA codes (Table S2 for IBA identification).

3.2. Contaminant distribution and characterization of IBAs

HCPC analysis was conducted to assess the fingerprint contamination of IBAs considering spatial data around 10 km around each sampling point. PCA presented a KMO measure of 0.79 indicating a good suitability of the data for the PCA analysis. Principal components 1 and 2 explained 28.9% and 13.2% of de total variance, respectively and grouped artificial and natural IBAs. Component 3 explained a relatively low percentage of the total variance, accounting for only 7.2 % of the total variability, and differentiated those IBAs with agricultural land-use affected by DDT, γ-HCH and β-HCH from those with natural surface and artificial surface and was relevant to distinguish agriculture as a pollution source. Agriculture occupies almost

half of the total surface in Spain, and the use of pesticides is extended to many different varieties of crops (Manjarres-López et al., 2021) which is an important factor that affects the quality of nearby IBAs. Due to its limited contribution, component 3 was not included in the clustering analysis but the results are indicated in Figure S2.

HCPC analysis differentiated soils samples in three clusters (Figure 2, Table S6). Cluster 1 grouped the 115 IBAs with significantly ($p<0.05$) lower mean concentrations of contaminants and low percentage of artificial surface and density of roads around the sampling areas. This pattern with no clear profile of contaminants or identifiable sources is often indicative of a diffuse pollution. Cluster 2 grouped 7 IBAs with a significantly higher concentration of PCBs (101, 138, 180, 153), DEP, DiBP, pyrene, acenaphthene and benzo[ghi] perylene. According to the land-use information, the high prevalence of these compounds was also related with the presence of artificial land-use, roads, industrial sites, and incineration plants. Therefore, they can be characterized as sampling points with a predominant anthropogenic pressure. This cluster includes IBAs close to large cities, as Barcelona (IBA 140) and Madrid (IBAs 71 and 73). Cluster number 3 grouped IBAs with a high PAHs fingerprint and with high mean concentrations of DDTs and HCB. PAHs as fluorene, benzo[a] anthracene, benzo[a]pyrene, indeno[1,2,3-cd] pyrene, phenanthrene, anthracene, chrysene, benzo[ghi]perylene, benzo[b]fluoranthene, pyrene, fluoranthene, benzo[k]fluoranthene were the main contributors. As shown in Figure 2, principal component 2 is also related with IBAs with a higher percentage of natural surface and surface affected by wildfires, which are known to be an important sources of PAHs in terrestrial ecosystems (Campos and Abrantes, 2021). The maximum concentration of PAHs was found in IBA 424 (Soba - Castro Valnera – Ordunte, Burgos, Cantabria and Vizcaya, central north Spain), a sampling area that was affected by three different wildfires in less than one year before sampling. PCA also indicated that those samples also correspond to soils with a higher content of total organic carbon (TOC), which is a determinant factor in the retention of apolar compounds as PAHs (Łyszczarz et al., 2021).

Although the PCA analysis has been useful to assess the general patterns of compounds distribution, it is important to note that the explained variance is relatively low, accounting for only 49.3% of the total variability. This indicates that there are likely other environmental variables not included in the analysis that could contribute significantly to the spatial distribution of the target compounds. For example, specific soil properties including texture or pH have been found to affect the retention of compounds in soil (Wenzel et al., 2002). These factors, along with other variables, may have an important influence on the results, and further investigation is needed to identify sources and understand their contribution.

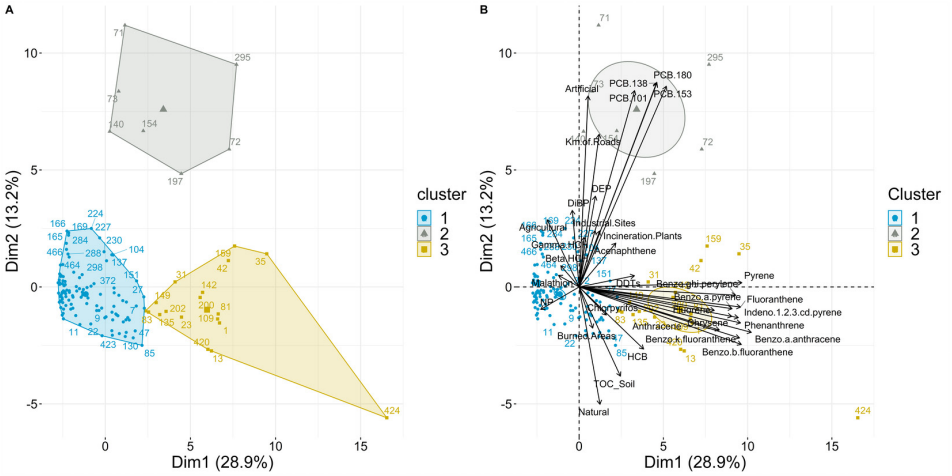


Figure 2. Soil HCPC analysis explaining 42.1% of the total variance, showing factor map of soil samples (A) and PCA (B). Numbers indicate IBA codes (Table S2 for IBA identification).

3.3. Environmental Risk Assessment of detected compounds

There is a lack of consensus in the model used for soil risk assessment in Europe. Different assumptions, approaches and acceptable risk levels exist between European member states, and therefore the legal Soil Screening Values (SSVs) for organic contaminants widely differ among countries. Variations exist not only in the number of SSVs considered in each regulation, but also in their specific values (EEA, 2022). The Spanish regulation sets Generic Reference Values (GRVs) (equivalent to SSVs) of contaminants in soils with toxicity thresholds below which they do not pose a risk to human or ecosystem health (BOE, 2005). However, those values only exist for 54 priority pollutants for ecosystem protection (including some legacy pesticides, PAHs, and industrial solvents), and only 10 correspond with the target compounds of our study.

Due to the lack of reference values for all compounds, we performed an ERA based on the proposed guidelines used by ECHA (European Commission, 2003), defining the PNEC values following the equilibrium partitioning method approach.

From the 140 soils samples analysed, 95 presented at least one compound at levels above the PNEC value, indicative of concentrations of high risk (RQ>1) for terrestrial organisms (Figure 3). PAHs were the most frequently detected compounds exceeding the PNEC values, followed by OCPs, OPPs, plasticizers, OPEs, and PCBs. The identification of clusters among soil samples are in accordance with the risk of the chemical families identified. The majority of IBAs corresponding to cluster 1 presented heterogeneous number of RQs values. IBAs from cluster 2 were characterized by having RQs corresponding

to plasticizers and IBA 295 (Llanos entre Cáceres y Trujillo-Aldea del Cano, Cáceres) was the only one with PCBs concentrations exceeding the PNEC values. This IBA has been included in the list of "IBAs in danger" by BirdLife International, which comprises areas where serious threats have been identified to put their natural values at risk. The main anthropogenic pressures of IBA 295 are the agricultural intensification and urbanization (BirdLife International, 2023). IBAs from cluster 3 were the group with the highest number of RQs, being most of them due to PAHs. RQs from OCPs and OPPs were distributed uniformly among IBAs clusters.

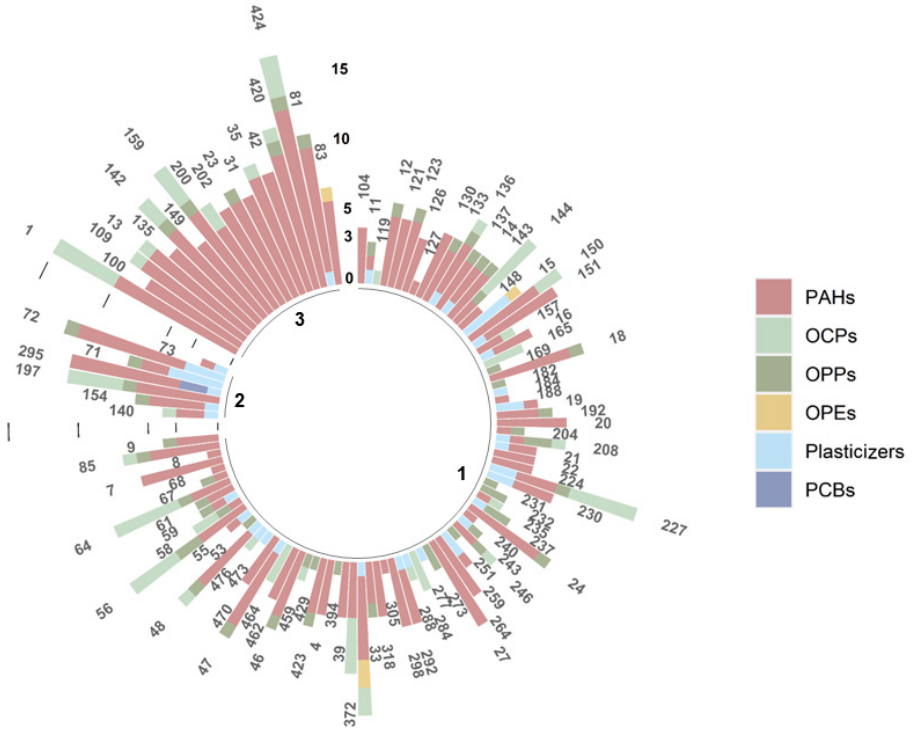


Figure 3. IBAs with total number of RQs > 1, classified according to identified clusters (1, 2, and 3). Numbers indicate IBA codes (Table S2 for IBA identification).

The $RQ_{f,i}$ described by Zhou et al. (2019) was used to prioritize the risk compounds in IBAs soils (Table 2). Among these compounds, the OPPs chlorpyrifos and malathion were particularly concerning, as all detected residues exceed the established PNEC values. Chlorpyrifos and malathion are among the most employed agricultural pesticides and their continued use has led to a world-wide contamination in soils (Mali et al., 2023). Both insecticides are considered highly hazardous compounds due to their neurotoxicity in non-target vertebrates (Sabzevari and Hofman, 2022). The

use of chlorpyrifos was banned in 2020 by the European Commission due to toxicity concerns (European Commission, 2020). However, our samples were collected before this ban, although a recent report has pointed out the use of this pesticide after its derogation (Pesticide Action Network, 2023). Malathion is still commercialized but only allowed in greenhouses applications (European Commission, 2023). Finally, benzo[b]fluoranthene was the cause of the highest percentage of IBAs with high-risk concentrations (54%). This is in accordance with previous studies where this PAHs was found frequently exceeding the security thresholds limit values in soils from protected areas in Poland (Kicińska and Dmytrowski, 2023).

Contamination is nowadays ubiquitous and should be taken into consideration for the conservation of habitats and biodiversity. Soil contamination can lead to its degradation and generate chain effects on biodiversity, and consequently losses of ecosystem services provided by such as food production. The first impact of soil contamination is observed in soil organisms. For instance, the presence of pesticides in soils has been related to alterations in the reproduction and behaviour of earthworms, nematodes and arthropods potentially affecting the structure and functioning of soil (Gunstone et al., 2021). The exposure to plasticizers has been related to alterations in the reproduction and behaviour of earthworms in agricultural soils, due to their endocrine disruption, oxidative stress effects and DNA damage (Berenstein et al., 2022; Song et al., 2019). Moreover, soil contamination is also concerning as it is an entrance of contaminants to the whole terrestrial ecosystem, as bioacumulative contaminants such as OCPs and PCBs are biomagnified along the trophic chain, reaching higher concentrations in terrestrial top predators such as mammals and birds (Cao et al., 2023; Wu et al., 2022). Overall, contaminants present in natural areas that are hotspots of biodiversity deteriorate the ecosystems and affect the species well-being and survival, which indirectly also affects human beings. Under the actual conditions of climate change, water scarcity and human intrusion, preserving the IBAs is of outmost importance to protect and sustain innate natural richness. By establishing soil monitoring networks in IBAs, an initial assessment of the pollution status will provide useful information to implement conservation and pollution mitigation actions. These would include sustainable agriculture by minimizing the release and use of agrochemicals to IBA sites, minimizing the discharge of plastic waste, reducing and control the affluence of tourists and minimizing road infrastructures and traffic. By performing regular monitoring campaigns in water (Dulsat-Masvidal et al., 2023) and soil, along with protection actions, the concentration of contaminants can be reduced in the long term. Altogether this means reducing the anthropogenic footprint in natural areas which are biodiversity hotspots.

Table 2. Prioritization of concerning compounds in soil samples according to RQfi value and percentage (%) of IBAs (n=140) with environmental risk for each compound.

Compound	Group	% RQ<0.01	% 0.01>RQ<0.1	% 0.1<RQ<1	% RQ>1	RQf,i
Chlorpyrifos	OPP	72.9	0.0	0.0	27	8354
Malathion	OPP	87.9	0.0	0.0	12	681
Benzo[b]fluoranthene	PAH	46.4	0.0	0.0	54	260
Benzo[a]pyrene	PAH	82.1	0.0	0.0	18	11.2
2,4' -DDT	OCP	91.4	0.0	0.0	9.0	6.10
Benzo[ghi]perylene	PAH	55.0	0.0	0.0	45	2.91
Fluoranthene	PAH	57.9	0.0	0.0	42	1.21
Indeno[1,2,3-cd]pyrene	PAH	72.9	0.0	0.0	27	0.39
Benzo[k]fluoranthene	PAH	47.1	0.0	14.3	39	0.29
HCHs	OCP	60.7	24.3	7.1	8.0	0.15
Pyrene	PAH	39.3	1.4	33.6	26	0.10
4,4'-DDE	OCP	23.6	28.6	32.9	15	0.10
Chrysene	PAH	73.6	0.0	3.6	23	0.07
4,4'-DDD	OCP	72.1	5.7	10.7	11	0.06
DiBP	Plasticizer	88.6	0.0	0.0	11	0.02

4. CONCLUSIONS

Soils from IBAs are impacted by organic contaminants. Legacy compounds as OCPs and PCBs were widespread in soils but present at trace levels, indicative of historic releases. In contrast, PAHs and plasticizers were found at higher concentrations, suggesting more recent pollution. OPPs and OPEs were present in specific sampling points. Three clusters with different contamination patters were determined using PCA and the relationships with land-use were identified. Most of the IBAs were affected by diffuse pollution, as no clear sources of chemical patterns were distinguished. However, IBAs with the highest urban pressures showed the highest concentrations of PCBs and plasticizers, IBAs from north Spain had a specific PAHs contribution and agricultural IBAs were impacted by DDT, γ-HCH and β-HCH.

In this study, we list PNEC values based on the chemical properties and ecotoxicological information of studied contaminants in soils to evaluate the risk. A total of 95 out of 140 analysed soils presented at least one compound at high-risk concentrations. The most concerning compounds were OPPs and PAHs, which were frequently detected at concentrations exceeding the PNEC values. Overall, the results describe for the first time the extent of soil contamination in IBAs from Spain, evidencing a widespread distribution of some contaminants in these natural areas. Further research is needed to identify the sources of contamination and propose mitigation actions to minimize the release and impact of contaminants in areas of high ecological interest.

REFERENCES

Abdul Hussain, B., Westgate, J.N., Hayward, S.J., Shunthirasingham, C., Brown, T.N., Hung, H., Lei, Y.D., Wania, F., 2019. Polycyclic aromatic hydrocarbons and polychlorinated biphenyls in soils and atmosphere of Western Canadian mountains: The role of source proximity, precipitation, forest cover and mountain cold-trapping. Atmos. Environ. X 1, 100004. https://doi.org/https://doi.org/10.1016/j.aeaoa.2018.100004

Aichner, B., Bussian, B., Lehnik-Habrink, P., Hein, S., 2013. Levels and Spatial Distribution of Persistent Organic Pollutants in the Environment: A Case Study of German Forest Soils. Environ. Sci. Technol. 47, 12703–12714. https://doi.org/10.1021/es4019833

Berenstein, G., Hughes, E.A., Zalts, A., Basack, S., Bonesi, S.M., Montserrat, J.M., 2022. Environmental fate of dibutylphthalate in agricultural plastics: Photodegradation, migration and ecotoxicological impact on soil. Chemosphere 290, 133221. https://doi.org/https://doi.org/10.1016/j.chemosphere.2021.133221

Beriro, D.J., Vane, C.H., Cave, M.R., Nathanail, C.P., 2014. Effects of drying and comminution type on the quantification of Polycyclic Aromatic Hydrocarbons (PAH) in a homogenised gasworks soil and the implications for human health risk assessment. Chemosphere 111, 396–404. https://doi.org/https://doi.org/10.1016/j.chemosphere.2014.03.077

BirdLife International, 2023. Important Bird Areas factsheet: Plain between Cáceres and Trujillo-Aldea del Cano. [WWW Document]. URL https://datazone.birdlife.org/site/factsheet/plain-between-c%C3%A1ceres-and-trujillo-aldea-del-cano-iba-spain/text (accessed 8.30.23).

BOE, 2005. Real Decreto 9/2005, de 14 de enero, por el que se establece la relación de actividades potencialmente contaminantes del suelo y los criterios y estándares para la declaración de suelos contaminados.

Campos, I., Abrantes, N., 2021. Forest fires as drivers of contamination of polycyclic aromatic hydrocarbons to the terrestrial and aquatic ecosystems. Curr. Opin. Environ. Sci. Heal. 24, 100293. https://doi.org/https://doi.org/10.1016/j.coesh.2021.100293

Cao, X., Lu, R., Xu, Q., Zheng, X., Zeng, Y., Mai, B., 2023. Distinct biomagnification of chlorinated persistent organic pollutants in adjacent aquatic and terrestrial food webs. Environ. Pollut. 317, 120841. https://doi.org/https://doi.org/10.1016/j.envpol.2022.120841

de Brogniez, D., Ballabio, C., Stevens, A., Jones, R.J.A., Montanarella, L., van Wesemael, B., 2015. A map of the topsoil organic carbon content of Europe generated by a generalized additive model. Eur. J. Soil Sci. 66, 121–134. https://doi.org/https://doi.org/10.1111/ejss.12193

Degrendele, C., Audy, O., Hofman, J., Kučerik, J., Kukučka, P., Mulder, M.D., Přibylová, P., Prokeš, R., Šáňka, M., Schaumann, G.E., Lammel, G., 2016. Diurnal Variations of Air-Soil Exchange of Semivolatile Organic Compounds (PAHs, PCBs, OCPs, and PBDEs) in a Central European Receptor Area. Environ. Sci. Technol. 50, 4278–4288. https://doi.org/10.1021/acs.est.5b05671

Donald, P.F., Fishpool, L.D.C., Ajagbe, A., Bennun, L.A., Bunting, G., Burfield, I.J., Butchart, S.H.M., Capellan, S., Crosby, M.J., Dias, M.P., Diaz, D., Evans, M.I., Grimmett, R., Heath, M., Jones, V.R., Lascelles, B.G., Merriman, J.C., O'brien, M., Ramírez, I., Waliczky, Z., Wege, D.C., 2019. Important Bird and Biodiversity Areas (IBAs): the development and characteristics of a global inventory of key sites for biodiversity. Bird Conserv. Int. 29, 177–198. https://doi.org/10.1017/S0959270918000102

Dulsat-Masvidal, M., Ciudad, C., Infante, O., Mateo, R., Lacorte, S., 2023. Water pollution threats in important bird and biodiversity areas from Spain. J. Hazard. Mater. 448, 130938. https://doi.org/https://doi.org/10.1016/j.jhazmat.2023.130938

Dziuban, C.D., Shirkey, E.C., Peeples, T.O., 1979. An Investigation of Some Distributional Characteristics of the Measure of Sampling Adequacy. Educ. Psychol. Meas. 39, 543–549. https://doi.org/10.1177/001316447903900304

EEA, 2022. Soil monitoring in Europe. Indicators and thresholds for soil health assessments.

EPA, U., 2012. Estimation Programs Interface SuiteTM for Microsofts Windows.

European Comission, 2020. Caring for soil is caring for life : ensure 75% of soils are healthy by 2030 for food, people, nature and climate : report of the Mission board for Soil health and food. Publications Office. https://doi.org/doi/10.2777/821504

European Commission, 2023. Active substance: Malathion [WWW Document]. URL https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/start/screen/active-substances/details/273 (accessed 4.22.23).

European Commission, 2020. Commission implementing regulation (EU) 2020/18 of 10 January 2020 concerning the non-renewal of the approval of the active substance chlorpyrifos, in accordance with Regulation (EC) No 1107/2009. Off. J. Eur. Union L 7/14.

European Commission, 2003. Technical Guidance Document on Risk Assessment.

FAO, 2015. Status of the World's Soil Resources: Main Report. FAO, Rome, Italy.

Ferreira, C.S.S., Seifollahi-Aghmiuni, S., Destouni, G., Ghajarnia, N., Kalantari, Z., 2022. Soil degradation in the European Mediterranean region: Processes, status and consequences. Sci. Total Environ. 805, 150106. https://doi.org/https://doi.org/10.1016/j.scitotenv.2021.150106

García, M.G., Sánchez, J.I.L., Bravo, K.A.S., Cabal, M.D.C., Pérez-Santín, E., 2022. Presence, distribution and current pesticides used in Spanish agricultural practices. Sci. Total Environ. 157291. https://doi.org/https://doi.org/10.1016/j.scitotenv.2022.157291

Gómez-Lavín, S., San Román, M.F., Ortiz, I., Fernández, J., de Miguel, P., Urtiaga, A., 2018. Dioxins and furans legacy of lindane manufacture in Sabiñánigo (Spain). The Bailín landfill site case study. Sci. Total Environ. 624, 955–962. https://doi.org/10.1016/j.scitotenv.2017.12.162

Greaves, A.K., Letcher, R.J., 2017. A review of organophosphate esters in the environment from biological effects to distribution and fate. Bull. Environ. Contam. Toxicol. 98, 2–7. https://doi.org/https://doi.org/10.1007/s00128-016-1898-0

Gunstone, T., Cornelisse, T., Klein, K., Dubey, A., Donley, N., 2021. Pesticides and soil invertebrates: A hazard assessment. Front. Environ. Sci. 9, 122.

Hu, X.C., Ge, B., Ruyle, B.J., Sun, J., Sunderland, E.M., 2021. A Statistical Approach for Identifying Private Wells Susceptible to Perfluoroalkyl Substances (PFAS) Contamination. Environ. Sci. \& Technol. Lett. 8, 596–602. https://doi.org/10.1021/acs.estlett.1c00264

Kicińska, A., Dmytrowski, P., 2023. Anthropogenic impact on soils of protected areas—example of PAHs. Sci. Rep. 13, 1524. https://doi.org/10.1038/s41598-023-28726-6

Kim, D., Kwak, J. Il, An, Y.-J., 2019. Physiological response of crop plants to the endocrine-disrupting chemical nonylphenol in the soil environment. Environ. Pollut. 251, 573–580. https://doi.org/https://doi.org/10.1016/j.envpol.2019.04.101

Kim, J.-T., Choi, Y.-J., Barghi, M., Kim, J.-H., Jung, J.-W., Kim, K., Kang, J.-H., Lammel, G., Chang, Y.-S., 2021. Occurrence, distribution, and bioaccumulation of new and legacy persistent organic pollutants in an ecosystem on King George Island, maritime Antarctica. J. Hazard. Mater. 405, 124141. https://doi.org/https://doi.org/10.1016/j.jhazmat.2020.124141

Li, X., Wang, Q., Jiang, N., Lv, H., Liang, C., Yang, H., Yao, X., Wang, J., 2023. Occurrence, source, ecological risk, and mitigation of phthalates (PAEs) in agricultural soils and the environment: A review. Environ. Res. 220, 115196. https://doi.org/https://doi.org/10.1016/j.envres.2022.115196

Liu, Y.-R., van der Heijden, M.G.A., Riedo, J., Sanz-Lazaro, C., Eldridge, D.J., Bastida, F., Moreno-Jiménez, E., Zhou, X.-Q., Hu, H.-W., He, J.-Z., Moreno, J.L., Abades, S., Alfaro, F., Bamigboye, A.R., Berdugo, M., Blanco-Pastor, J.L., de los Ríos, A., Duran, J., Grebenc, T., Illán, J.G., Makhalanyane, T.P., Molina-Montenegro, M.A., Nahberger, T.U., Peñaloza-Bojacá, G.F., Plaza, C., Rey, A., Rodríguez, A., Siebe, C., Teixido, A.L., Casado-Coy, N., Trivedi, P., Torres-Díaz, C., Verma, J.P., Mukherjee, A., Zeng, X.-M., Wang, L., Wang, J., Zaady, E., Zhou, X., Huang, Q., Tan, W., Zhu, Y.-G., Rillig, M.C., Delgado-Baquerizo, M., 2023. Soil contamination in nearby natural areas mirrors that in urban greenspaces worldwide. Nat. Commun. 14, 1706. https://doi.org/10.1038/s41467-023-37428-6

Łyszczarz, S., Lasota, J., Staszel, K., Błońska, E., 2021. Effect of forest and agricultural land use on the accumulation of polycyclic aromatic hydrocarbons in relation to soil properties and possible pollution sources. For. Ecol. Manage. 490, 119105. https://doi.org/https://doi.org/10.1016/j.foreco.2021.119105

Mali, H., Shah, C., Raghunandan, B.H., Prajapati, A.S., Patel, D.H., Trivedi, U., Subramanian, R.B., 2023. Organophosphate pesticides an emerging environmental contaminant: Pollution, toxicity, bioremediation progress, and remaining challenges. J. Environ. Sci. 127, 234–250. https://doi.org/https://doi.org/10.1016/j.jes.2022.04.023

Mishra, A., Kumari, M., Swati, Kumar, R., Iqbal, K., Thakur, I.S., 2022. Persistent organic pollutants in the environment: Risk assessment, hazards, and mitigation strategies. Bioresour. Technol. Reports 19, 101143. https://doi.org/https://doi.org/10.1016/j.biteb.2022.101143

Narizzano, R., Risso, F., Magherini, A., Magi, E., Giampieri, M., Devia, C., Venturelli, G., Carlini, E., 2013. Effect of drying conditions during sample pre-treatment on the determination of polycyclic aromatic hydrocarbons in soils. Anal. Bioanal. Chem. 405, 1115–1121. https://doi.org/10.1007/s00216-012-6304-0

NORMAN, 2022. NORMAN Ecotoxicology Database - Lowest PNECs [WWW Document]. URL https://www.norman-network.com/nds/ecotox/lowestPnecsIndex.php (accessed 3.16.22).

Pesticide Action Network, 2023. Banned Pesticides still in use in the EU.

Prasad, B., 2021. Phthalate pollution: environmental fate and cumulative human exposure index using the multivariate analysis approach. Environ. Sci. Process. Impacts 23, 389–399.

Qu, C., Albanese, S., Li, J., Cicchella, D., Zuzolo, D., Hope, D., Cerino, P., Pizzolante, A., Doherty, A.L., Lima, A., De Vivo, B., 2019. Organochlorine pesticides in the soils from Benevento provincial territory, southern Italy: Spatial distribution, air-soil exchange, and implications for environmental health. Sci. Total Environ. 674, 159–170. https://doi.org/https://doi.org/10.1016/j.scitotenv.2019.04.029

Ren, J., Wang, X., Gong, P., Wang, C., 2019. Characterization of Tibetan Soil As a Source or Sink of Atmospheric Persistent Organic Pollutants: Seasonal Shift and Impact of Global Warming. Environ. Sci. Technol. 53, 3589–3598. https://doi.org/10.1021/acs.est.9b00698

Rengarajan, T., Rajendran, Peramaiyan, Nandakumar, N., Lokeshkumar, B., Rajendran, Palaniswami, Nishigaki, I., 2015. Exposure to polycyclic aromatic hydrocarbons with special focus on cancer. Asian Pac. J. Trop. Biomed. 5, 182–189. https://doi.org/https://doi.org/10.1016/S2221-1691(15)30003-4

Rico, A., Dafouz, R., Vighi, M., Rodríguez-Gil, J.L., Daam, M.A., 2021. Use of Postregistration Monitoring Data to Evaluate the Ecotoxicological Risks of Pesticides to Surface Waters: A Case Study with Chlorpyrifos in the Iberian Peninsula. Environ. Toxicol. Chem. 40, 500–512. https://doi.org/10.1002/etc.4927

Rivier, P.-A., Havranek, I., Coutris, C., Norli, H.R., Joner, E.J., 2019. Transfer of organic pollutants from sewage sludge to earthworms and barley under field conditions. Chemosphere 222, 954–960. https://doi.org/https://doi.org/10.1016/j.chemosphere.2019.02.010

Rossini, P., Guerzoni, S., Matteucci, G., Gattolin, M., Ferrari, G., Raccanelli, S., 2005. Atmospheric fall-out of POPs (PCDD-Fs, PCBs, HCB, PAHs) around the industrial district of Porto Marghera, Italy. Sci. Total Environ. 349, 190–200. https://doi.org/https://doi.org/10.1016/j.scitotenv.2005.01.011

Sabzevari, S., Hofman, J., 2022. A worldwide review of currently used pesticides' monitoring in agricultural soils. Sci. Total Environ. 812, 152344. https://doi.org/https://doi.org/10.1016/j.scitotenv.2021.152344

Schuster, J.K., Gioia, R., Moeckel, C., Agarwal, T., Bucheli, T.D., Breivik, K., Steinnes, E., Jones, K.C., 2011. Has the Burden and Distribution of PCBs and PBDEs Changed in European Background Soils between 1998 and 2008? Implications for Sources and Processes. Environ. Sci. Technol. 45, 7291–7297. https://doi.org/10.1021/es200961p

Shukla, S., Khan, R., Bhattacharya, P., Devanesan, S., AlSalhi, M.S., 2022. Concentration, source apportionment and potential carcinogenic risks of polycyclic aromatic hydrocarbons (PAHs) in roadside soils. Chemosphere 292, 133413. https://doi.org/https://doi.org/10.1016/j.chemosphere.2021.133413

Silva, V., Mol, H.G.J., Zomer, P., Tienstra, M., Ritsema, C.J., Geissen, V., 2019. Pesticide residues in European agricultural soils – A hidden reality unfolded. Sci. Total Environ. 653, 1532–1545. https://doi.org/https://doi.org/10.1016/j.scitotenv.2018.10.441

Song, P., Gao, J., Li, X., Zhang, C., Zhu, L., Wang, Jinhua, Wang, Jun, 2019. Phthalate induced oxidative stress and DNA damage in earthworms (Eisenia fetida). Environ. Int. 129, 10–17. https://doi.org/https://doi.org/10.1016/j.envint.2019.04.074

Stockholm Convention, 2019. All POPs listed in the Stockholm Convention [WWW Document]. URL http://chm.pops.int/TheConvention (accessed 6.20.23).

Velázquez-Gómez, M., Hurtado-Fernández, E., Lacorte, S., 2018. Comprehensive method for the analysis of multi-class organic micropollutants in indoor dust. Sci. Total Environ. 635, 1484–1494. https://doi.org/https://doi.org/10.1016/j.scitotenv.2018.04.186

Wang, X., Zhu, Q., Yan, X., Wang, Y., Liao, C., Jiang, G., 2020. A review of organophosphate flame retardants and plasticizers in the environment: Analysis, occurrence and risk assessment. Sci. Total Environ. 731, 139071. https://doi.org/https://doi.org/10.1016/j.scitotenv.2020.139071

Wang, Y., Zhang, Z., Bao, M., Xu, Y., Zhang, L., Tan, F., Zhao, H., 2021. Characteristics and risk assessment of organophosphate esters and phthalates in soils and vegetation from Dalian, northeast China. Environ. Pollut. 284, 117532. https://doi.org/10.1016/j.envpol.2021.117532

Wenzel, K.-D., Manz, M., Hubert, A., Schüürmann, G., 2002. Fate of POPs (DDX, HCHs, PCBs) in upper soil layers of pine forests. Sci. Total Environ. 286, 143–154. https://doi.org/https://doi.org/10.1016/S0048-9697(01)00972-X

Wu, X., Zheng, X., Yu, L., Lu, R., Zhang, Q., Luo, X.-J., Mai, B.-X., 2022. Biomagnification of Persistent Organic Pollutants from Terrestrial and Aquatic Invertebrates to Songbirds: Associations with Physiochemical and Ecological Indicators. Environ. Sci. Technol. 56, 12200–12209. https://doi.org/10.1021/acs.est.2c02177

Yadav, I.C., Devi, N.L., Li, J., Zhang, G., 2018. Organophosphate ester flame retardants in Nepalese soil: Spatial distribution, source apportionment and air-soil exchange assessment. Chemosphere 190, 114–123. https://doi.org/https://doi.org/10.1016/j.chemosphere.2017.09.112

Yadav, I.C., Devi, N.L., Li, J., Zhang, G., 2017. Polychlorinated biphenyls in Nepalese surface soils: Spatial distribution, air-soil exchange, and soil-air partitioning. Ecotoxicol. Environ. Saf. 144, 498–506. https://doi.org/https://doi.org/10.1016/j.ecoenv.2017.06.057

Zhang, Q., Wang, Y., Zhang, C., Yao, Y., Wang, L., Sun, H., 2022. A review of organophosphate esters in soil: Implications for the potential source, transfer, and transformation mechanism. Environ. Res. 204, 112122. https://doi.org/https://doi.org/10.1016/j.envres.2021.112122

Zhang, Y., Lyu, L., Tao, Y., Ju, H., Chen, J., 2022. Health risks of phthalates: A review of immunotoxicity. Environ. Pollut. 313, 120173. https://doi.org/https://doi.org/10.1016/j.envpol.2022.120173

Zhou, S., Di Paolo, C., Wu, X., Shao, Y., Seiler, T.-B., Hollert, H., 2019. Optimization of screening-level risk assessment and priority selection of emerging pollutants – The case of pharmaceuticals in European surface waters. Environ. Int. 128, 1–10. https://doi.org/https://doi.org/10.1016/j.envint.2019.04.034

SUPPLEMENTARY INFORMATION

Table S1. Compounds studied and standard supplier.

Group	Compound	Supplier
PAH	PAH solution mix : naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benz(a) anthracene, chrysen, benzo[b]fluoranthene, benzo[k]fluoranthen, benzo[a]pyrene indeno[1,2,3-cd]pyrene, dibenz[a,h]anthracene, benzo[ghi] perylene	AccuStandard (New Haven, CT, USA)
OCPs	Hexachlorobutadiene (HCBD)	Sigma-Aldrich (Darmstadt, Germany and St. Louis, MO, USA)
	Alfa Hexachlorocyclohexane (α-HCH)	Dr. Ehrenstrofer
	Beta Hexachlorocyclohexane (β-HCH)	Dr. Ehrenstrofer
	Gamma Hexachlorocyclohexane (γ-HCH)	Dr. Ehrenstrofer
	Delta Hexachlorocyclohexane (δ-HCH)	Dr. Ehrenstrofer
	Hexachlorobenzene (HCB)	Sigma-Aldrich (Darmstadt, Germany and St. Louis, MO, USA)
	Pesticide mix 164: 2,4'-DDE, 4,4'-DDE, 2,4'-DDD, 2,4'-DDT, 4,4'-DDD, 4,4'-DDT	Dr. Ehrenstrofer
	α-Endosulfan	Dr. Ehrenstrofer
	β-Endosulfan	Dr. Ehrenstrofer
OPPs	Chlorpyrifos	Dr. Ehrenstrofer
	Malathion	Sigma-Aldrich (Darmstadt, Germany and St. Louis, MO, USA)
	Chlorfenvinphos	Sigma-Aldrich (Darmstadt, Germany and St. Louis, MO, USA)
PCBs	PCB Mix 3: PCB 28, PCB 52, PCB 101, PCB 118, PCB 138, PCB 153, PCB 180-	Dr. Ehrenstrofer
Plastizicers	4-nonylphenol (NP)	Dr. Ehrenstrofer
	Dimethyl phthalate (DMP)	Sigma-Aldrich (Darmstadt, Germany and St. Louis, MO, USA)
	Diethyl phthalate (DEP)	Sigma-Aldrich (Darmstadt, Germany and St. Louis, MO, USA)
	Dibutyl phthalate (DBP)	Sigma-Aldrich (Darmstadt, Germany and St. Louis, MO, USA)
	Diisobutyl phthalate (DiBP)	Sigma-Aldrich (Darmstadt, Germany and St. Louis, MO, USA)
	Butyl benzyl phthalate (BBzP)	Sigma-Aldrich (Darmstadt, Germany and St. Louis, MO, USA)
	Bis (2-ethylhexyl) phthalate (DEHP)	Sigma-Aldrich (Darmstadt, Germany and St. Louis, MO, USA)
	Bisphenol A (BPA)	Dr. Ehrenstrofer
OPEs	Tris (1,3-dichloroisopropyl) phosphate (TDCPP)	Sigma-Aldrich (Darmstadt, Germany and St. Louis, MO, USA)
	Tris(2-chloroethyl) phosphate (TCEP)	Sigma-Aldrich (Darmstadt, Germany and St. Louis, MO, USA)
	Tris (2-butoxyethyl) phosphate (TBOEP)	Sigma-Aldrich (Darmstadt, Germany and St. Louis, MO, USA)
	2-ethylhexyl diphenyl phosphate (EHDPHP)	Sigma-Aldrich (Darmstadt, Germany and St. Louis, MO, USA)

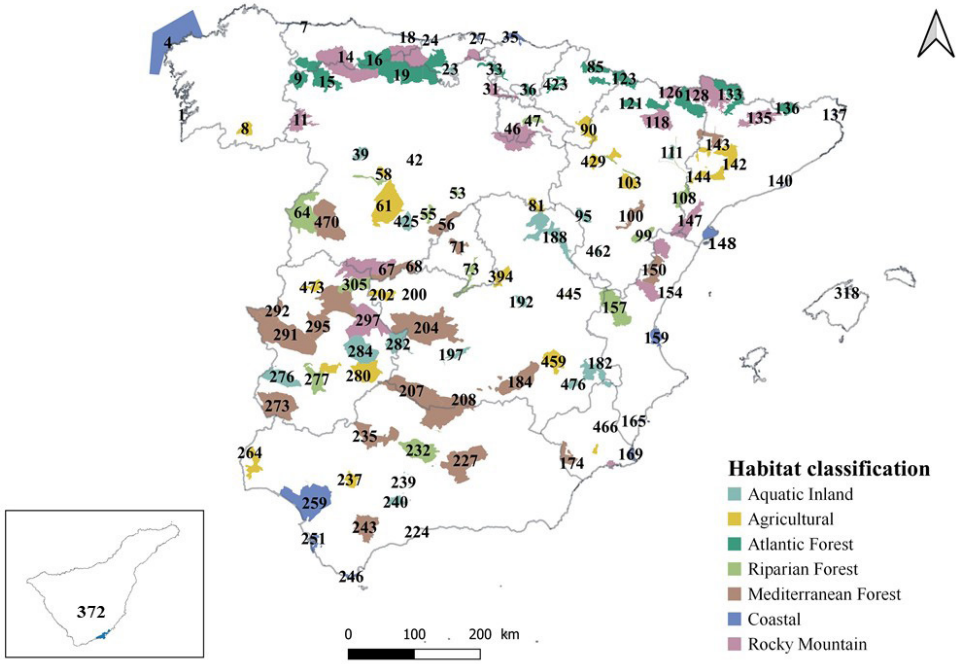


Figure S1. Map of the selected Important Bird and Biodiversity Areas from Spain. Numbers indicate IBA code.

Table S2. List of selected IBAs indicating name, province, habitat type and code. The table S2 is available as electronic supplementary material due to its large size. Please scan the QR code below to display the table.

Table S3. Quality parameters of the method, indicating type of compounds, the internal standard (IS) used, retention time, response factor, linear range, Instrumental detection limits (IDL), percentage recovery with standard error (%R±SE), method detection limits (MDL), and inter-day precision (%, n=5). Coefficient of determination (r^2) was 0.99 for all compounds. (*) Compounds which MDL were set at 3 times blanks contribution except for compounds contributing in the blanks (indicated in the main text). Compounds ordered by retention time.

Type	Compound	IS used	Retention time (min)	Response factor	Linearity (ng/µL)	IDL (ng)	%Recovery (± SE)	MDL (ng/g)	Interday (%RSD)
PAHs	Naphthalene	Naphthalene-d_8	8.63	0.17	0.001-0.2	0.17	85±1	0.30	0.8
OCPs	HCBD	Naphthalene-d_8	9.41	0.51	0.001-0.6	0.02	95±17	0.05	0.5
PAHs	Acenaphthylene	Acenaphthylene-d_{10}	14.52	1.30	0.001-0.8	0.02	83±6	1.37	0.9
Plasticizers	DMP	Acenaphthylene-d_{10}	14.62	1.24	0.001-0.4	0.02	28±12	5.25*	0.3
PAHs	Acenaphthene	Acenaphthylene-d_{10}	15.25	0.50	0.001-0.2	0.04	116±14	0.08	1.1
PAHs	Fluorene	Acenaphthylene-d_{10}	17.20	1.55	0.001-0.2	0.03	104±1	0.43	1.0
Plasticizers	DEP	Acenaphthylene-d_{10}	17.43	0.78	0.001-0.2	0.04	42±10	514*	4.3
OCPs	α-HCH	Phenanthrene-d_{10}	19.46	0.08	0.001-0.8	0.84	106±6	1.77	1.9
OCPs	HCB	Phenanthrene-d_{10}	19.74	0.50	0.001-0.8	0.08	72±1	0.02	1.6
OCPs	β-HCH	Phenanthrene-d_{10}	20.63	0.08	0.001-0.8	0.01	118±2	0.02	1.6
OCPs	γ-HCH	Phenanthrene-d_{10}	20.90	0.08	0.001-0.8	0.02	105±1	0.02	1.8
OPEs	TDCEP	Phenanthrene-d_{10}	20.94	0.01	0.001-0.2	0.56	83±5	1.19*	27.2
PAHs	Phenanthrene	Phenanthrene-d_{10}	21.33	0.26	0.001-0.8	0.09	110±3	0.17	0.9
PAHs	Anthracene	Phenanthrene-d_{10}	21.59	0.24	0.001-0.8	0.05	88±1	0.19	1.2
OCPs	δ-HCH	Phenanthrene-d_{10}	22.22	0.06	0.001-0.8	0.03	109±5	0.25	2.2
Plasticizers	NP	Phenanthrene-d_{10}	23.90	0.03	0.001-0.2	0.66	111±17	2.86*	15.4
Plasticizers	DiBP	Phenanthrene-d_{10}	23.99	0.12	0.1-0.8	0.03	88±6	165*	9.1
PCBs	PCB 28	Phenanthrene-d_{10}	24.15	0.75	0.001-0.6	0.01	97±6	0.07	2.0
PCBs	PCB 52	Phenanthrene-d_{10}	26.26	0.26	0.001-0.8	0.02	77±3	0.02	2.0
Plasticizers	DBP	Phenanthrene-d_{10}	26.90	0.04	0.001-0.8	0.09	27±5	87.2*	11.4
OCPs	Malathion	Phenanthrene-d_{10}	27.34	0.01	0.001-0.2	0.03	119±2	0.38	1.8
OCPs	Chlorpyrifos	Phenanthrene-d_{10}	27.82	0.05	0.001-0.2	4.35	118±5	5.71	0.5
PAHs	Fluoranthene	Phenanthrene-d_{10}	29.50	0.90	0.001-0.2	0.02	128±21	3.98	2.1
OCPs	Chlorfenvinphos	Phenanthrene-d_{10}	30.43	0.01	0.001-0.6	0.28	129±4	1.20	7.6
PAHs	Pyrene	Phenanthrene-d_{10}	31.05	0.84	0.001-0.2	0.02	117±8	0.20	2.6
OCPs	2,4'-DDE	Chrysene-d_{12}	31.43	0.37	0.001-0.8	0.01	106±8	0.08	5.2

Type	Compound	IS used	Retention time (min)	Response factor	Linearity (ng/µL)	IDL (ng)	%Recovery (± SE)	MDL (ng/g)	Interday (%RSD)
OCPs	α-Endosulfan	Chrysene-d_{12}	31.56	0.07	0.001-0.2	0.1	118±9	0.08	6.1
PCBs	PCB 101	Chrysene-d_{12}	31.58	1.04	0.001-0.2	0.01	101±7	0.03	5.9
Plasticizers	BPA	Chrysene-d_{12}	33.13	0.05	0.001-0.2	2	114±12	32.5*	54.1
OCPs	4,4′-DDE	Chrysene-d_{12}	33.39	0.69	0.001-0.2	0.04	121±3	0.07	8.2
OCPs	2,4′-DDD	Chrysene-d_{12}	33.85	1.02	0.001-0.2	0.05	95±6	0.05	6.4
OCPs	β-Endosulfan	Chrysene-d_{12}	34.98	0.01	0.001-0.2	0.26	94±14	1.03	5.4
PCBs	PCB 118	Chrysene-d_{12}	35.32	0.89	0.001-0.2	0.02	135±3	0.09	4.0
OCPs	2,4′-DDT	Chrysene-d_{12}	35.89	1.60	0.001-0.2	0.05	114±6	1.14	5.2
OCPs	4,4′-DDD	Chrysene-d_{12}	36.02	1.21	0.001-0.2	0.09	109±7	0.06	5.5
PCBs	PCB 153	Chrysene-d_{12}	36.69	0.73	0.001-0.2	0.01	113±4	0.08	3.5
OPEs	TDCPP	Chrysene-d_{12}	37.78	0.01	0.001-0.2	0.08	43±14	1.82*	5.9
OCPs	4,4′-DDT	Chrysene-d_{12}	38.09	0.38	0.001-0.2	0.13	101±8	2.51	4.0
Plasticizers	BBZP	Chrysene-d_{12}	38.18	0.02	0.005-0.2	0.29	38±13	20.1*	24.6
PCBs	PCB 138	Chrysene-d_{12}	38.29	0.69	0.001-0.2	0.01	115±2	0.07	3.6
OPEs	TBOEP	Chrysene-d_{12}	40.24	0.01	0.001-0.2	1.87	78±8	5.60*	0.4
PAHs	Chrysene	Chrysene-d_{12}	40.38	16.00	0.001-0.2	0.17	89±6	3.55	4.7
OPEs	EHDPHP	Chrysene-d_{12}	40.42	0.17	0.001-0.2	0.03	58±4	111*	9.1
PAHs	Benzo[a]anthracene	Chrysene-d_{12}	40.65	12.40	0.001-0.2	0.05	100±5	2.53	2.1
PCBs	PCB 180	Chrysene-d_{12}	42.26	0.31	0.001-0.2	0.02	126±1	0.11	4.3
Plasticizers	DEHP	Chrysene-d_{12}	43.46	0.02	0.005-0.4	0.36	95.00	1161*	17.3
PAHs	Benzo[b] fluoranthene	Perylene-d_{12}	47.10	1.99	0.001-0.2	0.11	89±6	0.97	10.1
PAHs	Benzo[k] fluoranthene	Perylene-d_{12}	47.12	3.88	0.001-0.1	0.1	118±10	1.29	9.6
PAHs	Benzo[a]pyrene	Perylene-d_{12}	48.53	2.34	0.001-0.1	0.1	62±5	13.80	6.2
PAHs	Indeno[1,2,3-cd] pyrene	Perylene-d_{12}	52.05	1.05	0.001-0.1	0.06	118±10	7.03	5.4
PAHs	Dibenzo[a,h] anthracene	Perylene-d_{12}	52.20	2.33	0.001-0.1	0.05	32±3	0.07	6.0
PAHs	Benzo[ghi]perylene	Perylene-d_{12}	52.72	1.25	0.001-0.1	0.2	20±2	0.04	10.6

Table S4. Spatial data around 10 Km soils sampling points used for HCPC analysis. The table S4 is available as electronic supplementary material due to its large size. Please scan the QR code below to display the table.

Table S5. Data used for the calculation of PNEC values for soils. And final PNEC values estimated for the target compounds in soil samples.

Compound	Koc (L/Kg)	PNEC Freshwater Norman (µg/L)	Henry Pa.m3. mol^{-1} (EPIWEB)	K soil-water	PNEC Soil ng/g (ww)	PNEC Soil ng/g (dw)
Naphthalene	731	2.00	53.3	22.1	26.0	29.4
Acenaphthylene	2625	0.53	55.6	79.0	24.6	27.8
Acenaphthene	2522	3.70	28.6	75.9	165	187
Fluorene	4241	0.25	17.0	127	18.7	21.2
Phenanthrene	7421	0.50	5.20	223	65.5	74.1
Anthracene	7274	0.10	5.20	218	12.8	14.5
Fluoranthene	30060	0.01	0.83	902	3.34	3.78
Pyrene	17180	0.03	0.84	516	8.49	9.60
Benzo[a]anthracene	99700	0.02	0.05	2991	42.2	47.7
Chrysene	110200	0.00	0.51	3306	5.64	6.37
Benzo[b]fluoranthene	103800	0.00	0.08	3114	0.31	0.35
Benzo[k]fluoranthene	200700	0.00	0.08	6021	7.44	8.40
Benzo[a]pyrene	208800	0.00	0.08	6264	0.63	0.71
Indeno[1,2,3-cd]pyrene	652400	0.00	0.01	19572	4.14	4.68
Dibenzo[a,h]anthracene	473900	0.01	0.05	14217	83.6	94.5
Benzo[ghi]perylene	567300	0.00	0.01	17019	1.70	1.92
HCBD	14070	0.10	1090	422	24.8	28.1
α-HCH	3915	0.02	25.9	118	1.38	1.56
β-HCH	3915	0.02	25.9	118	1.38	1.56
γ-HCH	3915	0.02	25.9	118	1.38	1.56
δ-HCH	3915	0.09	25.9	118	5.95	6.73
HCB	17340	0.01	90.4	520	2.39	2.70
2,4'-DDE	161100	0.00	3.56	4833	2.16	2.44
4,4'-DDE	446300	0.00	3.56	13389	3.15	3.56
2,4'-DDD	167600	0.00	4.40	5028	1.48	1.67
2,4'-DDT	781000	0.00	1.55	23430	0.08	0.09

Compound	Koc (L/Kg)	PNEC Freshwater Norman (µg/L)	Henry Pa.m3. mol^{-1} (EPIWEB)	K soil-water	PNEC Soil ng/g (ww)	PNEC Soil ng/g (dw)
4,4'-DDD	167600	0.00	4.40	5028	1.48	1.67
4,4'-DDT	992600	0.01	1.55	29778	175	198
Malathion	102	0.01	0.00	3.25	0.01	0.01
Chlorpyrifos	7901	0.00	0.26	237	0.06	0.07
Chlorfenvinphos	640	0.10	0.01	19.4	1.14	1.29
PCB 28	15070	0.11	0.17	452	29.3	33.1
PCB 52	27420	0.02	12.6	823	10.2	11.5
PCB 101	67730	0.01	9.36	2032	11.9	13.5
PCB 118	101800	0.01	9.36	3054	18.0	20.3
PCB 153	227100	0.00	6.94	6813	18.4	20.8
PCB 138	153000	0.00	6.94	4590	9.45	10.7
PCB 180	440400	0.00	5.14	13212	10.9	12.3
NP	18970	0.25	0.61	569	83.7	94.6
DMP	48	192	0.02	1.63	184	208
DEP	136	73.0	0.04	4.27	183	207
DBP	1919	10.0	0.12	57.8	340	384
DiBP	1168	1.11	0.12	35.2	23.0	26.0
BBZP	2572	5.20	0.00	77.4	237	267
DEHP	99470	1.30	1.20	2984	2282	2579
BPA	1245	0.24	0.00	37.6	5.30	5.99
TDCPP	1115	1.10	0.00	33.7	21.8	24.6
TCEP	67	65.0	0.00	2.20	84.3	95.3
TBOEP	678	24.0	0.00	20.5	290	327
EHDPHP	7384	0.02	0.03	222	2.35	2.65

Table S6. HCPC output, indicating the statistics that define cluster 1, 2 and 3 for soils samples.

Cluster 1	v test	Mean in category	Overall mean	SD in category	Overall SD	p-value
DDTs	-2.30E+00	-9.07E-02	7.33E-17	9.58E-01	9.96E-01	2.13E-02
km of Roads	-3.02E+00	-1.19E-01	-8.65E-18	9.05E-01	9.96E-01	2.55E-03
Artificial	-3.57E+00	-1.40E-01	-1.64E-17	6.35E-01	9.96E-01	3.63E-04
PCB 101	-6.21E+00	-2.45E-01	9.16E-18	2.08E-01	9.96E-01	5.22E-10
Anthracene	-6.42E+00	-2.53E-01	2.36E-17	2.91E-01	9.96E-01	1.39E-10
PCB 138	-6.42E+00	-2.53E-01	-3.19E-17	1.83E-01	9.96E-01	1.35E-10
Benzo[k]fluoranthene	-6.58E+00	-2.59E-01	5.50E-17	7.56E-01	9.96E-01	4.78E-11

Cluster 1	v test	Mean in category	Overall mean	SD in category	Overall SD	p-value
PCB 180	-6.60E+00	-2.60E-01	7.78E-18	2.28E-01	9.96E-01	3.99E-11
PCB 153	-7.00E+00	-2.76E-01	-6.65E-18	3.13E-01	9.96E-01	2.47E-12
Benzo[b]fluoranthene	-7.21E+00	-2.84E-01	7.95E-18	6.93E-01	9.96E-01	5.40E-13
Fluorene	-7.23E+00	-2.85E-01	-2.14E-17	1.13E-01	9.96E-01	4.77E-13
Fluoranthene	-7.32E+00	-2.88E-01	3.19E-17	7.36E-01	9.96E-01	2.57E-13
Chrysene	-7.66E+00	-3.02E-01	3.57E-17	6.57E-01	9.96E-01	1.89E-14
Phenanthrene	-7.67E+00	-3.02E-01	5.41E-17	6.34E-01	9.96E-01	1.77E-14
Pyrene	-7.77E+00	-3.06E-01	7.48E-17	7.04E-01	9.96E-01	7.84E-15
Benzo[ghi]perylene	-7.96E+00	-3.14E-01	6.43E-17	6.91E-01	9.96E-01	1.75E-15
Indeno[1,2,3-cd]pyrene	-8.09E+00	-3.19E-01	-3.73E-17	6.13E-01	9.96E-01	5.98E-16
Benzo[a]anthracene	-8.31E+00	-3.27E-01	2.87E-17	5.78E-01	9.96E-01	9.82E-17
Benzo[a]pyrene	-8.55E+00	-3.37E-01	-3.93E-17	4.65E-01	9.96E-01	1.26E-17
Cluster 2	**v test**	**Mean in category**	**Overall mean**	**SD in category**	**Overall SD**	**p-value**
PCB 138	9.89E+00	3.64E+00	-3.19E-17	1.47E+00	9.96E-01	4.83E-23
PCB 180	9.78E+00	3.60E+00	7.78E-18	1.51E+00	9.96E-01	1.41E-22
PCB 153	9.62E+00	3.54E+00	-6.65E-18	1.32E+00	9.96E-01	6.72E-22
PCB 101	9.46E+00	3.48E+00	9.16E-18	2.13E+00	9.96E-01	3.19E-21
Artificial	7.38E+00	2.72E+00	-1.64E-17	2.30E+00	9.96E-01	1.57E-13
km of Roads	4.56E+00	1.68E+00	-8.65E-18	1.41E+00	9.96E-01	5.15E-06
DEP	4.07E+00	1.50E+00	6.34E-18	1.61E+00	9.96E-01	4.75E-05
DiBP	2.91E+00	1.07E+00	-1.74E-17	1.68E+00	9.96E-01	3.64E-03
Pyrene	2.69E+00	9.90E-01	7.48E-17	1.13E+00	9.96E-01	7.23E-03
Industrial Sites	2.53E+00	9.34E-01	-2.00E-17	1.68E+00	9.96E-01	1.13E-02
Acenaphthene	2.45E+00	9.02E-01	1.51E-17	9.92E-01	9.96E-01	1.44E-02
Incineration Plants	2.30E+00	8.48E-01	-9.71E-18	1.84E+00	9.96E-01	2.14E-02
Benzo[ghi]perylene	2.15E+00	7.91E-01	6.43E-17	8.93E-01	9.96E-01	3.18E-02
Cluster 3	**v test**	**Mean in category**	**Overall mean**	**SD in category**	**Overall SD**	**p-value**
Benzo[a]pyrene	9.40E+00	2.07E+00	-3.93E-17	9.46E-01	9.96E-01	5.72E-21
Benzo[a]anthracene	9.04E+00	1.99E+00	2.87E-17	7.81E-01	9.96E-01	1.50E-19

Cluster 3	v test	Mean in category	Overall mean	SD in category	Overall SD	p-value
Indeno[1,2,3-cd]pyrene	8.75E+00	1.93E+00	-3.73E-17	7.36E-01	9.96E-01	2.15E-18
Chrysene	8.31E+00	1.83E+00	3.57E-17	7.98E-01	9.96E-01	9.20E-17
Phenanthrene	7.99E+00	1.76E+00	5.41E-17	9.90E-01	9.96E-01	1.35E-15
Benzo[b]fluoranthene	7.97E+00	1.75E+00	7.95E-18	8.50E-01	9.96E-01	1.62E-15
Benzo[ghi]perylene	7.71E+00	1.70E+00	6.43E-17	7.84E-01	9.96E-01	1.28E-14
Fluorene	7.56E+00	1.66E+00	-2.14E-17	2.00E+00	9.96E-01	3.94E-14
Fluoranthene	7.36E+00	1.62E+00	3.19E-17	7.07E-01	9.96E-01	1.81E-13
Pyrene	7.14E+00	1.57E+00	7.48E-17	7.79E-01	9.96E-01	9.22E-13
Anthracene	6.97E+00	1.53E+00	2.36E-17	2.03E+00	9.96E-01	3.11E-12
Benzo[k]fluoranthene	6.97E+00	1.53E+00	5.50E-17	1.02E+00	9.96E-01	3.20E-12
HCB	2.13E+00	4.69E-01	-1.99E-17	2.07E+00	9.96E-01	3.31E-02
DDTs	2.06E+00	4.53E-01	7.33E-17	1.05E+00	9.96E-01	3.94E-02

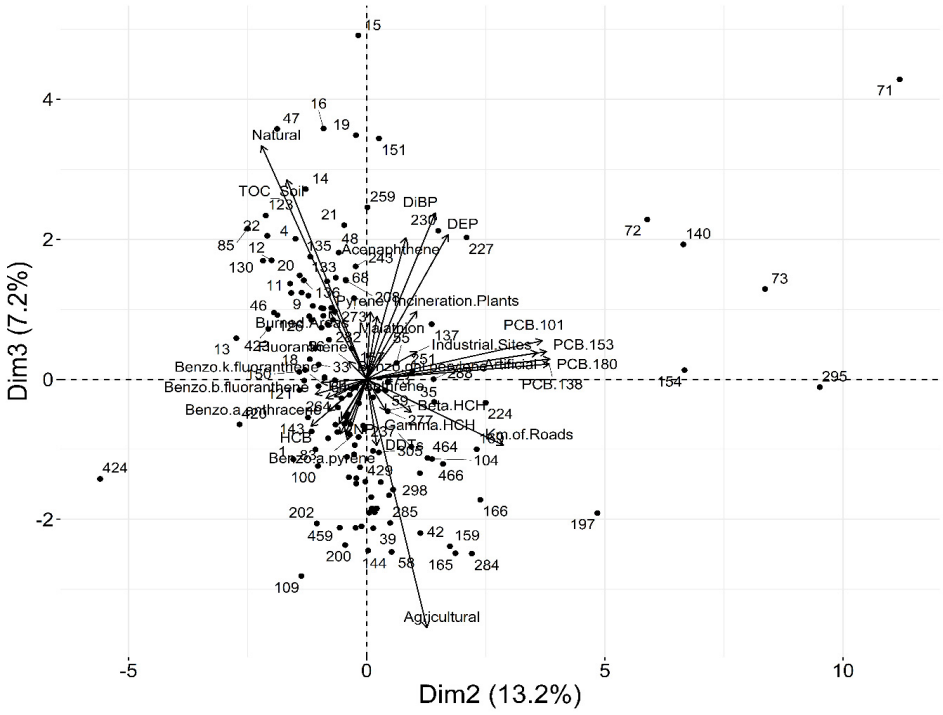
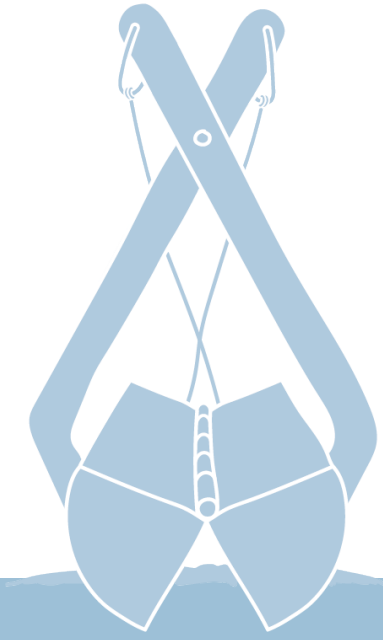


Figure S2. PCA analysis showing components 2 and 3, explaining a total variance of 20.4%. Numbers indicates IBA codes (Table S2 for IBA identification).



CHAPTER IV

ASSESSING SEDIMENTS CONTAMINATION STATUS IN IMPORTANT BIRD AND BIODIVERSITY AREAS

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ABSTRACT

River sediments constitute the physical habitat of aquatic ecosystems and are an important food resource for species and are a source of life. Yet, sediments are sinks of chemicals. The objective of this study is to determine the presence and risk of 52 legacy and emerging organic pollutants in sediments of 140 Important Bird and Biodiversity Areas (IBAs) located throughout the Spanish territory. In each IBAs, landscape observations including the existence of wastewater treatment plant (WWTP) discharges, picnic areas, landfills, agriculture, industry, urban areas, and human-generated waste (trash) abandoned in nature were recorded and served to determine main pressures and impact. The ∑contaminants in sediments ranged from 0.07 to 31076 ng/g and the most ubiquitous pollutants were polycyclic aromatic hydrocarbons (PAHs), DDTs and polychlorinated biphenyls (PCBs), while plasticizers and organophosphate esters (OPEs) were detected at the highest concentration attributed to their recent use and dumping of plastics associated with garbage. In-use pesticides were seldom detected. The concentrations detected have been compared to sediment quality standards and Predicted No-Effect Concentrations (PNEC) to evaluate the potential risk for the aquatic ecosystem. Risk compounds and pollution hotspots have been identified and mapped. Thresholds concentrations are provided to protect habitats and species that live in IBAs, and we highlight the need of sediment monitoring to preserve biodiversity.

1. INTRODUCTION

In the actual context of climate change and water scarcity, the impact of chemicals on the environment become more apparent, and the effects they can produce on biota and biodiversity are alarming. This has been evidenced by the recent published figures on biodiversity loss. According to the Europe Environmental Agency (EEA, 2021), the EU did not meet the 2020 target of improving the conservation status of EU protected species and habitats. Only 15% of habitats have good conservation status, 44.9% are in a poor state and 35.8% are in a bad state. The ambitious goals and targets of Kunming-Montreal Global Biodiversity Framework (EU, 2022) to protect and restore nature include the "reduction of pollution risks and negative impacts of pollution from all sources by 2030 to levels that are not harmful to biodiversity and ecosystems". There is an urgent need to protect natural areas against chemicals to ensure the survival of Europe's most valuable species and habitats.

Important Bird and Biodiversity Areas (IBAs) constitute the largest and most comprehensive global network of sites that are significant for the global persistence of biodiversity, including the conservation of both fauna and vegetation, and especially for the preservation of long-term bird populations (Donald et al., 2019). The IBA programme identifies, documents and protects over 13000 IBAs sites (Waliczky et al., 2019) using standardized and internationally agreed criteria (Trainor et al., 2008). The IBA network comprises terrestrial, aquatic, and marine habitats. Around 40% of the IBAs worldwide receive some form of protection, however many others are seriously threatened (BirdLife International, 2020). According to BirdLife International database, pollution leads to habitat degradation which affects 11% of all threatened bird species (BirdLife International, 2017). The main pressures in IBAs include agriculture, human disturbances, hunting, energy production and mining, dams and water management, residential and commercial development, invasive species, pollution, climate change, transport, logging, ecosystem modifications, fire and fire suppression, fisheries, gathering terrestrial plants and geological events (BirdLife International, 2023). These activities directly or indirectly produce the discharge of trash and chemicals to the environment, and inland wetlands, forests, grassland, coastal and agriculture have been identified as the most vulnerable habitats (SEO/ BirdLife, 2021).

Sediments are key components of the aquatic ecosystems and are essential to maintain the biodiversity in rivers, lakes, estuaries, wetlands and coastal habitats. They also provide important ecosystem services as balancing the riverine and coastal morphology, connect surface and ground waters, increase soil fertility, contribute to natural water purification and mitigate extreme flow events. However, sediments are sinks of chemical pollution and

human activities impact the quality and quantity of sediments and affect their ecological and chemical status (Brils, 2008). Despite the little information available on the quality of sediments in protected areas, especially inland, there is clear evidence of sediment pollution and their impact in these sites. For instance, the past use of organochlorine pesticides in agriculture affected surface sediments from four main Ramsar wetland sites in iSimangaliso Wetland Park (South Africa, a World Heritage site), posing ecotoxicological threats to the most important habitats for hippos, crocodiles, and aquatic birds of the African continent (Buah-Kwofie and Humphries, 2017). Sediments from protected tropical forest, mangroves and wetlands in northern Belize Nature Reserve contained neonicotinoids pesticides applied in agricultural fields at concentrations that posed a risk to aquatic invertebrates and benthic organisms by chronic and acute exposure (Bonmatin et al., 2019). Other emerging pressures are onshore wind, hydropower and solar photovoltaic generation that can modify sediments and impact ~30% of protected areas and Key Biodiversity Areas (KBA), compromising wilderness areas by ~60% (Rehbein et al., 2020). Even in polar areas, legacy and emerging contaminants start to leave their footprint (Azcune et al., 2022). Despite it is clear that various anthropogenic activities have an impact in protected or natural areas, a systematic sediment monitoring scheme in IBAs or KBA to determine contamination patterns, pollution hotspots and potential risk has not been undertaken.

Despite the key role of sediments to guarantee the quality of habitats, sediment environmental quality standards (EQS) are not prescribed except for a few Persistent Organic Pollutants. The United States Environmental Protection Agency (US EPA) provides Sediment Quality Guidelines (SQGs) to interpret historical data, identify potential problem chemicals or areas, design monitoring programs, classify hot spots and rank sites, and make decisions for more detailed studies (Long and MacDonald, 1998). The Canadian interim sediment quality guidelines (ISQGs) and probable effect levels (PELs) provide a basic indication on the degree of contamination and likely impact on ecology (CCME, 2002, 2001, 1999a, 1999b, 1999c). In Europe, the Water Framework Directive (WFD) includes the management of sediment associated contamination to achieve a good status of all European waters. As only hexachlorobenzene and hexachlorobutadiene have explicit EQS, the EU provide Member States with the option to apply sediment EQSs for priority substances and to implement long-term sediment monitoring so that concentrations do not significantly increase over time (Annex 1 of Directive 2008/105/EC, as amended by Directive 2013/39/EU). In the context of the Water Framework Directive, Lower and Upper Thresholds Values (LTV and UTV) are proposed in the integrated sediment management Guidelines and good practices (IKSE, 2014; Old and Lofts, 2022). This procedure establish sediment EQS considering bioavailability and effect-based data obtained by

field inventory, and may improve the assessment of the Ecological Status in waterbodies (Old and Lofts, 2022).

The objectives of the present study were to undertake a sediment monitoring study to determine the pollution status of 140 IBAs in Spain. First, landscape observations were undertaken to identify the main anthropogenic pressures affecting sediment quality, including the presence of wastewater treatment plants (WWTP), land use (agriculture, urban, industrial) and presence of trash (plastic, wipes, tires, etc.). Then, a set of 52 legacy and emerging organic contaminants were analysed to identify pollution patterns and the main pollution hotspots. Finally, a risk assessment was performed to identify main risk compounds according to Predicted No-Effect Concentrations (PNEC) and other sediment guidelines or regulations. Overall, this study provides information on the concentration of several contaminants in sediments from IBAs to be used as thresholds and contribute to the sustainable management of natural resources and ensure wildlife protection.

2. MATERIALS AND METHODS

2.1. Sampling and analysis

Sediments were collected from 140 IBAs representative of the main 7 habitats: agricultural, Atlantic Forest, Mediterranean forest, riparian forest, rocky mountain, inland aquatic, and coastal. Main pressures within the boundaries of the IBAs were identified using land-use information and by extensive visual inspection during sampling and comprised the following: wastewater treatment plant (WWTP) discharges, proximity to industry poles, agriculture, landfills, livestock, picnic area, recreational areas, and others) were recorded. Also, the presence of solid trash (urban, agricultural waste, plastic, wipes, and papers) was compiled. Sampling was georeferenced. Table 1 indicates the IBAs analysed in each habitat and the main pressures in each. Surface sediment samples were collected with a van Veen drag (1.4 L). Each sample was composed of a minimum of 8 subsamples collected in an area of the riverbed of approximately 10 m^2 and pooled and stored in a 1 kg glass recipient. Samples were sent refrigerated to the laboratory, freeze-dried, and sieved through 125 µm mesh and preserved in amber glass vials at 4ºC until analysis.

The 52 compounds studied (16 PAHs, 14 OCPs, 3 OPPs, 7 marker PCBs congeners, 4 OPEs, 6 phthalates, bisphenol A (BPA) and 4-nonylphenol (NP)) are listed in the Supplementary Information (SI) of chapter III. Analysis was performed with solid-liquid extraction using hexane: dichloromethane (1:1) and clean up was performed with 5 g Florisil Solid Phase Cartridges (Bond Elut, Agilent Technologies, Santa Clara, CA, USA) using hexane: dichloromethane as conditioning and eluting solvents. Samples were analysed using an Agilent

7890A GC gas chromatography coupled to 7000A tandem mass spectrometry (GC-MS/MS) (Agilent Technologies, Santa Clara, CA, USA). Quality control parameters of the method used are described in a previous study on the same contaminants in soils (Chapter III).

2.2. Data analysis and Environmental Risk Assessment

A data matrix was built containing compounds as columns (52) and sampling sites (140 IBAs) as rows, making 7280 data values. Compounds not detected were given a zero value in order not to overestimate their concentration (Dulsat-Masvidal et al., 2023). Descriptive statistical analysis was performed to determine the average, mean and maximum concentrations, number of occurrences and detection frequency. All values are expressed in dry weight (dw).

The dataset containing the target compounds and the georeferentiated sampling sites was used to assess the geographical distribution of contaminants using Inverse Distance Weighting (IDW). The interpolated values were calculated using "2" as an inverse distance power parameter. Interpolated data were then visualized on maps for the most detected families. Maps were elaborated with open-source software QGIS (version 3.18.2).

The contamination level of sediments was assessed comparing the detected concentrations with available SQG, aiming to determine the number of samples that could be considered polluted. SQGs provide concentrations below which sediments can be considered unpolluted, and values above which the contamination can be considered toxic for organisms. These thresholds differ in both nomenclature and magnitude depending on the guidelines. For instance, the US EPA adopted the consensus values of lower Threshold Effects Concentration (TEC) and upper Probable Effects Concentration (PEC), from MacDonald et al., (2000) (Ingersoll and MacDonald, 2002). The Canadian Council of Ministers of the Environment (CCME) sets Canadian ISQG and PEL (CCME, 2002, 2001, 1999a, 1999b, 1999c). And in the European context, the International Commission for the Protection of the Elbe River (ICPER) suggested LTV and UTV applied in Elbe river basin (Germany) (IKSE, 2014; Old and Lofts, 2022). It must be noted that there are no EQS for sediment in Europe.

For the Environmental Risk Assessment, individual Risk Quotients (RQs) for each compound were calculated as the ratio between the Measured Environmental Concentration (MEC) and the lowest Predicted Non-Effect Concentration (PNEC) in sediments extracted from the Norman network database (https://www.norman-network.com). Norman lowest PNECs are based on experimental eco-toxicity data of aquatic organisms (algae, daphnid

or fish) or Quantitative Structure-Activity Relationship (QSAR) predictions in case of lack of empirical data. RQs were classified as: no risk (RQ < 0.01), low risk (0.01 ≤ RQ <0.1), medium risk (0.1 ≤ RQ <1), and high risk (RQ ≥ 1). The ∑RQs in each IBAs was used to classify the risk levels observed among study areas according to no risk (∑RQs <1), low risk (1 ≤ RQ <10), medium risk (10 ≤ RQ <100), and high risk (∑RQ≥100). RQs values for each compound and sample were represented in a heatmap elaborated with ggplot2 package in R studio (R version 4.0.3).

3. RESULTS

3.1. Landscape observation to identify main pressures in Spanish IBAs

Table 1 lists the main pressures in each habitat category. It was observed that all IBAs are affected by diverse human activities, ranging from agriculture, livestock, discharge of WWTP effluents, roads, urban areas and recreational areas to more specific as aquaculture, military camps, industry poles, mines, thermal plant, landfills or airport. Land use information on the sampling site allowed to quantify the impact of these general activities. A total of 31% of IBAs were affected by WWTP discharges, 43% by agriculture surface, 38% by urban areas, and 8% had industrial poles close to the sampling site. These activities have an important impact in IBAs, and proof of it is the large amount of solid trash visualized during sampling. It is evident that the activities undertaken in these natural areas are a source of trash and organic contaminants to those environments. Around half of the analysed IBAs (79) presented trash residues documented during field sampling. This trash was either of urban origin (wipes, papers, plastic bags, candy papers, aluminium foil), agricultural (pesticide containers, greenhouse mulch) or industrial or landfill (plastic pieces). The presence of trash was found in 65% of the coastal IBAs, in 60% of rocky mountain and riparian habitats, in 55% of agricultural and Atlantic forest IBAs, and in 50% of Mediterranean forest and inland aquatic IBAs (Table 1). The widespread human waste indicates the poor behavioural attitude of visitors and inadequate management of residues within the IBAs. Figure 1 shows some pictures exemplifying the trash detected in IBAs.

Table 1. IBAs analysed, organized by habitat, IBA codes and name with water body name in brackets, and main pressures identified in the sampling area.

Habitat	IBAs	Pressures
Agricultural	8- Orense (Limia)	Agriculture, farms, WWTP discharge, wipes
	58- Valladolid (Bajoz)	Agriculture, a lot of trash, very dirty water
	61- Salamanca, Ávila (Almar)	Agriculture, livestock, aggregate extraction, shooting field, trash
	81- Soria/Guadalajara (Jalón)	Road, aquaculture, periurban site, some trash
	90- Navarra/Zaragoza (Limas)	Agriculture
	103- Zaragoza (Ginel)	Agriculture, shooting field, periurban
	114- Zaragoza (Val)	Agriculture, military area
	142- Lleida/Huesca (Sió)	Agriculture, WWTP discharge, picnic, periurban area, crops, trash
	144- Lleida (Set)	Agriculture, periurban area
	175- Murcia (Guadalentín)	Industry, road, farm, agriculture
	202- Toledo (Orco)	Agricultural waste
	237- Sevilla (Saladillo)	WWTP discharge (very dirty water), agriculture, highway
	264- Huelva (Cobica)	Agriculture, road, close to mine (Cu, S and pyrite), trash
	280- Badajoz (Cañada Real)	Well preserved
	285- Badajoz (Guadiana)	Well preserved
	394- Guadalajara (Tajo)	Agriculture, industry, aquaculture water discharge, trash
	429- Zaragoza (Umbria)	Agriculture, agricultural waste
	445- Cuenca (Guadazaón)	Agriculture, agricultural waste, livestock
	459- Albacete (Don Juan)	Agriculture, agricultural waste
	473- Cáceres (Alagón)	Agriculture, WWTP discharges, trash
Atlantic forest	9- León/Lugo (Burbia)	Periurban, road through river (no bridge), trash
	12- Asturias (Muniellos)	WWTP discharge, plastic waste
	13- Asturias/León (Cerredo)	Coal mines, WWTP discharge, livestock
	15- León (Pedroso)	Illegal landfill, plastics and agricultural waste
	16- Asturias/León (Nalón)	Urban area, sewage grid discharges, Picnic, waste
	19- León (Esla)	Livestock, agriculture, sewage grid discharge
	21- Palencia (Carrillón)	WWTP discharge, thermal plant, periurban area
	22- Cantabria/Burgos (Saja)	Picnic, recreative area, farms, urban
	33- Burgos/Álava (Cerneja)	Limestone extraction, trash, drinking water catchment area
	36- Álava/Burgos (Izki)	Picnic, trash
	85- Navarra (Irati)	Urban, agriculture
	86- Navarra (Salazar)	WWTP discharge, agriculture, farms, trash
	123- Navarra/Huesca (Esca)	WWTP discharge, urban, trash
	119- Huesca (Bailín, Gállego)	Area close to landfill of lindane
	121- Huesca (Aragon)	Urban, agriculture, trash
	127- Huesca (Cinqueta)	Urban, agriculture, trash
	129- Huesca (Isabena)	Urban, agriculture
	133- Alt Pallars (Noguera Pallaresa)	Urban, agriculture
	136- Girona (Ter)	WWTP discharge, periurban
	423- Navarra (Urederra)	WWTP discharge, some trash

Habitat	IBAs	Pressures
Mediterranean forest	56- Segovia (Eresma)	Recreational area
	68- Ávila (Tietar)	Livestock, roads
	71- Madrid (Manzanares)	Recreational area, trash
	100- Teruel (Martin)	Urban, marble industry, trash
	143- Lleida/Huesca (Segre)	Recreational area, periurban area, trash
	150- Castellón (Lucena)	WWTP discharge, trash
	174- Murcia (Lucheno)	Picnic area
	184- Ciudad Real/Albacete (Jabalón)	WWTP discharge, dirty water, agriculture, periurban area
	204- Ciudad Real/Toledo (Bullaque)	Recreational area, picnic, livestock, trash
	207- Ciudad Real/Córdoba (Guadalmez)	Dam, livestock, trash
	208- Jaén/Ciudad Real (Jándula)	Trash
	227- Jaén/Granada (Jaen)	Agriculture (olives), trash
	235- Córdoba (Guadiato)	Trash
	243- Cádiz/Málaga (Guadalete)	Dam
	273- Badajoz (dry river)	Pig farm
	291- Badajoz (Gévora)	Trash
	292- Cáceres (Salor)	No pressure
	295- Cáceres (Guardiloba)	Agriculture, WWTP discharge
	298- Cáceres (Porquerizo)	Agriculture (tobacco)
	470- Salamanca (Huebra)	Urban, livestock, trash
Riparian forest	42- Palencia/Valladolid (Pisuerga)	WWTP discharge, trash, road
	47- La Rioja (Iregua)	WWTP discharge, industry, periurban, trash
	53- Segovia (Duraton)	No impact
	55- Segovia (Moros)	Agriculture
	59- Valladolid/Zamora (Duero)	WWTP discharge, periurban, lots of trash
	64- Salamanca (Yeltes)	Urban, picnic, spa
	72- Toledo (Tajo)	Thermal plant, trash
	73- Madrid (Jarama)	WWTP, trash
	99- Teruel (Guadalope)	Mines, trash
	104- Zaragoza (Ebro)	WWTP discharge, close to large city
	108- Zaragoza/Teruel (Matarraña)	WWTP discharge, agriculture (fruit trees), farms
	109- Huesca/Zaragoza (Cinca)	Industrial waste (lindane), trash
	154- Castellón (Mijares)	WWTP discharge, agriculture, trash
	157- Valencia/Cuenca (Turia)	WWTP discharge, picnic, recreational,
	200- Toledo (Tajo)	Industry, sewage grid discharge, periurban, trash
	230- Jaén (Guadalquivir)	WWTP discharge, trash
	232- Córdoba/Jaén (Guadajoz)	Urban, agriculture (olive trees)
	277- Badajoz (Matachel)	Picnic, fishing area
	288- Badajoz (Guadiana)	WWTP discharge, fishing area, trash
	305- Cáceres (Tiétar)	Picnic, swimming pool, trash

Habitat	IBAs	Pressures
Rocky mountain	11- León/Orense (Bibei)	Village, livestock, fire
	14- Asturias/León (Somiedo)	Urban, picnic, sport installation, WWTP discharge, trash
	20- Cantabria/León (Fuente Deva)	WWTP discharge, pine cultivation, trash
	31- Burgos/Álava (Oroncillo)	Urban, agriculture
	46- Soria/Burgos (Duero)	WWTP discharge, livestock, trash
	48- La Rioja (Cidacos)	WWTP discharge, livestock, periurban
	67- Ávila/Salamanca (Tornes)	Fish farm, picnic, camping, trash
	83- Navarra (Arga)	WWTP discharge, village
	118- Huesca (Vero)	Camping, trash
	126- Ordesa (Cinca)	Urban, trash
	128- Posets (Esera)	Urban, trash
	130- Estanys de St. Maurici (Garona)	Trash
	135- Girona/Lleida (Llobregat)	WWTP discharge, livestock, tourist path, trash
	147- Teruel/Castellón (Ulldemo)	WWTP discharge, farms, village
	149- Castellón (Bergantes)	WWTP discharge, livestock, urban, trash
	151- Castellón (Mijares)	Bath area, picnic, sports camp, trash
	172- Murcia (Rambla Cañar)	WWTP discharge
	297- Cáceres (Ruecas)	Dam
	420- León (Torio)	Picnic
	424- Burgos/Cantabria (Gandara)	Direct urban discharges, trash
Inland aquatic	23- Cantabria (Ebro river, Reinosa)	WWTP discharge (dirty water), urban, trash, agricultural waste
	39- Zamora (Salado)	Agriculture, urban and agricultural waste, picnic area, trash
	89- Navarra/Zaragoza (Pitillas lagoon)	Agriculture, horse farm
	95- Teruel, Zaragoza (Gallocanta lagoon)	Trash, tractors, urban area, agriculture, fruit trees
	111- Huesca (rice fields Cinca)	Farms (pig), fruit trees
	165- Alicante (Levante wáter canal)	Agriculture, greenhouses, agricultural waste
	182- Albacete/Murcia (Rambla La-vadero)	WWTP discharge (dirty water), agricultural waste
	188- Guadalajara (Tajuña)	Picnic (dirty), urban area
	192- Cuenca (Zancara)	Agriculture (sunflowers, garlic, grain), urban area, trash
	197- Ciudad Real (Guadiana)	WWTP discharge (dirty water), agriculture, livestock, plastic trash
	231- Córdoba (Cañaveral)	Olive trees
	239- Cordoba/Sevilla (Genil)	Olive trees, fruit trees
	240- Málaga (Rincón creek)	Pig farm, landfill, olive trees, grain
	276- Badajoz (Olivenza)	Agriculture
	282- Badajoz/Ciudad Real (Bohonal)	Trash, urban area
	284- Cáceres (Dam Orellana)	Illegal landfill, lots of trash
	425-Avila (Adaja)	Agriculture (grain, pine trees), livestock
	462- Teruel (Jiloca)	Agriculture, livestock, farms
	466- Murcia (Campotejar lagoon)	WWTP discharge, fruit trees
	476- Albacete (Ontalafia lagoon)	Agriculture, WWTP discharge, industrial pole (wood), trash

Habitat	IBAs	Pressures
Coastal habitats	1- Islas Cies (Lago dos Nenos)	Tourism, past applications of pesticides for eucalyptus treatment
	4- Costa de Morte (San Ameido)	Urban
	7- Asturias/Lugo (Navia)	Paper factory, urban, roads
	18- Asturias/Cantabria (Sella)	WWTP discharge, livestock, periurban, agriculture, trash
	24- Cantabria (Peña)	Roads, trash
	27- Cantabria (Clarín)	WWTP discharge, farms, fish farm, periurban, trash
	35- Vizcaya (Oka)	WWTP discharge, periurban, rural, trash
	137- Girona (Muga)	WWTP discharge, picnic, tourist path, agriculture, periurban, trash
	140- Barcelona (Llobregat)	Airport, Industry, agriculture, trash
	148- Tarragona (llacuna Ebre)	Agriculture (rice fields)
	159- Valencia (Albufera)	Agriculture (rice fields), trash
	166- Alicante (Vinolopo)	Landfill, roads, livestock (sheep), urban, trash
	169- Murcia (Rambla Albujón)	Urban, roads, trash
	224- Málaga (Guadalhorce)	WWTP discharge, roads, industrial, trash
	246- Cádiz (de la Vega)	Periurban, agriculture (grain), trash
	251- Cádiz (San Pedro)	University area, wharf, trash
	259- Huelva/Cadiz (Palmoso, el Rocío)	Greenhouses (strawberry), livestock, trash
	318- S'Albufera Mallorca (Gran Canal)	Tourism
	372- El Medano Canarias (Los Bastianes)	Camping
	464- Alicante (Lagina Clot de Galvany)	WWTP discharge

Figure 1. Examples of sampling areas from IBAs affected by human waste.

3.2. Geographical distribution of contaminants in sediments

A total of 49 out of 52 target compounds studied were detected in 135 out of 140 sediment samples. IBAs not affected by chemical pollution were 55 (Segovia), 90 (Navarra/Zaragoza), 276 (Badajoz), 429 (Zaragoza) and 476 (Albacete). Table 2 lists the contaminants detected in sediments, the number of IBAs affected and detection frequency, the mean, minimum and maximum concentrations. According to the frequency of detection, the chemical families followed the order: PAHs > OCPs> plasticizers and OPEs> PCBs > OPPs. However, plasticizers were by far the chemical family detected at the highest concentrations, probably related to plastic trash. As no specific contamination pattern was observed among IBAs or habitats, the following section indicates the concentrations of the different families of contaminants in IBAs, compares the concentrations with existing sediment guidelines, maps their geographical distribution, and identifies potential contamination sources according to the pressures recorded in each sampling area.

Table 2. Target compounds in sediment samples (n=140) from IBAs ordered by chemical family, indicating number of occurrences (N) and detection frequency (%), mean ± S.E. (Standard Error), minimum and maximum concentrations, and number of samples and percentage (%) exceeding proposed threshold values by sediment guidelines from US, Canada, and Europe. All values expressed in ng/g dw, nd= no detected. aEQS value from Directive 2008/105/EC.

Compounds	N (%)	Mean ± S.E.	Min.	Max.	US EPA guidelines				Canadian guidelines				IKSE (Elbe river basin)				PNEC
					TEC	N (%) > TEC	PEC	N (%) > PEC	ISQG	N (%) > ISQG	PEL	N (%) > PEL	LTV	N (%) > LTV	UTV	N (%) > UTV	
Pyrene (HMW)	109 (78)	27.2 ± 4.1	0.57	283	195	3 (2)	1520	0	53	20 (14)	875	0					110
Phenanthrene (LMW)	107 (76)	13.5 ± 2.1	0.92	181	204	0	1170	0	41.9	9 (6)	515	0					552
Benzo[b]fluoranthene (HMW)	97 (69)	18.2 ± 4.6	0.98	562					-		-						3.52
Fluoranthene (HMW)	90 (64)	26.8 ± 4.2	4.11	425	423	1 (1)	2230	0	111	7 (5)	2355	0	180	4 (3)	250	1 (1)	19.7
Benzo[k]fluoranthene (HMW)	76 (54)	12 ± 2.5	1.54	308					-		-						2.29
Benzo[a]anthracene (HMW)	74 (53)	10.7 ± 1.7	2.82	132	108	1 (1)	1050	0	31.7	16 (11)	385	0					237
Chrysene (HMW)	63 (45)	9.94 ± 1.3	3.89	92.1	166	0	1290	0	57.1	3 (2)	862	0					29.3
Naphthalene (LMW)	58 (41)	21.2 ± 4.0	0.77	309	176	4 (3)	561	0	34.6	23 (16)	391	0					93.5
Indene[123-cd] pyrene (HMW)	55 (39)	11.3 ± 2.7	7.2	317					-		-						28
Benzo[ghi]perylene (HMW)	47 (34)	9.31 ± 2.5	2.58	286					-		-						0.34
Benzo[a]pyrene (HMW)	38 (27)	10.8 ± 2.3	13.8	228	150	1 (1)	1450	0	31.9	15 (11)	782	0	10	53 (38)	600	0	7.47
Fluorene (LMW)	37 (26)	1.86 ± 0.5	0.72	63.5	77.4	0	536	0	21.2	2 (1)	144	0					62.3
Anthracene (LMW)	33 (24)	1.62 ± 0.4	1.43	45.8	57.2	0	845	0	46.9	0	245	0	30	1 (1)	310	0	101
Acenaphthene (LMW)	15 (11)	1.5 ± 0.4	1.58	44					6.71	11 (8)	88.9	0					718
Dibenzo[a,h]anthracene (HMW)	13 (9)	1.19 ± 0.4	1.68	40.1	33	1 (1)	-	0	6.22	9 (6)	135	0					819
Acenaphthylene (LMW)	13 (9)	0.63 ± 0.2	1.95	27.6					20.2	1 (1)	201	0					149
∑PAH	122 (87)	178 ± 26	1.24	2193	1610	2 (1)	22800		-		-						-
4,4'-DDE	85 (61)	1.11 ± 0.3	0.07	31.3	3.16*	11 (8)	31.3*	1 (1)	1.42*	25 (18)	6.75*	3 (2)	0.31	56 (40)	6.8	3 (2)	1.31
4,4'-DDD	46 (33)	0.23 ± 0.1	0.06	6.39	4.88**	3 (2)	28**	0	3.54**	3 (2)	8.51**	1	0.06	45 (32)	3.2	2 (1)	0.4
HCBD	34 (24)	0.38 ± 0.1	0.11	5.75											55[a]		8.61
γ-HCH	26 (19)	0.09 ± 0.03	0.02	2.99	2.37	1 (1)	4.99	0	0.94	2 (1)	1.38	1 (1)	0.5	5 (4)	1.5	1 (1)	1.49
β-HCH	15 (11)	0.05 ± 0.02	0.13	1.55									5	0	5	0	1.49
HCB	10 (7)	0.04 ± 0.02	0.02	2.73									0.0004	10 (7)	17	0	2.13
2,4'-DDD	13 (9)	0.15 ± 0.1	0.25	13	4.88**	3 (2)	28**	0	3.54**	3 (2)	8.51**	1					0.4
4,4'-DDT	4 (3)	0.2 ± 0.1	2.84	14.9	4.16***	4 (3)	62.9***	0	1.19***	5 (4)	4.77***	4 (3)	1	4 (3)	3	1 (1)	100
2,4'-DDT	4 (3)	0.06 ± 0.03	1.37	3.59	4.16***	4 (3)	62.9***	0	1.19***	5 (4)	4.77***	4 (3)					0.04
δ- HCH	3 (2)	0.05 ± 0.03	0.55	3.41													1.49
α-HCH	1 (1)	-	4.64	4.64									0.5	1 (1)	1.5	1 (1)	1.49

Compounds	N (%)	Mean ± S.E.	Min.	Max.	US EPA guidelines				Canadian guidelines				IKSE (Elbe river basin)				PNEC
					TEC	N (%) > TEC	PEC	N (%) > PEC	ISQG	N (%) > ISQG	PEL	N (%) > PEL	LTV	N (%) > LTV	UTV	N (%) > UTV	
2,4'-DDE	6 (4)	0.03 ± 0.02	0.1	2.68	3.16 *	11 (1)	31.3*	1 (1)	1.42*	25 (18)	6.75*	3 (2)					11.5
α-Endosulfan	nd	-	-	-													-
β-Endosulfan	nd	-	-	-													-
∑OCP	95 (68)	2.41 ± 0.4	0.7	37.6													-
NP	53 (38)	1473 ± 268	75.5	20708					1400	40 (29)							87.3
DEP	36 (26)	424 ± 78	585	5584													367
DMP	19 (14)	2.54 ± 0.6	7.42	48.3													685
DBP	18 (13)	18.7 ± 4.5	88.8	324													699
BPA	15 (11)	13 ± 5.4	38.6	692													17.4
DiBP	12 (9)	21 ± 5.9	169	382													77.3
BBZP	9 (6)	3.01 ± 1.5	20.5	197													1353
DEHP	9 (6)	254 ± 122	18.5	14661													5574
∑Plasticizers	60 (43)	2209 ± 391	18.5	30278													
PCB 138	37 (26)	0.42 ± 0.1	0.1	12									1	10 (7)	20	0	144
PCB 180	28 (20)	0.36 ± 0.1	0.11	10.1									0.44	14 (10)	20	0	57.4
PCB 52	21 (15)	0.1 ± 0.05	0.07	7.45									0.1	19 (14)	20	0	139
PCB 101	20 (14)	0.14 ± 0.05	0.07	4.8									0.54	10 (7)	20	0	82.6
PCB 153	14 (10)	0.43 ± 0.1	0.08	15.1									1.5	11 (8)	20	0	87.4
PCB 28	10 (7)	0.23 ± 0.1	0.39	20.2									0.04	10 (7)	20	1 (1)	239
PCB 118	1 (1)	-	4.92	4.92									0.44	1 (1)	20	0	112
∑PCBs	43 (31)	1.72 ± 0.61	0.08	69.6	59.8	1	-	-	34.1	1 (1)	277	0			140	0	
TDCPP	27 (19)	1.45 ± 0.4	1.49	33.5													20.5
TBOEP	12 (9)	7.83 ± 2.4	48.2	196													14729
TDCEP	1 (1)	-	13	13													151
EHDPHP	1 (1)	-	292	292													2.24
∑OPEs	31 (22)	11.5 ± 3.3	1.49	298													
Malathion	2 (1)	0.05 ± 0.03	3.17	3.2													0.08
Chlorpyrifos	1 (1)	-	140	140													0.11
Chlorfenvinphos	nd	-	-	-													1.62
∑OPP	3 (2)	1.05 ± 1	3.17	140													-

(*) Sum of 4,4-DDE and 2,4'-DDE; (**) Sum of 4,4'-DDD and 2,4'-DDD; (***) Sum of 4,4'-DDT and 2,4'-DDT.

3.2.1. PAHs

∑PAHs were the most ubiquitous chemical group, present in 87% of the analysed samples at concentrations ranging from 1.24 to 2193 ng/g. PAHs are relatively persistent compounds and sediments act as their ultimate sink (Mesquita et al., 2014). Because of this, most of the sediment samples were affected by diffuse sources of PAHs. Figure 2a maps the distribution of ∑PAHs according to the <50th, 50-90th and >90th percentile concentrations. The median PAHs concentration across IBAs was of 71.9 ng/g. Sediments with PAHs concentrations exceeding the 90th percentile (from 513 to 2193 ng/g) are highlighted in red and correspond to IBAs with important anthropogenic pressures comprising the area of Valladolid (IBAs 42, 58), central/south Spain including Cáceres (IBAs 295, 473), Ciudad Real (IBAs 184), Toledo (IBA 72) and Huelva (IBA 264). Some northern IBAs with coal mining and industries (13, 23, 46), and Mediterranean coastal IBAs in the Valencia community (IBAs 159, 165, 150) with strong agricultural surface had also a high concentration of PAHs. IBA 109 in the Cinca river close to an industrial landfill had also a high concentration of PAHs.

Pyrene was the most frequently detected PAHs present in 78% of the samples at concentrations from 0.57 to 283 ng/g, followed by phenanthrene found in 76% of the samples ranging from 0.92 to 181 ng/g (Table 2). Other PAHs detected are indicated in table 2. HMW PAHs (pyrene, benzo[b]fluoranthene, fluoranthene, benzo[k]fluoranthene and benzo[a]anthracene) presented a higher prevalence (frequency of detection >50%) than LMW PAHs (Table 2). HMW PAHs are apolar and are more likely retained in sediment organic matter than LMW PAH (Liu et al., 2017). The pattern dominated by HMW PAHs indicate a pyrogenic source attributed to the combustion of organic matter including fossil fuels, coal and forest fires (Marris et al., 2020), also associated to domestic activities (waste incineration, traffic), industry (iron, aluminium and steel production, cement manufacturing, dye manufacturing, asphalt industries, rubber tire manufacturing, exhaust from refineries, power production, coal gasification, electric furnace, oxygen furnace, diesel engine, and gasoline-powered engines of large machineries) (Srogi, 2007) and agriculture (fungicide and insecticide production and use) (Mojiri et al., 2019).

The US EPA considers a sediment polluted by ∑PAHs when the concentrations exceed the PEC value of 22800 ng/g (Table 2), which was not the case in any sample of the present study. Individual PAHs were below the PEC value and only 1-3% of the samples exceeded the TEC values (Table 2). The Canadian ISQGs represent concentrations below which adverse biological effects will rarely occur. Contrary, the PELs indicates the concentrations above which adverse effects frequently occurs can be used as reference to identify potentially high levels of contamination (CCME, 1999a). The ISQGs and PELs available for PAHs are detailed in Table 2. PAHs exceeding the ISQG in more

than 10% of the samples were naphthalene in 23 IBAs, pyrene in 20 IBAs, benzo[a]anthracene in 16 IBAs, and benzo[a]pyrene in 15 IBAs. The PEL values were never surpassed. According to the thresholds applied in the Elbe river basin (IKSE, 2014; Old and Lofts, 2022), some individual PAHs presented values exceeding the LVT but below the UTV, this was the case for 1 sample for anthracene, 4 for fluoranthene, and 53 for benzo[a]pyrene, and only one sample (IBA 159, Albufera de Valencia marshes, Valencia) exceeded the UTV for fluoranthene.

Not much information is available on PAHs in continental river sediments. Previous studies report PAHs in sediments collected along the Ebro river in Spain at mean concentrations of 4.37 to 147 ng/g, with pyrene, chrysene and benzo[a]pyrene being the most ubiquitous (Navarro-Ortega et al., 2010). PAHs were detected in 53 Mediterranean shallow lakes in the NW of Spain surrounded by important agricultural activity with a profile dominated by benzo[a]anthracene, chrysene, fluoranthene, fluorene, naphthalene and phenanthrene detected at 3-20 ng/g, while benzo[a]pyrene, indeno[1,2,3-cd] perylene and benzo[g,h,i]perylene were detected at high concentrations (up to 266 ng/g) but with a very low detection frequency (2-6% of the samples) (Hijosa-Valsero et al., 2016). PAHs are widespread in lake sediments, although often below the SQG (Du and Jing, 2018).

In this study, we have analysed sediments from 20 costal IBAs and revealed the presence of PAHs in 19 coastal IBAs at concentrations ranging from 14.9 to 1753 ng/g, as shown in Figure 2 for the different chemical families. Marine coastal areas suffer multiple anthropogenic pressures (harbours, discharges, industry, aquaculture, etc.) that impact on the quality of sediment. ∑PAHs were detected at 25.9 ng/g to 3777 ng/g in marine sediments from an estuarine river in Vigo (Galicia Atlantic coast, NW Spain), an area affected by the Prestige oil spill in 2002, and the most abundant PAHs were pyrene, indene[c,d]perylene and fluoranthene (Monaco et al., 2017). In Ria Arousa in Pontevedra (NW Spain), ∑PAHs ranged from 11.6 to 30272 ng/g, with a mean value of 3907 ng/g and was associated to intensive mussel cultivation in the area (Pérez-Fernández et al., 2018). ∑PAHs were also detected at mean concentrations of 50.9 and 65.0 ng/g in the Mar Menor lagoon sediments (south east Spain) and a pyrolytic origin was observed except in urban areas or close to main ports where a petrogenic origin prevailed (León et al., 2017). In fact, coastal sediments from urban areas as Barcelona, Valencia, and Tarragona were highly impacted by PAHs (León et al., 2014). PAHs were also detected in coastal sediments collected along the Spanish coasts at mean concentrations from 77 to 4974 ng/g, with the highest levels in Cantabria (north coast) associated to the significant industrial activity, urban development, mining and navigation (León et al., 2020). Rocha and Palma (2019) detected naphthalene and phenanthrene in sediments collected in docks with industrial influence from the Tagus River (Portugal). These PAHs

are associated with a petrogenic sources (Adeniji et al., 2019; Wu et al., 2019).

Despite the little information on PAHs in sediments from protected areas, there is clear evidence on PAHs contamination. ∑PAH in sediment from a Caribbean coastal area ranged from 9 to 1723 ng/g, and gasoline and diesel fuel combustion were the main sources despite being a Ramsar wetland (Balgobin and Ramroop Singh, 2019). PAHs were also widespread in marine protected areas of Latin America and Caribbean comprising 9 countries, with ∑PAHs up to 32203 ng/g (Nunes et al., 2021). The Brisbane river in Australia is a natural area but land-use and urbanization were a main cause of PAHs sediment contamination, with prevalence of HMW PAHs (Liu et al., 2017). Overall, PAH contamination is globally diffused affecting both, urban and pristine areas (Jesus et al., 2022). This widespread contamination was also visualized in Spanish IBAs.

3.2.2. OCPs

∑OCPs pesticides were detected in 68% of the samples at concentrations ranging from 0.07 to 37.6 ng/g, with mean concentration of 2.41 ng/g (Table 2). The most prevalent OCPs were ∑DDTs, found in 61% of the samples ranging from 0.07 to 37.4 ng/g. In Figure 2b, IBAs with ∑DDTs concentrations > 90th percentile (5 to 37.4 ng/g) are highlighted in red, and the most impacted IBAs were IBA 372 in Canary Islands (37.4 ng/g), and IBA 109 in Huesca (34.9 ng/g) in an area affected by industrial and urban waste. Other samples above 90th percentile correspond to northern IBAs (127, 83), near coastal areas (140, 149 and 464), central Spain (42, 58), and central/south Spain (184,197, 237, 239, 288) (Figure 2b). The DDT metabolite 4,4'-DDE was found at 61% of the samples (from 0.07 to 31.3 ng/g) followed by 4,4'-DDD detected in 33% of the samples (from 0.06 to 6.39 ng/g). The concentrations of ∑DDE were found above the TEC values in 11 samples and in 1 (IBA 109, Huesca) above PEC thresholds. Considering the CCME values, 25 IBAs presented higher concentration than the ISQG but the PEL values were surpassed only in 3 IBAs: 109 (Huesca), 237 (Sevilla) and 372 (Canary Islands). The same IBAs also surpassed the UTV for 4,4'-DDE (Table 2). However, 56 IBAs had concentrations higher than the 0.31 ng/g LTV threshold. ∑DDD was not detected above the PEC, and only found above the PEL in IBA 197 (Ciudad Real). 4,4'-DDD exceeded the UTV in IBA 42 (Valladolid) and 372 (Canary Islands). 4,4'-DDT was only detected in 5 samples, specifically in IBAs 42 in Valladolid, 83 in Navarra, 127 in Huesca, 197 in Ciudad Real, and 372 in Canarias, at concentrations from 2.84 to 14.9 ng/g. In all cases, the ∑DDT concentrations exceeded the ISQG value of 1.19 ng/g, and 4 of these samples (IBAs 42, 83, 127, and 372) also surpassed the PEL. Four IBAs surpassed the LTV for 4,4'-DDT and IBA 42 also exceeded the UTV.

The DDT isomer ratio ([DDE+DDD]/DDT) is used to distinguish between recent and historical DDT inputs. Values higher than 0.5 are indicative of historical inputs, whereas values lower than 0.5 are indicative of recent input of DDTs (Hong et al., 1999; Peng et al., 2020). From the 140 samples, only in two IBAs 83 (Navarra) and 127 (Huesca), the calculated ratio was associated to recent uses of DDTs. Additionally, the 2,4'-DDT/4,4'-DDT ratio is employed to differentiate between the technical DDT and or dicofol contamination. Ratios around 7 signify dicofol contamination, while the values 0.2 to 0.3 are more likely to be related with technical DDT use (Peng et al., 2020). In the sediment samples the 2,4'-DDT/4,4'-DDT ratio was <0.3 in IBA 127 and IBA 83, suggesting the presence of technical DDT in both cases. The DDT levels in Ebro river sediment monitored in 2004-2006 ranged from 0.34 to 500 ng/g (Navarro-Ortega et al., 2010) and DDT was detected at higher concentrations than DDE. In our study, DDE was the most prevalent isomer, and maybe this indicates a general lowing trend due to use restrictions and environmental degradation. However, DDTs were detected in coastal sediments of IBA 159 (Albufera de Valencia), indicating past pollution. DDTs have been previously detected in coastal sediments from Spain at concentrations from 0.2 to 2.3 ng/g) (León et al., 2020).

Other OCPs present in sediments from IBAs were HCBD, detected in 24% of the samples at mean concentrations of 0.38 ± 0.08 ng/g (Table 1), with a maximum concentration of 5.75 ng/g in IBA 7 in Ribadeo (Asturias, N Spain) which is a commercial harbour in expansion. Its presence is basically associated with its used in the chemical industry as a solvent for rubber and other polymers and also as a pesticide. HCBD has an EQS of 55 ng/g according to Directive 2008/105/EC but none of the samples surpassed this limit. It was included in the Stockholm convention in 2011 (Stockholm Convention, 2019), and the low concentrations and detection frequency reflects the restriction on its use. The reported concentrations are similar to the ones found in sediments from the Óbidos Lagoon (Portugal) that ranged from 0.3 to 11.1 ng/g (Pinto et al., 2016).

Another important group of OCPs are ∑HCHs detected in 1-19% of the sediments at concentrations ranging from 0.02 to 8.04 ng/g (Table 1). The 90th percentile was of 0.36 ng/g and the highest concentrations were in IBA 119 (Huesca) close to the landfill containing OCPs residues in Sabiñanigo. Other sediments above 90th percentile were in other northern IBAs (13, 20, 85, 142), and central/south Spain (39, 58, 73, 184, 197, 200, 277, 295, 473) (Figure 2c). γ-HCH (lindane) was the most abundant HCHs, detected in 19% of the samples at concentrations ranging from 0.02 to 2.99 ng/g and only IBAs 13 (Asturias, Leon) and 197 (Ciudad Real) surpassed the TEC value of 2.37 ng/g and the ISQG value of 0.94 ng/g, and the latter also exceeded the PEL of 1.38 ng/g. Five samples had concentrations higher than LTV and IBA 197 was also the only site with levels above the UTV for γ-HCH and α-HCH (Table 2). Other

HCHs were sporadically detected and at trace concentrations as β-HCH was detected at 11% of the samples at concentrations ranging from 0.13 to 1.55 ng/g and δ- HCH and α-HCH were only detected at 2 and 1% of the samples, respectively. Only IKSE guidelines are available for these isomers (Table 2).

Finally, HCB was detected in 10 IBAs at mean concentrations of 0.04 ng/g, in all of them surpassing the LTV, but below the UTV of 17 ng/g (Table 2) or the EQS of 10 ng/g proposed in Directive 2008/105/EC. Endosulfans were never detected (Table 2).

3.2.3. Plasticizers

∑Plasticizers were detected in 60 IBAs (43% detection frequency) at concentrations ranging from 18.5 to 30278 ng/g (mean of 2209 ng/g). The distribution of plasticizers in IBAs is represented in figure 2D and sites with concentrations > 90th percentile (7043 ng/g) corresponded to IBAs with the highest urban pressure as detailed in Table 1. The most impacted were IBA 184 (Ciudad Real/Albacete, ∑plasticizers 30278 ng/g), affected by WWTP discharges, agriculture, and urban pressure, also the water was visibly of poor quality (Table 1), IBA 259 (Huelva/Cadiz, ∑plasticizers 22915 ng/g) affected by intensive strawberries cultivation and livestock, and trash residues (Table 1) and IBA 35 (Vizcaya, ∑plasticizers 17893 ng/g) affected by urban pressure, WWTP discharge and trash (Table 1). Other sediments with levels above 90th percentile corresponded to sites with trash at the sampling area such as IBAs 192, 202, 71, 149, 165, 424, and 264. Also, the high levels of plasticizers were found in sediments impacted by WWTPs discharges which was the case of IBAs 13,149, and 295. Concentrations above 90th percentile were also found in sediments next to picnic area (IBA 22, Cantabria/Burgos) or also close to urban areas and roads (IBA 7, Asturias) (Figure 2D).

NP was the compound detected at the highest concentrations, ranging from 75.5 to 20708 ng/g in 53 samples, from which 40 exceeded the ISQG values (Table 2). No other guidelines are reported. These levels are much higher than those reported in Spanish coastal sediment that ranged from 62-229 ng/g (León et al., 2020). NP is associated to the degradation of non-ionic surfactants in industrial and household applications, it has been found to be ubiquitous in freshwaters ecosystems worldwide as it is a high-volume production compound and an estimated half-life in sediments of more than 60 years (Bhandari et al., 2021). Therefore, despite use restrictions due to its endocrine disrupting effects (Directive 2003/53/EC), NP is still ubiquitous in sediments.

Regarding phthalates, DEP was detected in 26% of the samples at concentrations ranging from 585 to 5584 ng/g. DEP is used as cleaning products and household care and laundry and fabric treatment and in dry

cleaners, and is a main compound released by WWTPs (Bai et al., 2022). Other phthalates detected were DMP, DBP, DiBP, BBzP and DEHP detected in 6 to 14% of the samples at mean concentrations from 2.54 to 14661 ng/g, with DEHP the compound detected at the highest concentrations among phthalates (mean 254 ng/g). Phthalates are slowly degraded in sediment, and measured sediment half-lives for DBP, BBzP, DEHP and DNOP were reported to be 46, 2.9, 347 and 173 days, respectively, while DEHP exceeds 6 months (Kickham et al., 2012). No sediment guidelines are available for phthalates.

In Spain, phthalates (mainly DEP) have been reported in continental shelf sediments at mean concentrations from 400 to 2100 ng/g (León et al., 2020) and in Mar Menor from 72 to 3481 ng/g (Concha-Graña et al., 2021), and some studies indicate that phthalates also impact relatively pristine areas. In Italy, DEHP, BBP, DnBP, and DEP were recurrently detected in sediments from Lambro river, a tributary of the Po river, at mean concentrations ranging from 30.3 to 188 ng/g as a result of 50 years of chemical contamination discharged to the river, and in some sites exceeding the EU guideline values (Viganò et al., 2023). A recent review records the concentrations of 21 phthalates in river and costal sediments all around the world and indicate that DEHP, DnBP and DiBP, together with DMP and DEP as the main compounds with concentrations varying largely, but often reaching concentrations of 10000-15000 ng/g (Hidalgo-Serrano et al., 2022). WWTP effluents are a source of phthalates in river or coastal sediments (Vitali et al., 1997). They have been detected in sediments from southern Jiangsu Province (China) ranged from 2300 to 80100 ng/g, with mean concentration of 27800 ng/g in industrial areas, 8800 ng/g in urban and 3400 ng/g in agricultural, being DEHP and di-n-butyl phthalate (DnBP) the dominant compounds present in all samples (Wang et al., 2014). Similar results were obtained in coastal sediments from the Persian Gulf where significant higher concentration were detected in areas affected by industries or located near agricultural fields than in urban and natural areas, attributed to the use of plastic films in agricultures (e.g. for greenhouses, plastic mulching, fertilisers and packaging) and to the fact that the water used for irrigation usually comes from heavily polluted rivers located nearby (Arfaeinia et al., 2019). Urban land-use was also identified as an important source of phthalates in sediments from Australia, where DEHP was detected at mean concentrations of 1680 ng/g (maximum of 14000 ng/g) with a hazard quotient higher than 1, indicating potential high risk (Sardiña et al., 2019). However, the occurrence and impact of phthalates in sediments from IBAs or protected areas is largely unexplored and thus potential risks cannot be assessed.

3.2.4. PCBs

∑PCBs were detected at 43% of sediment samples at concentrations ranging from 0.08 to 69.6 ng/g. As it is detailed in Figure 2E, the 90th percentile of ∑PCBs is of 1.55 ng/g, indicating a generally low contamination of PCBs among all IBAs sediments. Individual PCBs were detected above the LTV from 1-19 IBAs (no EPA or Canadian guidelines available). The highest ∑PCBs concentration detected in IBA 73 (Jarama river, Madrid) surpassed the TEC and ISQG values for ∑PCBs, and was the only sample detected above the UTV for PCB 28 (Table 2). This river receives historical industrial drainage and fish population are affected by PCBs contamination (Nicola et al., 2014). Other sediments with ∑PCBs concentrations above 90th percentile corresponded to IBAs from north Spain (23, 35, 83), and northern-east areas (140, 149), and central Spain (42, 58, 72,197, 200, 282, 295, 473). In IBAs sediment, the profile was dominated by high chlorinated PCBs 138, 180 detected in 26 and 20% of the sediments at mean concentrations of 0.42 and 0.36 ng/g, and other PCBs were less prevalent and ranged from 0.07 to 4.92 ng/g.

Following the Stockholm Convention on POPs restrictions and production bans, the sediment concentration of PCBs have decreased compared to the late 1960 when PCBs were widely used in many industrial applications due to their insulating and fire-retardant properties (Ross, 2004; Vane et al., 2020). Still, PCBs residues are being detected in sediments worldwide (Avellan et al., 2022), including IBAs from Spain.

3.2.5. OPEs

Sediment is a major reservoir for OPEs which have been detected worldwide at concentrations that range from a few tens to hundreds of ng/g depending on the study area, and chlorinated OPEs are often the most frequently detected (Bekele et al., 2021). However, these compounds are likely to biodegrade and thus, the accumulation in sediment is low (Castro-Jiménez et al., 2022). Correspondingly, ∑OPEs were detected in 22% of sediments at levels from 1.49 to 298 ng/g, and tris(1,3-dichloro-2-propyl) phosphate (TDCPP) was the most prevalent compound detected in 19% of the samples at concentrations from 1.49 to 33.5 ng/g. TDCPP is widespread in the environment due to its massive use as a flame retardant but is a moderately polar and mobile compound in water with less affinity to sorb to sediment particles (Wang et al., 2020).

Compared to coastal sediments (Alkan et al., 2021), oceans (Xie et al., 2022) or very industrialized rivers (Giulivo et al., 2017), there is little information on OPEs occurrence in river sediments or river sediments from protected areas. OPEs have been reported in sediments from 3 rivers in Spain at concentrations between 153 and 824 ng/g in the industrialized and urbanized

Besos river basin, and 10 times lower in pristine sediments from the Nalon and Arga rivers, although no risk was observed (Cristale et al., 2013). The Guadalhorce River (166 km length and a basin of 3175 km^2) is located in the south of the Iberian Peninsula (Spain) and is affected by WWTP discharges, agriculture, cattle, industrial state and gas stations and OPEs were detected in surface and groundwater, and although sediments were not analysed, they presumably leached to aquifers (Llamas-Dios et al., 2021). A first study on OPEs in sediments from the Great Lakes in the United States averaged Σ14OPEs of 2.2, 4.7, and 16.6 ng/g in Lakes Superior, Michigan, and Ontario, respectively, and concentrations of chlorinated OPEs were estimated to double in 20 years and become a secondary source of contaminants to the water column (Cao et al., 2017). In the Qi'ao Island Mangrove Nature Reserve, in the Pearl river estuary in China, concentrations ranged from 23.5 to 187 ng/g, with TDCPP as main contaminant, and according to biota-sediment accumulation factor larger than 1, OPEs bioaccumulated in mangrove animals and translocated in plants, posing this area of ecological value at risk (Xie et al., 2022). As other contaminants, WWTP have been identified as a source of OPEs to river waters (Pantelaki and Voutsa, 2019) and these compounds can be accumulated in organisms and exert an effect (Pantelaki and Voutsa, 2020). Still, little information is available on the presence of these still unregulated contaminants in wildlife and the risk they may pose on biodiversity.

3.2.6. OPPs

Malathion and chlorpyrifos were detected only in 3 sediments at levels from 3.17 to 140 ng/g, and chlorfenvinphos was never detected. The low detection frequency is because either they were not applied or were degraded in the water-sediment system. Wei et al., (2021) reported fast degradation rates of OPPs in surface sediment compared to waterbodies, which is consistent with the low detection frequency of OPPs in sediment compared to the residues detected in water samples from IBAs (Dulsat-Masvidal et al., 2023). In Europe, chlorpyrifos was banned in 2020 (European Commission, 2020) and malathion is only allowed in greenhouse applications (European Commission, 2023), although many other countries worldwide continue to allow their use (FAO, 2022). However, chlorpyrifos was recurrently detected at 0.05 to 149.4 ng/g TOC in sediments from a protected area in northeast Argentina, reflecting its present use (Rolón et al., 2021).

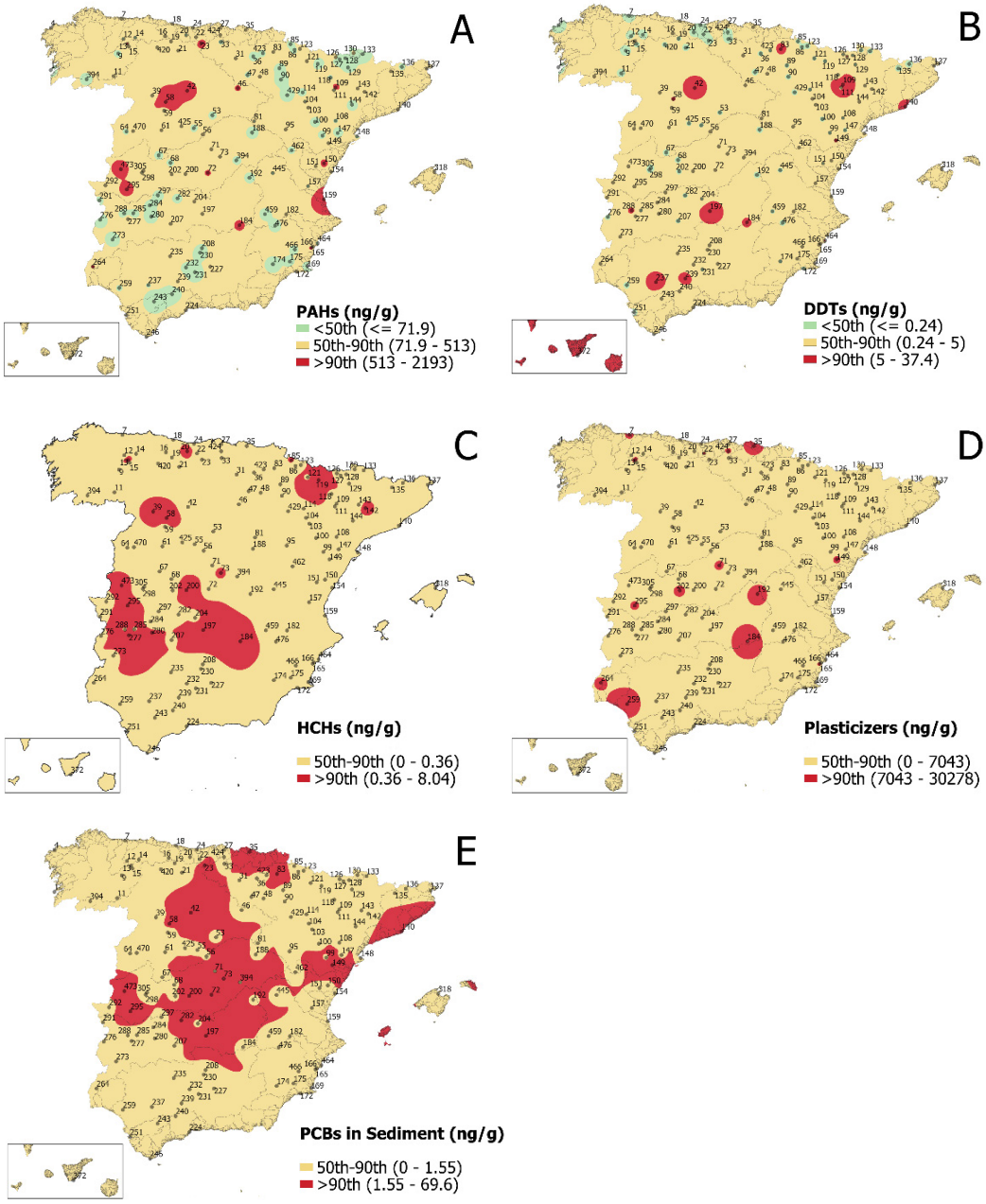

Figure 2. Maps of interpolated data showing the distribution of the different chemical families in sediments from Spanish IBAs: (A) PAHs, (B) DDTs, (C) HCHs, (D) plasticizers, (E) PCBs. Gradient colours according to the 10th, 50th and 90th percentiles of concentration data (ng/g).

4. ENVIRONMENTAL RISK ASSESSMENT OF CONTAMINANTS IN IBAS

There is a lack of harmonization among sediment guidelines regarding chemical included and concentration thresholds. For this reason, to identify the potential concentrations of risk in sediments, the Environmental Risk Assessment was performed using PNEC values from NORMAN database, which allowed the obtention of a standardized database for all target compounds (Table 2). We adopted the NORMAN guidelines as includes comprehensive values for freshwater sediments for all target compounds. Moreover, the same database was used in the risk assessment for freshwater in (Dulsat-Masvidal et al., 2023) (Chapter II). The RQ for each compound and IBAs are represented as heatmap in Figure 3. From the 140 IBAs, only 22 were found classified as no risk and 31 presented low risk concentrations considering the ∑risks <10. Following, 64 IBAs were classified as medium risk for presenting total sums of RQs between 10 and 100. And finally, 23 IBAs were identified of high-risk due to the ∑RQs above 100. As can be observed To identify concerning compounds in sediment samples, the frequency of detection and concentrations detected are considered.

Figure 4 plots results of PCA considering the contaminants detected and their concentrations, frequency of detection in IBAs and RQs higher than 1. PCA explained the 87.7% of the total variance. PC1 explained 58.1% of the variance and distinguished most prevalent compounds (high concentrations, detection frequency or RQs), compared to those seldom detected. PC2 explained 29.9% of the variance and separated those compounds that presented a high occurrence and a high RQs, mainly HMW PAHs and 4,4-DDE (top left quadrant) and compounds that were detected at the highest concentrations (bottom left quadrant), basically NP, DEP and DEHP.

Figure 3. Heatmap indicating the Risk Quotient (RQ) values for each target compound and in the 140 analysed sediment , indicated with IBA codes. The samples are ordered by the ∑Risk Quotient in each IBAs, classified from high risk to no risk.

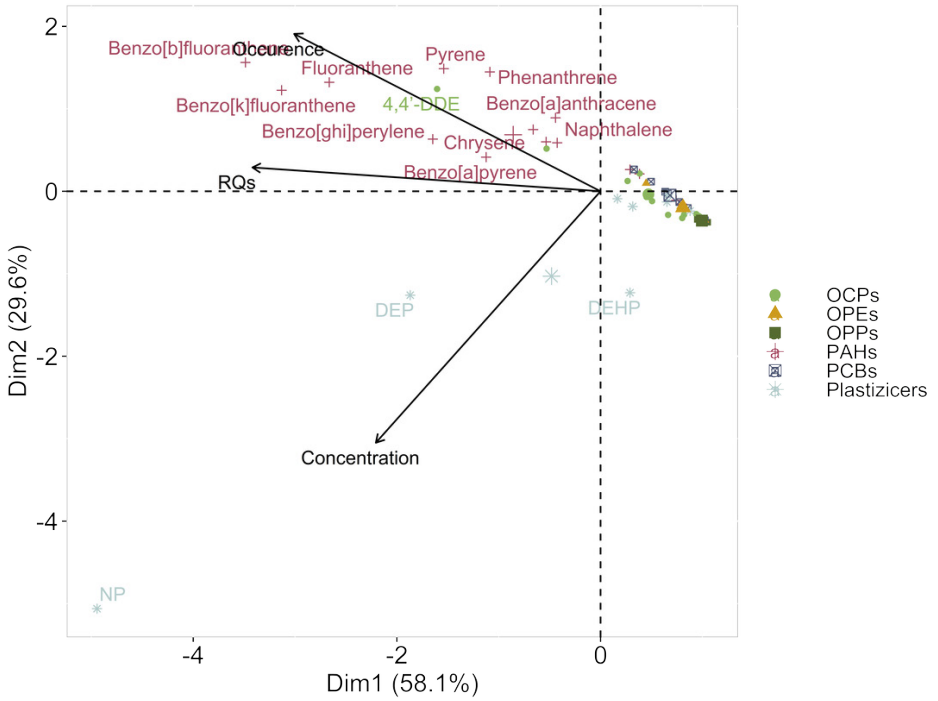

Figure 4. PCA identifying the most concerning compounds detected in IBAs sediments

Considering the variables of RQ, detection frequency and compounds detected at the highest concentrations, the impact of contaminants on freshwaters ecosystems may differ. First, sediments represent a primary route of exposure for infaunal and epibenthic species. Organic micropollutants detected at risk concentrations can interfere with the endocrine system of animals including fishes, amphibians, birds, and humans, affecting their development, growth, reproduction, and behaviour (Yan et al. 2010), and ultimately leading to population declines and habitat degradation. As an example, a negative relationship between the mussel population density and the PAHs tissue concentrations in Clinch River (USA), a biodiversity hotspot area has been reported (Cope et al., 2021). A decline of 67% of the abundance of freshwater mussels in Portugal in the past 20 years was related to the bad conservation of freshwater ecosystems (Lopes-Lima et al., 2023). Likewise, an alarming decline in freshwater species, including mussels and fish, have been observed in KBA from Spain (Nogueira et al., 2021). Moreover, benthic, and aquatic organisms constitute an important part of the freshwater trophic network and provide food for organisms from higher trophic levels, such as fish, birds, and mammals. As persistent contaminants biomagnify through the food web, high concentrations are detected in top predators, posing their wellbeing at risk (Goutte et al., 2020).

Compounds detected at high concentrations or those having a high detection frequency imply that either they are punctually discharged to the ecosystems or are either contaminants widely dispersed in the ecosystems from diffuse contamination sources. The former can be easily detected through sediment monitoring and actions to minimize their release can be implemented. Ubiquitous contaminants present in sediments impact the aquatic ecosystem health both by chronic exposure and long-term effects to organisms, yet unknown, or by affecting biodiversity and ecosystem services. Because of the complexity of controlling diffuse pollution sources, and the lack of sediment EQS, integrated management actions and regulatory measures would be needed to tackle sediment pollution.

This present study highlights the multiple anthropogenic pressures that affects the sediment quality of IBAs, which is an important resource for the conservation of freshwater ecosystems. These results, together with two recent studies in Spanish IBAs regarding water pollution due to the presence of pharmaceuticals, perfluoroalkyl substances, pesticides and plasticizers (Chapter II) and soil pollution by OCPS and PAHs (Chapter III) point to a poor conservation status of IBAs due to chemical pollution.

5. CONCLUSIONS

IBAs are natural areas of biodiversity importance but receive the impact of multiple human activities which include urbanization, WWTPs and the improper disposal of residues, that produce the discharge of trash and contaminants to the environment. Sediments were impacted by chemical pollution which can deteriorate the sediment quality. PAHs were the most ubiquitous chemical family detected in in 87% of the IBAs at concentrations ranging from 1.24 to 2193 ng/g. PAHs also corresponded to the compounds with a higher number of values exceeding toxicity thresholds of ISQG but also PNEC values. OCPs were detected in 68% of the sediment samples, and legacy DDTs were the most prevalent family detected in 61% of the samples at concentrations up to 37.4 ng/g, often exceeding sediment quality guidelines. Plasticizers were the chemical group detected at the highest concentrations, and their presence was associated to the improper disposal of trash and WWTP discharges at the studied IBAs. PCBs were detected in 31% of the samples, but at low concentrations indicating that their presence is mostly associated with historical pollution. Finally, OPEs and OPPs were the least detected chemical families, most probably associated with their degradation in the water-sediment system.

The present study highlights the impact of organic micropollutants in sediments from natural areas in Spain, providing evidence of the strong anthropogenic pressure that the freshwaters ecosystems are suffering,

and the importance to implement monitoring programs to evaluate the risk of contaminants in areas of great ecological interest. The knowledge and data acquired in this study stress out the need to mitigate the presence of trash and chemical pollution in these highly ecological value areas, and new strategies for the management and conservation of natural spaces are needed to preserve biodiversity.

REFERENCES

Adeniji, A.O., Okoh, O.O., Okoh, A.I., 2019. Levels of Polycyclic Aromatic Hydrocarbons in the Water and Sediment of Buffalo River Estuary, South Africa and Their Health Risk Assessment. Arch. Environ. Contam. Toxicol. 76, 657–669. https://doi.org/10.1007/s00244-019-00617-w

Alkan, N., Alkan, A., Castro-Jiménez, J., Royer, F., Papillon, L., Ourgaud, M., Sempéré, R., 2021. Environmental occurrence of phthalate and organophosphate esters in sediments across the Gulf of Lion (NW Mediterranean Sea). Sci. Total Environ. 760, 143412. https://doi.org/https://doi.org/10.1016/j.scitotenv.2020.143412

Arfaeinia, H., Fazlzadeh, M., Taghizadeh, F., Saeedi, R., Spitz, J., Dobaradaran, S., 2019. Phthalate acid esters (PAEs) accumulation in coastal sediments from regions with different land use configuration along the Persian Gulf. Ecotoxicol. Environ. Saf. 169, 496–506. https://doi.org/https://doi.org/10.1016/j.ecoenv.2018.11.033

Avellan, A., Duarte, A., Rocha-Santos, T., 2022. Organic contaminants in marine sediments and seawater: A review for drawing environmental diagnostics and searching for informative predictors. Sci. Total Environ. 808, 152012. https://doi.org/https://doi.org/10.1016/j.scitotenv.2021.152012

Azcune, G., Griffero, L., Pareja, L., Ríos, J.M., Galbán-Malagón, C., Pérez-Parada, A., 2022. Trends in the monitoring of legacy and emerging organic pollutants in protected areas. Trends Environ. Anal. Chem. 34, e00165. https://doi.org/https://doi.org/10.1016/j.teac.2022.e00165

Bai, L., Dong, X., Wang, F., Ding, X., Diao, Z., Chen, D., 2022. A review on the removal of phthalate acid esters in wastewater treatment plants: from the conventional wastewater treatment to combined processes. Environ. Sci. Pollut. Res. 29, 51339–51353. https://doi.org/10.1007/s11356-022-20977-6

Balgobin, A., Ramroop Singh, N., 2019. Source apportionment and seasonal cancer risk of polycyclic aromatic hydrocarbons of sediments in a multi-use coastal environment containing a Ramsar wetland, for a Caribbean island. Sci. Total Environ. 664, 474–486. https://doi.org/https://doi.org/10.1016/j.scitotenv.2019.02.031

Bekele, T.G., Zhao, H., Yang, J., Chegen, R.G., Chen, J., Mekonen, S., Qadeer, A., 2021. A review of environmental occurrence, analysis, bioaccumulation, and toxicity of organophosphate esters. Environ. Sci. Pollut. Res. 28, 49507–49528. https://doi.org/10.1007/s11356-021-15861-8

Bhandari, G., Bagheri, A.R., Bhatt, P., Bilal, M., 2021. Occurrence, potential ecological risks, and degradation of endocrine disrupter, nonylphenol, from the aqueous environment. Chemosphere 275, 130013. https://doi.org/https://doi.org/10.1016/j.chemosphere.2021.130013

BirdLife International, 2023. Data Zone [WWW Document]. URL http://www.birdlife.org/datazone (accessed 7.11.23).

BirdLife International, 2020. Important Bird and Biodiversity Areas (IBAs) [WWW Document]. URL https://www.birdlife.org/worldwide/programme-additional-info/important-bird-and-biodiversity-areas-ibas. (accessed 4.20.20).

BirdLife International, 2017. Pollution from agriculture, forestry and industry has significant impacts on birds [WWW Document]. URL http://datazone.birdlife.org/sowb/casestudy/pollution-from-agriculture-forestry-and-industry-has-significant-impacts-on-birds (accessed 4.29.20).

Bonmatin, J.-M., Noome, D.A., Moreno, H., Mitchell, E.A.D., Glauser, G., Soumana, O.S., Bijleveld van Lexmond, M., Sánchez-Bayo, F., 2019. A survey and risk assessment of neonicotinoids in water, soil and sediments of Belize. Environ. Pollut. 249, 949–958. https://doi.org/https://doi.org/10.1016/j.envpol.2019.03.099

Brils, J., 2008. Sediment monitoring and the European Water Framework Directive. Ann. Ist. Super. Sanita 44, 218–223.

Buah-Kwofie, A., Humphries, M.S., 2017. The distribution of organochlorine pesticides in sediments from iSimangaliso Wetland Park: Ecological risks and implications for conservation in a biodiversity hotspot. Environ. Pollut. 229, 715–723. https://doi.org/https://doi.org/10.1016/j.envpol.2017.07.031

Cao, D., Guo, J., Wang, Y., Li, Z., Liang, K., Corcoran, M.B., Hosseini, S., Bonina, S.M.C., Rockne, K.J., Sturchio, N.C., Giesy, J.P., Liu, J., Li, A., Jiang, G., 2017. Organophosphate Esters in Sediment of the Great Lakes. Environ. Sci. Technol. 51, 1441–1449. https://doi.org/10.1021/acs.est.6b05484

CCME, 2002. Canadian sediment quality guidelines for the protection of aquatic life: Nonylphenol and its ethoxylates. Winnipeg.

CCME, 2001. Canadian sediment quality guidelines for the protection of aquatic life: Polychlorinated biphenyls (PCBs). Winnipeg.

CCME, 1999a. Canadian sediment quality guidelines for the protection of aquatic life: Polycyclic aromatic hydrocarbons (PAHs). Winnipeg.

CCME, 1999b. Canadian sediment quality guidelines for the protection of aquatic life: Lindane (hexachlorocyclohexane). Winnipeg.

CCME, 1999c. Canadian sediment quality guidelines for the protection of aquatic life: DDT, DDE, and DDD. Winnipeg.

Concha-Graña, E., Moscoso-Pérez, C., Fernández-González, V., López-Mahía, P., Gago, J., León, V.M., Muniategui-Lorenzo, S., 2021. Phthalates, organotin compounds and per-polyfluoroalkyl substances in semiconfined areas of the Spanish coast: Occurrence, sources and risk assessment. Sci. Total Environ. 780, 146450. https://doi.org/https://doi.org/10.1016/j.scitotenv.2021.146450

Cope, W.G., Bergeron, C.M., Archambault, J.M., Jones, J.W., Beaty, B., Lazaro, P.R., Shea, D., Callihan, J.L., Rogers, J.J., 2021. Understanding the influence of multiple pollutant stressors on the decline of freshwater mussels in a biodiversity hotspot. Sci. Total Environ. 773, 144757. https://doi.org/10.1016/j.scitotenv.2020.144757

Cristale, J., García Vázquez, A., Barata, C., Lacorte, S., 2013. Priority and emerging flame retardants in rivers: Occurrence in water and sediment, Daphnia magna toxicity and risk assessment. Environ. Int. 59, 232–243. https://doi.org/10.1016/J.ENVINT.2013.06.011

Directive 2003/53/EC of The European Parliment And Of The Council of 18 June 2003 amending for the 26th time Council Directive 76/769/EEC relating to restrictions on the marketing and use of certain dangerous substances and preparations, 2003.

Directive 2008/105/EC of the European Parliament and of the Council of 16 December 2008 on environmental quality standards in the field of water policy, amending and subsequently repealing Council Directives 82/176/EEC, 83/513/EEC, 84/156/EEC, 84/491/EEC, 2008.

Donald, P.F., Fishpool, L.D.C., Ajagbe, A., Bennun, L.A., Bunting, G., Burfield, I.J., Butchart, S.H.M., Capellan, S., Crosby, M.J., Dias, M.P., Diaz, D., Evans, M.I., Grimmett, R., Heath, M., Jones, V.R., Lascelles, B.G., Merriman, J.C., O'brien, M., Ramírez, I., Waliczky, Z., Wege, D.C., 2019. Important Bird and Biodiversity Areas (IBAs): the development and characteristics of a global inventory of key sites for biodiversity. Bird Conserv. Int. 29, 177–198. https://doi.org/10.1017/S0959270918000102

Du, J., Jing, C., 2018. Anthropogenic PAHs in lake sediments: a literature review (2002–2018). Environ. Sci. Process. Impacts 20, 1649–1666. https://doi.org/10.1039/C8EM00195B

Dulsat-Masvidal, M., Ciudad, C., Infante, O., Mateo, R., Lacorte, S., 2023. Water pollution threats in important bird and biodiversity areas from Spain. J. Hazard. Mater. 448, 130938. https://doi.org/https://doi.org/10.1016/j.jhazmat.2023.130938

EEA, 2021. Conservation status of habitats under the EU Habitats Directive [WWW Document]. URL https://www.eea.europa.eu/ims/conservation-status-of-habitats-under (accessed 9.11.23).

EU, 2022. EU at COP15 global biodiversity conference [WWW Document]. URL https://environment.ec.europa.eu/topics/nature-and-biodiversity/eu-cop15-global-biodiversity-conference_en (accessed 9.11.23).

European Commission, 2023. Active substance: Malathion [WWW Document]. URL https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/start/screen/active-substances/details/273 (accessed 4.22.23).

European Commission, 2020. Commission implementing regulation (EU) 2020/18 of 10 January 2020 concerning the non-renewal of the approval of the active substance chlorpyrifos, in accordance with Regulation (EC) No 1107/2009. Off. J. Eur. Union L 7/14.

FAO, 2022. Pesticides and environmental incidents: Rotterdam Convention on the prior informed consent procedure for certain hazardous chemicals and pesticides in international trade. Rome, Italy. https://doi.org/https://doi.org/10.4060/cb6761en

Giulivo, M., Capri, E., Kalogianni, E., Milacic, R., Majone, B., Ferrari, F., Eljarrat, E., Barceló, D., 2017. Occurrence of halogenated and organophosphate flame retardants in sediment and fish samples from three European river basins. Sci. Total Environ. 586, 782–791. https://doi.org/https://doi.org/10.1016/j.scitotenv.2017.02.056

Goutte, A., Alliot, F., Budzinski, H., Simonnet-Laprade, C., Santos, R., Lachaux, V., Maciejewski, K., Le Menach, K., Labadie, P., 2020. Trophic transfer of micropollutants and their metabolites in an urban riverine food web. Environ. Sci. Technol. 54, 8043–8050.

Hidalgo-Serrano, M., Borrull, F., Marcé, R.M., Pocurull, E., 2022. Phthalate esters in marine ecosystems: Analytical methods, occurrence and distribution. TrAC Trends Anal. Chem. 151, 116598. https://doi.org/https://doi.org/10.1016/j.trac.2022.116598

Hijosa-Valsero, M., Bécares, E., Fernández-Aláez, C., Fernández-Aláez, M., Mayo, R., Jiménez, J.J., 2016. Chemical pollution in inland shallow lakes in the Mediterranean region (NW Spain): PAHs, insecticides and herbicides in water and sediments. Sci. Total Environ. 544, 797–810. https://doi.org/https://doi.org/10.1016/j.scitotenv.2015.11.160

Hong, H., Chen, W., Xu, L., Wang, X., Zhang, L., 1999. Distribution and fate of organochlorine pollutants in the Pearl River Estuary. Mar. Pollut. Bull. 39, 376–382. https://doi.org/10.1016/S0025-326X(99)00081-8

IKSE, 2014. Sedimentmanagementkonzept der IKSE - Vorschläge für eine gute Sedimentmanagementpraxis im Elbegebiet zur Erreichung überregionaler Handlungsziele.

Ingersoll, G.C., MacDonald, D.D., 2002. A Guidance Manual to Support the Assessment of Contaminated Sediments in Freshwater Ecosystems Volume III – Interpretation of the Results of Sediment Quality Investigations. Chicago.

Jesus, F., Pereira, J.L., Campos, I., Santos, M., Ré, A., Keizer, J., Nogueira, A., Gonçalves, F.J.M., Abrantes, N., Serpa, D., 2022. A review on polycyclic aromatic hydrocarbons distribution in freshwater ecosystems and their toxicity to benthic fauna. Sci. Total Environ. 820, 153282. https://doi.org/https://doi.org/10.1016/j.scitotenv.2022.153282

Kickham, P., Otton, S.V., Moore, M.M., Ikonomou, M.G., Gobas, F.A.P.C., 2012. Relationship between biodegradation and sorption of phthalate esters and their metabolites in natural sediments. Environ. Toxicol. Chem. 31, 1730–1737. https://doi.org/10.1002/etc.1903

León, V., Viñas, L., Concha-Graña, E., Fernández-González, V., Salgueiro-González, N., Moscoso-Pérez, C., Muniategui-Lorenzo, S., Campillo, J.A., 2020. Identification of contaminants of emerging concern with potential environmental risk in Spanish continental shelf sediments. Sci. Total Environ. 742, 140505. https://doi.org/https://doi.org/10.1016/j.scitotenv.2020.140505

León, V.M., García, I., Martínez-Gómez, C., Campillo, J.A., Benedicto, J., 2014. Heterogeneous distribution of polycyclic aromatic hydrocarbons in surface sediments and red mullet along the Spanish Mediterranean coast. Mar. Pollut. Bull. 87, 352–363. https://doi.org/https://doi.org/10.1016/j.marpolbul.2014.07.049

León, V.M., Moreno-González, R., García, V., Campillo, J.A., 2017. Impact of flash flood events on the distribution of organic pollutants in surface sediments from a Mediterranean coastal lagoon (Mar Menor, SE Spain). Environ. Sci. Pollut. Res. 24, 4284–4300. https://doi.org/10.1007/s11356-015-4628-y

Liu, A., Duodu, G.O., Mummullage, S., Ayoko, G.A., Goonetilleke, A., 2017. Hierarchy of factors which influence polycyclic aromatic hydrocarbons (PAHs) distribution in river sediments. Environ. Pollut. 223, 81–89. https://doi.org/https://doi.org/10.1016/j.envpol.2016.12.034

Llamas-Dios, M.I., Vadillo, I., Jiménez-Gavilán, P., Candela, L., Corada-Fernández, C., 2021. Assessment of a wide array of contaminants of emerging concern in a Mediterranean water basin (Guadalhorce river, Spain): Motivations for an improvement of water management and pollutants surveillance. Sci. Total Environ. 788, 147822. https://doi.org/https://doi.org/10.1016/j.scitotenv.2021.147822

Long, E.R., MacDonald, D.D., 1998. Recommended uses of empirically derived, sediment quality guidelines for marine and estuarine ecosystems. Hum. Ecol. Risk Assess. 4, 1019–1039.

Lopes-Lima, M., Reis, J., Alvarez, M.G., Anastácio, P.M., Banha, F., Beja, P., Castro, P., Gama, M., Gil, M.G., Gomes-dos-Santos, A., Miranda, F., Nogueira, J.G., Sousa, R., Teixeira, A., Varandas, S., Froufe, E., 2023. The silent extinction of freshwater mussels in Portugal. Biol. Conserv. 285, 110244. https://doi.org/https://doi.org/10.1016/j.biocon.2023.110244

MacDonald, D.D., Ingersoll, C.G., Berger, T.A., 2000. Development and evaluation of consensus-based sediment quality guidelines for freshwater ecosystems. Arch. Environ. Contam. Toxicol. 39, 20–31.

Marris, C.R., Kompella, S.N., Miller, M.R., Incardona, J.P., Brette, F., Hancox, J.C., Sørhus, E., Shiels, H.A., 2020. Polyaromatic hydrocarbons in pollution: a heart-breaking matter. J. Physiol. 598, 227–247. https://doi.org/10.1113/JP278885

Mesquita, S.R., L. van Drooge, B., Barata, C., Vieira, N., Guimarães, L., Piña, B., 2014. Toxicity of atmospheric particle-bound PAHs: an environmental perspective. Environ. Sci. Pollut. Res. 21, 11623–11633. https://doi.org/10.1007/s11356-014-2628-y

Mojiri, A., Zhou, J.L., Ohashi, A., Ozaki, N., Kindaichi, T., 2019. Comprehensive review of polycyclic aromatic hydrocarbons in water sources, their effects and treatments. Sci. Total Environ. 696, 133971. https://doi.org/10.1016/j.scitotenv.2019.133971

Monaco, D., Chianese, E., Riccio, A., Delgado-Sanchez, A., Lacorte, S., 2017. Spatial distribution of heavy hydrocarbons, PAHs and metals in polluted areas. The case of "Galicia", Spain. Mar. Pollut. Bull. 121, 230–237. https://doi.org/https://doi.org/10.1016/j.marpolbul.2017.06.003

Navarro-Ortega, A., Tauler, R., Lacorte, S., Barceló, D., 2010. Occurrence and transport of PAHs, pesticides and alkylphenols in sediment samples along the Ebro River Basin. J. Hydrol. 383, 5–17. https://doi.org/https://doi.org/10.1016/j.jhydrol.2009.12.031

Nicola, G.G., Parra, I., Sáez, M., Almodóvar, A., Jiménez, B., 2014. Evaluation of PCBs and DDTs in endemic Iberian barbel Barbus bocagei (Steindachner, 1864) populations. Sci. Total Environ. 479–480, 221–226. https://doi.org/https://doi.org/10.1016/j.scitotenv.2014.01.013

Nogueira, J.G., Sousa, R., Benaissa, H., De Knijf, G., Ferreira, S., Ghamizi, M., Gonçalves, D. V, Lansdown, R., Numa, C., Prié, V., Riccardi, N., Seddon, M., Urbańska, M., Valentini, A., Vikhrev, I., Varandas, S., Teixeira, A., Lopes-Lima, M., 2021. Alarming decline of freshwater trigger species in western Mediterranean key biodiversity areas. Conserv. Biol. 35, 1367–1379. https://doi.org/10.1111/cobi.13810

Nunes, B.Z., Zanardi-Lamardo, E., Choueri, R.B., Castro, Í.B., 2021. Marine protected areas in Latin America and Caribbean threatened by polycyclic aromatic hydrocarbons. Environ. Pollut. 269, 116194. https://doi.org/https://doi.org/10.1016/j.envpol.2020.116194

Old, G., Lofts, S., 2022. Integrated sediment management: guidelines and good practices in the context of the Water Framework Directive.

Pantelaki, I., Voutsa, D., 2020. Occurrence, analysis and risk assessment of organophosphate esters (OPEs) in biota: A review. Mar. Pollut. Bull. 160, 111547. https://doi.org/https://doi.org/10.1016/j.marpolbul.2020.111547

Pantelaki, I., Voutsa, D., 2019. Organophosphate flame retardants (OPFRs): A review on analytical methods and occurrence in wastewater and aquatic environment. Sci. Total Environ. 649, 247–263. https://doi.org/https://doi.org/10.1016/j.scitotenv.2018.08.286

Peng, S., Kong, D., Li, L., Zou, C., Chen, F., Li, M., Cao, T., Yu, C., Song, J., Jia, W., Peng, P., 2020. Distribution and sources of DDT and its metabolites in porewater and sediment from a typical tropical bay in the South China Sea. Environ. Pollut. 267, 115492. https://doi.org/https://doi.org/10.1016/j.envpol.2020.115492

Pérez-Fernández, B., Viñas, L., Bargiela, J., 2018. Occurrence and toxicological assessment of polycyclic aromatic hydrocarbons (PAHs) in marine sediments under mussel farming influence. Environ. Sci. Pollut. Res. 25, 15862–15872. https://doi.org/10.1007/s11356-018-1737-4

Pinto, M.I., Vale, C., Sontag, G., Noronha, J.P., 2016. Pathways of priority pesticides in sediments of coastal lagoons: The case study of Óbidos Lagoon, Portugal. Mar. Pollut. Bull. 106, 335–340. https://doi.org/https://doi.org/10.1016/j.marpolbul.2016.03.028

Rehbein, J.A., Watson, J.E.M., Lane, J.L., Sonter, L.J., Venter, O., Atkinson, S.C., Allan, J.R., 2020. Renewable energy development threatens many globally important biodiversity areas. Glob. Chang. Biol. 26, 3040–3051. https://doi.org/https://doi.org/10.1111/gcb.15067

Rocha, A.C., Palma, C., 2019. Source identification of polycyclic aromatic hydrocarbons in soil sediments: Application of different methods. Sci. Total Environ. 652, 1077–1089. https://doi.org/https://doi.org/10.1016/j.scitotenv.2018.10.014

Rolón, E., Ondarza, P.M., Miglioranza, K.S.B., Rosso, J.J., Mabragaña, E., Volpedo, A. V, Avigliano, E., 2021. Multi-matrix approach reveals the distribution of pesticides in a multipurpose protected area from the Atlantic Rainforest: potential risk for aquatic biota and human health? Environ. Sci. Pollut. Res. 28, 34386–34399. https://doi.org/10.1007/s11356-021-12699-y

Ross, G., 2004. The public health implications of polychlorinated biphenyls (PCBs) in the environment. Ecotoxicol. Environ. Saf. 59, 275–291. https://doi.org/https://doi.org/10.1016/j.ecoenv.2004.06.003

Sardiña, P., Leahy, P., Metzeling, L., Stevenson, G., Hinwood, A., 2019. Emerging and legacy contaminants across land-use gradients and the risk to aquatic ecosystems. Sci. Total Environ. 695, 133842. https://doi.org/https://doi.org/10.1016/j.scitotenv.2019.133842

SEO/BirdLife, 2021. Ciencia LIBERA, Análisis de la contaminación difusa en los espacios naturales. Madrid.

Srogi, K., 2007. Monitoring of environmental exposure to polycyclic aromatic hydrocarbons: a review. Environ. Chem. Lett. 5, 169–195. https://doi.org/10.1007/s10311-007-0095-0

Stockholm Convention, 2019. All POPs listed in the Stockholm Convention [WWW Document]. URL http://chm.pops.int/TheConvention (accessed 6.20.23).

Trainor, C.R., Santana, F., Pinto, P., Xavier, A.F., Safford, R., Grimmett, R., 2008. Birds , birding and conservation in Timor-Leste. Calidris 9, 16–45.

Vane, C.H., Turner, G.H., Chenery, S.R., Richardson, M., Cave, M.C., Terrington, R., Gowing, C.J.B., Moss-Hayes, V., 2020. Trends in heavy metals, polychlorinated biphenyls and toxicity from sediment cores of the inner River Thames estuary, London, UK. Environ. Sci. Process. Impacts 22, 364–380. https://doi.org/10.1039/C9EM00430K

Viganò, L., Guzzella, L., Marziali, L., Mascolo, G., Bagnuolo, G., Ciannarella, R., Roscioli, C., 2023. The last 50 years of organic contamination of a highly anthropized tributary of the Po River (Italy). J. Environ. Manage. 326, 116665. https://doi.org/https://doi.org/10.1016/j.jenvman.2022.116665

Vitali, M., Guidotti, M., Macilenti, G., Cremisini, C., 1997. Phthalate esters in freshwaters as markers of contamination sources—A site study in Italy. Environ. Int. 23, 337–347. https://doi.org/https://doi.org/10.1016/S0160-4120(97)00035-4

Waliczky, Z., Fishpool, L.D.C., Butchart, S.H.M., Thomas, D., Heath, M.F., Hazin, C., Donald, P.F., Kowalska, A., Dias, M.P., Allinson, T.S.M., 2019. Important Bird and Biodiversity Areas (IBAs): their impact on conservation policy, advocacy and action. Bird Conserv. Int. 29, 199–215. https://doi.org/10.1017/S0959270918000175

Wang, C., Chen, H., Li, H., Yu, J., Wang, X., Liu, Y., 2020. Review of emerging contaminant tris(1,3-dichloro-2-propyl) phosphate: Environmental occurrence, exposure, and risks to organisms and human health. Environ. Int. 143, 105946. https://doi.org/https://doi.org/10.1016/j.envint.2020.105946

Wang, J., Bo, L., Li, L., Wang, D., Chen, G., Christie, P., Teng, Y., 2014. Occurrence of phthalate esters in river sediments in areas with different land use patterns. Sci. Total Environ. 500–501, 113–119. https://doi.org/https://doi.org/10.1016/j.scitotenv.2014.08.092

Wei, G., Wang, C., Niu, W., Huan, Q., Tian, T., Zou, S., Huang, D., 2021. Occurrence and risk assessment of currently used organophosphate pesticides in overlying water and surface sediments in Guangzhou urban waterways, China. Environ. Sci. Pollut. Res. 28, 48194–48206. https://doi.org/10.1007/s11356-021-13956-w

Wu, H., Sun, B., Li, J., 2019. Polycyclic Aromatic Hydrocarbons in Sediments/Soils of the Rapidly Urbanized Lower Reaches of the River Chaohu, China. Int. J. Environ. Res. Public Health 16. https://doi.org/10.3390/ijerph16132302

Xie, J., Pei, N., Sun, Y., Chen, Z., Cheng, Y., Chen, L., Xie, C., Dai, S., Zhu, C., Luo, X., Zhang, L., Mai, B., 2022. Bioaccumulation

and translocation of organophosphate esters in a Mangrove Nature Reserve from the Pearl River Estuary, South China. J. Hazard. Mater. 427, 127909. https://doi.org/https://doi.org/10.1016/j.jhazmat.2021.127909

Xie, Z., Wang, P., Wang, X., Castro-Jiménez, J., Kallenborn, R., Liao, C., Mi, W., Lohmann, R., Vila-Costa, M., Dachs, J., 2022. Organophosphate ester pollution in the oceans. Nat. Rev. Earth Environ. 3, 309–322. https://doi.org/10.1038/s43017-022-00277-w

PART II: BIOMONITORING CONTAMINANTS IN BIRDS

CHAPTER V

A REVIEW OF CONSTRAINTS AND SOLUTIONS FOR COLLECTING RAPTOR SAMPLES AND CONTEXTUAL DATA FOR A EUROPEAN RAPTOR BIOMONITORING FACILITY

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ABSTRACT

The COST Action 'European Raptor Biomonitoring Facility' (ERBFacility) aims to develop pan-European raptor biomonitoring in support of better chemicals management in Europe, using raptors as sentinel species. This presents a significant challenge involving a range of constraints that must be identified and addressed. The aims of this study were to: (1) carry out a comprehensive review of the constraints that may limit the gathering in the field of raptor samples and contextual data, and assess their relative importance across Europe; and (2) identify and discuss possible solutions to the key constraints that were identified. We applied a participatory approach to identify constraints and to discuss feasible solutions. Thirty-one constraints were identified, which were divided into four categories: legal, methodological, spatial coverage, and skills constraints. To assess the importance of the constraints and their possible solutions, we collected information through scientific workshops and by distributing a questionnaire to stakeholders in all the countries involved in ERBFacility. We obtained 74 answers to the questionnaire, from 24 of the 39 COST participating countries. The most important constraints identified were related to the collection of complex contextual data about sources of contamination, and the low number of existing raptor population national/regional monitoring schemes and ecological studies that could provide raptor samples. Legal constraints, such as permits to allow the collection of invasive samples, and skills constraints, such as the lack of expertise to practice necropsies, were also highlighted. Here, we present solutions for all the constraints identified, thus suggesting the feasibility of establishing a long-term European Raptor Sampling Programme as a key element of the planned European Raptor Biomonitoring Facility.

1. INTRODUCTION

There is growing concern in the European Union (EU) and worldwide about the negative impacts of various chemicals on the environment (Krabbenhoft and Sunderland 2013, Hallman et al., 2014, Malaj et al., 2014, Jepson and Law 2016) and on human health (Movalli et al., 2018).

The European Union (EU) aims to achieve a non-toxic environment, and a wide range of legislation has been implemented to reduce these negative impacts on the environment and human health. This includes Regulation EC 1907/2006 and amendments (REACH—Registration, Evaluation, Authorisation & Restriction of Chemicals) concerning industrial substances, Regulation EC 1107/2009 concerning the authorisation of plant protection products, Regulation EC 726/2004 concerning the authorisation of human and veterinary pharmaceuticals, and the Biocidal Product Regulation (BPR, EU Regulation 528/2012). However, legal restrictions on the use of chemicals should be accompanied by effective monitoring methods, to provide early warning of emerging contaminant problems in the environment, inform substance risk assessments and evaluate the effectiveness of risk management measures (Shore and Taggart 2019, Rodríguez-Estival and Mateo 2019, García-Fernández 2020). Biomonitoring with sentinel species is an important tool for early detection of negative impacts of chemicals on all ecosystems, with potentially strong links to human health (Smits and Kimberly 2013; García-Fernández et al., 2020). Raptors (defined here as birds belonging to the orders Accipitriformes, Falconiformes and Strigiformes) are especially suitable for monitoring persistent substances in the environment because: (a) they are generally long-lived apex predators; (b) they effectively integrate contaminant exposure over time and over relatively large spatial areas; (c) they can be sampled without a need to sacrifice or harm the birds by sampling of feathers, blood, preen oil and/or addled/deserted eggs; (d) as charismatic birds, raptors found dead or injured are frequently delivered to wildlife rehabilitation centres or museums by the general public, providing good sources of tissue samples (internal organs, muscles, bones), and (e) their populations can be relatively easily monitored and quantified (Movalli et al., 2008, Gómez-Ramírez et al., 2014, Espín et al., 2016, Movalli et al., 2017, García-Fernández et al., 2020). Monitoring contaminants using raptors can usefully complement biomonitoring in humans within a One Health approach, , which acknowledges the interconnection between the health of people, domestic animals, and our shared environment, including wildlife and plants (Duke 2008, Walker et al., 2008, Berny et al., 2015, Movalli et al., 2018, Badry et al., 2020, García-Fernández et al., 2020).

With this in mind, the COST Action European Raptor Biomonitoring Facility (hereafter ERBFacility; https://erbfacility.eu/ and https://www.cost.eu/actions/CA16224/) was established with the aim to design and build key elements of

a “Facility” (or framework) for pan-European raptor biomonitoring, in order to enhance the evaluation of effectiveness of chemicals regulations and conventions, improve risk assessment of specific chemicals and provide early warning of emerging contaminant problems. Under this Facility, samples from key species would be collected, transported, stored, and analysed following harmonized methodologies. The three key elements of ERBFacility are: a European Raptor Sampling Programme, which gathers raptor samples and related ‘contextual data’ from the field; a distributed European Raptor Specimen Bank which stores these samples and related data; and a European Raptor Biomonitoring Scheme, which analyses raptor samples for contaminants (Movalli et al., 2019, Badry et al., 2020, Espín et al., 2020).

The creation of ERBFacility presents a significant challenge, with a number of constraints to be addressed. These constraints relate to the ‘field arena’ where samples are gathered, to the ‘collections arena’ where samples are stored, and to the ‘analysis arena’ where samples are analysed for contaminants. This paper addresses constraints relating to the first of these arenas, and the establishment of a European Raptor Sampling Programme as a key element of the planned Facility. This covers the process from collecting samples from raptors in the field up to the point of arrival of the samples at a collection (e.g. a natural history museum or environmental specimen bank or research collection) or an analytical laboratory.

Constraints relate both to the gathering of samples, and to the gathering and interpretation of reliable ‘contextual data’, that links the sample to other relevant data, e.g., on population parameters. Such contextual data provide the individual, population and ecological context for the better interpretation of contaminant data in raptor samples.

Previous work has illuminated some of the potential constraints in this regard. Raptor population monitoring schemes, which offer important potential for gathering raptor samples and contextual data, are not uniformly spread across Europe, apply diverse methods and are conducted at varying scales, from intensive academic research projects to broad-scale volunteer surveys (Kovács et al., 2008, Vrezec et al., 2012). However, we also know that there is an important number of raptor population monitoring schemes, widely distributed across Europe (Derlink et al., 2018). Alongside these, several existing monitoring programmes focus on contaminants in raptors populations (García-Fernández et al., 2008, Gómez-Ramírez et al., 2014, Carneiro et al., 2015, Espín et al., 2016). Many natural history museums, a small number of environmental specimen banks and some other research institutes hold substantial collections of frozen raptor carcasses and/or tissues suitable for contaminant monitoring (Movalli et al., 2017, 2018; Ramello et al., Unpublished results). In addition, wildlife rehabilitation centres and other institutions have potential as suppliers if raptor samples. These

previous studies demonstrate the wealth of existing activity on which the planned Facility can be build.

Beyond identifying constraints to implementation of the ERBFacility, it is crucial to identify effective and realistic solutions to address these constraints. Accordingly, we set for the present study two major objectives: (1) to conduct a comprehensive review of the constraints that may limit the collection of raptor samples and contextual data and assess their relative importance across Europe; and (2) to identify and discuss possible solutions to the key constraints that were identified.

While this paper focuses on constraints faced in the field arena, other work under ERBFacility addresses constraints in the collections arena (e.g., Ramello et al., Unpublished results, Sbokos et al., Unpublished results., Vlachopoulos et al., Unpublished results.) and in the analysis arena (e.g., Badry et al., 2020, Espin et al., 2021)

2. METHODS

This study focused on all 39 Member and Cooperating Member countries in the European Cooperation in Science & Technology network (COST, https://www.cost.eu/who-we-are/members/), including the 28 member states of the European Union plus Near Neighbour and International Partner Countries. We used a participative approach, to make effective use of the opinion of experts and people involved in collecting raptor samples and contextual data.

In order to accomplish the first objective (i.e., a comprehensive review of the relative importance of constraints that may limit the gathering in the field of raptor samples and contextual data), we drafted a preliminary list of potential constraints through a bibliographic review and use of expert knowledge (based on a questionnaire to a smaller group of experts and a workshop). As a further step, we created a second questionnaire for a larger group of experts to classify the relative importance of each constraint, by constraint type (i.e., legal, methodological, skills, and spatial coverage) and by the different categories of involved actors. In order to accomplish the second objective (i.e., identifying possible solutions to major constraints) we implemented sought expert opinion through a second workshop involving experts from several participating countries.

For the purpose of this study, we use the term "raptor samples" to mean: (1) non-invasive samples that do not require manipulation of birds (e.g. carcasses of birds found dead, moulted feathers, addled/deserted eggs, regurgitate pellets) but that may generate disturbance in some circumstances (e.g. when collecting them from active nests), and (2) invasive (but non-destructive) samples that require manipulation of live birds (e.g. blood or plasma,

pulled feathers, preen oil). All raptor sampling must be done under relevant permit, where applicable. We use the term "contextual data" to include all the information related to the sample, individual, or the population that can provide relevant context for the interpretation of the contamination levels detected in a given sample (see Table S1 in Supplementary Information).

2.1. Identification of potential constraints

The first step to identify potential constraints consisted of a literature review of scientific papers on contaminant monitoring studies using raptors as study species. We initially searched for papers using Google Scholar, published between 2000 and 2019, with the search terms: birds of prey, contaminant, contamination, eagle, ecotoxicology, falcon, owl, raptor, or their combinations. We limited the year interval of our search to avoid an excessive number of articles but also to avoid identifying potentially outdated constraints. We looked for additional relevant papers by inspecting the list of references in each paper. Overall, 66 papers were reviewed in detail to find any mention of possible constraints associated with the process of collecting and analysing samples.

The second step involved building a list of potential constraints based on expert opinion. We designed three short surveys, distributed via email to a group of 29 experienced researchers in raptor ecology and ecotoxicology from 19 different European countries to identify further constraints. These researchers were chosen among ERBFacility collaborators in order to ensure a broad country coverage, but also the representativeness of different institution types (universities, research institutions, natural history museums, non-governmental organizations, wildlife rehabilitation centres).

The third step was to discuss the list of potential constraints with a group of 46 experts working with raptors and owls during an ERBFacility workshop in Thessaloniki, Greece (February 2019) (ERBFacility, 2019a). These experts represented 20 participating countries. The participants were asked to provide contributions about the completeness of the list of constraints regarding their experience in the countries for which they had knowledge. The constraints were then grouped within four types: (1) legal; (2) methodological; (3) skills; and (4) spatial coverage constraints.

2.2. Classification of the importance of the constraints

Once we reached a final list of potential constraints, the fourth step was to design an online anonymous questionnaire with the aim of obtaining a classification of the relative importance of each constraint as it is perceived in different countries and by different groups of people involved in collecting

raptor samples and contextual data. The questionnaire was divided into three main parts, all with facultative questions (Table S2 in Supplementary information). The first part was designed to characterize the respondents, in terms of their professional role, expertise with raptors, and specific skills and permits held to work with raptors. In the second part of the questionnaire, respondents were given 45 questions in which they were asked to rate the importance of general and case-study-specific constraints. We used a classification from 1 (not a constraint) to 5 (strong constraint). Finally, in the third part of the questionnaire, we asked participants to select the five most relevant ways to address constraints and thereby improve the collection of raptor samples and contextual data in their country from a list of nine suggestions. In addition, we asked an open question allowing them to suggest further solutions.

The questionnaire was initially distributed to the 69 ERBFacility Management Committee Members and Alternate Members, representing 27 participating countries. In turn, these national representatives distributed the questionnaire to individuals involved in the collection of raptor samples and contextual data (researchers, bird ringers, non-governmental organisation workers, wildlife rehabilitation centre workers, museums curators, veterinarians, among others) in their respective countries. Considering the people to whom we first sent the questionnaire and the number of people we know to have been contacted by the national representatives, we estimate that the questionnaire was received by at least 150 people. We obtained 74 answers to the questionnaire, from 24 of the 39 COST countries.

2.3. Identification of potential solutions

Building on the list of key constraints, we drafted a list of possible solutions to each constraint. The solutions were divided into five types of action: (1) best practice guidance, (2) capacity building, (3) coordination, (4) species and contaminant prioritization, and (5) funding. The draft list of solutions was then presented and discussed at a ERBFacility workshop in Florence, Italy (March 2019) (ERBFacility, 2019b), involving 23 experts in raptor sampling, ecology, and ecotoxicology. The participants were divided into groups based on the five types of action. Each group was asked to discuss the most suitable solution to solve the potential constraints, including practicalities on how to implement the solutions, which actors should be involved and the time needed for implementation.

2.4. Data analysis

The results from the questionnaire were compared using non-parametric tests – Wilcoxon rank sum test; and Kruskal-Wallis rank sum test followed by

a post-hoc Dunn test (library "dunn.test"). Significance value was set at $p < 0.05$. Analyses were carried out using the statistical software R version 4.0.2 (R Core Team, 2020).

3. RESULTS AND DISCUSSION

3.1. What are the potential constraints for a European Raptor Sampling Programme?

Using our participative approach, we identified a total of 31 potential constraints to collecting raptor samples and contextual data. Six of these constraints concerned legal aspects, 13 were methodological constraints, 5 were related to the skills of participants, and 7 were related to spatial coverage (Table 1, see a detailed description of the constraints in Table S3 in Supplementary Information).

Table 1. Summary of constraints and solutions for collecting raptor samples and contextual data.

#	Constraint	Possible Solutions
Legal constraints		
1	Legal restrictions on transportation of samples within country	Provide best practice guidance. Improve knowledge of the best shipping conditions. Establish national coordinators
2	Legal restrictions to holding and storing raptor samples (carcass, feathers, eggs)	Provide training/guidance to obtain licences for storing raptor samples
3	Legal restrictions for sampling blood or other invasive samples	Provide training to field workers to obtain licences for collecting invasive samples
4	Legal restrictions for handling live wild birds	Provide training to obtain licences for handling live wild birds
5	Legal restrictions for visiting active nests	Provide training to obtain licences to visit active nests
6	Legal restrictions to access private property	Provide best practice guidance. Establish national coordinators ambassadors
Methodological constraints		
7	Difficulty in collecting contextual data on potential sources of contamination	Increase knowledge on local contamination sources
8	Difficulty in collecting contextual data on diet	Provide guidance and training to study diet
9	Difficulty in collecting contextual data on reproductive performance	Provide guidance and training on how to collect breeding parameters

#	Constraint	Possible Solutions
10	Difficulty in collecting mandatory or high priority contextual data (age, sex, feather type)	Provide guidance and training on raptor identification and collecting contextual data
11	Lack of contextual data because of non-precise location of samples	Provide guidance on how to record locations
12	Lack of amount of sampled blood for nestlings	Use another sample matrix (e.g. nestling feathers) or pool blood samples from the same nest.
13	Lack of information on adequate protocols for collecting samples	Improve distribution of the existing protocols for sampling and increase access to training for field workers
14	Difficulty of providing sampling material	Provide the sampling material from reference laboratories (syringes, containers, anticoagulants, etc.)
15	Difficulty in Harmonisation of contextual data related with the sample	Improve data flux and organization. Creation of a suitable database (application or software).
16	Difficulty in adequate short-term storage of the samples	Best practice guidance and increased capacity building for storage
17	Difficulty to relate sample to specific contextual data	Design specific ID code
18	Difficulty to find an institution to send the sample for analysis	Establish National Coordinators that coordinate with different institutions
19	Difficulty to support the shipping cost or ensure correct transportation	Funding for the expenses to be covered by the European Raptor Biomonitoring Facility. Having a national coordinator that can pick up samples and provide transportation protocols
Spatial coverage constraints		
20	Focal raptor population with very low abundance or uneven distribution	Consider monitoring a set of raptors with similar diet and habitat
21	Low number of monitoring schemes and ecological studies to provide access to raptors samples	Increase the number of projects working with raptors
22	Low number of monitoring schemes and ecological studies to provide complex contextual data	Increase the number of projects working with raptors
23	Low number of suitable sampling areas in the country	Consider monitoring in a set of similar habitats

#	Constraint	Possible Solutions
24	Difficulty to access raptor breeding areas	Increase efforts to get samples without necessity to access breeding areas e.g., moulted feathers or carcasses. Work with species that are easy to access
25	Difficulty to access to the nests	Increase efforts to get samples without necessity to access nests e.g., moulted feathers or carcasses
26	Lack of institutions to participate in the Sampling Programme	Collaborate with a neighbouring country. Motivate the participation of more institutions
Skills constraints		
27	Lack of skilled people for field sampling	Provide training and guidance for fieldwork
28	Lack of means for capacity building by field coordination institutions	Increase the funding for capacity building
29	Lack of motivation among field workers	Improve feedback. Establish national ambassadors
30	Lack of skills required for post-processing of carcasses (necropsies)	Improve training and guidance for necropsies
31	Lack of skills to collect complex contextual data	Improve training and guidance to collect contextual data

Legal constraints – There are many regulations and laws aimed at protecting raptors. At the international level, CITES (Convention on Trade in Endangered Species) has the purpose of ensuring that no species of wild fauna or flora becomes or remains subject to unsustainable exploitation because of international trade. CITES plays an important role in regulating the transportation of raptor samples between countries (CITES, 1984). In the EU, the Birds Directive (Directive 2009/147/EC) aims to protect all wild bird species naturally occurring in the EU and regulates the handling of any readily recognisable parts or derivatives of such birds. Each Member State must transcribe this into national legislation or administrative measures. Because of variations in transcription, constraints under this Directive may therefore differ between countries, but generally all countries limit actions that can disturb or harm raptors, particularly during the breeding period. Legal constraints often exist at the outset of collecting raptor samples and contextual data in the field in terms of gaining access to private property (e.g. when a raptor breeds or dies on private land). These constraints vary between countries and locations with the varying percentage of private land among European countries and the varying willingness of landowners to

allow access for research purposes. Many countries restrict visits to active raptor nests; in some cases, active nests are protected by legislation in order to prevent persecution or disturbance or other potential damage to threatened and sensitive bird species. In addition, there are legal restrictions for handling live raptors. Across Europe, handling usually requires evidence of specific training and experience and proper facilities in order to obtain the appropriate licence. Restrictions are even stricter for sampling of blood or other samples when involving manipulation of live birds (namely Directive 2010/63/EU as amended by Regulation EU 2019/1010). There are moreover national and international legal restrictions that apply to the transportation of sample material within a country (and between countries), and to the storing of raptor samples. The possession and transport of biological samples, and especially those from protected species such as raptors, may be subject to legal restrictions including under CITES convention, the Nagoya Protocol on Access and Benefit-sharing (www.cbd.int/abs), IATA Dangerous Goods Regulations (DGR), the UN European Agreement concerning the International Carriage of Dangerous Goods by Road (ADR), and country-specific regulations for national postal services. The complexity and lack of knowledge of the legislation, or the logistical difficulties it raises, may hamper development of a European Raptor Sampling Programme as a key element of the European Raptor Biomonitoring Facility. Legal constraints relating to the shipment of samples, and measures to address them, are tackled by a separate ERBFacility study (Sbokos et al., in prep).

Methodological constraints – All raptor samples should be collected following adequate protocols that allow for subsequent rigorous chemical analyses and interpretation, as well as ensuring the safety of both fieldworkers and birds (Espin et al., 2020). Despite the existence of field and sampling protocols specifically for raptors (e.g. Bird and Bildstein 2007, Hardy et al., 2013, Espín et al., 2014, 2020), the insufficient dissemination and awareness of these protocols may be an important constraint to a European Raptor Biomonitoring Scheme. When a sample is collected it may be necessary to carry out short-term storage before it is sent for long-term storage in natural history museums, environmental specimen banks or other research collections. Unsuitable short-term storage (e.g. high temperatures, inadequate containers, incorrect conservation method) or improper sample collection (e.g. insufficient sample amount, cross-contamination) may result in sample deterioration or the sample not being suitable for chemical and biomarker analysis (Espín et al., 2014, 2020). Samples must be sent as soon as possible to a collection for appropriate long-term storage or alternatively to an ecotoxicology laboratory for chemical analysis. Field workers may not be aware of the most suitable institutions to which to send the samples, in order to make them available for biomonitoring. Moreover, samples must be transported following adequate transport protocols, and considerable

associated shipping costs might discourage the participation of fieldworkers in the Sampling Programme. If these constraints are not solved, they could lead to the loss of a great number of potential samples and/or cause an under-representation of some regions of Europe in sampling.

All collected samples must have at least basic contextual data relating directly to the sample itself, such as: species, age group, sampling location, matrix type, and date. In the case of carcasses, it is relevant to obtain the information needed to estimate the time of death (Valverde et al., in press). If this information is missing, a sample is unlikely to be suitable for use in the Biomonitoring Scheme. Additional contextual data about the individual and the population from which it is known to derive, such as diet composition, habitat, moulting or migration patterns can be important for the interpretation of the results (Elliot et al., 2007, Lourenço et al., 2011, Lodenius and Solonen, 2013, Bustnes et al,. 2013, Roque et al., 2017). Some contextual data, such as diet and reproductive performance, can be particularly relevant depending on the aims of the Biomonitoring Scheme (Palma et al., 2005; Schipper et al., 2012, Badry et al., 2019) but recording these data often entails considerable time investment and expertise. Finally, in many case studies it is relevant and valuable to have information available on contamination sources local to the area of sample collection (Elliot et al., 2007, Espín et al., 2014, Badry et al,. 2019).

Skills constraints – Specific skills and experience are necessary to obtain and process raptor samples (particularly taking blood or carrying out a necropsy) and to collect complex contextual data (e.g., determine sex and age of raptors, carry out rigorous population monitoring). Most frequently, field workers have good raptor identification skills but may lack training in sample collection. To be able to train field workers it is first necessary to develop capacity building among field coordination institutions. Contributing to a European Raptor Sampling Programme will often be a voluntary action, and through time there can be a loss of motivation to participate without effective work from a coordinating organisation. For a successful Programme, it will be important to keep fieldworkers well motivated to obtain raptor samples and collect relevant contextual data.

Spatial coverage constraints – One of the greatest challenges of a European Raptor Sampling Programme as proposed by ERBFacility is ensuring wide geographical coverage. There are several candidate raptor species that could be selected as priorities for a European Raptor Biomonitoring Scheme (Badry et al., 2020) but among these some species may have a low abundance in some European countries, or an uneven distribution within a country (especially in countries with large territories), leading to unrepresentative monitoring or high costs/effort needed to obtain a minimum number of samples. Several species that are underrepresented in existing monitoring studies

within Europe are mainly common and widespread species (e.g. Common Buzzard *Buteo buteo*, European Honey Buzzard *Pernis apivorus*, Northern Goshawk *Accipiter gentilis*, Eurasian Sparrowhawk *A. nisus*) and species breeding predominantly in southern and eastern Europe (e.g. Long-legged Buzzard *Buteo rufinus*, Booted Eagle *Hieraaetus pennatus*, Short-toed Snake Eagle *Circaetus gallicus*) (Vrezec et al., 2012). The lack of ongoing population monitoring schemes and ecological studies may hamper the collection of raptor samples and contextual data (e.g. diet, reproductive performance, population trends, behaviour). Moreover, some contaminants are associated with specific habitats or land-uses (e.g. a specific plant protection product), and sampling needs to take into account that these areas may be poorly or not represented, or be very localized, in some countries. In addition, the access of fieldworkers to some regions where raptors occur may be difficult or impossible, for example in remote or roadless areas, isolated islands, or restricted areas (e.g. military zones). Some raptor nests may also be difficult to monitor, (e.g. on high cliffs or in treetops). Nest visits are essential to obtain several sample types (e.g. eggs, feathers, pellets, nestling feathers or blood) and certain contextual data (e.g. some measures of reproductive performance or diet composition). Finally, the lack of institutions to store and ship samples in one or more countries/regions may limit spatial coverage.

3.2. Which are the strongest constraints for collecting raptor samples and data?

Responses to our questionnaire to the strength of the constraints included reasonable representation from the various groups involved in field work with raptors. Of the 74 respondents, 64% worked with raptors as their professional job, 26% as both professional job and volunteers, and 10% as volunteers. Regarding the institutions in which respondents carry out their work with raptors, 50% exclusively work in governmental institutions (e.g. universities, research institutes, natural history museums), 27% work exclusively for non-governmental organizations (NGOs) or as volunteers (e.g. ringers), 20% work for both governmental research institutions and NGOs (or as volunteers), and 3% work for private companies or as independent professionals. According to the profile, we grouped the actors involved in collecting raptor samples and contextual data into two types: (1) governmental – people having as main institution a governmental organization dedicated to research, either in zoology, ecology or ecotoxicology, including universities, research institutes, and natural history museums (62%, n = 46); and (2) non-governmental people working professionally or as volunteers in NGOs, private companies, or as independent workers (i.e. without any connection with governmental organizations; 38%, n = 28).

Among respondents, southern European countries were more represented than northern and eastern European countries (Figure 1). This spatial bias is similar to that obtained in a previous study that assessed the existing monitoring programmes measuring contaminants in raptor samples until 2012 (Gómez-Ramírez et al., 2014). There was a significant gap in participation of central and eastern European countries, such as Poland, Latvia and Lithuania, despite our efforts to involve specific expertise from co-authors and workshops participants from the countries less well represented.

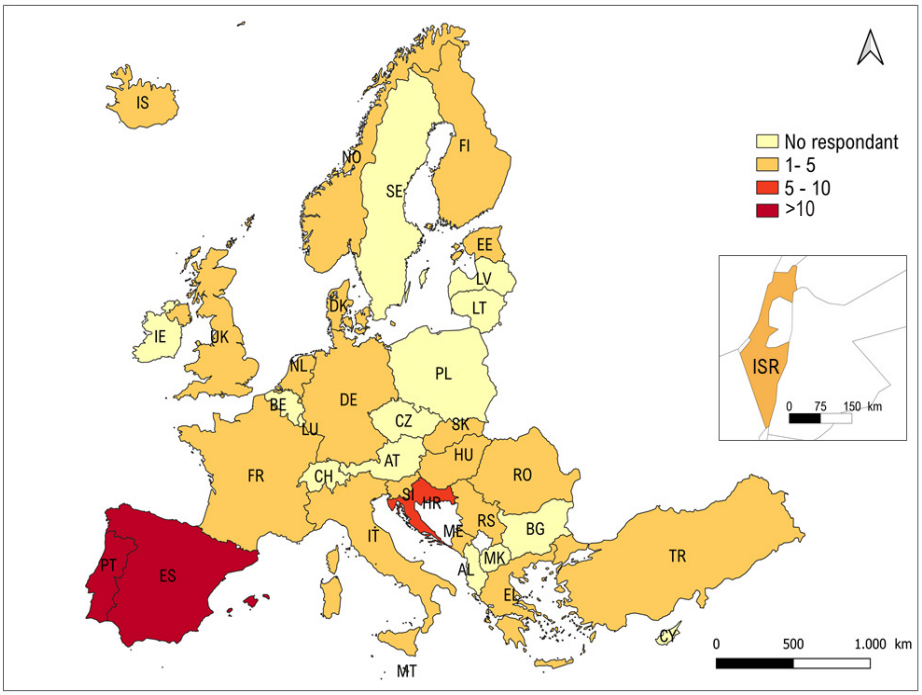

Figure 1. Number of questionnaire responses received per country (two letter abbreviation of country names).

According to the questionnaire results, the most common skill among governmental and non-governmental workers is the skill of carrying out field surveys and monitoring of raptor populations, e.g. collecting data on basic population or reproduction parameters (Figure 2). Both types of actors have similar skills in monitoring raptors populations, including permits to handle birds. However, for collecting invasive samples (e.g. blood) there is a greater number of governmental workers with the required skills in comparison to non-governmental workers. The skills related with the shipping of samples are also more common among governmental actors. The capacity to carry out necropsies is the least common skill, held only by governmental respondents.

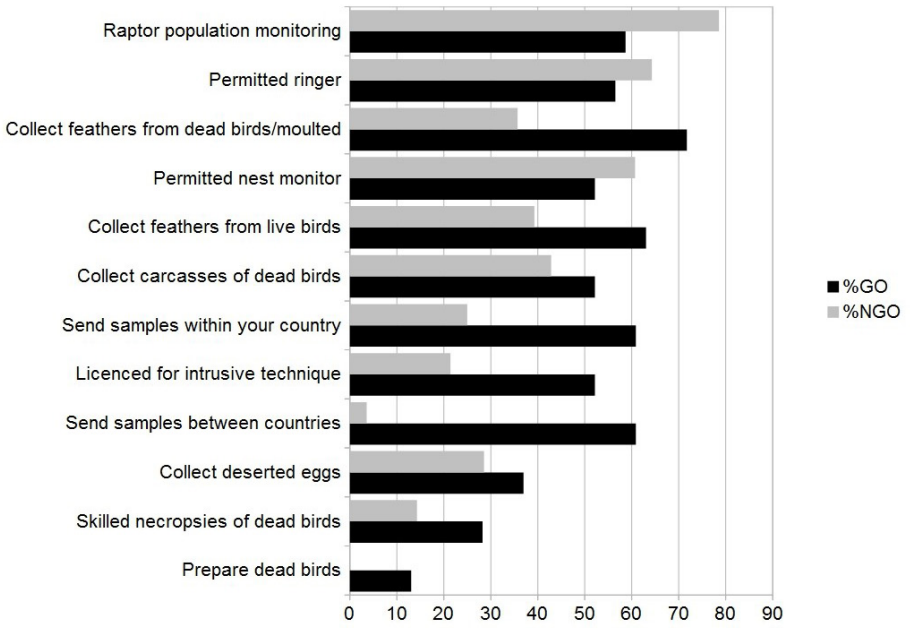

Figure 2. Skills of respondents (n=74) to the questionnaire according to actor type - governmental institutions (GOs) or non-governmental organizations (NGOs).

3.2.1. Classification of constraints by actor type

The questionnaire covered different kinds of actors likely to be involved in a Sampling Programme. There were in general significant differences in the scores given between actors carrying out their work with support of non-governmental versus governmental institutions (Wilcoxon rank sum test: $W = 645796$; $p < 0.001$). Non-governmental field workers generally gave higher scores to the questions on constraints than governmental field workers (Figure 3). Methodological, spatial, and skills constraints seem to represent stronger limitations for collecting samples and contextual data by field workers supported by non-governmental organizations. Despite experiencing more difficulties to obtain raptor samples, non-governmental institutions may provide valuable knowledge about complex contextual data, as more than 60% of species population monitoring schemes are run by non-governmental organisations and more than half of all species schemes rely on greater than 50% volunteer effort (Derlink et al., 2018).

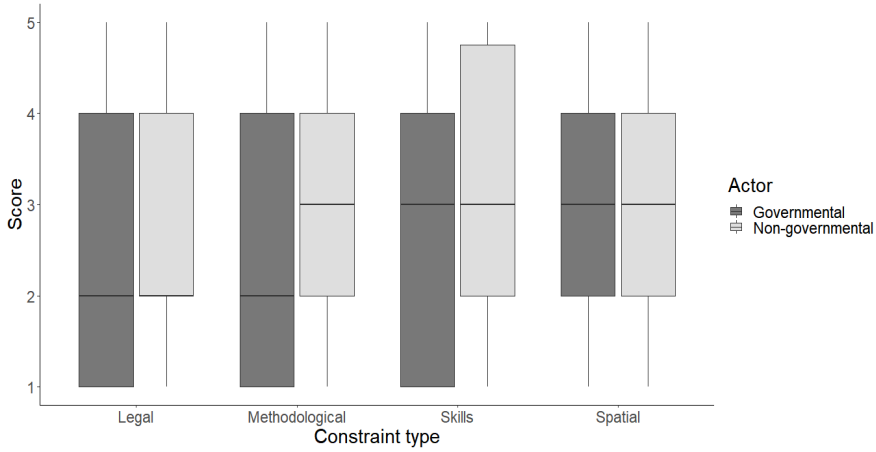

Figure 3. Difference in scoring of the four types of constraints (legal, methodological, skills, and spatial coverage) according to actor type:field workers with support from governmental or non-governmental organizations. Boxplots showing median, quartiles and range.

3.2.2. Perceived relevance of each constraint type

We found a difference in the mean scores given by respondents to the four types of constraints (Kruskal-Wallis rank sum test: chi-squared = 13.77, df = 3, p = 0.003; ; Post-hoc Dunn test: legal-methodological: P = 0.001; legal-skills: P = 0.008; legal-spatial: P < 0.001; methodological-skills: P = 0.19; methodological-spatial: P = 0.27; skills-spatial: P = 0.30). Amongst the respondents to the questionnaire, the set of legal constraints was less relevant than the constraints related to methodological aspects, skills, or spatial coverage (Figure 4).

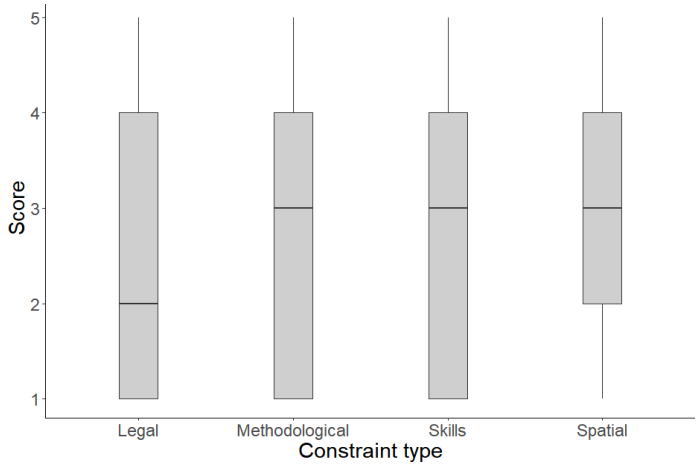

Figure 4. Classification of the different types of constraints by respondents to the survey (median and 95% confidence intervals; scores range from 1 (low) to 5 (high) relevance of constraint). Boxplots showing median, quartiles and range.

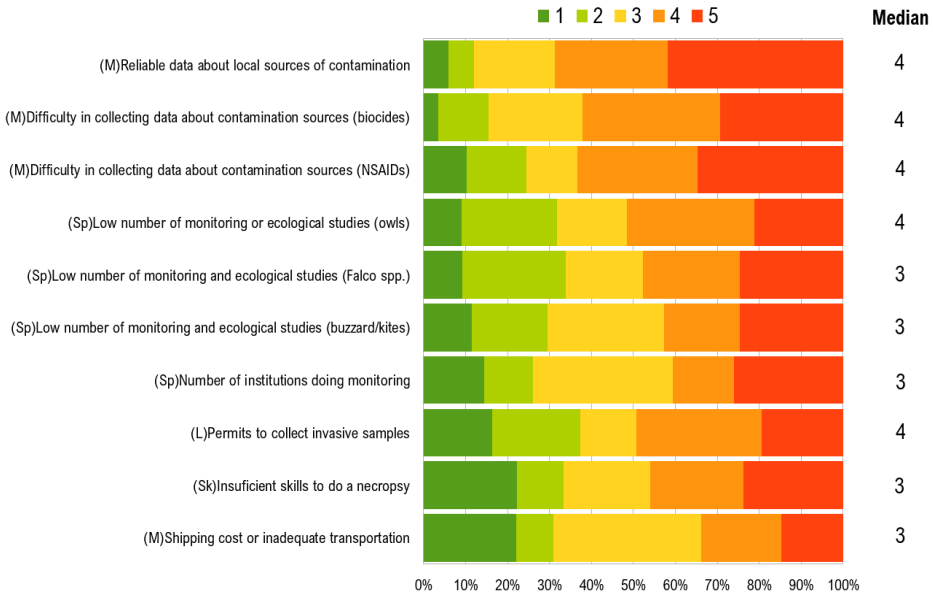

Figure 5. Scoring of the top ten questions regarding constraints to the sampling of raptors. Scores indicating the importance of constraints from 1 (low importance) to 5 (high importance). Letters in brackets preceding the constraint indicate its type: (L) Legal; (M) Methodological, (Sk) Skills; (Sp) Spatial coverage. Complete figure of questions about constraints detailed in Figure S1 in Supplementary Material ESM1.

The top ten constraints perceived to be the most important with median scores above 3 (Figure 5) included all four types of constraints. The top three constraints were related to methodological limitations to obtain reliable data on local contamination, including general sources of contamination (e.g. pesticides used, hunting practices) and more specific examples as biocides and non-steroidal anti-inflammatory drugs (NSAIDs). The respondents also highlighted other methodological constraints, such as the shipping cost or the inadequate transportation of samples. Also, in the top 10 were spatial coverage constraints relating to the low number of existing monitoring schemes and ecological studies and the low number of institutions involved in contaminant biomonitoring. The legal constraint with the highest score was the collection of invasive samples (e.g. blood from nestlings or adults). The lack of skills to do a necropsy was identified in the top 10, as an important constraint to obtain raptor samples. The abundance of raptors seemed to be the least relevant constraint (median=2 for all species, see Figure S1 in Supplementary Information).

3.3. How can we solve the constraints related to biomonitoring with raptors?

Once the constraints were identified (Table 1), a list of potential feasible solutions was discussed among experts. The potential solutions were classified into five topics of action: (1) best practice; (2) capacity building; (3) coordination; (4) selection of focal species and contaminants; and (5) projects and funding.

Disseminating best practice – 23% of the constraints identified may be solved by a consolidation of best practices for field sampling across Europe. To achieve this, it is necessary to provide and disseminate protocols to harmonize sampling methods, thus improving the potential for pan-European comparison of results. Preferably, all materials (e.g. protocols, related audio-visual materials) to provide guidance on collecting raptor samples and contextual data should be provided or indicated in an "advice hub" – i.e. an online platform where new guidance to fill gaps, and links to existing good practice guidance could be provided to a broad public. Some examples of best practice guidance required include: (1) identifying the most adequate sampling material, (2) defining the minimum/optimum sample size for analysis, (3) specifying the required short-term storage conditions, and (4) defining comparable methods to collect contextual data (e.g. breeding parameters, diet; see Table S1 in Supplementary Material ESM1). Important steps towards providing this guidance have already been taken (e.g. Hardey et al., 2009; Espín et al., 2014, 2016, 2020, Valverde et al., 2020) (see also https://www.sertoxmur.com/?page_id=5322), but there is a need for existing protocols and training audio-visual material to be more widely distributed, made more readily available (e.g. by translation into national languages; being available in stable and permanent online webpages), and established as reference guidelines to sample raptors for monitoring pollutants. In addition, there is a need for a European Raptor Specimen Database that captures relevant data on available (frozen) specimens, and to link this to a raptor tissue sample database (for tissues samples destined for contaminant analysis) and databases of contaminant data arising from these tissues. The attachment of a unique identifier to each specimen and to tissues arising from each specimen will permit association of contextual field data with contaminant data and therefore enable more informed interpretation of contaminant data. Separate work is ongoing under ERBFacility on the design of a raptor specimen database (Vlachopoulos et al., Unpublished results), aligned with the Distributed System of Scientific Collections DiSSCo (www.dissco.eu). These databases and guidance must be maintained and updated in order to promote their use as relevant sources for future needs.

Promote capacity building and training – Another set of solutions involve increasing the availability of training activities across countries, as this would

help to solve 39% of methodological, legal and skills constraints. These activities are necessary to allow people involved in collecting samples to obtain specific skills and knowledge. These new competences will often be complementary to people's skills, and include for example, how to record basic contextual data (e.g. identification of species, age, sex) and complex contextual data (e.g. diet, behaviour, reproductive performance, survival, population trend, geographic distribution range), and how to collect samples, with special focus on sampling from live birds and on performing adequate necropsies to obtain samples from carcasses. Access to specific training is usually essential to obtain relevant permits to sample raptors, such as permits to visit nests, handle birds, collecting invasive samples or to hold and store samples that are of a restricted nature. It is therefore highly recommended that regular training activities are provided across Europe prior to, and during, the implementation of a European Raptor Sampling Programme. These can be carried out at a national level and international level in "training camps" for people involved in collecting samples but also perhaps most usefully to train up trainers who can themselves go on to offer training more locally.

Improve coordination – To successfully implement a long-term biomonitoring European Raptor Sampling Programme, it is important to improve coordination between individual researchers and institutions in order to facilitate sample and data flux and storage, and thus increase the number of raptor samples available for analysis. As a solution to improve coordination within ERBFacility, we suggest establishing a role such as a national/regional coordinator should be established. These coordinators could play a pivotal role in the ERBFacility and facilitate in each country access to crucial information such as best practice guidelines, sampling protocols, guidance on legislation, and contact between relevant stakeholders. They could promote coordination between institutions and provide guidance on the flux of samples to the most adequate destinations: museums, collections, laboratories or ecotoxicology researchers. Depending on their logistic capability, coordinators could also help with storage of samples for short periods, assuming a centralizing and distributing role. Coordinators associated with environmental specimen banks and natural history museums might also be able to ensure the long-term storage of samples within their country or region (this issue of long-term storage is addressed more fully by related work under ERBFacility on development of a distributed European Raptor Specimen Bank). Coordinators could be very useful to help to solve several key constraints, centralizing questions and providing expertise and consistent solutions within their geographical area of operation (e.g. facilitating information on short-term storage and shipping of samples, advising on their country's legal framework for collecting samples) and could also be valuable in providing more local feedback on the results of the European Raptor Biomonitoring Scheme to fieldworkers, thus motivating

participants in the longer term. Finally, coordinators could also centralize and facilitate information that may be difficult to access, such as potential sources of contamination, and practical information such as where to get the specific materials needed to collect samples.

Selection of the most suitable focal species and contaminants (prioritization) – To solve constraints relating to spatial coverage, it will be important to select focal species that can maximise the representativeness of different countries and regions (Badry et al. 2020). The most suitable focal species will vary depending on the chemicals targeted by the Monitoring Scheme. A suitable set of focal species should also minimize potential spatial gaps in data resulting from: incomplete coverage by the Sampling Programme; raptors with uneven distributions; and difficulties in accessing breeding areas. Whether it is possible to obtain an adequate amount of the matrix (e.g. blood, liver) from the focal species should also be considered, and, if not, larger species will need to be selected or samples pooled for analysis. The choice of focal species should take into account spatial representativeness but also the susceptibility of the species (high probability of exposure) to the focal chemical; species and population traits, such as distribution, diet composition and food web, foraging behaviour and habitats, and migratory movements, i.e. migratory versus resident need consideration here (Lourenço et al., 2011, Badry et al., 2019, 2020).

Badry et al., (2020) indicated that common buzzard (*Buteo buteo*) and tawny owl (*Strix aluco*) are suitable species for a European Raptor Biomonitoring Scheme for many contaminants, because of their wide distribution and abundance. Although other species may be regionally better suited for particular chemical threats, such as the golden eagle (*Aquila chrysaetos*) for lead, the northern goshawk (*Accipiter gentilis*) for mercury across areas including Northern Europe, or vultures for non-steroidal anti-inflammatory drugs (NSAIDs).

Increase the number of monitoring actions (projects and funding) – Finally, there will be a need to implement measures that contribute to an increase in the number of raptor monitoring projects that can work as national or regional support to a European Raptor Sampling Programme. This can be achieved by a coordinated support from national or regional funds, but also by promoting international consortia supported by EU funds. Indirectly, a greater number of contaminant and raptor monitoring projects would also contribute to increase spatial coverage and to reduce skills constraints as well as improve pan-European accessibility of raptor samples. Some countries have good examples of long-term monitoring schemes (e.g. Berny et al., 2015, Vrezec et al., 2014, Walker et al., 2008) that bring valuable experience to bear on development of the European Raptor Biomonitoring Facility. Questionnaire respondents were asked to identify the most important solutions to the

constraints for sampling raptors. The most frequently highlighted solution by the questionnaire respondents was increasing the overall number of monitoring schemes and projects (30%) (Figure 6). Best practice guidance, including the dissemination of protocols to collect and process samples, were also highly scored solutions (27%). In contrast, capacity building activities related to training in ringing and handling live birds (9%) were the solutions least prioritised by respondents.

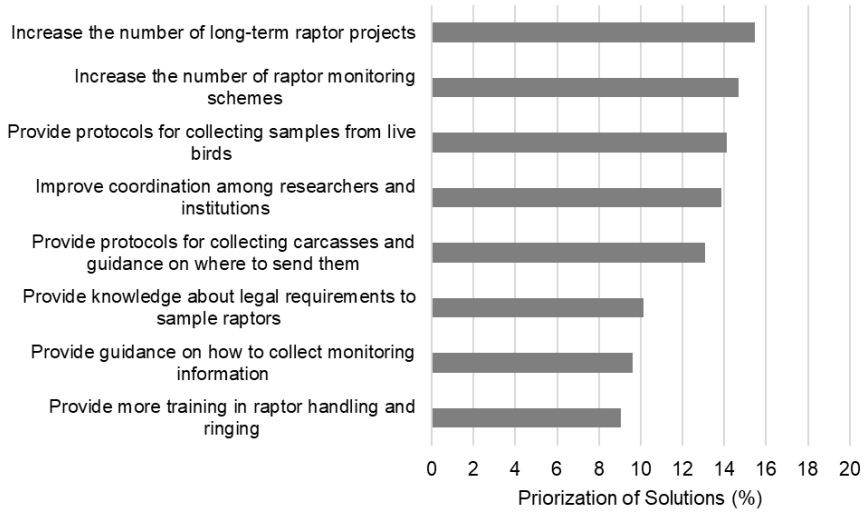

Figure 6. Frequency of the most prevalent solutions to constraints for sampling raptors, as identified by questionnaire respondents.

3.4. Limitations of the study

The approaches used to identify constraints and solutions present some limitations. The number of respondents from each country was not balanced, and in some cases, we only had one respondent from a country, which limits the comparison of constraints between countries. Also, the number of respondents working on research in universities was higher than for other relevant professional occupations and the results of the questionnaire could have a slight bias towards the situation of researchers working in southern European countries, who were the most frequently represented group in the study. We made an effort to compensate for this bias by including the specific experience of workshop participants and the manuscript co-authors, which covered some of the least represented countries in the questionnaire.

4. CONCLUSIONS

Our participatory approach, combining the opinion of experts and participants involved in collecting raptor samples and contextual data, has provided important information on the constraints associated with implementing a European Raptor Sampling Programme in support of a European Raptor Biomonitoring Scheme, both key elements of a European Raptor Biomonitoring Facility. The approach presented here might be applied elsewhere prior to the development of long-term biomonitoring schemes so that constraints can be anticipated and mitigated with effective solutions. Our approach provides information on the actors that can potentially be involved in sampling programmes and about their current capacity to provide raptor samples. Among the constraints to collecting raptor samples and contextual data, legal constraints appear of less importance to respondents than methodological, skills, and spatial coverage constraints. This is somewhat reassuring, as legal constraints, if they exist, could be more difficult to resolve than those in the other constraint categories. Most constraints highlighted refer to methodological aspects of collecting samples and contextual data. The lack of protocols to collect invasive samples in a harmonized way has been identified as one of the most important constraints to be solved. Disseminating existing protocols could be an effective way to harmonize methodological procedures to obtain raptor samples for contaminant monitoring from across Europe. However, national adaptation of international protocols may face additional legal and linguistic barriers. Increasing the number of raptor contaminant monitoring schemes that can contribute to create the necessary network of people and institutions at national and regional level that may ensure the long-term collection of both samples and complex contextual data will demand funding and effective sharing of knowledge from existing schemes. Our approach suggests that establishing a long-term European Raptor Sampling Programme as a key element of a European Raptor Biomonitoring Facility is feasible considering that all the constraints that we identified may be solved by reasonable solutions.

REFERENCES

Badry, A., Palma, L., Beja, P., Ciesielski, T.M., Dias, A., Lierhagen, S., Munro Jenssen, B., Stuararo, N., Eulaers, I., Jaspers, V.L.B., 2019. Using an apex predator for large-scale monitoring of trace element contamination: associations with environmental, anthropogenic and dietary proxies. Sci. Total Environ. 676, 746–755. https://doi.org/10.1016/j.scitotenv.2019.04.217

Badry, A., Krone, O., Jaspers, V.L.B., Mateo, R., García-Fernández, A., Leivits, M., Shore, R.F., 2020. Towards harmonisation of chemical monitoring using avian apex predators: identification of key species for pan-European biomonitoring. Sci. Total Environ. 731:139198. https://doi.org/10.1016/j.scitotenv.2020.139198

Berny, P., Vilagines, L., Cugnasse, J.M., Mastain, O., Chollet, J.Y., Joncour, G., Razin, M., 2015. VIGILANCE POISON: Illegal poisoning and lead intoxication are the main factors affecting avian scavenger survival in the Pyrenees (France). Ecotoxicol. Environ. Saf. 118, 71—82. https://doi.org/10.1016/j.ecoenv.2015.04.003

Bird DM, Bildstein KL (Eds.) 2007. Raptor research and management techniques. Raptor Research Foundation. Hancock House Publishers, Surrey.

Bustnes, J.O., Bårdsen, B.J., Herzke, D., Johnsen, T. V., Eulaers, I., Ballesteros, M., Hanssen, S.A., Covaci, A., Jaspers, V.L.B., Eens, M., Sonne, C., Halley, D., Moum, T., Nøst, T.H., Erikstad, K.E., Ims, R.A., 2013. Plasma concentrations of organohalogenated pollutants in predatory bird nestlings: Associations to growth rate and dietary tracers. Environ. Toxicol. Chem. 32, 2520–2527. https://doi.org/10.1002/etc.2329

Carneiro, M., Colaço, B., Brandão, R., Azorín, B., Nicolas, O., Colaço, J., Pires, M.J., Agustí, S., Casas-Díaz, E., Lavin, S., Oliveira, P.A., 2015. Assessment of the exposure to heavy metals in Griffon vultures (*Gyps fulvus*) from the Iberian Peninsula. Ecotoxicol. Environ. Saf. 113, 295-301. https://doi.org/10.1016/j.ecoenv.2014.12.016

CITES, 1984. Convention on international trade in endangered species of wild Fauna and Flora. Environmental Policy and Law, 13(3–4), 130. https://doi.org/10.1016/S0378-777X(84)80087-6

Council Directive 92/43/EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora. Available at: http://data.europa.eu/eli/dir/1992/43/oj

Derlink, M., Wernham, C., Bertoncelj, I., Kovács, A., Saurola, P., Duke, G., Movalli, P., Vrezec, A., 2018. A review of raptor and owl monitoring activity across Europe: its implications for capacity building towards Pan-European monitoring. Bird Study 65, S4–S20. https://doi.org/10.1080/00063657.2018.1447546

Directive 2009/147/EC of the European Parliament and of the Council of 30 November 2009 on the conservation of wild birds. Available at: http://data.europa.eu/eli/dir/2009/147/oj

Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes Text with EEA relevance. Available at: http://data.europa.eu/eli/dir/2010/63/oj

Duke, G., 2008. The EU environmental policy context for monitoring for and with raptors in Europe. Ambio 37, 397–400. https://doi.org/10.1579/0044-7447(2008)37[397:TEEPCF]2.0.CO;2

Elliott, J.E., Morrissey, C.A., Henny, C.J., Inzunza, E.R., Shaw, P., 2007. Satellite telemetry and prey sampling reveal contaminant sources to pacific northwest ospreys. Ecol. Appl. 17, 1223–1233. https://doi.org/10.1890/06-1213

ERBFacility. 2019a. WG4 meeting on best practice raptor sampling in the field. https://erbfacility.eu/events/wg4-meeting-best-practice-raptor-sampling-field (Accessed 3 October 2021)

ERBFacility. 2020. European Raptor Biomonitoring Facility. https://erbfacility.eu/

ERBFacitlity. 2019b. ERBFacility 2nd General Meeting (GM2). https://erbfacility.eu/events/erbfacility-2nd-general-meeting-gm2 (Accessed 3 October 2021)

Espin, S., García Fernández, A.J., Herzke, D., Shore, R.F., van Hattum, B., Martinez-López, E., Coeurdassier, M., Eulaers, I., Fritsch, C., Gómez-Ramírez, P., Jaspers, V.L.B., Krone, O., Duke, G., Helander, B., Mateo, R., Movalli, P., Sonne, C., van den Brink, N.W., 2014. Sampling and contaminant monitoring protocol for raptors. EURAPMON, 34. http://www.eurapmon.net/ (Accessed 3 October 2021)

Espín, S., García-Fernández, A. J., Herzke, D., Shore, R. F., van Hattum, B., Martínez-López, E., Coeurdassier, M., Eulaers, I., Fritsch, C., Gómez-Ramírez, P., Jaspers, V. L. B., Krone, O., Duke, G., Helander, B., Mateo, R., Movalli, P., Sonne,

C., & van den Brink, N. W. (2016). Tracking pan-continental trends in environmental contamination using sentinel raptors-what types of samples should we use? Ecotoxicology, 25(4), 777–801. https://doi.org/10.1007/s10646-016-1636-8

Espín, S., Andevski, J., Duke, G., Eulaers, I., Gómez-Ramírez, P., Hallgrimsson, G.T., Helander, B., Herzke, D., Jaspers, V.L.B., Krone, O., Lourenço, R., María-Mojica, P., Martínez-López, E., Mateo, R., Movalli, P., Sánchez-Virosta, P., Shore, R.F., Sonne, C., van den Brink, N.W., van Hattum, B., Vrezec, A., Wernham, C., García-Fernández, A.J., 2020. A schematic sampling protocol for contaminant monitoring in raptors. Ambio. https://doi.org/10.1007/s13280-020-01341-9

García-Fernández, A.J., 2020. Ecotoxicological risk assessment in the context of different EU regulations. In: Roy, K. (Ed.), Ecotoxicological QSARs, Methods in Pharmacology and Toxicology. Springer Protocols, Humana, New York, pp. 3-25.

García-Fernéndez, A.J., Calvo, J.F., Martínez-López, E., Maria-Mojica, P., Martínez, J.E., 2008. Raptor ecotoxicology in Spain: A review on persistent environmental contaminants. Ambio 37, 432-439. https://doi.org/10.1579/0044-7447(2008)37[432:REISAR]2.0.CO;2

García-Fernández, A.J., Espín, S., Gómez-Ramírez, P., Martínez-López, E., Navas, I. 2020. Wildlife sentinels for human and environmental health hazards in ecotoxicological risk assessment. In: Roy K (Ed.). Ecotoxicological QSARs. Methods in Pharmacology and Toxicology. Springer Protocols, Humana, New York, pp 77-94.

Gómez-Ramírez, P., Shore, R.F., van den Brink, N.W., van Hattum, B., Bustnes, J.O., Duke, G., Fritsch, C., García-Fernández, A.J., Helander, B.O., Jaspers, V., Krone, O., Martínez-López, E., Mateo, R., Movalli, P., Sonne, C., 2014. An overview of existing raptor contaminant monitoring activities in Europe. Environ. Int. 67, 12-21. https://doi.org/10.1016/j.envint.2014.02.004

Hallmann, C.A., Foppen, R.P.B., Van Turnhout, C.A.M., De Kroon, H., Jongejans, E., 2014. Declines in insectivorous birds are associated with high neonicotinoid concentrations. Nature 511, 341–343. https://doi.org/10.1038/nature13531

Jepson, P.D., Law, R.J., 2016. Persistent pollutants, persistent threats. Science (80-.). 352, 1388–1389. https://doi.org/10.1126/science.aaf9075

Kovács, A., Mammen, U.C.C., Wernham, C. V., 2008. European monitoring for raptors and owls: State of the art and future needs. Ambio 37, 408-412. https://doi.org/10.1579/0044-7447(2008)37[408:EMFRAO]2.0.CO;2

Krabbenhoft, D.P., Sunderland, E.M., 2013. Global Change and Mercury. Science (80-.). 341, 1457–1458. https://doi.org/10.1126/science.1242838

Lodenius, M., Solonen, T., 2013. The use of feathers of birds of prey as indicators of metal pollution. Ecotoxicology 22, 1319–1334. https://doi.org/10.1007/s10646-013-1128-z

Lourenço, R., Tavares, P.C., Del Mar Delgado, M., Rabaça, J.E., Penteriani, V., 2011. Superpredation increases mercury levels in a generalist top predator, the eagle owl. Ecotoxicology 20, 635-642. https://doi.org/10.1007/s10646-011-0603-7

Malaj, E., von der Ohe, P.C., Grote, M., Kühne, R., Mondy, C.P., Usseglio-Polatera, P., Brack, W., Schäfer, R.B., 2014. Organic chemicals jeopardize the health of freshwater ecosystems on the continental scale. Proc. Natl. Acad. Sci. 111, 9549-9554. https://doi.org/10.1073/pnas.1321082111

Movalli, P., Duke, G., Osborn, D., 2008. Introduction to monitoring for and with raptors. Ambio 37, 395—396. https://doi.org/10.1579/0044-7447(2008)37[395:itmfaw]2.0.co;2

Movalli, P., Dekker, R., Koschorreck, J., Treu, G., 2017. Bringing together raptor collections in Europe for contaminant research and monitoring in relation to chemicals regulations. Environ. Sci. Pollut. Res. 24, 24057–24060. https://doi.org/10.1007/s11356-017-0096-x

Movalli, P., Krone, O., Osborn, D., Pain, D., 2018. Monitoring contaminants, emerging infectious diseases and environmental change with raptors, and links to human health. Bird Study 65, S96–S109. https://doi.org/10.1080/00063657.2018.150673

Movalli, P., Duke, G., Ramello, G., Dekker, R., Vrezec, A., Shore, R.F., García-fernández, A., Wernham, C., Krone, O.,

Alygizakis, N., Badry, A., 2019. Progress on bringing together raptor collections in Europe for contaminant research and monitoring in relation to chemicals regulation 20132-20136.

Palma, L., Beja, P., Tavares, P.C., Monteiro, L.R., 2005. Spatial variation of mercury levels in nesting Bonelli's eagles from Southwest Portugal: Effects of diet composition and prey contamination. Environ. Pollut. 134, 549–557. https://doi.org/10.1016/j.envpol.2004.05.017

R Core Team. 2020. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Ramello, G., Duke, G., Dekker, R., van der Mije, S., Movalli P., (Unpublished results). A survey of raptor collections in Europe and their potential to provide samples for pan-European contaminant monitoring.

Rodríguez-Estival, J., Mateo, R., 2019. Exposure to anthropogenic chemicals in wild carnivores: a silent conservation threat demanding long-term surveillance. https://doi.org/10.1016/j.coesh.2019.06.002

Roque, I., Lourenço, R., Marques, A., Coelho, J.P., Coelho, C., Pereira, E., Rabaça, J.E., Roulin, A., 2016. Barn owl feathers as biomonitors of mercury: sources of variation in sampling procedures. Ecotoxicology 25, 469–480. https://doi.org/10.1007/s10646-015-1604-8.

Sbokos, J., Duke, G., Hosner, P., Kristensen J.B., Movalli, P. (in prep.) Legal constraints to international shipping of raptor samples in Europe.

Schipper, A.M., Wijnhoven, S., Baveco, H., Van Den Brink, N.W., 2012. Contaminant exposure in relation to spatio-temporal variation in diet composition: A case study of the little owl (*Athene noctua*). Environ. Pollut. 163, 109–116. https://doi.org/10.1016/j.envpol.2011.12.020

Shore, R.F., Taggart, M.A., 2019. Population-level impacts of chemical contaminants on apex avian species. Curr. Opin. Environ. Sci. Heal. 11, 65–70. https://doi.org/https://doi.org/10.1016/j.coesh.2019.06.007

Smits, J.E.G., Fernie, K.J., 2013. Avian wildlife as sentinels of ecosystem health. Comp. Immunol. Microbiol. Infect. Dis. 36, 333–342. https://doi.org/10.1016/j.cimid.2012.11.007

Valverde I, Espín S, María-Mojica P, García-Fernández AJ. 2020. Protocol to classify the stages of carcasses decomposition and estimate the time of death in small-size raptors. European Journal of Wildlife Research (in press).

Vlachopoulos, K., Dekker, R., Duke, G., Koureas, D., Islam, S., van der Mije, S., Movalli, P. (Unpublished results). Towards a European Raptor Specimen Database

Vrezec, A., Duke, G., Kovács, A., Saurola, P., Wernham, C., Burfield, I., Movalli, P., Bertoncelj, I., 2014. Overview of raptor monitoring activities in Europe. Acrocephalus 33, 145-157. https://doi.org/10.2478/v10100-012-0003-y

Walker, L.A., Shore, R.F., Turk, A., Pereira, M.G., Best, J., 2008. The Predatory Bird Monitoring Scheme: identifying chemical risks to top predators in Britain. Ambio 37, 466–471. https://doi.org/10.1579/0044-7447(2008)37[469:tpbmsi]2.0.co;2

SUPLEMENTARY INFORMATION

Table S1. Examples of contextual data that may be collected for raptor samples under the framework of the European Raptor Biomonitoring Facility (ERBFacility). Most items in this list are collected for the specific individual (i.e., specimen contextual data), while "Regional population trends" is collected for the local/regional population (i.e. population contextual data). Reproductive performance, diet composition, known contamination sources, and behavioural data may be collected both at specimen and population levels.

Contextual data	**Priority**
Date	Mandatory
Raptor species	Mandatory
Sampling location (coordinates or detailed description)	Mandatory
Individual condition (dead/alive)	Mandatory
Matrix type (e.g. carcass, liver, blood, feather, pellet, egg)	Mandatory
Age	Mandatory
Name of the collector	High
Sex	High
Condition of the bird (alive; e.g. lean, emaciated, etc.)	High
Type of feather (flight, tail, body)	High
Estimation of time of death	High
Reproductive performance (breeding, clutch size, hatching success)	High
Diet composition (insects, mammals, birds, etc.)	Medium to high
Measurements (weight, wing length, body length, etc.)	Medium
Land use (agricultural, urban, natural, etc.)	Medium
Known contamination sources in the area (pesticides use, proximity to urban areas, etc.)	Medium
Ring number (if ringed)	Medium
Morph type/ plumage pattern	Medium
Regional population trends of the focal species	Low/optional
Behavioural data	Low/optional
Bait (specific for poisoning events)	Low/optional
Entomofauna (specific for poisoning events)	Low/optional
Water and soil	Low/optional
Blood slide for biomarkers	Low/optional
Photos (of the individual, of the habitat)	Low/optional
Measurements (weight, wing length, body length, etc.)	Medium

Table S2. Questionnaire used to classify the importance of the constraints in raptor sampling. The table S2 is available as electronic supplementary material due to its large size. Please scan the QR code below to display the table.

Table S3. Full description of the potential constraints to a biomonitoring scheme using raptors to detect contaminants at the pan-European scale.

#	Constraint type	Constraint	Explanation
1	Legal	Legal restrictions to access private property	Some samples may have to be collected in private land, for example when a raptor nest or a carcass dies is located in private land. Therefore, the lack of permission to access private land may be a limitation to collect samples. The legal frame and proportion of private land is considerably variable across European countries.
2	Legal	Legal restrictions for visiting active nests	Active raptor nests are often protected by legislation in order to prevent disturbance of breeding birds. Visiting active nests is an important activity necessary to obtain samples and contextual data, therefore it may be constrained by legal restrictions in some cases may limit the samples obtention.
3	Legal	Legal restrictions for handling live birds	Permits are required for handling live birds, namely raptors for which specific permits may be needed. Thus, the lack of permits for handling live raptors may limit the capacity to collect samples and contextual data acquisition.
4	Legal	Legal restrictions for sampling blood or other invasive samples	Collecting invasive samples, such as blood, in raptors, requires specific permits and skills. The lack of permits may constrain fieldworkers from obtaining these kinds of samples.
5	Legal	Legal restrictions on transportation of samples within country	Most countries are under international legislation that regulate the transportation of biological samples. In addition, every country has its own legislation for shipping biological samples. In some countries, transportation of samples is a complex process and may require specific permits and procedures.
6	Legal	Legal restrictions to holding and storing raptor samples (carcasses, feathers, eggs)	Raptors species are protected across Europe, and therefore holding and storing raptor samples, such as carcasses, feathers, eggs or other matrix types are restricted by national and international legislation. Thus legal restrictions might affect the sampling program. The participation of fieldworkers and institutions will thus be limited if they do not have the specific permits required to hold raptor specimens and samples.

#	Constraint type	Constraint	Explanation
7	Methodological	Lack of information on adequate protocols for collecting samples	All samples must be obtained following adequate sampling protocols to ensure comparability. The lack of sampling protocols or its insufficient distribution is an important limitation to adequately collecting samples.
8	Methodological	Difficulty of providing material for sampling	Sample collecting may require specific material, as for example hypodermic needles, syringes for blood sampling, or specific containers to store the samples. The inaccessibility of the correct materials for sampling may dissuade field workers to collect samples or may contaminate the samples if the material used is not adequate.
9	Methodological	Lack of amount of sampling blood of nestlings	Blood is a matrix that reflects the most recent exposure to contaminants; therefore, it may be relevant to some ecotoxicological studies. However, the amount of blood that is possible to collect from live birds is limited according to the weight of the bird, and sometimes the amount may not be enough for a correct chemical analysis.
10	Methodological	Difficulty for correct short-term storage of the samples	When a sample is collected it may need be stored for a short period before it is sent for a long-term storage. An incorrect short-term storage of the sample may cause its deterioration and unsuitability for analysis (e.g. high temperature, inadequate containers).
11	Methodological	Difficulty to support the shipping cost or ensure a correct transportation	Samples must be sent following the adequate packaging, shipping and transportation protocols. The shipping cost can discourage the participation of fieldworkers in the monitoring program.
12	Methodological	Difficulty to find an institution to send the sample	When a sample is collected it must be sent as soon as possible to an institution for long-term storage or chemical analysis. However, fieldworkers may not know which the most suitable institution is to send the samples for the monitoring program.
13	Methodological	Difficulty in collecting mandatory or high priority contextual data (age, sex, feather type)	All collected samples must have contextual data to be related with concentration values of chemicals. At least mandatory or high priority contextual data such as raptor species, location of the sample, matrix, or date, must be recorded and send with the sample in order to process it. The lack of contextual data can impair the interpretation of analytical results.
14	Methodological	Difficulty in collecting contextual data- potential sources of contamination	Knowing the potential sources of contamination can be helpful for understanding the chemical analysis results. However, this information is difficult to obtain in some cases because local people may not be interested to collaborate by for example informing about the use of pesticides or hunting practices.
15	Methodological	Difficulty in collecting contextual data – diet	Diet is an important input of contaminants, and variations in diet cause different patterns of accumulation of contaminants among individuals, populations, or species. Knowing the diet of a species (or population) requires a great investment of time and specific knowledge to identify diet remains. Therefore, despite its interest, determining the diet of the raptor individuals can sometimes be a constraint.

#	Constraint type	Constraint	Explanation
16	Methodological	Difficulty in collecting contextual data – reproductive performance	Reproductive performance is an important population contextual data to record as it can reflect contaminants accumulation and it is important to detect possible effects of contaminants on reproduction. Reproductive performance is difficult to record because it entails time and specific knowledge. Usually it requires a monitoring scheme ongoing.
17	Methodological	No land-use information as contextual data because of non-precise location	Land use is related with the different kinds of pollutants of an area. For this reason, it is important to provide information about the location of the sample and its land uses. Chemical analysis results may be explained by the land uses where the sample was collected or where the individual raptor forages.
18	Methodological	Difficulty in the storage of contextual data related with the sample	Contextual data must be stored in order to be unequivocally linked to a specific sample and thus provide useful information about it. An inefficient organization can be a problem for contextual data storage, which could result in the loss of essential information for the monitoring scheme.
19	Methodological	Difficulty to relate sample with contextual data	Contextual data is basic to understand analysis results of the sample, for this reason all samples should be clearly related with contextual data. Difficulties in linking contextual data to contaminant concentrations would constrain the results of a biomonitoring scheme.
20	Spatial Coverage	Focal raptor population with very low abundance or uneven distribution	Some focal raptors species chosen by the monitoring scheme may not have a very high abundance in all countries of Europe, or their distribution may be uneven. This can create coverage gaps that may constrain overall results. Accordingly, not all focal raptor species may be suitable for all countries.
21	Spatial Coverage	Low number of monitoring schemes and ecological studies to provide access to raptors samples	National and regional raptor monitoring schemes and ecological studies can provide an important number of raptor samples to a pan-European biomonitoring scheme. An overall low number of these schemes may limit the number of samples collected.
22	Spatial Coverage	Low number of monitoring schemes and ecological studies to provide complex contextual data	National and regional raptor monitoring schemes and ecological studies are the main sources of specific and complex contextual data, for example information about diet, reproductive performance, behavioural data, or population trend. A low number of monitoring schemes may lead to insufficient population contextual data.
23	Spatial Coverage	Low habitat areas in the country	Some habitats may be particularly relevant or useful for monitoring a focal contaminant. For example, a specific crop pesticide. The absence of a specific habitat within a country or region may prevent the focal contaminant from being adequately monitored or may hamper widespread comparisons.
24	Spatial Coverage	Difficulty to access to breeding areas	The access to the areas where raptors are breeding is fundamental to collect raptor samples and contextual data. Some of these areas are difficult to access, for example if the species is breeding in an isolated island, very remote areas, or with very steep relief.

#	Constraint type	Constraint	Explanation
25	Spatial Coverage	Difficulty to access to the nests	Visiting raptor nests is a very important activity into sampling raptors, since several samples can be obtained there such as addled/deserted eggs, feathers, pellets, and samples from nestlings. Moreover, important contextual data can be obtained during visiting to nests, such as reproductive performance parameters. Some raptor species nests are located in difficult sites with difficult access, such as cliffs or in the top of trees. Therefore, the difficulty of accessing to the raptor nests may be a limitation to obtaining samples and contextual data from raptors.
26	Spatial Coverage	Lack of institutions to participate in the biomonitoring scheme	Several national institutions should play an important role in a pan-European biomonitoring scheme using raptors. This includes institutions that can provide samples, contextual data, long-term storage, analysis capacity and an ambassador or focal role. An insufficient number of institutions in a country or region may cause a gap in spatial coverage.
27	Skills	Lack of skilled people for raptor sampling	Specific skills and experience are needed to carry out raptor sampling. A limited number of skilled fieldworkers is thus an important constraint to obtain samples.
28	Skills	Lack of means for capacity building by field coordination institutions	In order to have enough skilled fieldworkers it is necessary to promote capacity building among field coordination institutions. The lack of means to carry out capacity building is an important constraint for collecting raptor samples and contextual data.
29	Skills	Lack of motivation among field workers	Field work requires considerable time and economic effort. Collecting samples for a biomonitoring scheme may represent additional work to that carried out regularly by most fieldworkers and therefore their lack of motivation to participate may constrain the overall number of samples that are collected.
30	Skills	Lack of skills required for post-processing of carcasses (necropsies)	Raptor carcasses represent an important source of samples in ecotoxicological studies (e.g. liver, kidney, muscle, bone, fat). In order to extract different matrices from carcasses, specific skills to do necropsies are required. Thus, the lack of people skilled to do necropsies is a potential constraint in a pan-European biomonitoring scheme.
31	Skills	Lack of skills to collect complex contextual data	Collecting contextual data may require specific skills, for example, to determine sex, age, or even identify similar species of raptors. The lack of skilled people to collect contextual data is a limitation to obtaining information crucial for the interpretation of analytical results.

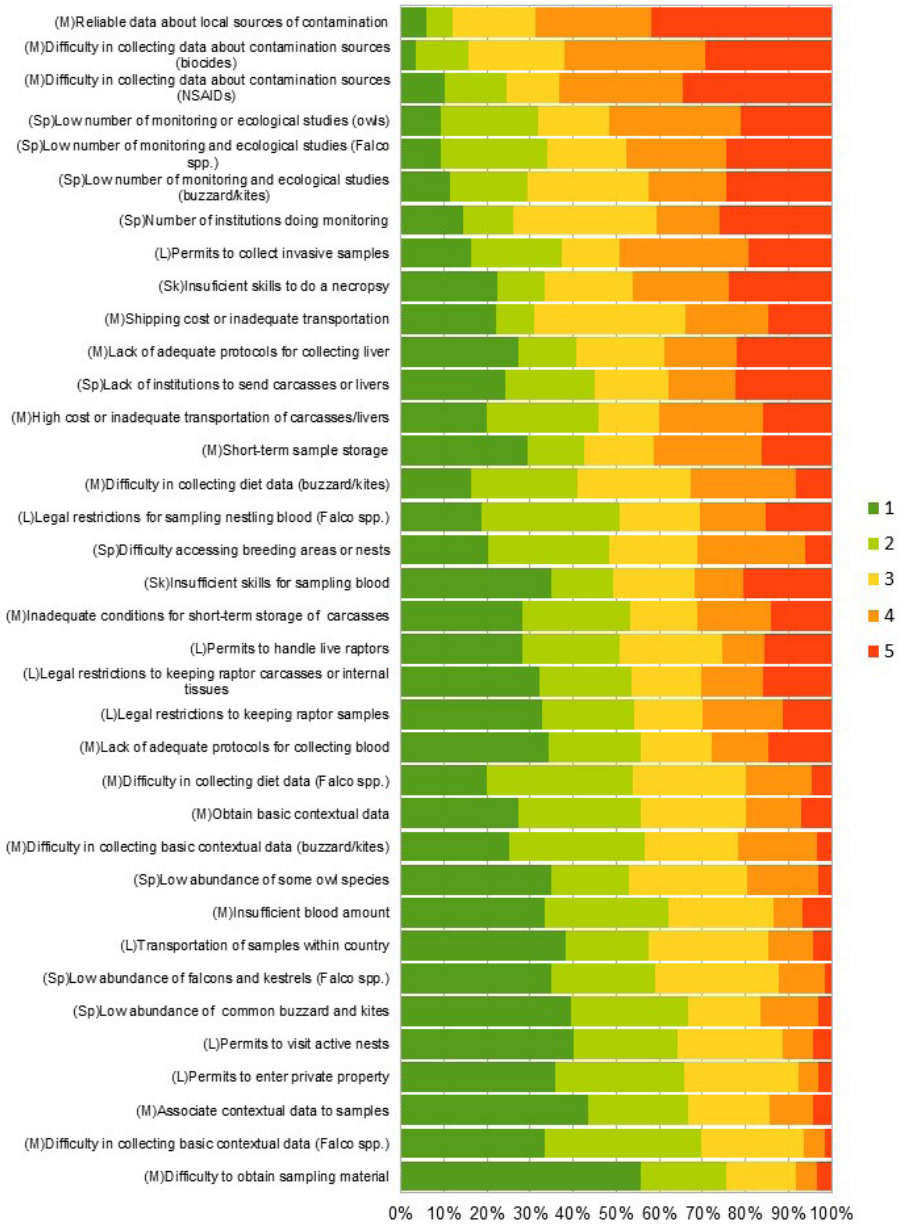

Figure S1. Scoring of the questions regarding constraints to the sampling of raptors. Scores indicating the importance of constraints from 1 (low importance) to 5 (high importance). Letters in brackets preceding the constraint indicate its type: (L) Legal; (M) Methodological, (Sk) Skills; (Sp) Spatial coverage.

CHAPTER VI

LEGACY AND EMERGING CONTAMINANTS IN FLAMINGOS' CHICKS' BLOOD FROM THE EBRO DELTA NATURAL PARK

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ABSTRACT

The Ebro delta is a wetland of international importance for waterbird conservation but severally affected by intensive agriculture, toxic waste discharges from a past chloro-alkali industry and affluence of tourism. The discharge of contaminants associated to these activities pose waterbirds breeding in the Ebro delta at risk. The aim of this study is to evaluate the exposure of 91 emerging and legacy micropollutants in flamingo chicks (*Phoenicopterus roseus*), an emblematic species of the area. Fifty chicks of 45-60 days were captured, biometric parameters measured and whole blood collected. Compounds analyzed included perfluoroalkyl substances (PFASs), pharmaceuticals, organophosphate esters (OPEs), in-use pesticides, polychlorinated biphenyls (PCBs), organochlorine pesticides (OCs), and polycyclic aromatic hydrocarbons (PAHs). The results indicate a multi-exposure of flamingo's chicks from a very young age. PFASs were the most ubiquitous compounds with ∑PFASs ranging from 9.34 to 576 ng/mL, being PFOA, PFOS and PFHxS detected in all samples. ∑PAHs ranged from 0.19 to 423 ng/mL, ∑PCBs from 0.5 to 15.6 ng/mL and ∑OCs from 1.35 to 37.8 ng/mL. Pharmaceuticals, OPEs and in-use pesticides were not detected. The flamingo's filtering behavior on mud and maternal ovo-transference are the more likely routes of exposure of organic micropollutants to flamingos' chicks. The reported levels of micropollutants were not associated with any alteration in the body condition of chicks. This is the first study to describe flamingos' chicks' exposure to multiple contaminants, highlighting the importance of biomonitoring for wildlife conservation and biodiversity preservation.

1. INTRODUCTION

The Ebro delta is one of the most important wetlands systems of the western Mediterranean and of international significance for waterbird conservation. With a relatively small surface of 320 km^2, it hosts more than 300 species of birds, within 100 breeding species (Ibáñez and Caiola, 2018). It has been catalogued as a Natural Park since 1983, a Special Protection Area for Birds, and it was included in the Ramsar list of international importance wetlands. The Ebro delta is classified as an Important Bird and Biodiversity Area (IBA) in danger by Birdlife International due intense agriculture, urbanism, tourism, and past chloro-alkali industry waste discharges (BirdLife International, 2022). These anthropogenic pressures have been identified as sources of a large number of organic contaminants in the area, including pharmaceuticals in wastewaters (Čelić et al., 2019), perfluorinated and perfluoroalkyl substances (PFASs) in water, sediments and fish (Pignotti et al., 2017), in-used pesticides in water (Barbieri et al., 2021), and Persistent Organic Pollutants (POPs) such as polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCs) including dichlorodiphenyltrichloroethane (DDTs), cycloidians and hexachlorocyclohexanes (HCHs) in water (Gómez-Gutiérrez et al., 2006). Contaminants in the area can pose a conservation threat due to their bioaccumulation potential and toxic effects on wildlife. Several studies have reported the presence of organic pollutants in biota from the Ebro delta, as invertebrates (Álvarez-Muñoz et al., 2019), fish (Pignotti et al., 2017), and reptiles (Santos et al., 1999). POPs have also been detected in several bird species dwelling in the area. PCBs, DDTs, and HCHs have been reported in eggs of purple heron (Huertas et al., 2016) and also in gull species (Morales et al., 2012). Other studies have also reported mercury (Pereira et al., 2019) and new emerging contaminants such as dechloranes in gulls eggs (Ayala-Cabrera et al., 2021). These studies demonstrate the benefits of using birds as biomonitors of environmental pollutants as they reflect the distribution patterns of contaminants in a given habitat.

The Greater flamingo (*Phoenicopterus roseus*) is an excellent sentinel species in brackish marshlands and lagoons due to its mud filtering feeding behavior (Borghesi et al., 2011; Johnoson and Cezillly, 2007). Despite being currently relatively common, flamingos are vulnerable species due to wetland loss throughout its distribution range and the low number of breeding sites that vary depending on the location, weather conditions, terrestrial predators, human disturbance, and pollution (Ancora et al., 2008; Geraci et al., 2012). In the western Mediterranean, Greater flamingos constitute a metapopulation with breeding dispersal among colonies, with more than 90% of the population breeding in less than 10 sites (Balkız et al., 2007). The flamingos of the Ebro delta are one of the few stable colonies of this species in the western Mediterranean, and are one of the most emblematic breeding waterbirds of the area. Their population is of 2985 breeding pairs,

according to 2019 census (population controlled by the Natural Park staff since 2004). The flamingo breeding colony is well preserved as the affluence of tourism and intrusion of invasive species or predators is minimized, but little is known on the impact of contaminants that historically affect the area and can represent a new threat to the survival and well-being of this species. Moreover, exposure to mixtures of micropollutants can have additive effects, especially in chicks, which are especially vulnerable to pollution and crucial for the maintenance of the colony.

In this study, we have analyzed for the first-time a near one hundred compounds, including legacy OCs, PCBs, polycyclic aromatic hydrocarbons (PAHs) and emerging contaminants as high consumption pharmaceuticals, PFASs, in-use pesticides and organophosphate esters (OPEs) in blood from two months old chicks of Greater flamingos with the aim to evaluate their occurrence and potential impact on body indexes. At this early age flamingos are fed by their parents, therefore, micropollutants in their blood evidence in a great measure local contamination reaching chicks via ovo-transfer or through the diet during the first 2 months of life. The specific objectives of this research are: (i) to develop 3 extraction and analytical protocols for analyzing 91 legacy and emerging contaminants in blood; (ii) to screen the contamination patterns of Ebro Delta Greater flamingos; and (iii) to assess the health of the breeding colony by measuring the biometric data of individuals. This is to our knowledge the first study to evaluate the early exposure of both legacy and emerging contaminants in flamingos' chicks. The study is relevant internationally because early exposure to organic contaminants can have detrimental effects towards the development and survival of many bird species. Considering that many bird species are affected by various anthropogenic pressures and climate change, it is important to evaluate the occurrence and impact of a large number of contaminants of different chemical families in bird species dwelling in areas which are heavily affected by environmental pollution but yet are of high conservation relevance.

2. METHODS

2.1. Sampling

Flamingos' chicks from the Ebro Delta breeding colony (Figure 1) are ringed annually with the collaboration of 200 volunteers under the organization of Ebro Delta Natural Park. Under this ringing activity, 50 two months old individuals were collected for contaminants analysis on the 4th of August 2019 (sampling was authorized by the Ebro delta Natural Park). Blood samples were collected following the good practices guidelines described by Espín et al., 2020. Two mL of blood were collected from tarsal vein by veterinarians, using a 5 mL syringe with 25 gauge size needle and placed into 2 mL Eppendorf

tubes. Samples were kept refrigerated in the field, stored at -21ºC and freeze-dried. Blood was split in three parts to perform three analytical procedures; in three samples the amount of blood was not enough to do all the analysis. Therefore, 47 samples were analysed for Method 1 (PFASs) and 50 samples were analysed for method 2 (OCs, PCBs and PAHs) and 3 (Pharmaceuticals, pesticides and OPEs). Biometric measurements were taken for each chick including weight (± 50 g), wing and tarsus (± 1 mm) and culmen (± 0.1 mm). Scaled Mass Indexes (SMI) were calculated following the method proposed by Peig and Green, 2009.

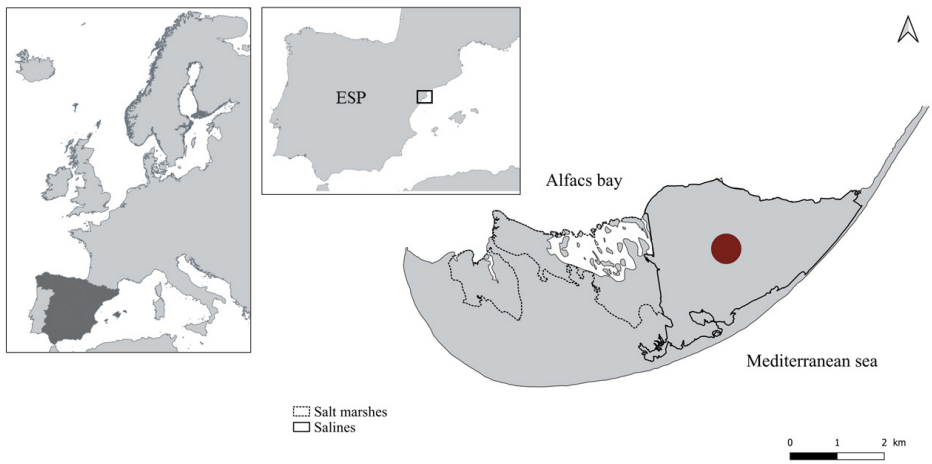

Figure 1. Location of greater flamingos breeding colony in Ebro delta Natural Park at the Alfacs peninsula (South Catalonia, Spain).

2.2. Sample preparation

2.2.1. Standards and chemicals

Target compounds included 17 PFASs, 7 marker PCBs congeners, 16 PAHs, 14 OCs, 17 pharmaceuticals, 17 polar pesticides and 3 OPEs. Pharmaceuticals and pesticides selected were high consumption volume chemicals and compounds detected in Important Biodiversity Areas (Dulsat-Masvidal et al., 2022). Certified standards of 98-99% purity were purchased from Wellington laboratories Inc. (Guelph, ON, Canada), AccuStandard (New Haven, CT, USA), Sigma-Aldrich (St. Louis, USA) and Dr. Ehrenstorfer (Augsburg, Germany). The surrogate standards used were triphenyl phosphate-d_{15} (TPhP-d_{15}), acetaminophen-methyl-d_3, carbamazepine-d_2, lidocaine-diethyl-d_{10}, sulfamethoxazole-d_4, isoproturon-d_6, estrone-d_2, M-PFOA, M-PFOS, and the deuterated PAHs solution mix (naphthalene-d_8, acenaphthene-d_{10}, phenanthrene-d_{10}, chrysene-d_{12} and perylene-d_{12}). Further details of the analyte's standards are provided in the Supplementary Information (SI) (Table

S1). Solvents used were acetonitrile (ACN) purchased from Fisher Scientific Chemical (Bridgewater, USA), methanol (MeOH) and HPLC grade water from Merck (Darmstadt, Germany). Ammonium formate (NH_4HCO_2), ammonium acetate ($NH_4CH_3CO_2$) and formic acid (HCOOH) were from Sigma-Aldrich.

2.2.2. Analysis of perfluoroalkyl substances (Method 1)

The extraction method was adapted from a previous study analyzing PFASs in gull eggs (Colomer-Vidal et al., 2022). Briefly, 50 mg of whole freeze-dried blood were placed in a 2 mL polypropylene Eppendorf vial (Eppendorf, Hamburg, Germany), spiked with 50 ng of M-PFOA and M-PFOS and 1.5 mL of acetonitrile were added. Samples were vortexed (1 min) and ultrasonicated (10 min) and this procedure was repeated 3 times without changing the solvent, and then centrifuged (10 min, 3500 rpm). The supernatant was collected and 25 mg of active carbon and 50 µL of glacial acid were added. The extract was vortexed (1 min) and centrifuged again (10 min), and extracts were filtered through 13 mm x 0.2 µm nylon syringe filter (Clarify, Phenomenex, USA). Samples were evaporated to dryness and reconstituted with 100 µL of methanol and 100 µL of HPLC water with 10 mM ammonium acetate. The analysis was performed by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) with an electrospray ion source (Waters, Milford, USA). An Acquity BEH C18 analytical column (100 mm x 2.1 mm internal diameter, 1.7 µm particle size) with a BEH C18 VanGuard pre-column (5 mm x 2.1 mm internal diameter, 1.7 µm particle size) was deployed (Acquity, Waters, Ireland). An Acquity BEH C18 trap column (30 mm x 2.1 mm internal diameter, 1.7 µm particle size) was placed before the injector to avoid PFASs contamination from the chromatographic system. The mobile phase consisted of (A) a mixture of methanol and acetonitrile (80:20) with 10 mM of ammonium acetate and (B) water with 10 mM of ammonium acetate. Gradient elution started at 50% A and 50% B (condition kept for 3 min), increased to 100% A in 7 min and returned to initial conditions in 5 min. All compounds were measured under negative electrospray (ESI-). The data was processed through Mass Lynx software.

2.2.3. Analysis of organochlorine compounds and PAHs (Method 2)

Fifty mg of whole freeze-dried blood were weighed in 2 mL Eppendorf vial. The samples were spiked with 50 ng of internal standards solution containing naphthalene-d_8, acenaphthene-d_{10}, phenanthrene-d_{10}, chrysene-d_{12} and perylene-d_{12}. The extraction consisted of adding 1.5 mL of hexane: dichloromethane (1:1), then the samples were vortexed for 1 min and ultrasonicated for 10 min, and this procedure was repeated 3 times without changing the solvent. Samples were centrifuged (10 min, 3500 rpm)

and the supernatant was collected. Clean-up was performed with 5 g Florisil Bond Elut cartridges (Agilent Technologies, USA) using 30 mL of hexane: dichloromethane (1:1) as conditioning and elution solvent. The extracts were evaporated near dryness and reconstituted with 250 µL of hexane. Samples were analyzed by gas chromatography coupled to tandem mass spectrometry (GC-MS/MS) using a HP-5MS Agilent column (30 m length x 0.25 mm internal diameter x 0.25 µm film thickness) (Agilent Technologies, Santa Clara, CA, USA) according to a previous method (Velázquez-Gómez et al., 2018). The initial temperature was set at 70ºC and kept for 1 min, then increased at 175ºC in 4 min, from 175°C to 235°C in 20 min, and to 305ºC in 8 min. The data was processed using the Mass Hunter Quantitative software.

2.2.4. Analysis of pharmaceuticals, pesticides, and OPEs (Method 3)

A single extraction method was optimized to analyze pharmaceuticals, pesticides and OPEs. Fifty mg of freeze-dried blood were weighed in 2 mL Eppendorf vial and spiked with 50 ng of internal standards: acetaminophen-d_{13}, lidocaine-d_{10}, isoproturon-d_6, TPhP-d_{15}, sulfamethoxazole-d_4 and carbamazepine-d_2. Then, 1.5 mL of acetonitrile was added, samples were vortexed and ultrasonicated for 10 min (procedure repeated 3 times without changing the solvent), centrifuged (10 min, 3500 rpm) and the supernatant collected. Samples were directly purified with 30 mg Oasis PRIME HLB cartridges (1 cm^3 cartridge tube, Waters, USA) without prior conditioning and elution was performed with 1.5 mL of acetonitrile-water (80:20) solution. The samples were evaporated until dryness and reconstituted with 200 µL of ACN: Water (1:1). The analysis was performed by LC-MS/MS with an electrospray ion source (Waters, Milford, USA). An Acquity BEH C18 analytical column (100 mm x 2.1 mm internal diameter, 1.7 µm particle size) with a VanGuard C18 pre-column was deployed (Acquity, Waters, Ireland). The mobile phase consisted of (A) acetonitrile with 0.1% formic acid and (B) water with 0.1% formic acid. Gradient elution started at 5% A and 95% B (3 min in hold time), and increased to 100% of A in 21 min (1 min hold time). All compounds were measured under ESI+ except diclofop and furosemide that were measured with ESI-. The data was processed through Mass Lynx software.

2.3. Method validation / quality control

Quality control analysis was performed to ensure the method suitability for all compounds studied. Human whole blood was spiked with a mixture of the 91 contaminants at 50 ng/g and extracted using the 3 protocols described above. Unspiked blood was also analyzed to determine initial contaminant contribution. Procedural blanks were performed without any matrix by spiking the extraction solvent with 50 ng of the internal standards.

The calibration curve was built from 0.001 to 0.8 ng/µL with IS at 0.05 ng/µL for GC-MS/MS analysis and from 0.001 to 0.3 ng/µL with IS at 0.05 ng/µL for LC-MS/MS methods. Concentrations were calculated using internal standard quantification. Instrumental limits of detection (IDL) were calculated as the amount of analyte that gave a S/N ratio of 3 using the standard solution at 0.001 ng/µL and method detection limits (MDL) as the concentration that gave a S/N ratio of 3 using spiked blood at 50 ng/g. Values below MDL were given a value of zero in order not to overestimate the concentrations detected. Quality parameters including recoveries and MDL of each analyte are displayed in Tables S2 to S4 of the SI. All methods were effective, sensitive and robust. Blood samples were weighed before and after freeze drying to express results in ng/mL wet weight (ww). The average of water in blood was 86%.

2.4. Data analysis

Data were log x+1 transformed. As not all variables followed a normal distribution, non-parametric test Kruskal-Wallis and Dunn's post-hoc test were used to assess differences between chemical families' total concentrations of detected compounds (PFASs, PAHs, PCBs, and OCs). Spearman's correlations were used to assess the correlation between compounds. For statistical purposes, compounds detected in less than 20% of samples were excluded from the analysis.

Linear models were used to assess the effect of contaminants on the body condition of chicks. The multicollinearity of the variables was assessed using Variance Inflation Factors (VIF) <5 (James et al., 2013). Only independent variables were considered. Consequently, models were built with the total concentration of PFASs, PAHs, OCs and PCBs as explanatory variables. The top models were selected using Akaike's information criterion corrected for small sample size (AICc).

The level of significance was set at $\alpha = 0.05$ in all statistical tests. Statistical analyses were performed in the statistical software Rstudio version 4.0.2 (Core Team, 2020), and figures were elaborated using the ggplot2 library from the same software package and QGIS version 3.16.

3. RESULTS AND DISCUSSION

3.1. Concentrations detected in Greater flamingos' chicks

Contaminants have been detected in flamingo's chick blood for the first time, indicating early exposure to legacy POPs and PAHs, while in-use compounds such as pesticides, pharmaceuticals and OPEs were not detected in any sample. The total concentration ranged from 16.9 ng/mL to 623 ng/mL and according to the mean concentration followed the order PFASs > PAHs > OCs > PCBs. PFASs were detected at significantly higher concentrations ($p<0.05$) compared to the other chemical families. The presence of pollutants in chicks can be through maternal transfer and/or diet, and can vary according to the chemical families, as described below.

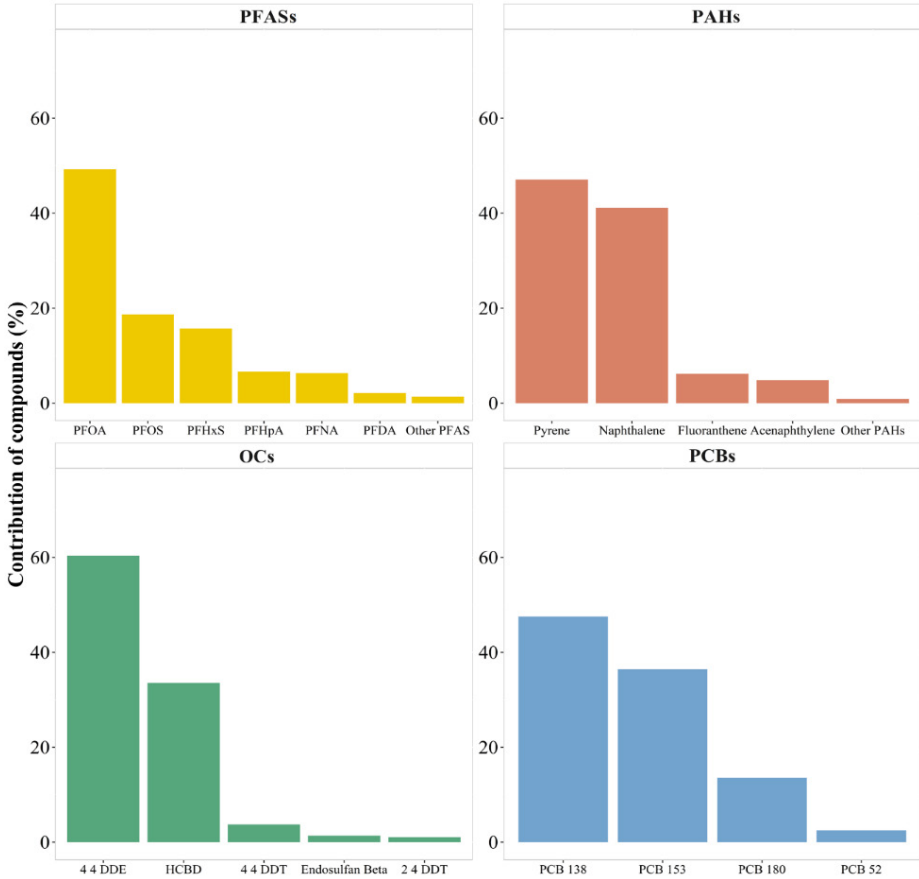

Figure 2. Percentage of contribution of compounds for each chemical family (PFASs, PAHs, OCs, PCBs).

Table 1. Detection frequency (%), mean ± SE (Standard Error), minimum and maximum concentrations and method detection limit (MDL) of detected compounds in flamingo's blood, expressed in ng/mL and ordered by detection frequency. Other compounds monitored but not detected are indicated in Table S1.

Compounds	Detection frequency (N)	Mean ± SE (ng/mL)	Min	Max	MDL (ng/mL)
PFOA	100 (47)	38.5 ± 8.3	3.04	366	0.06
PFOS	100 (47)	14.6 ± 0.9	3.83	35.6	0.09
PFHxS	100 (47)	12.3 ± 1.4	1.80	39.0	0.05
PFNA	94 (44)	4.93 ± 0.7	0.13	24.8	0.12
PFDA	74 (35)	1.66 ± 0.2	1.26	5.45	1.22
PFHpA	47 (22)	5.19 ± 3.9	1.68	122	0.18
PFTriDA	40 (19)	0.74 ± 0.2	1.31	2.63	0.21
PFUnA	30 (14)	0.30 ± 0.2	0.16	2.41	0.14
PFBA	2 (1)	-	0.20	0.20	0.14
PFBS	2 (1)	-	1.06	1.06	0.13
∑ PFASs	100 (47)	78.2 ± 12	9.34	576	
Naphthalene	84 (42)	12.8 ± 1.9	1.29	50.3	0.23
Pyrene	64 (32)	14.6 ± 6.8	0.18	333	0.11
Fluoranthene	52 (26)	1.91 ± 0.9	0.13	41.1	0.06
Acenaphthylene	50 (25)	1.50 ± 0.5	0.66	23.7	0.14
Benz[a]anthracene	10 (5)	0.21 ± 0.1	1.95	2.27	0.25
Benzo[ghi]perylene	2 (1)	-	2.40	2.40	1.25
∑PAHs	90 (45)	31.0 ± 8.9	0.19	423	
4,4'-DDE	90 (45)	5.12 ± 0.7	1.32	19.0	0.07
HCBD	40 (20)	2.85 ± 1.1	0.85	35.8	0.02
4,4'-DDT	18 (9)	0.32 ± 0.1	0.35	5.61	0.14
2,4'-DDT	4 (2)	0.09 ± 0.1	1.56	2.86	1.02
β-Endosulfan	4 (2)	0.12 ± 0.1	2.84	2.95	0.92
∑Organochlorine pesticides	92 (46)	8.58 ± 1.3	1.35	37.8	
PCB 153	46 (23)	0.81 ± 0.2	0.50	5.14	0.11
PCB 138	28 (14)	1.06 ± 0.3	1.57	7.99	0.14
PCB 180	28 (14)	0.30 ± 0.1	0.45	3.15	0.28
PCB 52	2 (1)	-	2.74	2.74	0.04
∑ PCBs	48 (24)	2.3 ± 0.5	0.50	15.6	

3.1.1. Perfluoroalkyl substances (PFASs)

PFASs were the most predominant chemical family in flamingo's samples. PFASs have been widely used in many products as water and stain repellents, pesticides, surfactants and fire-fighting foams (Custer et al., 2014). There are multiple sources of PFASs in the environment that might cause the exposure of those compounds to flamingos, including discharges from waste water treatment plants, sewage sludge, landfills or the application of fire-fighting foams (Stahl et al., 2018). PFASs are likely to be found in blood due to their affinity to plasma proteins (Briels et al., 2019; Jones et al., 2003), explaining the high concentrations found even at an early age. Ten out of 17 target PFASs were detected at mean concentrations ranging from 0.30 to 38.5 ng/mL. Compounds present in all analyzed samples and at the highest concentrations were PFOA (3.04 to 366 ng/mL, mean 38.5 ng/mL), PFOS (3.83 to 35.6 ng/mL, mean 14.6 ng/mL) and PFHxS (1.80 to 39, mean 12.3 ng/mL), which represented 49%, 19%, and 16% respectively of the ∑PFASs (Figure 2). PFNA and PFDA were detected in 94 and 77% of the samples at mean concentrations of 4.93 and 1.66 ng/mL, respectively (Table 1). Other compounds detected were odd-numbered PFCAs (Table 1). We found a higher predominance of long-chain PFASs than short-chain PFASs, as PFHxS was the only short-chain PFASs detected at relevant concentrations and prevalence. These results are consistent with previous studies reporting higher concentrations of long-chain and odd-numbered PFCAs and PFOS in ducks and inland birds (such as herons and egrets) owing to their higher potential for bioaccumulation compared to short-chain PFASs (Sharp et al., 2021; Hong et al., 2022). This long-chain PFASs pattern has also been observed in flamingos.

The main routes of exposure of PFASs to wild birds are maternal ovo-transfer and dietary exposure (Bertolero et al., 2015; Lopez-Antia et al., 2021). In waterbirds species, PFASs exposure is more likely to occur through ingestion of sediments rather than water ingestion (Larson et al., 2018). Benthic crustaceans are the main flamingo's food resources and are known to accumulate PFASs (Bertin et al., 2016; Haukås et al., 2007). This can directly enhance the accumulation of PFASs by adults due to their filtering feeding behavior on mud. Likewise, flamingos' chicks are exposed to PFASs through the regurgitation feeding. It has been reported that the presence of PFASs in sediment and benthic organisms due to firefighting foams applications in the Caribbean island of Bonaire was the most likely cause of flamingos decline in the area, due to the toxic effects of PFASs on their food resources (de Vries et al., 2017).

There are very few studies on PFASs in bird's blood from the Ebro delta to compare our results with. PFASs, predominantly PFOS, PFUnA and PFTriDA, have been reported in eggs from yellow-legged gull (*Larus michahellis*) and Audouin's gull (*Larus audouinii*) from the Ebro delta at higher concentrations

compared to other natural parks from the Iberian peninsula (Colomer-Vidal et al., 2022; Vicente et al., 2012). PFOS was detected in blood from *Larus michahellis* and *Larus audouinii* adults at mean concentrations of 46.8 ng/mL (from 8.92 to 142 ng/mL) and 45.5 ng/mL (from 27.4 to 107 ng/mL), respectively (Bertolero et al., 2015). These levels are higher than the ones found in flamingos' chicks, where the mean PFOS concentration was 14.6 ng/mL (from 3.83 to 35.6 ng/mL). PFOS is more accumulative in species with predominantly marine diet (Lopez-Antia et al., 2021), explaining lower levels in flamingos who are 2 months old and whose diet is based on benthic organisms, compared to gull species. Similar concentrations than the ones reported here of PFOS, PFHxS and PFNA were detected in great black-backed gull (*Larus marinus*) and lesser in black-backed gull (*Larus fuscus)* from Southwestern France (Sebastiano et al., 2021).

PFOA was the most abundant compound in flamingos' fledglings and the concentrations found in the present study were high when compared to other bird species (Figure 3). PFOA was detected at one order of magnitude higher concentrations than previously reported in Arctic and marine top predators (Herzke et al., 2009; Leat et al., 2013; Melnes et al., 2017; Sebastiano et al., 2020), and raptor species from various countries (Dykstra et al., 2021; Gómez-Ramírez et al., 2017; Hansen et al., 2020; Jouanneau et al., 2020; Shlosberg et al., 2011; Sun et al., 2020). Also, PFOA concentrations were much higher than the ones reported by Lopez-Antia et al., 2021 in plasma from nestling of the top-predator lesser black-backed gull (*Larus fuscus*) exposed to fluorochemical plants effluents in Belgium (2.6 to 38.8 ng/mL, mean of 7 ng/mL); also higher than the ones reported in tree swallows (*Tachycineta bicolor*) breeding in areas known to be highly polluted by PFASs (Custer et al., 2019, 2012; Custer et al., 2014). Likewise, European shags presented unexpected high concentrations of PFCAs with mean PFOA concentrations of 26.4 ng/mL in males and 12.9 ng/mL in females, strongly suggesting contamination of PFASs in water, sediment, and organisms of Isle of May (United Kingdom) (Carravieri et al., 2020). The only studies reporting higher concentrations than those found in flamingos from the Ebro delta are from great tits breeding within the boundaries of the 3M perfluorochemical plant in Belgium (Groffen et al., 2020; Lopez-Antia et al., 2019). Therefore, the PFOA concentrations reported in the present study are very high based on the previous reported levels. PFOA has a very short half-life of only 4.6 days in chicken plasma (Yoo et al., 2009), thus high concentrations detected in blood are indicative of recent exposure.

PFOS was included in annex B (restriction) of the Stockholm convention in 2009, while PFOA and PFHxS were included in annex A (elimination) in 2019 and 2022, respectively (Stockholm Convention, 2022). Despite being restricted, the use of firefighting foams containing PFASs was still permitted until end of existences, as far as 4th July 2020 (EC, 2020). Firefighting foams containing

PFOS and PFOA has led to contamination of waters and sediments in Bonaire (de Vries *et al*., 2017). The contamination profile dominated by PFHxS, PFOS and PFOA has been related to firefighting foam exposure through drinking water in the Swedish population (Xu et al., 2021) and fire fighters (Rotander et al., 2015). In June 2018, a major fire burned down a shipyard located in front of Alfacs bay where the colony inhabits and 2 other fires originated in the Ebro delta inland forest burning more than 200 ha in 2019. The firefighting foams could have contaminated the bay and reached flamingos' feeding and breeding area. Yet, the causes of the high concentrations of PFASs in flamingos remain unclear and further studies are needed, as multiple sources of PFASs may explain their presence in chicks.

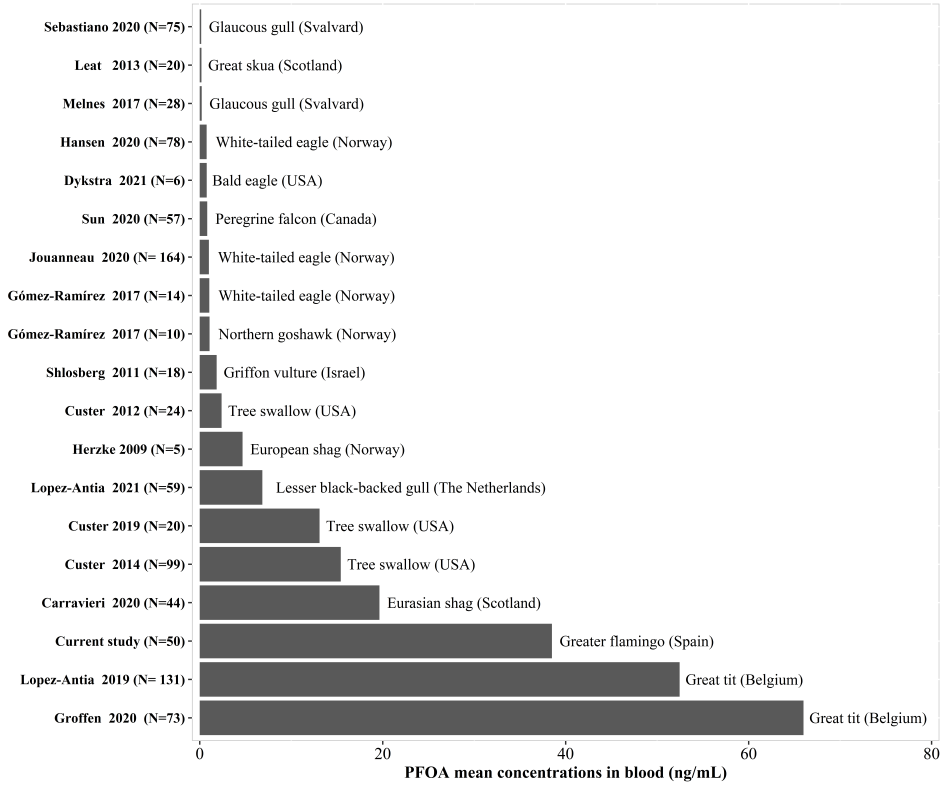

Figure 3. Mean concentrations of PFOA (ng/mL) in blood (whole and plasma) from several birds' species from different locations, ordered by concentrations levels.

3.1.2. PAHs

Seven out of 16 PAHs were detected in 90% of flamingos' samples. Naphthalene was detected in 84% of the samples with a mean concentration of 12.8 ± 1.9 ng/mL, representing 41% of ∑PAHs, followed by pyrene with

an occurrence of 64% of the samples and a mean concentration of 14.6 ± 6.7 ng/mL and contributing to 47% of ∑PAHs (Figure 2). Fluoranthene and acenaphthylene were found at lower concentrations in 52 and 50% of the samples, respectively (Table 1). PAHs may originate from traffic and motor-associated agricultural practices in the rice field.

PAHs are associated with solid particles and remain in sediments for many years (Waszak et al., 2021), and accumulate in some benthic invertebrates which have a poor capacity to metabolize PAHs (Rust et al., 2004). Flamingos filtering feeding behavior on benthic organisms could be an important route of exposure of these contaminants, as diet has been identified to be an important route of exposure of PAHs to birds (Custer *et al.*, 2017; Fernie *et al.,* 2018). However, the potential of biomagnification of PAHs is low due to the high metabolizing rates in vertebrates (Paruk et al., 2016). Scarce information is available on the levels of PAHs in bird's blood. Morin-crini et al., 2020 reported PAHs in whole blood of red kites (*Milvus milvus*) from north-west Spain, however the detected levels (∑PAHs= 1.7 ng/mL) were far lower than the ones detected in the current study. High prevalence of naphthalene was reported in blood from gull species after an oil spill accident (Pérez et al., 2008).

PAHs can be classified into high molecular weight (HMW), which presents four or more benzene rings, and low molecular weight (LMW), which presents less than four rings. HMW are associated with petrogenic sources such as petroleum and diesel fuel, while LMW are more volatile and are associated with pyrogenic processes like volcanoes and forest fires. Ratios between LMW and HMW compounds have been used as diagnostic sources of PAHs in birds' eggs, where a phenanthrene/anthracene ratio <10 accompanied by a fluoranthene/pyrene ratio >1 is indicative of a pyrogenic source, and a phenanthrene/anthracene ratio >15 combined with a fluoranthene/pyrene ratio <1 is indicative of a petrogenic source (Pereira et al., 2009). In our results, phenanthrene and anthracene were not detected while the fluoranthene/pyrene ratio was 0.13, therefore no clear diagnostic ratios were obtained. Altogether, the high prevalence of naphthalene and pyrene would point both to a petrogenic source and a pyrogenic one associated to fires occurring in the area.

3.1.3. OC pesticides and PCBs

OCs and PCBs were ubiquitous but present at trace concentrations, indicative of historic exposure. The most prevalent halogenated compound was the metabolite 4,4'-DDE, detected in 90% of the samples at concentrations ranging from 1.32 to 19 ng/mL and was the compound with the highest contribution to ∑OCs (60%). 4,4'-DDT was detected in 18%

of the samples (0.35 to 5.61 ng/mL). Recent inputs of DDTs could be due to dicofol production; the ratio between 2,4'-DDT and 4,4'- DDT > 0.2 has been proposed to be indicative of dicofol contamination (Li et al., 2015; Zapata et al., 2018). Yet, 2,4'-DDT/4,4'-DDT ratio obtained in blood was 0.27, which is not consistent enough to identify dicofol as the source of DDTs in flamingos. HCBD was present in 40% of the samples with concentrations from 0.85 to 35.8 ng/mL and contributed to 34% of ∑OCs. Other OCs were detected at lower concentrations and detection frequency (Table 1, Figure 2). PCBs mean concentration ranged from 0.3 to 1.06 ng/mL. The main congeners detected were PCB 153 (46% of the samples, from 0.5 to 5.14 ng/mL), followed by PCB 138 (28% of the samples, from 1.57 to 7.99 ng/mL) and PCB 180 (28% of the samples, from 0.45 to 3.15 ng/mL) (Table 1). PCB138 and PCBs 153 accounted for 47.5% and 36.4% of ∑PCBs, respectively (Figure 2). The concentrations of OCs and PCBs detected in flamingo's samples are similar to those reported in Egyptian vulture from NE Spain (*Neophron percnopterus*) (de la Casa-Resino et al., 2015; Ortiz-Santaliestra et al., 2019) or eggs of Greater flamingos from Doñana National Park (Guitart et al., 2005).

During the last century a choro-alkali plant dumped about 500,000 tons of sludge to the Ebro river, being and important source of POPs, including, DDTs, PCBs and HCHs.(Huertas et al., 2016). Ever since, several studies have reported the presence of these compounds in birds breeding in Ebro Delta. Huertas et al., 2016 reported ∑DDTs levels from 90 to 1700 ng/g (ww) and ∑PCBs from 74 to 520 ng/g (ww) in eggs from purple heron (*Ardea purpurea*) nesting around the chlor-alkali plant. Also, those compounds have been reported in gull colonies breeding close to the breeding colony of flamingos. Morales et al., 2012 found ∑PCBs ranging from 1.11 to 380 ng/g (ww) and ∑DDTs from 0.12 to 271 ng/g (ww) in eggs from *Larus michahellis,* and ∑PCBs from 0.58 to 429 ng/g (ww) and ∑DDTs from 0.22 to 213 ng/g ww in eggs from *Larus audouinii*. Years later, Zapata et al., 2018, found mean concentrations of ∑PCBs ranging from 0.11 to 253 ng/g (ww) and ∑DDTs from 0.05 to 218 ng/g (ww) in eggs of *Larus michahellis*, as well as ∑PCBs ranging from 0.20 to 304 ng/g (ww) and ∑DDTs from 0.02 to 760 ng/g (ww) in eggs of *Larus audouinii*. A monitoring study conducted on flamingos from Ebro Delta Natural Park reported higher concentrations of organochlorine pesticides (DDTs, HCBD and HCHs) in livers than those reported in blood in the present study (Gutiérrez et al., 1997). Differences in concentrations among matrices are due to the higher lipid content of livers, enhancing the accumulation of persistent compounds such as DDTs and PCBs compared to blood (Espín et al., 2016). Overall, our results are in accordance with previous studies on biomonitoring OCs and PCBs in the area, indicating that the Ebro Delta is still affected by the historic releases of OCs and PCBs and that flamingos, similar to other birds breeding in the area, are still exposed to these toxic contaminants.

3.2. Physical conditions related to contaminants' exposure.

This study has shown that flamingos are exposed to a mixture of compounds at a very early life stage, and for PFASs at unexpected high concentrations. 4,4'-DDE and PCBs 138, 153, 180 presented a strong correlation (p-value <0.05) indicating a similar source of exposure for these families of compounds (Figure 4). HCBD, used as a solvent for chlorine and as algaecide in industrial cooling systems had a strong correlation with PAHs. A weaker correlation was observed among PFASs and PAHs (Figure 4). The exposure to these multiple contaminants may represent a disadvantage as part of their energy will have to be invested in detoxification rather than growing or developing. Biometric and body indexes for the 50 flamingo chicks studied are indicated in Table S5. Lineal models did not show any association between the body condition of chicks (weight and SMI indexes) and the sum of the studied chemical families in Greater flamingos (Table S6). Associated effects of some PFASs on biometric parameters have been reported, as an increase of body mass and wing length in fledging's shearwaters (Szabo et al., 2021). However, the association between body condition and exposure to PFASs is yet to be clarified. Lopez-Antia et al., 2021 found positive correlations between PFOS levels and body condition indexes in four-weeks-old Larus fuscus, while Sebastiano et al., 2021 reported negative correlations in Larus fuscus and Larus marinus adults. Both studies pointed to sex differences as an important factor in the measurements. Although adult flamingos present sexual dimorphism, being males larger than females, flamingos' chicks cannot be sexed through biometric measurements at this early age (Boucheker et al., 2020), therefore sex could not be assessed as a factor in the analyses. However, no differences in the metabolism of contaminants are expected due to the similar development of both sexes at this age.

There is still a gap of knowledge about toxicity thresholds and how PFASs affects wildlife. There are evidence of the capacity of PFASs to cause cytotoxicity by interacting with DNA/RNA or secondary protein structures, lipids, and fatty acids (Gorrochategui et al., 2016). The exposure of PFASs was also associated to a potentially thyroid hormones disruption in several bird species (Sebastiano et al., 2021). At individual level, PFOS has been found to affect hepatic function and reproductive impairment at concentrations below the ones found at flamingos' chicks (Newsted et al., 2005). Moreover, PAHs effects on wildlife have been extensively recognized; they are known to be carcinogenic, endocrine disruptors and to produce embryotoxicity in birds (Wallace et al., 2020). OCs are known to cause a wide range of adverse effects, including endocrine disruption, impaired immune function, and neurotoxicity (Walker et al., 2012). Further biomonitoring and toxicity assessment studies are necessary to assess the impact of the reported mixture concentrations on the fitness of flamingos and long-term condition, including the effects at the population level.

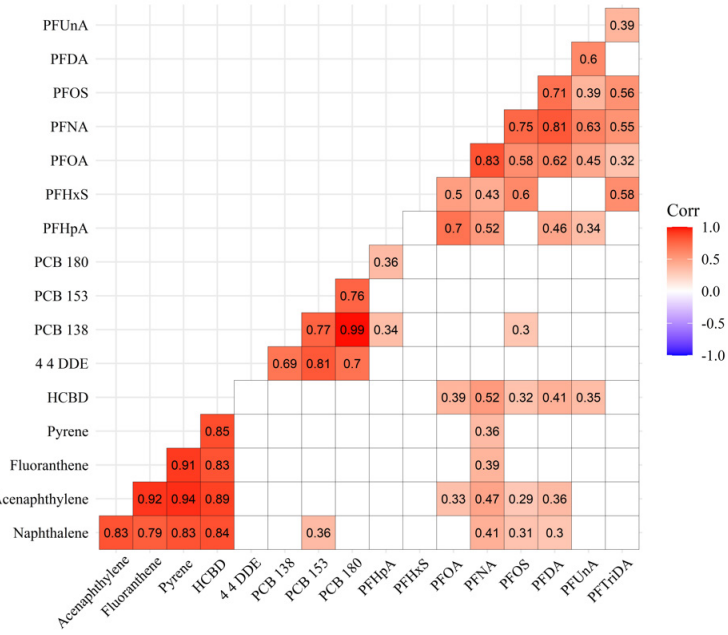

Figure 4. Spearman's correlation matrix for target compounds detected in >20% of the samples. Only significant results (p-value < 0.05) are shown.

4. CONCLUSIONS

Flamingos of the Ebro Delta breeding colony are chronically and multi-exposed to micropollutants from a very young age. The most polar compounds, including pharmaceuticals, in-used pesticides and polar OPEs were not detected in flamingo's blood either because they are not exposed to these contaminants or because they are metabolized. Contrarily, PFASs, PAHs, OCs and PCBs presented a high prevalence in flamingos' chicks. Flamingo's filtering behavior on mud and benthic organisms is a likely route of exposure to POPs. Maternal ovo-transfer is also an important route to consider and suggest that the Ebro Delta flamingo colony is chronically exposed to POPs, as has been observed in other species breeding within this area. However, PFOA was detected at high concentrations in all individuals and its presence may be attributed to the use of PFOA-containing firefighting foams used close to the flamingo's breeding colony. The exposure of chicks to micropollutants was not related to any alteration of the body condition based on biometric measurements. Overall, the study found that flamingos are exposed to a wide range of micropollutants at an early age, illustrating the significance of pollution as a threat to waterbird conservation. The present study highlights the importance of including biomonitoring studies to evaluate the pollution footprint in bird conservation actions.

REFERENCES

Álvarez-Muñoz, D., Rambla-Alegre, M., Carrasco, N., Lopez de Alda, M., Barceló, D., 2019. Fast analysis of relevant contaminants mixture in commercial shellfish. Talanta 205, 119884. https://doi.org/https://doi.org/10.1016/j.talanta.2019.04.085

Ancora, S., Bianchi, N., Leonzio, C., Renzoni, A., 2008. Heavy metals in flamingos (*Phoenicopterus ruber*) from Italian wetlands: The problem of ingestion of lead shot. Environ. Res. 107, 229–236. https://doi.org/10.1016/j.envres.2008.02.004

Ayala-Cabrera, J.F., Lacorte, S., Moyano, E., Santos, F.J., 2021. Analysis of Dechlorane Plus and related compounds in gull eggs by GC-HRMS using a novel atmospheric pressure photoionization source. Anal. Bioanal. Chem. 413, 3421–3431. https://doi.org/10.1007/s00216-021-03286-8

Balkız, Ö., Özesmi, U., Pradel, R., Germain, C., Sıkı, M., Amat, J.A., Rendén-Martos, M., Baccetti, N., Béchet, A., 2007. Range of the Greater Flamingo, *Phoenicopterus roseus*, metapopulation in the Mediterranean: New insights from Turkey. J. Ornithol. 148, 347–355. https://doi.org/10.1007/s10336-007-0136-2

Barbieri, M.V., Peris, A., Postigo, C., Moya-Garcés, A., Monllor-Alcaraz, L.S., Rambla-Alegre, M., Eljarrat, E., López de Alda, M., 2021. Evaluation of the occurrence and fate of pesticides in a typical Mediterranean delta ecosystem (Ebro River Delta) and risk assessment for aquatic organisms. Environ. Pollut. 274. https://doi.org/10.1016/j.envpol.2020.115813

Bertin, D., Labadie, P., Ferrari, B.J.D., Sapin, A., Garric, J., Geffard, O., Budzinski, H., Babut, M., 2016. Potential exposure routes and accumulation kinetics for poly- and perfluorinated alkyl compounds for a freshwater amphipod: *Gammarus* spp. (Crustacea). Chemosphere 155, 380–387. https://doi.org/10.1016/J.CHEMOSPHERE.2016.04.006

Bertolero, A., Vicente, J., Meyer, J., Lacorte, S., 2015. Accumulation and maternal transfer of perfluorooctane sulphonic acid in yellow-legged (*Larus michahellis*) and Audouin's gull (*Larus audouinii*) from the Ebro Delta Natural Park. Environ. Res. 137, 208–214. https://doi.org/10.1016/j.envres.2014.12.018

BirdLife International, 2022. Important Bird Areas factsheet: Ebro delta [WWW Document].

Borghesi, F., Andreotti, A., Baccetti, N., Bianchi, N., Birke, M., Migani, F., Dinelli, E., 2011. Flamingo feathers to monitor metal contamination of coastal wetlands: methods and initial results concerning the presence of mercury at six Mediterranean sites. Chem. Ecol. 27, 137–151. https://doi.org/10.1080/02757540.2011.625942

Boucheker, A., Nedjah, R., Prodon, R., Gillingham, M., Dechaume-Moncharmont, F.-X., Béchet, A., Samraoui, B., 2020. Cohort effect on discriminant rate: the case of greater flamingo (*Phoenicopterus roseus*) chicks sexed with morphological characters. Web Ecol. 20, 153–159. https://doi.org/10.5194/we-20-153-2020

Briels, N., Torgersen, L.N., Castaño-Ortiz, J.M., Løseth, M.E., Herzke, D., Nygård, T., Bustnes, J.O., Ciesielski, T.M., Poma, G., Malarvannan, G., Covaci, A., Jaspers, V.L.B., 2019. Integrated exposure assessment of northern goshawk (*Accipiter gentilis*) nestlings to legacy and emerging organic pollutants using non-destructive samples. Environ. Res. 178, 108678. https://doi.org/https://doi.org/10.1016/j.envres.2019.108678

Carravieri, A., Burthe, S.J., De La Vega, C., Yonehara, Y., Daunt, F., Newell, M.A., Jeffreys, R.M., Lawlor, A.J., Hunt, A., Shore, R.F., Pereira, M.G., Green, J.A., 2020. Interactions between Environmental Contaminants and Gastrointestinal Parasites: Novel Insights from an Integrative Approach in a Marine Predator. Environ. Sci. Technol. 54, 8938–8948. https://doi.org/10.1021/acs.est.0c03021

Čelić, M., Gros, M., Farré, M., Barceló, D., Petrović, M., 2019. Pharmaceuticals as chemical markers of wastewater contamination in the vulnerable area of the Ebro Delta (Spain). Sci. Total Environ. 652, 952–963. https://doi.org/10.1016/J.SCITOTENV.2018.10.290

Colomer-Vidal, P., Bertolero, A., Alcaraz, C., Garreta-Lara, E., Santos, F.J., Lacorte, S., 2022. Distribution and ten-year temporal trends (2009–2018) of perfluoroalkyl substances in gull eggs from Spanish breeding colonies. Environ. Pollut. 293, 118555. https://doi.org/10.1016/J.ENVPOL.2021.118555

Custer, C.M., Custer, T.W., Delaney, R., Dummer, P.M., Schultz, S., Karouna-Renier, N., 2019. Perfluoroalkyl Contaminant Exposure and Effects in Tree Swallows Nesting at Clarks Marsh, Oscoda, Michigan, USA. Arch. Environ. Contam. Toxicol. 77, 1–13. https://doi.org/10.1007/s00244-019-00620-1

Custer, C.M., Custer, T.W., Dummer, P.M., Etterson, M.A., Thogmartin, W.E., Wu, Q., Kannan, K., Trowbridge, A., McKann, P.C., 2014. Exposure and effects of perfluoroalkyl substances in tree swallows nesting in Minnesota and Wisconsin, USA. Arch. Environ. Contam. Toxicol. 66, 120–138. https://doi.org/10.1007/s00244-013-9934-0

Custer, C.M., Custer, T.W., Schoenfuss, H.L., Poganski, B.H., Solem, L., 2012. Exposure and effects of perfluoroalkyl compounds on tree swallows nesting at Lake Johanna in east central Minnesota, USA. Reprod. Toxicol. 33, 556–562.

Custer, M., Custer, T.W., Dummer, P.M., Etterson, M.A., Thogmartin, W.E., Wu, Q., Kannan, K., Trowbridge, A., McKann, P.C., 2014. Exposure and effects of perfluoroalkyl substances in tree swallows nesting in Minnesota and Wisconsin, USA. Arch. Environ. Contam. Toxicol. 66, 120–138.

Custer, T.W., Custer, C.M., Dummer, P.M., Goldberg, D., Franson, J.C., Erickson, R.A., 2017. Organic contamination in tree swallow (Tachycineta bicolor) nestlings at United States and binational Great Lakes Areas of Concern. Environ. Toxicol. Chem. 36, 735–748. https://doi.org/https://doi.org/10.1002/etc.3598

de la Casa-Resino, I., Hernández-Moreno, D., Castellano, A., Pérez-López, M., Soler, F., 2015. Chlorinated pollutants in blood of White stork nestlings (*Ciconia ciconia*) in different colonies in Spain. Chemosphere 118, 367–372. https://doi.org/10.1016/j.chemosphere.2014.10.062

de Vries, P., Slijkerman, D.M.E., Kwadijk, C.J.A.F., Kotterman, M.J.J., Posthuma, L., de Zwart, D., Murk, A.J., Foekema, E.M., 2017. The toxic exposure of flamingos to per- and Polyfluoroalkyl substances (PFAS) from firefighting foam applications in Bonaire. Mar. Pollut. Bull. 124, 102–111. https://doi.org/10.1016/J.MARPOLBUL.2017.07.017

Dulsat-Masvidal, M., Ciudad, C., Infante, O., Mateo, R., Lacorte, S., 2022. Pilot monitoring scheme of water pollutants in Important Bird and Biodiversity Areas. Ecotoxicol. Environ. Saf. 237, 113562. https://doi.org/10.1016/j.ecoenv.2022.113562

Dykstra, C.R., Route, W.T., Williams, K.A., 2021. Trends and Patterns of Perfluoroalkyl Substances in Blood Plasma Samples of Bald Eagle Nestlings in Wisconsin and Minnesota, USA. Environ. Toxicol. Chem. 40, 754–766. https://doi.org/https://doi.org/10.1002/etc.4864

EC, 2020. Commission Delegated Regulation (EU) 2020/784 of 8 April 2020 amending Annex I to Regulation (EU) 2019/1021 of the European Parliament and of the Council as regards the listing of perfluorooctanoic acid (PFOA), its salts and PFOA-related compounds.

Espín, S., Andevski, J., Duke, G., Eulaers, I., Gómez-Ramírez, P., Hallgrimsson, G.T., Helander, B., Herzke, D., Jaspers, V.L.B., Krone, O., Lourenço, R., María-Mojica, P., Martínez-López, E., Mateo, R., Movalli, P., Sánchez-Virosta, P., Shore, R.F., Sonne, C., van den Brink, N.W., van Hattum, B., Vrezec, A., Wernham, C., García-Fernández, A.J., 2021. A schematic sampling protocol for contaminant monitoring in raptors. Ambio 50, 95–100. https://doi.org/10.1007/s13280-020-01341-9

Espín, S., García-Fernández, A.J., Herzke, D., Shore, R.F., van Hattum, B., Martínez-López, E., Coeurdassier, M., Eulaers, I., Fritsch, C., Gómez-Ramírez, P., Jaspers, V.L.B., Krone, O., Duke, G., Helander, B., Mateo, R., Movalli, P., Sonne, C., van den Brink, N.W., 2016. Tracking pan-continental trends in environmental contamination using sentinel raptors—what types of samples should we use? Ecotoxicology 25, 777–801. https://doi.org/10.1007/s10646-016-1636-8

Fernie, K.J., Marteinson, S.C., Chen, D., Eng, A., Harner, T., Smits, J.E.G., Soos, C., 2018. Elevated exposure , uptake and accumulation of polycyclic aromatic hydrocarbons by nestling tree swallows (*Tachycineta bicolor*) through multiple exposure routes in active mining-related areas of the Athabasca oil sands region. Sci. Total Environ. 624, 250–261. https://doi.org/10.1016/j.scitotenv.2017.12.123

Geraci, J., Béchet, A., Cézilly, F., Ficheux, S., Baccetti, N., Samraoui, B., Wattier, R., 2012. Greater flamingo colonies around the Mediterranean form a single interbreeding population and share a common history. J. Avian Biol. 43, 341–354. https://doi.org/10.1111/j.1600-048X.2012.05549.x

Gómez-Gutiérrez, A.I., Jover, E., Bodineau, L., Albaigés, J., Bayona, J.M., 2006. Organic contaminant loads into the Western Mediterranean Sea: Estimate of Ebro River inputs. Chemosphere 65, 224–236. https://doi.org/10.1016/J.CHEMOSPHERE.2006.02.058

Gómez-Ramírez, P., Bustnes, J.O., Eulaers, I., Herzke, D., Johnsen, T.V., Lepoint, G., Pérez-García, J.M., García-Fernández, A.J., Jaspers, V.L.B., 2017. Per-and polyfluoroalkyl substances in plasma and feathers of nestling birds of prey from northern Norway. Environ. Res. 158, 277–285.

Gorrochategui, E., Lacorte, S., Tauler, R., Martin, F.L., 2016. Perfluoroalkylated Substance Effects in Xenopus laevis A6 Kidney Epithelial Cells Determined by ATR-FTIR Spectroscopy and Chemometric Analysis. Chem. Res. Toxicol. 29, 924–932. https://doi.org/10.1021/acs.chemrestox.6b00076

Groffen, T., Lasters, R., Bervoets, L., Prinsen, E., Eens, M., 2020. Are feathers of a songbird model species (The Great Tit, Parus major) suitable for monitoring perfluoroalkyl acids (PFAAs) in blood plasma? Environ. Sci. Technol. 54, 9334–9344.

Guitart, R., Clavero, R., Mateo, R., Máñez, M., 2005. Levels of Persistent Organochlorine Residues in Eggs of Greater Flamingos from the Guadalquivir Marshes (Doñana), Spain. J. Environ. Sci. Heal. Part B 40, 753–760. https://doi.org/10.1080/03601230500189394

Gutiérrez, J.M., Mateo, R., Prats, C., Guitart, R., 1997. Organochlorine residues in normal and lead poisoned greater flamingos: Relationships with the fatty acid composition. J. Environ. Sci. Heal. - Part A 32, 853–863. https://doi.org/10.1080/10934529709376582

Hansen, E., Huber, N., Bustnes, J.O., Herzke, D., Bårdsen, B.-J., Eulaers, I., Johnsen, T. V, Bourgeon, S., 2020. A novel use of the leukocyte coping capacity assay to assess the immunomodulatory effects of organohalogenated contaminants in avian wildlife. Environ. Int. 142, 105861.

Haukås, M., Berger, U., Hop, H., Gulliksen, B., Gabrielsen, G.W., 2007. Bioaccumulation of per- and polyfluorinated alkyl substances (PFAS) in selected species from the Barents Sea food web. Environ. Pollut. 148, 360–371. https://doi.org/10.1016/j.envpol.2006.09.021

Herzke, D., Nygård, T., Berger, U., Huber, S., Røv, N., 2009. Perfluorinated and other persistent halogenated organic compounds in European shag (*Phalacrocorax aristotelis*) and common eider (*Somateria mollissima*) from Norway: a suburban to remote pollutant gradient. Sci. Total Environ. 408, 340–348.

Hong, S.H., Reiner, J.L., Jang, M., Schuur, S.S., Han, G.M., Kucklick, J.R., Shim, W.J., 2022. Levels and profiles of perfluorinated alkyl acids in liver tissues of birds with different habitat types and trophic levels from an urbanized coastal region of South Korea. Sci. Total Environ. 806, 151263. https://doi.org/https://doi.org/10.1016/j.scitotenv.2021.151263

Huertas, D., Grimalt, J.O., Jover, L., Sanpera, C., Ruiz, X., 2016. Organochlorine compounds in Purple Heron eggs (*Ardea purpurea*) nesting in sites located around a chlor-alkali plant (Ebro River). Sci. Total Environ. 540, 211–220. https://doi.org/10.1016/j.scitotenv.2015.07.037

Ibáñez, C., Caiola, N., 2018. Ebro Delta (Spain), in: Finlayson, C.M., Milton, G.R., Prentice, R.C., Davidson, N.C. (Eds.), The Wetland Book: II: Distribution, Description, and Conservation. Springer Netherlands, Dordrecht, pp. 1113–1121. https://doi.org/10.1007/978-94-007-4001-3_145

James, G., Witten, D., Hastie, T., Tibshirani, R., 2013. An introduction to statistical learning. Springer.

Johnoson, A., Cezillly, F.C., 2007. The Greater Flamingo. T & AD Poyser, London.

Jones, P.D., Hu, W., De Coen, W., Newsted, J.L., Giesy, J.P., 2003. Binding of perfluorinated fatty acids to serum proteins. Environ. Toxicol. Chem. 22, 2639–2649. https://doi.org/10.1897/02-553

Jouanneau, W., Bårdsen, B.-J., Herzke, D., Johnsen, T.V., Eulaers, I., Bustnes, J.O., 2020. Spatiotemporal analysis of perfluoroalkyl substances in white-tailed eagle (*Haliaeetus albicilla*) nestlings from northern Norway—a ten-year study. Environ. Sci. Technol. 54, 5011–5020.

Larson, E.S., Conder, J.M., Arblaster, J.A., 2018. Modeling avian exposures to perfluoroalkyl substances in aquatic habitats impacted by historical aqueous film forming foam releases. Chemosphere 201, 335–341. https://doi.org/10.1016/j.chemosphere.2018.03.004

Leat, E.H.K., Bourgeon, S., Eze, J.I., Muir, D.C.G., Williamson, M., Bustnes, J.O., Furness, R.W., Borgå, K., 2013. Perfluoroalkyl substances in eggs and plasma of an avian top predator, great skua (Stercorarius skua), in the north Atlantic. Environ. Toxicol. Chem. 32, 569–576.

Li, L., Liu, J., Hu, J., 2015. Global inventory, long-range transport and environmental distribution of dicofol. Environ. Sci. Technol. 49, 212–222. https://doi.org/10.1021/es502092x

Lopez-Antia, A., Groffen, T., Lasters, R., Abdelgawad, H., Sun, J., Asard, H., Bervoets, L., Eens, M., 2019. Perfluoroalkyl Acids (PFAAs) Concentrations and Oxidative Status in Two Generations of Great Tits Inhabiting a Contamination Hotspot. Environ. Sci. Technol. 53, 1617–1626. https://doi.org/10.1021/acs.est.8b05235.

Lopez-Antia, A., Kavelaars, M.M., Müller, W., Bervoets, L., Eens, M., 2021. Understanding PFAAs exposure in a generalist seabird species breeding in the vicinity of a fluorochemical plant: Influence of maternal transfer and diet. Environ. Pollut. 271, 116355. https://doi.org/10.1016/j.envpol.2020.116355.

Melnes, M., Gabrielsen, G.W., Herzke, D., Sagerup, K., Jenssen, B.M., 2017. Dissimilar effects of organohalogenated compounds on thyroid hormones in glaucous gulls. Environ. Res. 158, 350–357.

Morales, L., Martrat, M.G., Olmos, J., Parera, J., Vicente, J., Bertolero, A., Ábalos, M., Lacorte, S., Santos, F.J., Abad, E., 2012. Persistent Organic Pollutants in gull eggs of two species (Larus michahellis and Larus audouinii) from the Ebro delta Natural Park. Chemosphere 88, 1306–1316. https://doi.org/10.1016/j.chemosphere.2012.03.106

Morin-crini, N., Scheifler, R., Amiot, C., Riols, R., 2020. Determination of polycyclic aromatic hydrocarbon (PAH) contents in micro-volumes of the whole blood and liver of Red Kite by a simplified GC-MS / MS method. Int. J. Environ. Anal. Chem. 00, 1–10. https://doi.org/10.1080/03067319.2020.1726899

Newsted, J.L., Jones, P.D., Coady, K., Giesy, J.P., 2005. Avian toxicity reference values for perfluorooctane sulfonate. Environ. Sci. Technol. 39, 9357–9362. https://doi.org/10.1021/es050989v

Ortiz-Santaliestra, M.E., Tauler-Ametller, H., Lacorte, S., Hernández-Matías, A., Real, J., Mateo, R., 2019. Accumulation of pollutants in nestlings of an endangered avian scavenger related to territory urbanization and physiological biomarkers. Environ. Pollut. 252, 1801–1809. https://doi.org/10.1016/j.envpol.2019.06.101

Paruk, J.D., Adams, E.M., Uher-Koch, H., Kovach, K.A., Long, D., Perkins, C., Schoch, N., Evers, D.C., 2016. Polycyclic aromatic hydrocarbons in blood related to lower body mass in common loons. Sci. Total Environ. 565, 360–368. https://doi.org/https://doi.org/10.1016/j.scitotenv.2016.04.150

Peig, J., Green, A.J., 2009. New perspectives for estimating body condition from mass/length data: the scaled mass index as an alternative method. Oikos 118, 1883–1891. https://doi.org/https://doi.org/10.1111/j.1600-0706.2009.17643.x

Pereira, M.G., Lawlor, A., Bertolero, A., Díez, S., Shore, R.F., Lacorte, S., 2019. Temporal and Spatial Distribution of Mercury in Gulls Eggs from the Iberian Peninsula. Arch. Environ. Contam. Toxicol. 76, 394–404. https://doi.org/10.1007/s00244-018-0584-0

Pereira, M.G., Walker, L.A., Wright, J., Best, J., Shore, R.F., 2009. Concentrations of polycyclic aromatic hydrocarbons (PAHs) in the eggs of predatory birds in Britain. Environ. Sci. Technol. 43, 9010–9015. https://doi.org/10.1021/es901805e

Pérez, C., Velando, A., Munilla, I., López-Alonso, M., Daniel, O., 2008. Monitoring polycyclic aromatic hydrocarbon pollution in the marine environment after the Prestige oil spill by means of seabird blood analysis. Environ. Sci. Technol. 42, 707–713. https://doi.org/10.1021/es071835d

Pignotti, E., Casas, G., Llorca, M., Tellbüscher, A., Almeida, D., Dinelli, E., Farré, M., Barceló, D., 2017. Seasonal variations in the occurrence of perfluoroalkyl substances in water, sediment and fish samples from Ebro Delta (Catalonia, Spain). Sci. Total Environ. 607–608, 933–943. https://doi.org/10.1016/j.scitotenv.2017.07.025

Rotander, A., Toms, L.L., Aylward, L., Kay, M., Mueller, J.F., 2015. Elevated levels of PFOS and PFHxS in fi re fi ghters exposed to aqueous fi lm forming foam (AFFF). Environ. Int. 82, 28–34. https://doi.org/10.1016/j.envint.2015.05.005

Rust, A.J., Burgess, R.M., Brownawell, B.J., McElroy, A.E., 2004. Relationship between metabolism and bioaccumulation of benzo[a]pyrene in benthic invertebrates. Environ. Toxicol. Chem. 23, 2587–2593. https://doi.org/10.1897/03-354

Santos, X., Pastor, D., Llorente, G.A., Albaigés, J., 1999. Organochlorine levels in viperine snake *Natrix maura* carcasses from the ebro delta (NE Spain): Sexual and size-related differences. Chemosphere 39, 2641–2650. https://doi.org/https://doi.org/10.1016/S0045-6535(99)00199-X

Sebastiano, M., Angelier, F., Blévin, P., Ribout, C., Sagerup, K., Descamps, S., Herzke, D., Moe, B., Barbraud, C., Bustnes, J.O., 2020. Exposure to PFAS is associated with telomere length dynamics and demographic responses of an arctic top predator. Environ. Sci. Technol. 54, 10217–10226.

Sebastiano, M., Jouanneau, W., Blévin, P., Angelier, F., Parenteau, C., Gernigon, J., Lemesle, J.C., Robin, F., Pardon, P., Budzinski, H., Labadie, P., Chastel, O., 2021. High levels of fluoroalkyl substances and potential disruption of thyroid hormones in three gull species from South Western France. Sci. Total Environ. 765, 144611. https://doi.org/10.1016/j.scitotenv.2020.144611

Sharp, S., Sardiña, P., Metzeling, L., McKenzie, R., Leahy, P., Menkhorst, P., Hinwood, A., 2021. Per- and Polyfluoroalkyl Substances in Ducks and the Relationship with Concentrations in Water, Sediment, and Soil. Environ. Toxicol. Chem. 40, 846–858. https://doi.org/https://doi.org/10.1002/etc.4818

Shlosberg, A., Rumbeiha, W.K., Lublin, A., Kannan, K., 2011. A database of avian blood spot examinations for exposure of wild birds to environmental toxicants: the DABSE biomonitoring project. J. Environ. Monit. 13, 1547–1558.

Stahl, T., Gassmann, M., Falk, S., Brunn, H., 2018. Concentrations and Distribution Patterns of Perfluoroalkyl Acids in Sewage Sludge and in Biowaste in Hesse, Germany. J. Agric. Food Chem. 66, 10147–10153. https://doi.org/10.1021/acs.jafc.8b03063

Stockholm Convention, 2022. Chemicals proposed for listing under the Convention [WWW Document]. URL http://www.pops.int/TheConvention/ThePOPs/ChemicalsProposedforListing

Sun, J., Letcher, R.J., Eens, M., Covaci, A., Fernie, K.J., 2020. Perfluoroalkyl acids and sulfonamides and dietary, biological and ecological associations in peregrine falcons from the Laurentian Great Lakes Basin, Canada. Environ. Res. 191, 110151. https://doi.org/10.1016/J.ENVRES.2020.110151

Szabo, D., Lavers, J.L., Shimeta, J., Green, M.P., Mulder, R.A., Clarke, B.O., 2021. Correlations between Per - and Poly fl uoroalkyl Substances and Body Morphometrics in Fledgling Shearwaters Impacted by Plastic Consumption from a Remote Paci fi c Island 40, 799–810. https://doi.org/10.1002/etc.4924

Velázquez-Gómez, M., Hurtado-Fernández, E., Lacorte, S., 2018. Comprehensive method for the analysis of multi-class organic micropollutants in indoor dust. Sci. Total Environ. 635, 1484–1494. https://doi.org/https://doi.org/10.1016/j.scitotenv.2018.04.186

Vicente, J., Bertolero, A., Meyer, J., Viana, P., Lacorte, S., 2012. Distribution of perfluorinated compounds in Yellow-legged gull eggs (*Larus michahellis) f*rom the Iberian Peninsula. Sci. Total Environ. 416, 468–475. https://doi.org/https://doi.org/10.1016/j.scitotenv.2011.11.085

Walker, C.H., Sibly, R.M., Hopkin, S.P., Peakall, D.B., 2012. Principles of Ecotoxicology, Fourth edi. ed. CRC Press, Boca Raton.

Wallace, S.J., de Solla, S.R., Head, J.A., Hodson, P. V., Parrott, J.L., Thomas, P.J., Berthiaume, A., Langlois, V.S., 2020. Polycyclic aromatic compounds (PACs) in the Canadian environment: Exposure and effects on wildlife. Environ. Pollut. 265, 114863. https://doi.org/10.1016/j.envpol.2020.114863

Waszak, I., Karolina, J.-S., Agnieszka, O., Zaniewicz, G., 2021. Estimation of native and alkylated polycyclic aromatic hydrocarbons (PAHs) in seabirds from the south coast of the Baltic Sea 4366–4376.

Xu, Y., Nielsen, C., Li, Y., Hammarstrand, S., Andersson, E.M., Li, H., Pineda, D., Lindh, C.H., Fletcher, T., Olsson, D.S., Engstr, K., Jakobsson, K., 2021. Serum perfluoroalkyl substances in residents following long-term drinking water contamination from firefighting foam in Ronneby , Sweden 147. https://doi.org/10.1016/j.envint.2020.106333

Yoo, H., Guruge, K.S., Yamanaka, N., Sato, C., Mikami, O., Miyazaki, S., Yamashita, N., Giesy, J.P., 2009. Depuration kinetics and tissue disposition of PFOA and PFOS in white leghorn chickens (*Gallus gallus)* administered by subcutaneous implantation. Ecotoxicol. Environ. Saf. 72, 26–36. https://doi.org/https://doi.org/10.1016/j.ecoenv.2007.09.007

Zapata, P., Ballesteros-Cano, R., Colomer, P., Bertolero, A., Viana, P., Lacorte, S., Santos, F.J., 2018. Presence and impact of Stockholm Convention POPs in gull eggs from Spanish and Portuguese natural and national parks. Sci. Total Environ. 633, 704–715. https://doi.org/10.1016/j.scitotenv.2018.03.081

SUPPLEMENTARY INFORMATION

Table S1. List of analytes and standards suppliers.

Type	Compound	Supplier
PFASs	Mix native PFAC-MXB	Wellington laboratories Inc. (Guelph, ON, Canada)
PAHs	PAH solution mix (naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benz(a)anthracene, chrysen, benzo[b]fluoranthene, benzo[k] fluoranthen, benzo[a]pyrene indeno[1,2,3-cd]pyrene, dibenz[a,h] anthracene, benzo[ghi]perylene)	AccuStandard (New Haven, CT, USA)
OC compounds	HCBD	Sigma-Aldrich (Darmstadt, Germany and St. Louis, MO, USA)
	α-HCH	Dr. Ehrenstorfer GmbH (Augsburg, Germany)
	β-HCH	Dr. Ehrenstorfer GmbH
	γ-HCH	Dr. Ehrenstorfer GmbH
	δ-HCH	Dr. Ehrenstorfer GmbH
	HCB	Sigma-Aldrich (Darmstadt, Germany and St. Louis, MO, USA)
	Pesticide mix 164 (2,4'-DDE, 4,4'-DDE, 2,4'-DDD, 2,4'-DDT, 4,4'-DDD, 4,4'-DDT)	Dr. Ehrenstorfer GmbH
	α-Endosulfan	Dr. Ehrenstorfer GmbH
	β-Endosulfan	Dr. Ehrenstorfer GmbH
	Chlorpyrifos	Dr. Ehrenstorfer GmbH
PCBs	PCB Mix 3 (PCB 28, PCB 52, PCB 101, PCB 118, PCB 138, PCB 153, PCB 180)	Dr. Ehrenstorfer GmbH
Pharmaceuticals	Metformin	Dr. Ehrenstorfer GmbH
	Nicotine	Sigma-Aldrich (St. Louis, USA)
	Alopurinol	Sigma-Aldrich (St. Louis, USA)
	Atenolol	Sigma-Aldrich (St. Louis, USA)
	Paracetamol	European Pharmacopea Reference Standard
	Levetiracetam	Sigma-Aldrich (St. Louis, USA)
	Gabapentin	Sigma-Aldrich (St. Louis, USA)
	Caffeine	Sigma-Aldrich (St. Louis, USA)
	Pentoxifylline	Sigma-Aldrich (St. Louis, USA)
	Tramadol	Sigma-Aldrich (St. Louis, USA)
	Sulfamethoxazole	Sigma-Aldrich (St. Louis, USA)
	Venlafaxine	Sigma-Aldrich (St. Louis, USA)
	Trazodone	Sigma-Aldrich (St. Louis, USA)
	Quetiapine	Sigma-Aldrich (St. Louis, USA)
	Losartan	Sigma-Aldrich (St. Louis, USA)
	Furosemide	Sigma-Aldrich (St. Louis, USA)
	Diclofenac	Sigma-Aldrich (St. Louis, USA)
	Atorvastatin	Sigma-Aldrich (St. Louis, USA)
	Ibuprofen	Sigma-Aldrich (St. Louis, USA)
	Carbamazepine	Sigma-Aldrich (St. Louis, USA)

Type	Compound	Supplier
Pesticides	Dimethoate	Sigma-Aldrich (St. Louis, USA)
	Chlorfenvinphos	Sigma-Aldrich (St. Louis, USA)
	Isoproturon	Sigma-Aldrich (St. Louis, USA)
	Metalaxyl	Sigma-Aldrich (St. Louis, USA)
	Triadimenol	Dr. Ehrenstorfer GmbH
	Tebuconazol	Sigma-Aldrich (St. Louis, USA)
	Kresoxim-methyl	Sigma-Aldrich (St. Louis, USA)
	Diclofop	Sigma-Aldrich (St. Louis, USA)
	Spinosad	Dr. Ehrenstorfer GmbH
	Pyraclostrobin	Dr. Ehrenstorfer GmbH
	Tebufenpyrad	Dr. Ehrenstorfer GmbH
	Prosulfocarb	Sigma-Aldrich (St. Louis, USA)
	Pendimethalin	Sigma-Aldrich (St. Louis, USA)
OPEs	TCEP	Sigma-Aldrich (St. Louis, USA)
	TBP	Sigma-Aldrich (St. Louis, USA)
	TPhP	Sigma-Aldrich (St. Louis, USA)
Internal standards	Triphenyl phosphate-d_{15} (TPhP-d_{15})	Sigma-Aldrich (St. Louis, USA)
	Acetaminophen-methyl-d_3	Sigma-Aldrich (St. Louis, USA)
	Carbamazepine-d_2	Sigma-Aldrich (St. Louis, USA)
	Lidocaine-diethyl-d_{10}	Sigma-Aldrich (St. Louis, USA)
	Sulfamethoxazole-d_4	Sigma-Aldrich (St. Louis, USA)
	Isoproturon-d_6	Sigma-Aldrich (St. Louis, USA)
	Estrone-d_2	Sigma-Aldrich (St. Louis, USA)
	M-PFOA	Wellington laboratories Inc. (Guelph, ON, Canada),
	M-PFOS	Wellington laboratories Inc. (Guelph, ON, Canada),
	PAH solution mix (naphthalene d-$_8$, acenaphthene d-$_{10}$, phenanthrene d-$_{10}$, chrysene d-$_{12}$ and perylene d-$_{12}$)	Sigma-Aldrich (St. Louis, USA)

Table S2. Quality parameters for PFASs (Method 1), indicating the response factor, linearity range, Instrumental detection limits (IDL), method detection limits (MDL), percentage recovery with standard deviation (%R±SD) and inter-day precision (%, n=5). Coefficient of determination (r^2) was 0.99 for all compounds.

Compound	F	Linearity (ng/µL)	IDL (ng)	MDL (ng/mL)	% Recovery (± SD)	Interday (%RSD)
PFBA	7.39	0.001-0.3	0.010	0.05	107± 3.6	3.84
PFPA	2.96	0.001-0.3	0.050	0.05	104 ± 2.2	5.54
PFBS	3.84	0.001-0.3	0.040	0.05	108 ± 2.80	6.71
PFHxA	0.28	0.001-0.3	0.050	0.1	96 ± 1.7	11.6
PFHpA	0.27	0.001-0.3	0.050	0.07	98± 3.8	7.92
PFHxS	3.94	0.001-0.3	0.030	0.02	108 ± 2.5	8.08
PFOA	0.35	0.001-0.3	0.020	0.02	96 ± 1.6	11.68
PFNA	1.91	0.001-0.3	0.040	0.04	97 ± 2.5	9.93
PFOS	1.06	0.001-0.3	0.030	0.03	101 ± 2.7	2.90
PFDA	1.10	0.001-0.3	0.030	0.44	100 ± 1.7	9.35
PFUnA	1.20	0.001-0.3	0.050	0.05	108 ± 3.3	10.78
PFDS	0.95	0.001-0.3	0.020	0.03	111 ± 1.2	3.81
PFDoA	1.06	0.001-0.3	0.030	0.04	111 ± 2.8	10.20
PFTriDA	2.35	0.001-0.3	0.110	0.07	117 ± 3.2	12.96
PFTeDA	1.00	0.001-0.3	0.080	0.05	113 ± 4.0	14.31
PFHxDA	1.55	0.001-0.1	0.140	0.08	119 ± 7.9	17.47
PFODA	0.49	0.001-0.1	0.370	0.16	190 ± 9.7	14.72

Table S3. Quality parameters for PAHs, OCs and PCBs (Method 2), indicating the response factor, linearity range, Instrumental detection limits (IDL), method detection limits (MDL), percentage recovery with standard deviation (%R±SD) and inter-day precision (%, n=5). Coefficient of determination (r^2) was 0.99 for all compounds.

Compound	Response factor	Linearity (ng/µL)	IDL (ng)	MDL (ng/mL)	% Recovery (± SD)	Interday (%RSD)
Naphthalene	0.09	0.001-0.2	0.200	0.27	61 ± 4.1	13.6
Acenaphthylene	0.98	0.001-0.2	0.026	0.21	56 ± 0.3	8.43
Acenaphthene	0.33	0.001-0.2	0.059	0.19	67 ± 1.1	4.61
Fluorene	0.67	0.001-0.2	0.087	1.83	71 ± 2.2	7.09
Phenanthrene	0.08	0.001-0.2	0.250	0.68	79 ± 0.2	4.86
Anthracene	0.07	0.001-0.2	0.250	0.58	77 ± 2.1	5.69
Fluoranthene	0.21	0.001-0.2	0.044	0.07	79 ± 3.6	13.2
Pyrene	0.22	0.001-0.8	0.049	0.13	68 ± 4.6	15.2
B(a)A	11.15	0.001-0.8	0.208	0.83	68 ± 1.7	6.89

Compound	Response factor	Linearity (ng/µL)	IDL (ng)	MDL (ng/mL)	% Recovery (± SD)	Interday (%RSD)
Chrysene	13.69	0.001-0.6	0.379	3.65	60 ± 0.2	6.64
B [b]F	7.03	0.001-0.8	1.115	3.60	53 ± 1.4	10.6
B[k]F	6.58	0.001-0.6	0.211	0.13	61 ± 2.0	11.6
B[a]P	4.89	0.001-0.2	0.082	0.25	73 ± 5.8	12.4
Ind[1,2,3-cd]P	2.50	0.001-0.2	0.368	10.53	83 ± 3.9	13.7
Dibenz[a,h]A	1.45	0.001-0.2	0.182	0.25	62 ± 3.6	18.3
B[ghi]P	3.13	0.001-0.2	0.082	1.25	50 ± 1.7	13.1
HCBD	0.19	0.001-0.8	0.012	0.03	86 ± 4.7	11.4
α-HCH	0.10	0.001-0.2	0.008	0.06	105 ± 3.2	8.32
β-HCH	0.01	0.001-0.2	0.085	1.53	114 ± 2	0.66
γ-HCH	0.01	0.001-0.8	0.100	0.99	104 ± 9.3	6.31
δ-HCH	0.01	0.001-0.2	0.030	0.64	87± 10.2	12.4
HCB	0.02	0.001-0.2	0.026	0.03	68 ± 4.2	5.77
2,4’-DDE	3.23	0.001-0.8	0.015	0.07	95 ± 7.5	17.6
4,4’-DDE	1.95	0.001-0.2	0.014	0.07	94 ± 7.5	14.5
2,4’-DDD	3.95	0.001-0.2	0.024	0.05	92 ± 6.6	11.2
2,4’-DDT	8.13	0.001-0.2	0.038	1.28	100 ± 7.6	10.3
4,4’-DDD	5.12	0.001-0.2	0.105	0.10	107 ± 9.3	17.4
4,4’-DDT	1.91	0.001-0.2	0.058	0.139	108 ± 8.1	21.2
α-Endosulfan	0.18	0.001-0.8	0.029	0.04	72 ± 5.0	16.6
β-Endosulfan	0.08	0.001-0.2	0.662	1.10	65 ± 3.8	11.6
Chlorpyrifos	1.03	0.001-0.6	0.110	0.06	90± 4.5	16.906
PCB 28	0.09	0.001-0.2	0.069	0.02	77± 2.9	18.8
PCB 52	2.01	0.001-0.8	0.003	0.05	74 ± 6.1	10.7
PCB 101	2.11	0.001-0.8	0.005	0.081	68 ± 1.4	17.0
PCB 118	2.31	0.001-0.8	0.079	0.09	71 ± 7.6	10.2
PCB 138	1.90	0.001-0.2	0.058	0.14	108 ± 8.1	21.2
PCB 153	1.70	0.001-0.6	0.005	0.11	74 ± 2.7	8.69
PCB 180	0.79	0.001-0.8	0.012	0.29	75 ± 9.6	9.11

Table S4. Quality parameters for pesticides and pharmaceuticals (Method 3), indicating the response factor, linearity range, Instrumental detection limits (IDL), method detection limits (MDL), percentage recovery with standard deviation (%R±SD) and inter-day precision (%, n=5). Coefficient of determination (r^2) was 0.99 for all compounds.

Compound	Response factor	Linearity (ng/µL)	IDL (ng)	MDL (ng/mL)	% Recovery (± SD)	Interday (%RSD)
Metformin	1.26	0.001-0.2	0.079	0.03	40 ± 4.4	n.d
Nicotine	0.64	0.001-0.3	0.110	0.07	105 ± 2.7	n.d
Alopurinol	0.02	0.001-0.2	1.370	0.46	156 ± 6.4	32.3
Atenolol	0.04	0.015-0.2	0.540	11.7	43 ± 15.3	55.3
Paracetamol	0.26	0.001-0.3	0.257	0.37	29 ± 1.9	47.7
Levetiracetam	0.10	0.005-0.3	2.222	0.52	65 ± 1.3	17.5
Gabapentin	3.47	0.001-0.2	0.935	0.2	27 ± 1.3	16.8
Caffeine	0.43	0.005-0.2	0.342	0.35	103 ± 3.3	18.2
Pentoxifylline	1.85	0.001-0.3	0.012	0.01	83 ± 1.9	6.9
Tramadol	0.72	0.005-0.3	0.520	0.43	115 ± 4.2	16.7
Sulfamethoxazole	0.46	0.001-0.3	0.318	0.27	106 ± 1.0	27.3
Venlafaxine	2.56	0.005-0.3	0.079	0.05	11 ± 1.6	32.6
Trazodone	2.80	0.001-0.3	0.067	0.03	32 ± 2.1	17.8
Quetiapine	1.28	0.001-0.3	0.121	0.1	50 ± 2.6	10.6
Losartan	3.63	0.001-0.2	0.06	0.04	55 ± 0.7	22.4
Furosemide	0.09	0.01-0.2	1.367	3.94	17 ± 1.8	14.1
Diclofenac	0.04	0.001-0.2	0.52	0.05	37 ± 1.2	15.1
Atorvastatin	1.32	0.005-0.3	0.48	0.34	37 ± 1.1	33.5
Ibuprofen	0.06	0.025-0.3	4.21	3.01	73 ± 5.7	16.1
Carbamazepine	8.30	0.001-0.2	0.416	0.1	86 ± 4.7	14.1
Dimethoate	0.06	0.005-0.3	0.060	0.19	120 ± 1.5	15.9
Chlorfenvinphos	0.23	0.001-0.3	0.13	0.13	107 ± 1.8	29.4
Isoproturon	0.44	0.001-0.2	0.10	0.1	100 ± 2.3	33.3
Metalaxyl	0.05	0.001-0.3	0.20	0.28	120 ± 2.2	12.1
Triadimenol	0.01	0.005-0.2	3.38	2.59	114 ± 19.3	17.7
Tebuconazol	0.30	0.01-0.2	0.13	0.09	99 ± 15.1	9.2
Kresoxim-methyl	0.03	0.015-0.3	0.87	15.3	118 ± 1.9	15.2
Diclofop	11.36	0.005-0.3	0.65	0.48	50 ± 2.1	32.3
Spinosad	0.07	0.001-0.3	0.23	0.19	15 ± 1.7	11.6
Pyraclostrobin	0.04	0.001-0.3	1.76	1.27	104 ± 0.6	8.2
Tebufenpyrad	0.03	0.001-0.3	4.65	0.36	106 ± 2.5	12.7
Prosulfocarb	0.03	0.001-0.3	2.73	0.19	95 ± 1.9	11.6
Pendimethalin	0.02	0.001-0.3	1.03	0.48	88 ± 2.2	28.6

Compound	Response factor	Linearity (ng/µL)	IDL (ng)	MDL (ng/mL)	% Recovery (± SD)	Interday (%RSD)
TCEP	0.01	0.005-0.2	1.832	1.15	96 ± 2.3	10.5
TBP	0.23	0.001-0.3	0.59	0.1	90 ± 4.8	6.5
TPhP	0.10	0.001-0.3	2.35	0.68	103 ± 2.0	9.2

Table S5. Biometric parameters and Scaled Mass Indexes of greater flamingos' chicks (n=50).

ID	Wing (mm)	Tarsus (mm)	Bill (mm)	Weight (g)	SMI Wing	SMI Tarsus	SMI Bill
F1	355	220	117	2400	1906	2574	2507
F2	325	214	112	1750	2762	1989	2102
F3	366	260	120	2550	1597	1922	2456
F4	318	223	111	2500	4675	2605	3090
F5	320	240	118	1700	3028	1517	1728
F6	340	255	122	2200	2445	1728	2010
F7	344	228	113	1900	1928	1889	2218
F8	362	236	118	2100	1433	1942	2134
F9	330	240	118	2000	2803	1785	2033
F10	343	240	126	1900	1972	1696	1565
F11	355	224	122	1995	1584	2059	1822
F12	285	190	106	1150	5043	1680	1647
F13	344	232	121	2100	2131	2013	1970
F14	340	230	120	2000	2222	1953	1926
F15	250	240	117	2100	25524	1874	2193
F16	310	220	111	2100	4788	2252	2596
F17	320	214	111	1950	3473	2217	2410
F18	357	223	119	2300	1749	2397	2275
F19	360	244	125	2300	1638	1982	1944
F20	375	237	123	2400	1244	2199	2136
F21	350	233	115	2400	2128	2280	2649
F22	350	250	127	2400	2128	1965	1928
F23	353	235	118	2250	1867	2099	2287
F24	330	240	116	2000	2803	1785	2147
F25	350	240	119	2200	1951	1963	2176
F26	358	240	126	2150	1599	1919	1771
F27	340	245	116	2150	2389	1837	2308
F28	320	250	115	2150	3829	1760	2373
F29	395	270	124	2550	883	1775	2211
F30	360	227	112	2150	1531	2158	2582
F31	352	240	122	2200	1866	1963	2010
F32	387	225	122	2150	872	2199	1964
F33	330	234	117	2100	2944	1977	2193
F34	325	225	116	2300	3631	2352	2469
F35	325	240	115	2300	3631	2053	2538
F36	350	240	119	2100	1862	1874	2077
F37	360	270	124	2300	1638	1601	1995

ID	Wing (mm)	Tarsus (mm)	Bill (mm)	Weight (g)	SMI Wing	SMI Tarsus	SMI Bill
F38	361	239	120	2050	1429	1846	1974
F39	370	236	117	2100	1209	1942	2193
F40	346	246	118	2050	1988	1737	2084
F41	352	235	121	2150	1824	2006	2016
F42	367	270	123	2350	1441	1636	2091
F43	385	222	120	2050	866	2157	1974
F44	298	232	117	1900	5889	1821	1984
F45	360	233	118	1950	1389	1852	1982
F46	385	260	126	2600	1098	1960	2142
F47	367	240	124	2450	1502	2186	2125
F48	365	265	124	2300	1472	1665	1995
F49	353	219	119	1900	1577	2057	1880
F50	289	225	110	2050	8066	2096	2608

Table S6. Lineal model estimates for biometric measurements and micropollutants concentrations.

Fixed Effects	Estimates	Std. Error	t-value	p
Weight				
(Intercept)	2216	1.82E+02	12.2	<0.001
∑PFASs	6.52	4.07E+01	0.16	0.87
∑OC Pesticides	-66.0	5.86E+01	-1.13	0.27
∑PCBs	31.1	5.59E+01	0.56	0.58
R^2 = 0.04				
BMI Tarsus				
(Intercept)	1868.7	1.88E+02	9.95	<0.001
∑PFASs	-4.1	4.60E+01	-0.09	0.93
∑OC Pesticides	31.7	4.05E+01	0.78	0.44
∑PAHs	29.4	2.17E+01	1.36	0.18
R^2 = 0.07				
BMI Wing				
(Intercept)	3291.4	3.02E+03	1.09	0.28
∑PFASs	-516.3	7.70E+02	-0.67	0.51
∑PCBs	911.5	6.35E+02	1.44	0.16
∑PAHs	402.9	3.56E+02	1.13	0.26
R^2 = 0.08				
BMI Bill				
(Intercept)	2321.8	2.15E+02	10.81	<0.001
∑PFASs	-62.8	5.26E+01	-1.20	0.24
∑OC Pesticides	39.5	4.63E+01	0.85	0.40
∑PAHs	7.4	2.48E+01	0.30	0.77
R^2 = 0.047				

CHAPTER VII

ASSESSING CONTAMINATION PROFILES IN LIVERS FROM ROAD-KILLED OWLS

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ABSTRACT

Raptors are recognised as valuable sentinel species for monitoring environmental contaminants owing to their foraging behaviour across terrestrial and aquatic food webs and their high trophic position. This study monitored environmental contaminants in livers from road-killed owls to evaluate differences in the exposure patterns due to factors as species, age, and sex of individuals. Carcasses of road-killed individuals of eagle owl (*Bubo bubo*), long-eared owl (*Asio otus*), little owl (*Athene noctua*), tawny owl (*Strix aluco*), and barn owl (*Tyto alba*) were collected in Alentejo (Portugal). Eighty-one organic contaminants were analyzed, including organochlorine pesticides (OCPs), perfluoroalkyl substances (PFASs), polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), pharmaceuticals, in-use pesticides, and organophosphate esters flame retardants (OPEs). Overall, 21 contaminants were detected. ∑OCPs were prevalent in all species at concentrations ranging from 3.24 to 4480 ng/g ww, followed by PFOS, the only PFASs detected (from 2.88 to 848 ng/g ww) and ∑PCBs (1.98 to 2010 ng/g ww). ∑PAHs were ubiquitous but detected at the lowest concentrations (from 7.35 to 123 ng/g ww). Differences among species were observed according to Principal Component Analysis. Eagle owl and long-eared owl presented the highest levels of ∑OCPs, ∑PCBs, and PFOS, consistently with its higher trophic position while ∑PAHs prevailed in tawny owl, barn owl and little owl, related with their frequent use of urban areas for nesting and roadsides for hunting. Adults presented higher concentrations of ∑OCPs and ∑PCBs than juveniles, while no differences were observed for PFOS and ∑PAHs among ages. Pharmaceuticals, in-use pesticides and OPEs were not detected. Overall, this study shows specific contamination patterns in 5 species with similar diet but with differences in habitat preferences.

1. INTRODUCTION

Birds are exposed to a myriad of organic contaminants, affecting the wellbeing of many species, and posing a serious threat to biodiversity and ecosystem services. Most studies focus on Persistent Organic Pollutants (POPs) such as organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs) and perfluorinated compounds (PFASs), which are persistent global environmental contaminants despite the efforts to regulate them (Stockholm Convention, 2019). Other contaminants affecting birds are polycyclic aromatic hydrocarbons (PAHs) that despite being metabolized, they are recurrently detected in bird species as a result of exposure to petroleum and combustion (Custer et al., 2001). Finally, the presence and impact of emerging contaminants such as pharmaceuticals, in-use pesticides or plasticizers in birds are still under discussion, as only a few studies report their presence (González-Rubio et al., 2021). Contaminants have been detected in eggs, feathers, blood, livers, and other birds' internal tissues (Espín et al., 2016), and evidence that they are bioaccumulated through the food web, affecting particularly top predators (Rodríguez-Jorquera et al., 2017).

Biomonitoring programmes that use birds as sentinel species have been proven to be a good approach for the early detection of adverse effects of contaminants in ecosystems and to assess the effectiveness of legislation (Smits and Fernie, 2013). Raptors have been described as good sentinel species for monitoring legacy and emerging contaminants (Gómez-Ramírez et al., 2014; Movalli et al., 2018), mostly because of their foraging habits through both terrestrial and aquatic food webs and high trophic position (Badry et al., 2020). Also, raptors are a widely studied group of birds with a great number of monitoring schemes that provide data about their population, reproduction, and potential adverse effects caused by contaminants (Derlink et al., 2018). However, working with raptors can also be challenging because they are protected species and sensitive to human disturbance. For this reason, the use of non-invasive and non-intrusive sampling methods to perform monitoring studies with raptors is strongly recommended (Espín et al., 2016). The collection of individuals found dead is a sampling approach that allows the analysis of internal tissues without animal disturbance. Internal tissues as livers or muscles are excellent matrices to analyse a wide number of pollutants, especially the most persistent ones (Espín et al., 2016).

Roads can represent feeding opportunities to some diurnal raptor species as road-killed animals or prey are abundant along road verges (Hanmer and Robinson, 2021; Meunier et al., 2000). However, for many owls (Strigiformes), roads represent a major threat due to frequent mortality caused by collision with vehicles (Gomes et al., 2009; Santos et al., 2013; van der Horst et al., 2019). Because of their large size, raptor carcasses are often easily detected along roadsides (berms and verges) and reported by citizens to authorities or wildlife rehabilitations centres. Owls are the most frequent victims

among raptors (Hanmer & Robinson, 2021). Therefore, road-killed owls can be a valuable source of samples and contextual information to assess the exposure of contaminants in a non-invasive approach.

The present study monitored 81 organic micropollutants, including emerging and legacy compounds, in 47 livers from road-killed owls collected in rural areas of Alentejo (Portugal). Differences in the exposure patterns were evaluated in 5 species: eagle owl (*Bubo bubo*), long-eared owl (*Asio otus*), little owl (*Athene noctua*), tawny owl (*Strix aluco*), and barn owl (*Tyto alba*). These species have similar biology but different habitat preferences, and we discuss the potential causes of exposure according to species and diet.

2. MATERIALS AND METHODS

2.1. Sample collection

Seventy-three road-killed owls found in Alentejo (Portugal) were collected from 2010 to 2019 during regular monitoring of road killings (Santos et al., 2013; van der Horst et al. 2019). Biometric data for all individuals were measured and included wing, tarsus, bill, mouth, weight, wingspan, body lentgth and ulna (data detailed in Table S1). Individuals were aged through the moulting pattern of feathers and sexed by biometric differences and gonad inspection (Martínez et al., 2002). Necropsies were conducted in all owls to obtain liver samples for chemical analysis. However, non-fresh livers or those with severe damage due to collision were excluded. From the initial 73 carcasses collected, 47 liver samples suitable for chemical analysis were obtained for five owl species breeding in the area: eagle owl (n = 7), long-eared owl (n = 5), little owl (n = 12), tawny owl (n = 12), and barn owl (n = 11). Samples were frozen at -21ºC, freeze-dried and the tissue was homogenized with a mortar and a pestle. Liver samples were weighted before and after freeze drying to determine the water content. The average content of water in livers was: 74% for eagle owl, 73% for long-eared owl, 72% for tawny owl, 69% for barn owl, and 71% for little owl.

2.2. Chemical standards

Compounds studied are indicated in Table S2 in Supplementary Material. A total of 81 organic compounds were analysed including 14 OCPs, 7 marker PCBs congeners, 16 PAH, 14 PFASs, 15 pharmaceuticals, 12 in-use pesticides and 3 OPEs. Certified standards of 98-99% purity were purchased from Wellington laboratories Inc. (Guelph, ON, Canada), AccuStandard (New Haven, CT, USA), Sigma-Aldrich (St. Louis, USA), and Dr. Ehrenstorfer (Augsburg, Germany). The surrogate standards used were triphenyl phosphate-d_{15} (TPhP-d_{15}), acetaminophen-methyl-d_3, carbamazepine-d_2, lidocaine-

diethyl-d_{10}, sulfamethoxazole-d_4, isoproturon-d_6, estrone-d_2, ^{13}C-PFOA (M-PFOA), ^{13}C-PFOS (M-PFOS), and a PAH solution mix (naphthalene-d_8, acenaphthene-d_{10}, phenanthrene-d_{10}, chrysene-d_{12} and perylene-d_{12}) (Table S2). Solvents used were acetonitrile (ACN) purchased from Fisher Scientific Chemical (Bridgewater, USA), methanol (MeOH) and HPLC grade water from Merck (Darmstadt, Germany), ammonium formate (NH_4HCO_2), ammonium acetate ($NH_4CH_3CO_2$) and formic acid (HCOOH) from Sigma-Aldrich.

2.3. Sample extraction and analysis

The analytical methodology developed to analyse contaminants in blood (Dulsat-Masvidal et al. 2023) was adapted for the analysis of liver samples. Three different extraction and analytical methods were used: Method A for the determination of OCPs, PCBs and PAH, Method B for PFASs, and Method C for the determination of pharmaceuticals, in-use pesticides and OPEs. Results are expressed as ng/g wet weight (ww) taking into account the % water in livers from each species, to be consistent with previous studies and compare our results according to the open bibliography.

2.3.1. Analysis of OCPs, PCBs and PAHs (Method A).

50 mg of each freeze-dried liver sample was weighed in 2 mL polypropylene vial (Eppendorf, Hamburg, Germany) and spiked with 50 ng of mixture of internal standards (naphthalene-d_8, acenaphthylene-d_{10}, phenanthrene- d_{10}, chrysene-d_{12} and perylene-d_{12}). A generic solid-liquid extraction was performed by adding 1.5 mL of hexane: dichloromethane (1:1) into the vials. Samples were vortexed (1 min) and ultrasonicated (10 min) and this procedure was repeated 3 times without changing solvent. Samples were centrifuged for 10 min at 2370 rcf and the supernatants were collected. Clean-up was performed with 5 g florisil Bond Elut cartridges (Agilent Technologies, USA) using 30 mL of hexane: dichloromethane (1:1) as conditioning and elution mixture solvent. The extracts were evaporated to near dryness and reconstituted with 250 µL of hexane. Samples were analysed by gas chromatography coupled to a triple quadrupole (QqQ) mass spectrometer (Agilent 7890A chromatograph and 7000A MS analyzer, from Agilent Technologies, Santa Clara, CA, USA) with electron ionisation (EI) at 70 eV. A HP-5MS Agilent column of 30 m length x 0.25 mm inner diameter x 0.25 µm film thickness (Agilent Technologies, Wilmington, DE, USA) was used for the separation of compounds according to a previous method (Dulsat-Masvidal et al., 2023). The initial temperature was set at 70 ºC and kept for 1 min, then increased to 175 ºC in 4 min, from 175 °C to 235 °C in 20 min, and to 305 ºC in 8 min. The data was processed through Mass Hunter Quantitative software.

2.3.2. Analysis of PFASs (Method B)

50 mg of each freeze-dried liver sample was weighed in 2 mL polypropylene vial (Eppendorf, Hamburg, Germany) and spiked with 50 ng of the internal standards M-PFOA and M-PFOS. Samples were solid-liquid extracted with 1.5 mL of acetonitrile. Samples were vortexed (1 min) and ultrasonicated (10 min), and this procedure was repeated 3 times without changing the solvent. After centrifugation (10 min, 2370 rcf), the supernatant was collected in a new vial and 25 mg of active carbon and 50 µL of glacial acid were added. The extract was vortexed (1 min) and centrifuged (10 min), and extracts filtered through 0.2 µm x 13 mm nylon syringe (Clarify, Phenomenex, USA). Samples were evaporated to dryness and reconstituted with 100 µL of methanol and 100 µL HPLC water with 10 mM ammonium acetate. The analysis was performed by liquid chromatography with tandem mass spectrometry (LC-MS/MS) with an electrospray ion source (Waters, Milford, USA). An ACQUITY BEH C18 analytical column (100 mm length x 2.1 mm inner diameter, 1.7 µm particle size) with a VanGuard pre-column was deployed (Acquity, Waters, Ireland). An ACQUITY BEH C18 trap column (30 mm length x 2.1 mm inner diameter, 1.7 µm particle size) was placed before the injector to avoid PFASs contamination from the chromatographic system. The mobile phase consisted of (A) a mixture of methanol and acetonitrile (80:20) with 10 mM of ammonium acetate and (B) water with 10 mM of ammonium acetate. Gradient elution started at 50% A and 50% B (condition kept for 3 min), increased to 100% B in 7 min and returned to initial conditions in 5 min. All compounds were measured under ESI-. The data was processed through Mass Lynx software.

2.3.3. Analysis of pharmaceuticals, pesticides, and OPEs (Method C)

50 mg of each freeze-dried liver sample was weighed in 2 mL polypropylene vial (Eppendorf, Hamburg, Germany) and spiked with 50 ng of internal standards (acetaminophen-d-13, lidocaine-d-10, isoproturon-d6, TPhP-d-15 and sulfamethoxazole-d-2). Then, 1.5 mL of acetonitrile was added, samples were vortexed and ultrasonicated for 10 min (procedure repeated 3 times without changing the solvent), centrifuged (10 min, 2370 rcf) and the supernatant collected. Samples were directly purified with 30 mg Oasis PRIME HLB cartridges (1 cm^3 cartridge tube, Waters, USA) without prior conditioning, and elution was performed with 1.5 mL of acetonitrile-water (80:20) solution. The samples were evaporated until dryness and reconstituted with 200 µL of ACN: water (1:1). The analysis was performed by LC-MS/MS with an electrospray ion source (Waters, Milford, USA). An Acquity BEH C18 analytical column (100 mm × 2.1 mm internal diameter, 1.7 µm particle size) with a VanGuard C18 pre-column was deployed (Acquity, Waters, Ireland). The mobile phase consisted of (A) acetonitrile with 0.1% formic acid and (B) water with 0.1% formic acid. Gradient elution started at 5% A and 95% B (3 min

in hold time) and increased to 100% of A in 21 min (1 min hold time). All compounds were measured under ESI + except diclofop and furosemide that were measured with ESI- in the same run. The data was processed through the Mass Lynx software.

2.4. Method validation/quality control

Commercial chicken liver was used as matrix for method validation to evaluate the extraction efficiency. Chicken liver (50 mg freeze-dried, n = 5) was spiked with 50 ng of the mixture of target analytes and extracted using the 3 methods described above. Unspiked chicken liver was also analysed to ensure the lack of initial contaminant contribution. Also, 3 procedural blanks (no matrix) were analysed to determine background contamination. All samples and quality controls were spiked with 50 ng of internal standards. The calibration curve was built in hexane from 0.001 to 0.8 ng/µL with IS at 0.05 ng/µL for GC-MS/MS analysis and in methanol and water from 0.001 to 0.3 ng/µL with IS at 0.05 ng/µL for LC-MS/MS methods. Concentrations were calculated using internal standard quantification. Instrumental limits of detection (IDL) were calculated as the amount of analyte that gave a S/N ratio of 3 using the standard solution at 0.001 ng/µL. Method detection limits (MDL) were calculated as the concentration that gave a S/N ratio of 3 using spiked liver. Values below MDL were given a value of zero in order not to overestimate the concentrations detected. Means for chemical families were calculated using zero values for non-detected contaminants not to overestimate the concentrations. All the quality parameters of the used methods are detailed in the Supplementary Material Table S3 for PAHs, OCPs, and PCBs, Table S4 for PFASs and Table S5 for pharmaceuticals, in-use pesticides and OPEs.

2.5. Data analysis

The data distribution was tested through normality plots (Figure S1); logarithmic transformation (log x+1) was used to obtain a normal distribution of the variables. Considering that ∑PCBs still presented some skewing in distribution (Figure S1), we used non-parametric test Kruskal-Wallis followed by post-hoc Dunn test to determine differences between mean chemical groups. When all tested variables exhibited a normal distribution, a two-way ANOVA followed by the Tukey test were used to assess differences between mean groups. Principal Component Analysis (PCA) was used to assess the different profiles of contamination between species. Kaiser–Meyer–Olkin (KMO) test was used to assess the usefulness of the PCA, where KMO > 0.5 indicates variables interdependent enough for using PCA (Dziuban et al., 1979). All statistical analysis were performed in R studio (R version 4.0.3), and the figures were elaborated using ggplot2 and factoextra package.

3. RESULTS AND DISCUSSION

3.1. Occurrence of contaminants in owl livers

All individuals analysed contained residues of contaminants in livers, indicating exposure to multiple contaminants of different chemical families. A total of 21 out of 81 target compounds were found in liver samples (Table 1). Figure S2 indicates all compounds detected according to the detection frequency considering all species. The ∑contaminants ranged from 28.2 to 4661 ng/g ww and according to the median values decreased following the order: eagle owl > long-eared owl > tawny owl > barn owl > little owl (Figure 1A). Different patterns of contaminants were observed among species, being ∑OCPs the most prevalent in all species, except barn owl, followed by ∑PFASs, ∑PCBs, and ∑PAHs (Figure 1B). Barn owl had a profile dominated by ∑PCBs, followed by ∑PFASs and ∑OCPs. Pharmaceuticals, in-use pesticides and OPEs were not detected in any sample, despite the method being effective to determine those emerging compounds (Table S5).

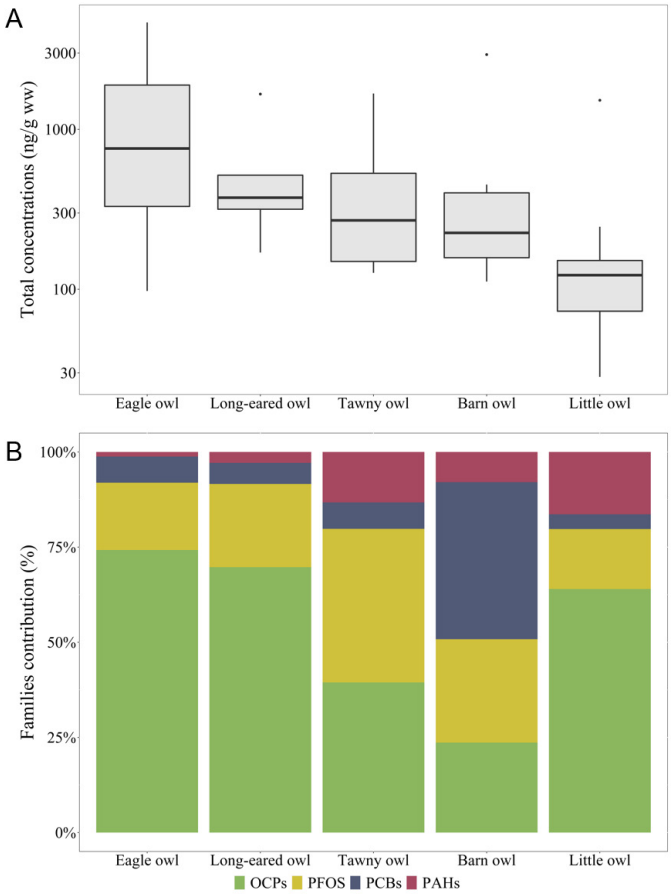

Figure 1. (A) Concentration of total contaminants in the 47 analysed owl livers from the different species and (B) Contamination profile in each species according to the chemical family.

Table 1. Compounds detected out of 81 studied and ∑chemical families in liver samples from 5 species of nocturnal raptors, indicating frequency of detection (f.d.) (%) and number of individuals with positive findings for each contaminant (n), mean with standard error (S.E.), minimum and maximum concentrations, expressed in ng/g wet weight. Compounds are ordered from the highest to lowest concentration and frequency of detection considering eagle owl.

Compounds	Eagle owl (n=7)				Long-eared owl (n=5)				Tawny owl (n=12)				Barn owl (n=11)				Little owl (n=12)			
	f.d. (n)	Mean ± S.E.	Min.	Max.	f.d. (n)	Mean ± S.E.	Min.	Max.	f.d. (n)	Mean ± S.E.	Min.	Max.	f.d. (n)	Mean ± S.E.	Min.	Max.	f.d. (n)	Mean ± S.E.	Min.	Max.
4,4'- DDE	100 (7)	1193 ± 738	19	4475	100 (5)	423 ± 235	43	1341	100 (12)	182 ± 94.4	14.9	1179	100 (11)	116 ± 56.9	2.19	636	100 (12)	144 ± 109	3.24	1342
Naphthalene	86 (6)	10.6 ± 2.39	6.47	17.5	100 (5)	13.8 ± 2.22	7.35	19.7	100 (12)	13.2 ± 1.53	8.21	27.7	91 (10)	18.2 ± 4.35	11.1	55.1	100 (12)	20.7 ± 1.99	13.4	38.6
Acenaphthene	71 (5)	4.78 ± 2.38	2.33	18	80 (4)	2.02 ± 0.81	1.05	4.83	100 (12)	47 ± 5.99	24.3	92.8	100 (11)	17.8 ± 2.63	3.45	34.9	100 (12)	12.8 ± 2.70	3.49	33.5
PFOS	86 (6)	285 ± 124	20.9	848	100 (5)	133 ± 38.9	8.89	245	100 (12)	187 ± 64.5	15.1	774	100 (11)	135 ± 31	37.2	306	83 (10)	36.1 ± 16.0	2.88	200
PCB 138	100 (7)	50.8 ± 20.7	1.98	131	100 (5)	17.3 ± 5.51	3.98	34.2	67 (8)	19.4 ± 14.3	2.64	176	82 (9)	57.2 ± 45.6	5.04	511	17 (2)	4.38 ± 3.86	6.03	46.5
Phenanthrene	29 (2)	0.43 ± 0.29	1.06	1.92	20 (1)	-	1.98	1.98	58 (7)	1.2 ± 0.33	1.19	3.12	91 (10)	3.41 ± 0.74	1.34	8.59	75 (9)	2.87 ± 0.61	1.72	6.1
PCB 180	71 (5)	24.8 ± 9.91	1.79	63.6	80 (4)	9.2 ± 3.65	5.18	21.2	42 (5)	11.1 ± 8.57	1.96	104	82 (9)	13.4 ± 7.6	2.66	87.3	8 (1)	-	21.8	21.8
PCB 153	57 (2)	26.9 ± 13.1	2.52	79.8	60 (3)	6.36 ± 3.04	4.85	13.9	17 (2)	1.72 ± 1.16	9.77	10.9	82 (9)	62 ± 55.3	2.95	615	17 (2)	2.51 ± 2.14	4.54	25.6
Lindane	57 (4)	1.79 ± 0.68	1.3	4.04	20 (1)	-	1.07	1.07	25 (3)	0.31 ± 0.16	1.11	1.4	27 (3)	0.6 ± 0.36	1.24	3.69	25 (3)	0.62 ± 0.33	2.17	2.74
HCB	71 (5)	2.1 ± 0.63	1.96	4.65	20 (1)	-	3.42	3.42	17 (2)	0.55 ± 0.37	2.9	3.7	18 (2)	0.69 ± 0.47	3.72	3.91	17 (2)	1.14 ± 0.8	5.12	8.61
α-endosulfan	-	-	-	-	-	-	-	-	33 (4)	0.05 ± 0.02	0.1	0.2	18 (2)	0.03 ± 0.02	0.12	0.23	17 (2)	0.03 ± 0.02	0.1	0.22
PCB 118	57 (4)	8.41 ± 3.89	2.12	23.6	40 (2)	0.82 ± 0.52	1.55	2.54	-	-	-	-	9 (1)	-	797	797	8 (1)	-	2.53	2.53
2,4'- DDD	29 (2)	0.07 ± 0.06	0.06	0.43	20 (1)	-	0.11	0.11	8 (1)	-	0.1	0.1	9 (1)	-	0.16	0.16	17 (2)	0.24 ± 0.21	0.32	2.58
Pyrene	-	-	-	-	20 (1)	-	3.73	3.73	8 (1)	-	2.15	2.15	-	-	-	-	25 (3)	0.91 ± 0.49	3.12	4.46
Acenaphthylene	57 (4)	2.69 ± 1.12	2.84	7.27	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2,4'- DDE	-	-	-	-	-	-	-	-	17 (2)	0.01 ± 0.01	0.07	0.1	-	-	-	-	8 (1)	-	0.09	0.09
4,4'- DDD	-	-	-	-	-	-	-	-		-	-	-	9 (1)	-	1.46	1.46	8 (1)	-	6.6	6.6
Fluoranthene	-	-	-	-	20 (1)	-	2.47	2.47	-	-	-	-	-	-	-	-	8 (1)	-	2.81	2.81
2,4'- DDT	-	-	-	-	-	-	-	-	-	-	-	-	9 (1)	-	2.67	2.67	-	-	-	-
Fluorene	14 (1)	-	6.43	6.43	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Benzo(a)anthracene	-	-	-	-	-	-	-	-	8 (1)	-	0.59	0.59	-	-	-	-	-	-	-	-
∑ OCPs	100 (7)	1197 ± 738	25.7	4480	100 (5)	424 ± 235	43.1	1341	100 (12)	183 ± 94.3	14.9	1179	100 (11)	117 ± 57.2	3.83	640	100 (12)	147 ± 110	3.24	1353
∑PFASs	86 (6)	285 ± 124	20.9	848	100 (5)	133 ± 38.9	8.89	245	100 (12)	187 ± 64.5	15.1	774	100 (11)	135 ± 31.0	37.2	306	83 (10)	36.1 ± 16.0	2.88	200
∑ PCBs	100 (7)	111 ± 47.3	1.98	298	100 (5)	33.7 ± 12.1	3.98	69.2	67 (8)	32.3 ± 22.9	3.53	280	82 (9)	205 ± 181	11.6	2010	17 (2)	8.92 ± 7.81	13.1	94
∑ PAHs	100 (7)	19.4 ± 3.21	8.57	30.5	100 (5)	17.4 ± 3.66	7.35	26.5	100 (12)	61.6 ± 7.42	32.5	123	100 (11)	39.4 ± 5.39	20.1	81.6	100 (12)	37.6 ± 4.32	21.4	68.5

Table 1 shows the detection frequency, the mean, minimum and maximum concentrations of each detected contaminant in the 5 species. Among chemical families, ∑OCPs were detected at concentrations from 3.24 to 4480 ng/g ww, with higher mean values in eagle owl (1197±738) compared to the other species (424±235 ng/g ww in long-eared owl to 117±57.2 ng/g ww in barn owl). The most ubiquitous OCP was 4,4'-DDE (the most stable and toxic metabolite of 4,4'-DDT), which was detected in all individuals at concentrations from 2.19 to 4475 ng/g ww (Table 1). 4,4'-DDE is a recognized legacy contaminant detected in biota (Turusov et al., 2002). 2,4'-DDD was detected in 8-29% of the analysed birds at concentrations from 0.06 to 2.58 ng/g ww and other DDT isomers were seldom detected (Table 1). 4,4'-DDT was never detected, indicating that it has not been used over the last decades, coinciding with the Stockholm Convention phase out of this compound (Stockholm Convention, 2019). Our results are consistent with those of Roque et al. (2022) who found 4,4'-DDE as the most prevalent OCPs in livers from road-killed barn owls in Portugal at mean concentrations from 1.93 to 162 ng/g ww, lower that the ones found in barn owls in the present study which were from 2.19 to 636 ng/g ww. 4,4'-DDE was also the main OCPs detected in livers from 18 diurnal birds of prey in Spain (van Drooge et al., 2008) and also in white-tailed eagles (*Haliaeetus albicilla*) in Germany (Badry et al., 2022). The minimum concentration associated with lethality in birds is 2000 ng/g (Beyer and Meador, 2011); in our study 2 adult eagle owls presented concentrations above these limits at 4475 ng/g ww (female) and 3577 ng/g ww (male). These concentrations are considered high enough to be the underlying cause of mortality, however concentrations 10 to 100 times lower than the lethal concentrations can also cause behavioural effects in birds including decreased aggression, impaired avoidance, as well as reduced defence and attentiveness in the nest (Hellou et al., 2013). 4,4'-DDE is also known as an endocrine disruptor in birds. High levels of 4,4'-DDE in eggs from eagle owls in Spain have been related with eggshell thinning potentially affecting the reproduction of the species (Gómez-Ramírez et al., 2012). Another OCPs detected in owl's liver was lindane, with a higher detection frequency and mean concentrations in eagle owl (57%, 1.79±0.68 ng/g ww) than the rest of species (20-27%, from 0.6±0.36 to 0.62±0.33 ng/g ww). HCB was detected in all species (17-71% of detection frequency depending on the species), with a special prevalence in eagle owl (71% of detection frequency, mean 2.1±0.63 ng/g ww) but with the highest concentrations in 2 little owl individuals (5.12 and 8.61 ng/g ww). Finally, α-endosulfan was only detected in tawny owl, barn own and little owl at concentrations from 0.10 to 0.23 ng/g ww.

Among PFASs, perfluorooctane sulfonate (PFOS) was the only one detected, present in 83 to 100% of the analysed birds at concentrations from 2.88 to 848 ng/g ww (Table 1). It was identified in all samples from long-eared owl, tawny owl and barn owl at mean concentrations between 133±38.9

and 187±64.5 ng/g ww. In eagle owl, the concentrations were much higher (86% detection frequency, mean 285±124 ng/g ww) and little owl was the least impacted species (83% detection frequency, mean 36.1±16 ng/g ww). PFOS is commonly reported in birds of prey due to its potential for bioaccumulation and biomagnification through the food web (Eriksson et al., 2016; Monclús et al., 2022). PFOS was also the main PFASs detected in livers from the top predator common buzzard (B*uteo buteo)* in Belgium, with mean concentrations between 41.8 and 67.1 ng/g ww (Groffen et al., 2023). High PFOS concentrations are reported in road-killed barn owls close to a PFASs chemical plant in Belgium, with median concentrations of 304 ng/g in the range of 42 to 992 ng/g ww (Jaspers et al., 2013). The toxic reference value (TRV) for PFOS in liver of avian top predators is estimated to be 600 ng/g ww (Newsted et al., 2005), in the present study one individual of eagle owl and one of tawny owl surpassed this limit.

∑PCBs were detected in all individuals of eagle owl and long eared owl at mean concentrations of 111±47.3 and 33.7±12.1 ng/g ww, respectively, in 67% of tawny owls and 82% of barn owls at mean concentrations of 32.3±22.9 ng/g ww and 205±181 ng/g ww, respectively, and in 17% of little owls at mean concentrations of 8.92±7.81 ng/g ww. Barn owls were the most impacted species by PCBs. Tawny owl and little owl were the species with the lowest detection frequency and concentrations. The highest concentrations were found in barn owls, which presented a ∑PCBs mean concentration of 205±181 ng/g ww (range 11.6 to 2010 ng/g ww). The highest levels were found in a juvenile female that presented ∑PCBs of 2010 ng/g due to high concentrations of PCB 138 (511 ng/g ww), PCBs 153 (615 ng/g ww), and PCBs 118 (797 ng/g ww). The levels found in barn owls are within the range or below the ones found in barn owls collected in Italy, with mean concentration of 651 ng/g (range from 55 to 2688 ng/g) (Naso et al., 2003). In all species, the PCBs profile was dominated by PCB 138, found in 17-100% of the birds at levels from 1.98 to 511 ng/g, followed by PCB 180 and PCB 153 present in 8-82% of the individuals depending on the species at concentrations ranging from 1.79 to 104 ng/g ww and from 2.52 to 615 ng/g ww, respectively. The PCBs profile is similar to the ones reported in livers from birds where high chlorinated PCBs (PCB 118, 138,153) were more abundant than low chlorinated PCBs (28, 52 and 101) (Buck et al. 2020). Due to the lack of unsubstituted adjacent meta- and para-positions on the biphenyl rings, high chlorinated PCBs are more resistant to metabolic cytochrome P450-mediated attacks and are more persistent and bioacumulative in soft tissues than low chlorinated PCBs, which are more rapidly metabolised and excreted by organisms (Tomza-Marciniak et al., 2019). The exposure of PCBs in birds has been associated with decreased nest attentiveness in glaucous gulls (*Larus hyperboreus*) (Bustnes et al., 2001) and disruption of feather colouration in American kestrels (*Falco sparverius*) (Bortolotti et al., 2003).

∑PAHs were detected in all samples although at low concentrations compared to other contaminants (from 7.35 to 123 ng/g ww). Naphthalene and acenaphthene were the most frequently PAHs found in the different species in 86-100% and 71-100% of the samples, respectively, followed by phenanthrene with a detection frequency of 20-91% (Table 1). The mean concentrations ranged from 17.4±3.66 to 61.6±7.42 ng/g ww, and in contrast to OCPs, eagle owl and long-eared owl had the lowest concentrations among studied species. Other PAHs sporadically detected are indicated in Table 1. Low-molecular-weight (LMW) as naphthalene and acenaphthene presented a higher contribution than high-molecular-weight (HMW) PAHs and is indicative of petrogenic PAHs sources including crude oil and petroleum products such as kerosene, gasoline, diesel fuel, lubricating oil, and asphalt (Saha et al., 2009). High concentrations of naphthalene, acenaphthene and phenanthrene has been related to the exposure of coal tar (McCormick et al., 2022). Luzardo et al. (2014) also reported a higher prevalence of LMW PAHs in livers from 6 species of birds of prey from Canary Islands (Spain), including barn owl and long-eared owl, being naphthalene the main compound detected. Similar to our results, barn owls presented higher levels of PAHs than long-eared owls, however the levels detected in the present study are lower than those reported by Luzardo et al. (2014).

3.2. Differences of exposure among owl species

The PCA grouped owl species according to the exposure profile to the different chemical families (Figure 2). Principal Component 1 explained 47.4% of the variance and is positively related with ∑PCBs, ∑OCPs and PFOS and is negatively related with ∑PAHs. Component 2 explained 24.2% of the variance, and it is explained by individuals with high contribution of ∑PAHs and PFOS and negatively related with ∑PCBs and ∑OCPs. Eagle owl and long-eared owl samples are distributed in the right principal component 1 axis, indicating a similar profile of contaminants. These 2 species are characterised by the prevalence of the most persistent compounds such as ∑OCPs, ∑PCBs and PFOS and low ∑PAHs concentrations. Tawny owl and barn owl samples are distributed in the center of the plot, showing intermediate levels of ∑OCPs, ∑PCBs, and PFOS, but also a high contribution of ∑PAHs. Little owl samples are grouped in the PCA bottom left quadrant, indicating a lower contribution of ∑OCPs, ∑PCBs, and PFOS, and a high contribution of PAHs.

Despite all species are nocturnal raptors and collected in the same Alentejo region in Portugal, they present differences in their diet, nesting site characteristics, lifespan, or habitat preferences, as summarized in Table 2. These differences represent a key factor explaining the different profiles of contamination among species. Eagle owl and long-eared owl are distinguished by feeding in higher trophic levels and hunting and nesting in

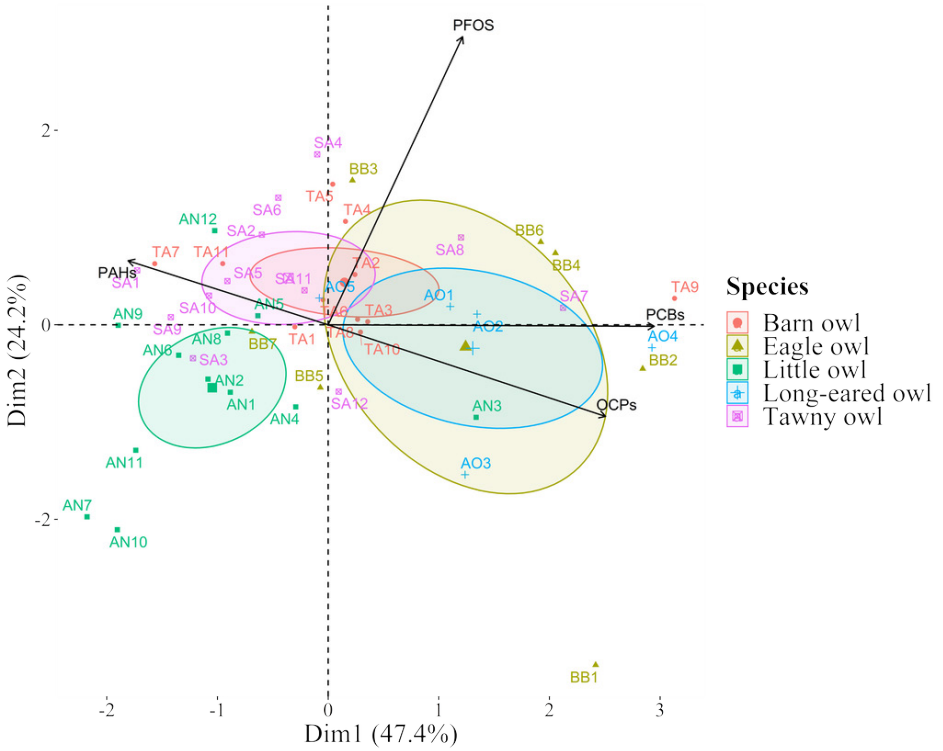

Figure 2. Principal component analysis of concentrations (logarithmic transformation log x+1) of compounds detected in owl's livers. Plot showing components 1 and 2, which explain 74.4% of the total variance. KMO = 0.53.

woodland areas (Table 2). Eagle owl is a long-lived super predator, and its diet is composed of medium sized mammals such as rabbits, hares, hedgehogs and rats and birds such as partridges, pigeons, and jays (Lourenço, 2006). Eagle owl was the species with the highest total concentration levels (Figure 1, Table 1), mainly due to the high levels of ∑OCPs (25.7 to 4480 ng/g ww), PFOS (20.6 to 848 ng/g ww) and ∑PCBs (1.98 to 298 ng/g ww). The high exposure of persistent compounds in eagle owls is due to its super predator diet which makes this species prone to a higher bioaccumulation of persistent compounds through the food web, compared to other species feeding on lower trophic levels (Lourenço et al., 2011). It must be considered that the eagle owl and long-eared owl were the less frequently road-killed carcasses found compared to the 3 other species. In the Alentejo region, eagle owl and long-eared owl inhabit and forage in areas further away from roads and urban areas (Lourenço et al. 2015), which could contribute to lower ∑PAHs levels.

Tawny owl, barn owl and little owl showed a contaminant profile characterised by the presence of higher concentrations of ∑PAHs than the other species (Figure S3). In birds, the exposure to PAHs usually occurs by

breathing air contaminated by coal tar or wild fires, or by eating polluted foods (González et al., 2002). Therefore, the foraging behaviour on small mammals or invertebrates in the roadsides or closer to urban areas could be a source of PAHs for nocturnal raptors which hunt or nest in these areas, explaining the higher exposure of ∑PAHs found in barn owl, tawny owl, and little owl in our study.

Table 2. Species studied, hunting habitat, nesting sites, lifespan, and diet of the sampled bird species in the study area (Alentejo, Portugal).

Species	Hunting habitat	Nesting sites	Lifespan[a] (years)	Diet	References
Eagle owl *Bubo bubo*	Woodlands, shrublands	Old quarries, river banks	27	Rabbits, hares, partridges, rats, hedgehogs, pigeons. Sometimes other mammals and birds.	R. Lourenço et al., 2015, 2021; R. F. Lourenço, 2002
Long-eared owl *Asio otus*	Woodlands, cereal fields	Nests from other birds (raptors, crows, etc.)	13-17	Mainly small mammals. Sometimes passerine birds.	R. Lourenço et al., 2015, 2021; R. F. Lourenço, 2002; Magalhaes, 1974
Tawny owl *Strix aluco*	Woodlands with varying densities	Old trees; sometimes buildings	22	Invertebrates, small mammals, and birds.	R. Lourenço et al., 2015; R. F. Lourenço, 2002; I. M. Roque et al., 2021; Santos, 1998; Silva et al., 2012; van der Horst et al., 2019
Barn owl *Tyto alba*	Pastures, cereal fields, open woodlands	Mostly human buildings (barns, old houses)	15-17	Mainly small mammals (mice, voles, shrew). Sometimes passerine birds.	R. Lourenço et al., 2015; R. F. Lourenço, 2002; I. M. Roque et al., 2021; Santos, 1998
Little owl *Athene noctua*	Pastures, cereal fields, olive groves	Human buildings, old trees	10-11	Mainly invertebrates. Sometimes small mammals.	R. Lourenço et al., 2015, 2021; R. F. Lourenço, 2002; Santos, 1998; Tomé et al., 2008

[a] Fransson et al. 2010.

3.3. Individual factors of exposure to contaminants

Individuals were classified as juveniles when the moult pattern indicated they were in their first calendar year (year of birth), and adults when the date and moult of flight feathers was indicative of the second or more calendar years (Table S1). From the 47 individuals analysed, 16 were juveniles and 26 were adults. Five individuals were not aged due to unclear plumage pattern. We obtained representatives of both age classes in all species except for long-eared owl, for which only adults were collected. In all species, adults presented significantly higher mean concentrations ($p < 0.05$) of ∑OCPs, PFOS and ∑PCBs than juveniles (Figure 3). This is expected since these compounds tend to bioaccumulate along the life of the birds, usually leading

to higher concentrations in adult individuals. ∑PAHs concentrations were not different among age classes (Figure 3), probably because although PAHs are lyophilic and therefore have potential to be bioaccumulated, they are also easily metabolised and excreted by vertebrates (Malcolm and Shore, 2003), thus limiting their bioaccumulation and explaining the similar concentrations between adults and juveniles.

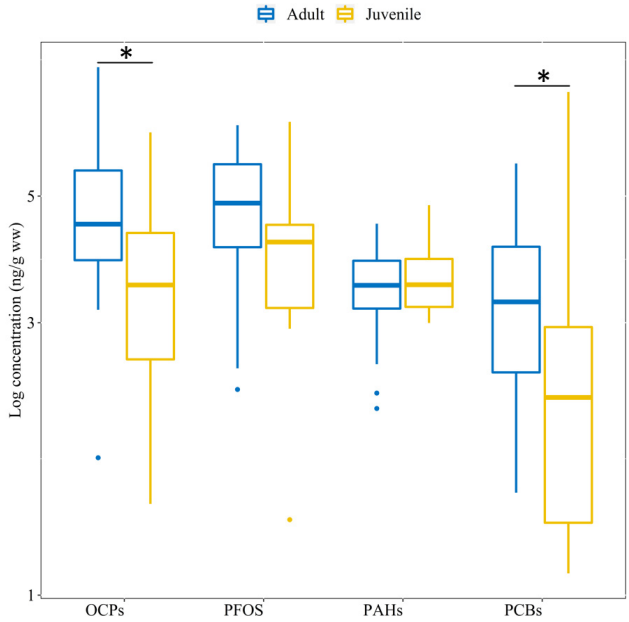

Figure 3. Total concentrations (logarithmic transformation log x+1) of each chemical group in adults (2nd calendar year or more) and juveniles (1st calendar year). * indicates significant differences between age classes; p<0.05.

The sex of the individuals is another important factor to consider when explaining contaminant exposure, as breeding females may have lower concentrations of lipophilic compounds compared to males, due to the contaminant deposition in eggs (Bustnes et al., 2017). However, sex could not be assessed as a factor of exposure to contaminants owing to the opportunistic sampling in this study. Of the 47 individuals, 19 were identified as females and 8 as males, but for a large number of individuals it was not possible to determine the sex (n = 20) due to unclear morphological differences in juvenile individuals and in the most deteriorated cadavers, with gonads severely damaged due to the collision. Individual contextual data are of interest to improve the interpretation of variations in exposure pattern between individuals. In the present study, this comparison was limited by the uneven dating and sexing of individuals between species due to opportunistic sampling based on carcass collection.

4. CONCLUSIONS

The use of road-killed birds is a useful approach to monitor the exposure of organic micropollutants without animal disturbance, which is preferred in sensitive species such as nocturnal raptors. Twenty-one contaminants were detected in livers from five nocturnal raptor species. Owls were found to be exposed to a variety of persistent compounds as OCPs, PFOS, PCBs, and PAHs, while pharmaceuticals, OPEs and, in-use pesticides were not detected. 4,4'-DDE was detected in all individuals at high concentrations (2.19 to 4475 ng/g ww), exceeding the minimum lethal concentrations in two adult eagle owls. PFOS was the only perfluorinated compound detected but was ubiquitous in all species and at concentrations up to 848 ng/g ww. ∑PCBs were detected in all species at concentrations ranging from 1.98 to 2010 ng/g ww, with an important prevalence in barn owl. ∑PAHs were also found in all individuals, being the LMW PAHs naphthalene and acenaphthene the most frequently detected PAHs. Age is an important factor to consider when assessing contaminant exposure in long-lived species such as raptors. Adults presented a higher concentration of ∑OCPs, ∑PCBs and PFOS than juveniles, but no statistical differences were found in ∑PAHs between age classes. Additionally, high levels of ∑OCPs, ∑PCBs, and PFOS were observed in woodland species feeding at higher trophic levels, particularly in the eagle owl but also in the long-eared owl. However, these species presented a significantly lower concentration of ∑PAHs than tawny owl, barn owl and little owl. The differences observed can be attributed to more regular foraging in roadsides of the latter species.

REFERENCES

Badry, A., Krone, O., Jaspers, V.L.B., Mateo, R., García-Fernández, A., Leivits, M., Shore, R.F., 2020. Towards harmonisation of chemical monitoring using avian apex predators: Identification of key species for pan-European biomonitoring. Sci. Total Environ. 731, 139198. https://doi.org/10.1016/j.scitotenv.2020.139198

Badry, A., Treu, G., Gkotsis, G., Nika, M.-C., Alygizakis, N., Thomaidis, N.S., Voigt, C.C., Krone, O., 2022. Ecological and spatial variations of legacy and emerging contaminants in white-tailed sea eagles from Germany: Implications for prioritisation and future risk management. Environ. Int. 158, 106934. https://doi.org/https://doi.org/10.1016/j.envint.2021.106934

Beyer, W.N., Meador, J.P., 2011. Environmental Contaminants in Biota: Interpreting Tissue Concentrations, 2nd ed. CRC Press, Boca Raton.

Bortolotti, G.R., Fernie, K.J., Smits, J.E., 2003. Carotenoid Concentration and Coloration of American Kestrels (*Falco sparverius*) Disrupted by Experimental Exposure to PCBs. Funct. Ecol. 17, 651–657.

Buck, A., Carrillo-Hidalgo, J., Camarero, P.R., Mateo, R., 2020. Organochlorine pesticides and polychlorinated biphenyls in common kestrel eggs from the Canary Islands: Spatiotemporal variations and effects on eggshell and reproduction. Chemosphere 261, 127722. https://doi.org/https://doi.org/10.1016/j.chemosphere.2020.127722

Bustnes, J.O., Bakken, V., Erikstad, K.E., Mehlum, F., Skaare, J.U., 2001. Patterns of incubation and nest-site attentiveness in relation to organochlorine (PCB) contamination in *Glaucous gulls*. J. Appl. Ecol. 38, 791–801. https://doi.org/https://doi.org/10.1046/j.1365-2664.2001.00633.x

Bustnes, J.O., Bårdsen, B.-J., Moe, B., Herzke, D., Hanssen, S.A., Sagerup, K., Bech, C., Nordstad, T., Chastel, O., Tartu, S., Gabrielsen, G.W., 2017. Temporal variation in circulating concentrations of organochlorine pollutants in a pelagic seabird breeding in the high Arctic. Environ. Toxicol. Chem. 36, 442–448. https://doi.org/10.1002/etc.3560

Custer, T.W., Custer, C.M., Dickerson, K., Allen, K., Melancon, M.J., Schmidt, L.J., 2001. Polycyclic aromatic hydrocarbons, aliphatic hydrocarbons, trace elements, and monooxygenase activity in birds nesting on the North Platte River, Casper, Wyoming, USA. Environ. Toxicol. Chem. 20, 624–631. https://doi.org/https://doi.org/10.1002/etc.5620200323

Derlink, M., Wernham, C., Bertoncelj, I., Kovács, A., Saurola, P., Duke, G., Movalli, P., Vrezec, A., 2018. A review of raptor and owl monitoring activity across Europe: its implications for capacity building towards pan-European monitoring. Bird Study 65, S4–S20. https://doi.org/10.1080/00063657.2018.1447546

Dulsat-Masvidal, M., Bertolero, A., Mateo, R., Lacorte, S., 2023. Legacy and emerging contaminants in flamingos' chicks' blood from the Ebro Delta Natural Park. Chemosphere 312, 18–26. https://doi.org/10.1016/j.chemosphere.2022.137205

Dziuban, C.D., Shirkey, E.C., Peeples, T.O., 1979. An Investigation of Some Distributional Characteristics of the Measure of Sampling Adequacy. Educ. Psychol. Meas. 39, 543–549. https://doi.org/10.1177/001316447903900304

Eriksson, U., Roos, A., Lind, Y., Hope, K., Ekblad, A., Kärrman, A., 2016. Comparison of PFASs contamination in the freshwater and terrestrial environments by analysis of eggs from osprey (*Pandion haliaetus*), tawny owl (*Strix aluco*), and common kestrel (*Falco tinnunculus*). Environ. Res. 149, 40–47. https://doi.org/https://doi.org/10.1016/j.envres.2016.04.038

Espín, S., García-Fernández, A.J., Herzke, D., Shore, R.F., van Hattum, B., Martínez-López, E., Coeurdassier, M., Eulaers, I., Fritsch, C., Gómez-Ramírez, P., Jaspers, V.L.B., Krone, O., Duke, G., Helander, B., Mateo, R., Movalli, P., Sonne, C., van den Brink, N.W., 2016. Tracking pan-continental trends in environmental contamination using sentinel raptors—what types of samples should we use? Ecotoxicology 25, 777–801. https://doi.org/10.1007/s10646-016-1636-8

Fransson, T., Kolehmainen, T., Kroon, C., Jansson, L., Wenninger, T., 2010. EURING list of longevity records for European birds. [WWW Document]. URL https://euring.org/data-and-codes/longevity-list

Gomes, L., Grilo, C., Silva, C., Mira, A., 2009. Identification methods and deterministic factors of owl roadkill hotspot locations in Mediterranean landscapes. Ecol. Res. 24, 355–370. https://doi.org/10.1007/s11284-008-0515-z

Gómez-Ramírez, P., Martínez-López, E., García-Fernández, A.J., Zweers, A.J., van den Brink, N.W., 2012. Organohalogen exposure in a Eurasian Eagle owl (*Bubo bubo*) population from Southeastern Spain: Temporal–spatial trends and risk assessment. Chemosphere 88, 903–911. https://doi.org/https://doi.org/10.1016/j.chemosphere.2012.03.014

Gómez-Ramírez, P., Shore, R.F., van den Brink, N.W., van Hattum, B., Bustnes, J.O., Duke, G., Fritsch, C., García-Fernández, A.J., Helander, B.O., Jaspers, V., Krone, O., Martínez-López, E., Mateo, R., Movalli, P., Sonne, C., 2014. An overview of existing raptor contaminant monitoring activities in Europe. Environ. Int. 67, 12–21. https://doi.org/10.1016/j.envint.2014.02.004

González-Rubio, S., Ballesteros-Gómez, A., Asimakopoulos, A.G., Jaspers, V.L.B., 2021. A review on contaminants of emerging concern in European raptors (2002−2020). Sci. Total Environ. 760, 143337. https://doi.org/https://doi.org/10.1016/j.scitotenv.2020.143337

González, A.S., Lage, Y., Simal, L.J., 2002. Determination of polycyclic aromatic hydrocarbons from buzzards (*Buteo buteo)* and tawny owl (*Strix aluco*) by liquid chromatography with fluorescence detection. J. AOAC Int. 85, 141–145.

Groffen, T., Bervoets, L., Eens, M., 2023. Temporal trends in PFAS concentrations in livers of a terrestrial raptor (*common buzzard; Buteo buteo*) collected in Belgium during the period 2000–2005 and in 2021. Environ. Res. 216, 114644. https://doi.org/https://doi.org/10.1016/j.envres.2022.114644

Hanmer, H.J., Robinson, R.A., 2021. Incidence of road mortality in ringed raptors and owls: a spatial analysis. British Trust for Ornithology.

Hellou, J., Lebeuf, M., Rudi, M., 2013. Review on DDT and metabolites in birds and mammals of aquatic ecosystems. Environ. Rev. 21, 53–69. https://doi.org/10.1139/er-2012-0054

Jaspers, V.L.B., Herzke, D., Eulaers, I., Gillespie, B.W., Eens, M., 2013. Perfluoroalkyl substances in soft tissues and tail feathers of Belgian barn owls (*Tyto alba*) using statistical methods for left-censored data to handle non-detects. Environ. Int. 52, 9–16.

Lourenço, R., 2006. The food habits of Eurasian eagle-owls in Southern Portugal. J. Raptor Res. 40, 297–300.

Lourenço, R., Moreira, S., Roque, I., Tomé, R., 2021. Estimating the population and distributions trends of owls in Portugal using citizen science data from Program NOCTUA-Portugal and other sources. Airo 29, 291–305.

Lourenço, R., Roque, I., Tomé, R., Sepúlveda, P., Atlas, E., Melo, C., Pereira, C., 2015. Current status and distribution of nocturnal birds (Strigiformes and Caprimulgiformes) in Portugal. Airo 23, 36–50.

Lourenço, R., Tavares, P.C., Del Mar Delgado, M., Rabaça, J.E., Penteriani, V., 2011. Superpredation increases mercury levels in a generalist top predator, the eagle owl. Ecotoxicology 20, 635–642. https://doi.org/10.1007/s10646-011-0603-7

Lourenço, R.F., 2002. The owl (order Strigiformes) assemblage in the north-eastern Algarve. Airo 12, 25–33.

Luzardo, O.P., Ruiz-Suárez, N., Henríquez-Hernández, L.A., Valerón, P.F., Camacho, M., Zumbado, M., Boada, L.D., 2014. Assessment of the exposure to organochlorine pesticides, PCBs and PAHs in six species of predatory birds of the Canary Islands, Spain. Sci. Total Environ. 472, 146–153. https://doi.org/https://doi.org/10.1016/j.scitotenv.2013.11.021

Magalhaes, C.M.P. de, 1974. Hábitos alimentares do bufo-pequeno: *Asio otus* (L.)-na Tapada de Mafra. Estud. e Divulg. Tec. Grup. A Secc. Zool. Florest. e Cineg. (Portugal).(1974). 21 p.

Malcolm, H.M., Shore, R.F., 2003. Effects of PAHs on Terrestrial and Freshwater Birds, Mammals and Amphibians, in: PAHs: An Ecotoxicological Perspective. John Wiley & Sons, Ltd, pp. 225–241. https://doi.org/https://doi.org/10.1002/0470867132.ch12

Martínez, J.A., Zuberogoitia, I., Alonso, R., 2002. Rapaces nocturnas: guıa para la identificación de la edad y el sexo en las estrigiformes ibéricas. Monticola ediciones, Madrid (in Spanish).

McCormick, S., Snawder, J.E., Chen, I.-C., Slone, J., Calafat, A.M., Wang, Y., Meng, L., Alexander-Scott, M., Breitenstein, M., Johnson, B., Meadows, J., Fairfield Estill, C., 2022. Exposure assessment of polycyclic aromatic hydrocarbons in refined coal tar sealant applications. Int. J. Hyg. Environ. Health 242, 113971. https://doi.org/https://doi.org/10.1016/j.ijheh.2022.113971

Meunier, F.D., Verheyden, C., Jouventin, P., 2000. Use of roadsides by diurnal raptors in agricultural landscapes. Biol. Conserv. 92, 291–298.

Monclús, L., Løseth, M.E., Dahlberg Persson, M.J., Eulaers, I., Kleven, O., Covaci, A., Benskin, J.P., Awad, R., Zubrod, J.P., Schulz, R., Wabakken, P., Heggøy, O., Øien, I.J., Steinsvåg, M.J., Jaspers, V.L.B., Nygård, T., 2022. Legacy and emerging organohalogenated compounds in feathers of Eurasian eagle-owls (*Bubo bubo*) in Norway: Spatiotemporal variations and associations with dietary proxies (δ13C and δ15N). Environ. Res. 204, 112372. https://doi.org/https://doi.org/10.1016/j.envres.2021.112372

Movalli, P., Krone, O., Osborn, D., Pain, D., 2018. Monitoring contaminants, emerging infectious diseases and environmental change with raptors, and links to human health. Bird Study 65, S96–S109. https://doi.org/10.1080/00063657.2018.1506735

Naso, B., Perrone, D., Ferrante, M.C., Zaccaroni, A., Lucisano, A., 2003. Persistent Organochlorine Pollutants in Liver of Birds of Different Trophic Levels from Coastal Areas of Campania, Italy. Arch. Environ. Contam. Toxicol. 45, 407–414. https://doi.org/10.1007/s00244-003-2201-z

Newsted, J.L., Jones, P.D., Coady, K., Giesy, J.P., 2005. Avian toxicity reference values for perfluorooctane sulfonate. Environ. Sci. Technol. 39, 9357–9362. https://doi.org/10.1021/es050989v

Rodríguez-Jorquera, I.A., Vitale, N., Garner, L., Perez-Venegas, D.J., Galbán-Malagón, C.J., Duque-Wilckens, N., Toor, G.S., 2017. Contamination of the Upper Class: Occurrence and Effects of Chemical Pollutants in Terrestrial Top Predators. Curr. Pollut. Reports 3, 206–219. https://doi.org/10.1007/s40726-017-0061-9

Roque, I., Lourenço, R., Marques, A., Martínez-López, E., Espín, S., Gómez-Ramirez, P., García-Fernández, A.J., Roulin, A., Rabaça, J.E., 2022. A First Record of Organochlorine Pesticides in Barn Owls (*Tyto alba*) from Portugal: Assessing Trends from Variation in Feather and Liver Concentrations. Bull. Environ. Contam. Toxicol. 109, 436–442. https://doi.org/10.1007/s00128-022-03576-6

Roque, I.M., Marques, A., Lourenço, R., Godinho, C., Pereira, P., Rabaça, J.E., 2021. TytoTagus Project: Common barn owl post-fledging dispersal and survival in the Tagus Valley, Portugal.

Saha, M., Togo, A., Mizukawa, K., Murakami, M., Takada, H., Zakaria, M.P., Chiem, N.H., Tuyen, B.C., Prudente, M., Boonyatumanond, R., Sarkar, S.K., Bhattacharya, B., Mishra, P., Tana, T.S., 2009. Sources of sedimentary PAHs in tropical Asian waters: Differentiation between pyrogenic and petrogenic sources by alkyl homolog abundance. Mar. Pollut. Bull. 58, 189–200. https://doi.org/https://doi.org/10.1016/j.marpolbul.2008.04.049

Santos, S.M., Lourenco, R., Mira, A., Beja, P., 2013. Relative effects of road risk, habitat suitability, and connectivity on wildlife roadkills: the case of tawny owls (*Strix aluco*). PLoS One 8, e79967. https://doi.org/https://doi.org/10.1371/journal.pone.0079967

Santos, S.M.L., 1998. Rapinas nocturnas (Aves, Strigiformes) do Parque Natural da Serra de São Mamede. Relatório publicado. Fac. Ciências da Univ. Lisboa, Lisboa.

Silva, C.C., Lourenço, R., Godinho, S., Gomes, E., Sabino-Marques, H., Medinas, D., Neves, V., Silva, C., Rabaça, J.E., Mira, A., 2012. Major roads have a negative impact on the Tawny Owl *Strix aluco* and the Little Owl *Athene noctua* populations. Acta Ornithol. 47, 47–54.

Smits, J.E.G., Fernie, K.J., 2013. Avian wildlife as sentinels of ecosystem health. Comp. Immunol. Microbiol. Infect. Dis. 36, 333–342. https://doi.org/10.1016/j.cimid.2012.11.007

Stockholm Convention, 2019. All POPs listed in the Stockholm Convention [WWW Document]. URL http://chm.pops.int/TheConvention (accessed 6.20.23).

Tomé, R., Catry, P., Bloise, C., Korpimaki, E., 2008. Breeding density and success, and diet composition of Little Owls Athene noctua in steppe-like habitats in Portugal. Ornis Fenn. 85, 22.

Tomza-Marciniak, A., Pilarczyk, B., Witczak, A., Rząd, I., Pilarczyk, R., 2019. PCB residues in the tissues of sea ducks wintering on the south coast of the Baltic Sea, Poland. Environ. Sci. Pollut. Res. 26, 11300–11313. https://doi.org/10.1007/s11356-019-04586-4

Turusov, V., Rakitsky, V., Tomatis, L., 2002. Dichlorodiphenyltrichloroethane (DDT): ubiquity, persistence, and risks. Environ. Health Perspect. 110, 125–128. https://doi.org/10.1289/ehp.02110125

van der Horst, S., Goytre, F., Marques, A., Santos, S., Mira, A., Lourenço, R., 2019. Road effects on species abundance and population trend: a case study on tawny owl. Eur. J. Wildl. Res. 65. https://doi.org/10.1007/s10344-019-1325-z

van Drooge, B., Mateo, R., Vives, Í., Cardiel, I., Guitart, R., 2008. Organochlorine residue levels in livers of birds of prey from Spain: Inter-species comparison in relation with diet and migratory patterns. Environ. Pollut. 153, 84–91. https://doi.org/https://doi.org/10.1016/j.envpol.2007.07.029

SUPPLEMENTARY INFORMATION

Table S1. Biometric measurments of the collected roadkilled owls. Individuals were aged following Euring codes where 3 corresponds to birds in the 1st calendar-year of (juveniles), and other codes corresponds to individuals in their second of above calendar-year (adults). X corresponds to individuals not considered for chemical analyisis as the liver was severely damaged.

Specie	Code	Wing (cm)	Tarsus (mm)	Bill (mm)	Mouth (mm)	Weight (g)	Wingspan (cm)	Body length (cm)	Ulna (mm)	Euring code	Sex
Eagle owl	BB2	44	79.4	45.2	35.7	176	138	61	186	A	M
Eagle owl	BB3	44	79.7	43.5	41.2	152	144	59	177	3	M
Eagle owl	BB4	48.5	78.6	50.3	45.4	1640	-	60	192	5	F
Eagle owl	BB5	46	86.4	45.4	42.5	164	-	53	191	7	F
Eagle owl	BB6								176	C	M
Eagle owl	BB7	43.5	88.2	-	-	1740	-	-	190	3	M
Eagle owl	X	48	87.3	49.8	-	2220	-	-	200	3	F
Eagle owl	X	45	91.3	-	-	1680	-	-	206	3	-
Long-eared owl	AO1	289	42.5	27.9	22.7	210	-	-	92.8	5	M
Long-eared owl	AO2	295	46	26.2	25.2	270	-	-	96.7	5	F
Long-eared owl	AO3	295	45.3	25.8	23.4	200	-	-	95.1	5	M
Long-eared owl	AO4	293	43.5	29	23.5	270	96	37.6	101	5	F
Long-eared owl	AO5	300	Broken	28.5	24.3	310	-	-	93	5	F
Long-eared owl	X	-	-	-	-	-	-	-	-	3	M
Tawny owl	SA1	272	54.8	33.1	28	450	85		100	3	F
Tawny owl	SA2	270	59.5	32.3	30.4	520	-		106	5	F
Tawny owl	SA3	264	47.7	29.1	32.1	390	-		97.8	3	F
Tawny owl	SA4	259	56.4	30.3	26.3	390	-		96.3	7	M
Tawny owl	SA5	262	56.3	32.2	27.2	410	-		96.2	5	-
Tawny owl	SA6	277	59.4	31.7	28.9	450	-		102	5	F
Tawny owl	SA7	265	50.7	29.4	26.6	390	92	39.5	9739	5	M
Tawny owl	SA8	268	52.4	32.6	27	460	-	39.5	102	9	-
Tawny owl	SA9	268	51.8	33	28.6	410	-	-	101	3	F
Tawny owl	SA10	276	53	32.8	28.7	440	92	40	100	3	F
Tawny owl	SA11	270	52.7	-	-	380	-	-	96	3	-
Tawny owl	SA12	274	53.4	32.8	27.2	500	95		106	6	-
Tawny owl	X	265	44.6	32.2	29	460	-	-	-	3	-
Tawny owl	X	275	54.4	32.6	27.9	420	92	37	104	3	-
Barn owl	TA1	291	66	32.2	23.7	290	-		100	5	-
Barn owl	TA2	285	64.4	31	24	250	-		101	3	M
Barn owl	TA3	293	68	34.4	23.4	430	-		103	5	F

Specie	Code	Wing (cm)	Tarsus (mm)	Bill (mm)	Mouth (mm)	Weight (g)	Wingspan (cm)	Body length (cm)	Ulna (mm)	Euring code	Sex
Barn owl	TA4	296	64.5	33.4	23.7	320	-		101	5	F
Barn owl	TA5	280	60.6	32.5	21.2	330	-		99.1	5	F
Barn owl	TA6	286	59.3	31	23.5	250	-		96.7	3	F
Barn owl	TA7	287	62.4	31.6	25	280	92	31	98	3	F
Barn owl	TA8	-	-	-	-	-	-	-	-	5	-
Barn owl	TA9	285	66.3	-	-	240	-	-	96.4	3	F
Barn owl	TA10	286	61.6	32.5	23.3	300	92		96.9	4	F
Barn owl	TA11	-	-	-	-	-	-	-	-	5	-
Barn owl	X	285	66	33.4	23.7	280	-		96	5	M
Barn owl	X	285	60.7	29.8	-	-	-		-	3	-
Barn owl	X	-	-	-	-	-	-	-	-	3	-
Barn owl	X	-	-	-	-	-	-	-	-	-	-
Barn owl	X	-	-	-	-	-	-	-	-	-	-
Little owl	AN1	152	36.5	19.4	19.6	130	52	22	56.4	3	M
Little owl	AN2	58	38.1	-	-	130	59	-	-	3	F
Little owl	AN3	-	-	-	-	-	-	-	-	-	-
Little owl	AN4	156	34.5	19.6	18.2	130	53	-	61	5	M
Little owl	AN5	159	40.1	-	-	110	-	-	62.5	-	-
Little owl	AN6	159	38.4	20.1	19.2	110	-	23	58.3	-	-
Little owl	AN7	-	-	-	-	-	-	-	-	-	-
Little owl	AN8	156	36.8	19.1	18.3	140	53.5	23	58.8	4	-
Little owl	AN9	158	37.7	18.4	18.5	140	55	24	62.1	3	-
Little owl	AN10	137	32.2	18.7	-	110	50.5	-	57.2	3	M
Little owl	AN11	157	38.8	19.2	-	-	-	-	62.1	3	-
Little owl	AN12	-	-	-	-	-	-	-	-	-	-
Little owl	X	-	-	-	-	-	-	-	-	-	-
Little owl	X	-	-	-	-	-	-	-	-	-	-
Little owl	X	-	-	-	-	-	-	-	-	-	-
Little owl	X	-	-	-	-	-	-	-	-	3	-
Little owl	X	-	-	-	-	-	-	-	-	-	-
Little owl	X	-	-	-	-	-	-	-	-	-	-

Table S2. List of analytes and standards suppliers.

Type	Compound	Supplier
PFASs	Mix native PFAC-MXB	Wellington laboratories Inc. (Guelph, ON, Canada),
PAHs	PAH solution mix (naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benz(a)anthracene, chrysen, benzo[b] fluoranthene, benzo[k]fluoranthen, benzo[a] pyrene indeno[1,2,3-cd]pyrene, dibenz[a,h] anthracene, benzo[ghi]perylene)	AccuStandard (New Haven, CT, USA)
OCPs	Hexachlorobutadiene (HCBD)	Sigma-Aldrich (Darmstadt, Germany and St. Louis, MO, USA)
OCPs	α-HCH	Dr. Ehrenstorfer (Augsburg, Germany)
OCPs	β-HCH	Dr. Ehrenstorfer
OCPs	γ-HCH	Dr. Ehrenstorfer
OCPs	δ-HCH	Dr. Ehrenstorfer
OCPs	Hexachlorobenzene (HCB)	Sigma-Aldrich (Darmstadt, Germany and St. Louis, MO, USA)
OCPs	Pesticide mix 164 (2'4-DDE, 4'4-DDE, 2'4-DDD, 2'4-DDT, 4'4-DDD, 4'4-DDT)	Dr. Ehrenstorfer
OCPs	α-Endosulfan	Dr. Ehrenstorfer
OCPs	β-Endosulfan	Dr. Ehrenstorfer
PCBs	PCB Mix 3 (PCB 28, PCB 52, PCB 101, PCB 118, PCB 138, PCB 153, PCB 180)	Dr. Ehrenstorfer
Pharmaceuticals	Metformin	Dr. Ehrenstorfer
Pharmaceuticals	Nicotine	Sigma-Aldrich (St. Louis, USA)
Pharmaceuticals	Alopurinol	Sigma-Aldrich (St. Louis, USA)
Pharmaceuticals	Atenolol	Sigma-Aldrich (St. Louis, USA)
Pharmaceuticals	Paracetamol	European Pharmacopea Reference Standard
Pharmaceuticals	Levetiracetam	Sigma-Aldrich (St. Louis, USA)
Pharmaceuticals	Gabapentin	Sigma-Aldrich (St. Louis, USA)
Pharmaceuticals	Caffeine	Sigma-Aldrich (St. Louis, USA)
Pharmaceuticals	Pentoxifylline	Sigma-Aldrich (St. Louis, USA)
Pharmaceuticals	Tramadol	Sigma-Aldrich (St. Louis, USA)
Pharmaceuticals	Sulfamethoxazole	Sigma-Aldrich (St. Louis, USA)
Pharmaceuticals	Venlafaxine	Sigma-Aldrich (St. Louis, USA)
Pharmaceuticals	Trazodone	Sigma-Aldrich (St. Louis, USA)
Pharmaceuticals	Quetiapine	Sigma-Aldrich (St. Louis, USA)
Pharmaceuticals	Losartan	Sigma-Aldrich (St. Louis, USA)
Pharmaceuticals	Furosemide	Sigma-Aldrich (St. Louis, USA)
Pharmaceuticals	Diclofenac	Sigma-Aldrich (St. Louis, USA)

Type	Compound	Supplier
Pharmaceuticals	Atorvastatin	Sigma-Aldrich (St. Louis, USA)
Pharmaceuticals	Ibuprofen	Sigma-Aldrich (St. Louis, USA)
Pharmaceuticals	Carbamazepine	Sigma-Aldrich (St. Louis, USA)
Pesticides	Dimethoate	Sigma-Aldrich (St. Louis, USA)
Pesticides	Chlorfenvinphos	Sigma-Aldrich (St. Louis, USA)
Pesticides	Isoproturon	Sigma-Aldrich (St. Louis, USA)
Pesticides	Metalaxyl	Sigma-Aldrich (St. Louis, USA)
Pesticides	Triadimenol	Dr. Ehrenstorfer
Pesticides	Tebuconazol	Sigma-Aldrich (St. Louis, USA)
Pesticides	Kresoxim-methyl	Sigma-Aldrich (St. Louis, USA)
Pesticides	Diclofop	Sigma-Aldrich (St. Louis, USA)
Pesticides	Spinosad	Dr. Ehrenstorfer
Pesticides	Pyraclostrobin	Dr. Ehrenstorfer
Pesticides	Tebufenpyrad	Dr. Ehrenstorfer
Pesticides	Prosulfocarb	Sigma-Aldrich (St. Louis, USA)
Pesticides	Pendimethalin	Sigma-Aldrich (St. Louis, USA)
Pesticides	Chlorpyrifos	Dr. Ehrenstorfer
OPEs	TCEP	Sigma-Aldrich (St. Louis, USA)
OPEs	TBP	Sigma-Aldrich (St. Louis, USA)
OPEs	TPhP	Sigma-Aldrich (St. Louis, USA)
Internal standards	Triphenyl phosphate-d_{15} (TPhP-d_{15})	Sigma-Aldrich (St. Louis, USA)
	Acetaminophen-methyl-d_3	Sigma-Aldrich (St. Louis, USA)
	Carbamazepine-d_2	Sigma-Aldrich (St. Louis, USA)
	Lidocaine-diethyl-d_{10}	Sigma-Aldrich (St. Louis, USA)
	Sulfamethoxazole-d_4	Sigma-Aldrich (St. Louis, USA)
	Isoproturon-d_6	Sigma-Aldrich (St. Louis, USA)
	Estrone-d_2	Sigma-Aldrich (St. Louis, USA)
	^{13}C-PFOA (M-PFOA)	Wellington laboratories Inc. (Guelph, ON, Canada),
	^{13}C-PFOS (M-PFOS)	Wellington laboratories Inc. (Guelph, ON, Canada),
	Deuterated PAH solution mix (naphthalene-d_8, acenaphthene-d_{10}, phenanthrene-d_{10}, chrysene-d_{12} and perylene-d_{12})	Sigma-Aldrich (St. Louis, USA)

Table S3. Quality parameters for Method A to determine OCPs, chlorpyrifos, PAHs, and PCBs, ordered by families and retention times (R.T., min), indicating response factor, linearity range (coefficient of determination (r^2) was > 0.99 for all compounds), Instrumental Detection Limits (IDL), Method Detection Limits (MDL), percentage recovery with standard deviation (%R±SD) and inter-day precision (%, n=5). Ordered by retention time and chemical family.

Family	Compound	R.T.	F	Linearity (ng/µL)	IDL (ng)	MDL (ng/g)	%R ± S.D.	Inter-day (%RSD)
OCPs	HCBD	9.28	0.19	0.001-0.8	0.012	0.23	95 ± 29.8	11.4
OCPs	α-HCH	19.3	0.10	0.001-0.2	0.008	5.52	100 ± 3.21	8.3
OCPs	HCB	19.6	0.02	0.001-0.2	0.026	0.07	67 ± 2.34	5.77
OCPs	β-HCH	20.5	0.01	0.001-0.8	0.100	0.14	100 ± 5.93	6.31
OCPs	γ-HCH (Lindane)	20.5	0.01	0.001-0.2	0.085	0.09	105 ± 17.6	0.66
OCPs	δ-HCH	22.0	0.01	0.001-0.2	0.030	0.92	91 ± 17.4	12.5
OCPs	2,4′-DDE	31.2	3.23	0.001-0.8	0.015	0.08	82 ± 8.68	17.5
OCPs	4,4′- DDE	33.1	1.95	0.001-0.2	0.014	0.14	84 ± 9.15	14.5
OCPs	2,4′-DDD	33.6	3.95	0.001-0.2	0.024	0.09	83 ± 15.8	11.2
OCPs	2,4′- DDT	35.6	8.13	0.001-0.2	0.038	2.77	86 ± 5.64	10.3
OCPs	4,4′- DDD	35.7	5.12	0.001-0.2	0.105	0.18	91 ± 5.99	17.4
OCPs	4,4′- DDT	37.8	1.91	0.001-0.2	0.058	0.24	105 ± 3.01	21.2
OCPs	α-Endosulfan	31.3	0.18	0.001-0.8	0.029	0.07	64 ± 5.6	16.6
OCPs	β-Endosulfan	34.7	0.08	0.001-0.2	0.662	2.11	56 ± 3.02	11.6
OPPs	Chlorpyrifos	27.5	1.03	0.001-0.6	0.110	2.41	76 ± 3.26	16.9
PAHs	Naphthalene	8.51	0.09	0.001-0.2	0.200	0.63	50.1 ± 36	13.65
PAHs	Acenaphthylene	14.4	0.98	0.001-0.2	0.026	0.20	54 ± 1.24	8.43
PAHs	Acenaphthene	15.1	0.33	0.001-0.2	0.059	0.25	69 ± 3.55	4.61
PAHs	Fluorene	17.1	0.67	0.001-0.2	0.087	4.93	76 ± 12.6	7.09
PAHs	Phenanthrene	21.4	0.08	0.001-0.2	0.250	1.09	76 ± 16.2	4.86
PAHs	Anthracene	21.4	0.07	0.001-0.2	0.250	1.16	77 ± 16.6	5.69
PAHs	Fluoranthene	29.3	0.21	0.001-0.2	0.044	0.20	82 ± 18.3	13.2
PAHs	Pyrene	30.8	0.22	0.001-0.8	0.049	0.28	73 ± 7.32	15.2
PAHs	Benz(a)anthracene	40.4	11.15	0.001-0.8	0.208	0.54	66 ± 2.75	6.89
PAHs	Chrysene	40.1	13.7	0.001-0.6	0.379	5.08	62 ± 7.4	6.64
PAHs	Benzo[b]fluoranthene	47.0	7.03	0.001-0.8	1.115	7.67	53 ± 10.6	10.6
PAHs	Benzo[k]fluoranthene	47.0	6.58	0.001-0.6	0.211	0.18	59 ± 14.1	11.6
PAHs	Benzo[a]pyrene	48.1	4.89	0.001-0.2	0.082	0.52	65 ± 6.8	12.4
PAHs	Indeno[1,2,3-cd] pyrene	51.9	2.50	0.001-0.2	0.368	13.0	89 ± 6.6	13.7
PAHs	Dibenz[a,h]anthracene	52.0	1.45	0.001-0.2	0.182	0.45	66 ± 4.53	18.3
PAHs	Benzo[ghi]perylene	52.6	3.13	0.001-0.2	0.082	0.40	61 ± 5.19	13.1
PCBs	PCB 28	23.9	0.09	0.001-0.2	0.069	0.18	83 ± 23.9	18.8
PCBs	PCB 52	26.0	2.01	0.001-0.8	0.003	0.11	62 ± 8.56	10.7

Family	Compound	R.T.	F	Linearity (ng/µL)	IDL (ng)	MDL (ng/g)	%R ± S.D.	Inter-day (%RSD)
PCBs	PCB 101	31.3	2.11	0.001-0.8	0.005	0.10	60 ± 8.78	17.0
PCBs	PCB 118	35.0	2.31	0.001-0.8	0.079	0.11	55 ± 7.57	10.2
PCBs	PCB 138	38.0	1.90	0.001-0.2	0.058	0.18	58 ± 4.08	21.2
PCBs	PCB 153	36.4	1.70	0.001-0.6	0.005	0.17	63 ± 12.3	8.69
PCBs	PCB 180	42.0	0.79	0.001-0.8	0.012	0.33	62 ± 11.5	9.11

Table S4. Quality parameters for Method B analysing PFASs, ordered by retention time (R.T.), indicating response factor, linearity range (coefficient of determination (r^2) was > 0.99 for all compounds), Instrumental Detection Limits (IDL), Method Detection Limits (MDL), percentage recovery with standard deviation (%R±SD) and inter-day precision (%, n=5).

Family	Compound	R.T.	F	Linearity (ng/µL)	IDL (ng)	MDL (ng/g)	% R ± S.D.	Inter-day (%RSD)
PFASs	PFBA	1.71	7.39	0.001-0.3	0.010	0.24	112 ± 2.69	3.84
PFASs	PFPA	2.36	2.96	0.001-0.3	0.050	0.24	112 ± 2.69	5.54
PFASs	PFBS	2.66	3.84	0.001-0.3	0.040	0.48	95.5 ± 3.98	6.71
PFASs	PFHxA	3.77	0.28	0.001-0.3	0.050	0.48	96.3 ± 1.9	11.6
PFASs	PFHpA	6.71	0.27	0.001-0.3	0.050	0.96	79.8 ± 1.15	7.92
PFASs	PFHxS	7.86	3.94	0.001-0.3	0.030	0.30	88.4 ± 4.8	8.08
PFASs	PFOA	9.37	0.35	0.001-0.3	0.020	0.30	95.9 ± 1.63	11.7
PFASs	PFNA	10.4	1.91	0.001-0.3	0.040	0.60	168 ± 19.1	9.93
PFASs	PFOS	10.6	1.06	0.001-0.3	0.030	0.48	102 ± 3.12	2.90
PFASs	PFDA	11.1	1.10	0.001-0.3	0.030	1.08	225 ± 8.51	9.35
PFASs	PFUnA	11.6	1.20	0.001-0.3	0.050	16.86	138 ± 15.01	10.8
PFASs	PFDS	11.7	0.95	0.001-0.3	0.020	0.36	21.6 ± 9.68	3.81
PFASs	PFDoA	12.0	1.06	0.001-0.3	0.030	0.78	83.8 ± 7.87	10.2
PFASs	PFTriDA	12.5	2.35	0.001-0.3	0.110	1.02	44.4 ± 13.5	12.9

Table 5. Quality parameters for Method C determining pharmaceuticals, pesticides and OPEs, ordered by chemical family and retention time (R.T.), indicating response factor, linearity range (coefficient of determination (r^2) was > 0.99 for all compounds), Instrumental Detection Limits (IDL), Method Detection Limits (MDL), percentage recovery with standard deviation (%R±SD) and inter-day precision (%, n=5).

Family	Compound	R.T.	F	Linearity (ng/µL)	IDL (ng)	MDL (ng/g)	%R ± S.D.	Inter-day (%RSD)
Pharmaceuticals	Atenolol	0.99	0.04	0.015-0.2	0.540	1.5	43 ± 21.2	55.3
Pharmaceuticals	Paracetamol	2.85	0.26	0.001-0.3	0.257	1.44	142 ± 13.9	47.7
Pharmaceuticals	Caffeine	5.81	0.43	0.005-0.2	0.342	2.52	150 ± 18.3	18.2
Pharmaceuticals	Pentoxifylline	8.31	1.85	0.001-0.3	0.012	0.24	141 ± 9.8	6.9
Pharmaceuticals	Tramadol	8.53	0.72	0.005-0.3	0.520	0.84	149 ± 9.3	16.7
Pharmaceuticals	Sulfamethoxazole	8.64	0.46	0.001-0.3	0.318	0.78	123 ± 11.6	27.3
Pharmaceuticals	Venlafaxine	10.1	2.56	0.005-0.3	0.079	0.54	261 ± 19.5	32.6
Pharmaceuticals	Trazodone	10.4	2.80	0.001-0.3	0.067	0.12	111 ± 8.27	17.8
Pharmaceuticals	Quetiapine	10.6	1.28	0.001-0.3	0.121	0.18	124 ± 13.5	10.6
Pharmaceuticals	Furosemide	11.5	0.09	0.01-0.2	1.367	1.14	34 ± 3.66	14.1
Pharmaceuticals	Carbamazepine	11.6	8.30	0.001-0.2	0.416	0.06	178 ± 15.9	14.1
Pharmaceuticals	Losartan	12.6	3.63	0.001-0.2	0.061	0.3	83 ± 8.81	22.4
Pharmaceuticals	Diclofenac	16.8	0.04	0.001-0.2	0.524	0.24	40 ± 6.36	15.1
Pharmaceuticals	Atorvastatin	16.8	1.32	0.005-0.3	0.483	0.42	77 ± 8.61	33.5
Pharmaceuticals	Ibuprofen	17.2	0.06	0.025-0.3	4.211	1.44	142 ± 13.9	16.1
Pesticides	Dimethoate	8.49	0.06	0.005-0.3	0.060	0.6	82 ± 2.3	15.9
Pesticides	Chlorfenvinphos	12.4	0.23	0.001-0.3	0.134	0.72	20 ± 1.73	29.4
Pesticides	Isoproturon	13.1	0.44	0.001-0.2	0.104	0.42	109 ± 1.13	33.3
Pesticides	Metalaxyl	13.2	0.05	0.001-0.3	0.199	0.84	132 ± 2.97	12.1
Pesticides	Triadimenol	15.1	0.01	0.005-0.2	3.376	4.62	131 ± 1.98	17.7
Pesticides	Tebuconazol	16.8	0.30	0.01-0.2	0.126	0.3	58 ± 3.12	9.2
Pesticides	Kresoxim-methyl	18.2	0.03	0.015-0.3	0.874	0.78	51 ± 2.64	15.2
Pesticides	Spinosad	18.7	0.07	0.001-0.3	0.227	0.78	71 ± 1.51	11.6
Pesticides	Pyraclostrobin	19.0	0.04	0.001-0.3	1.761	0.36	15 ± 2.63	8.2
Pesticides	Tebufenpyrad	20.4	0.03	0.001-0.3	4.649	0.6	82 ± 2.3	12.7
Pesticides	Prosulfocarb	20.5	0.03	0.001-0.3	2.725	0.9	27 ± 1.66	11.6
OPEs	TCEP	12.3	0.01	0.005-0.2	1.832	1.02	102 ± 2.38	10.5
OPEs	TBP	18.7	0.23	0.001-0.3	0.593	1.14	55 ± 1.78	6.5
OPEs	TPhP	18.7	0.10	0.001-0.3	2.350	1.14	54 ± 1.68	9.2

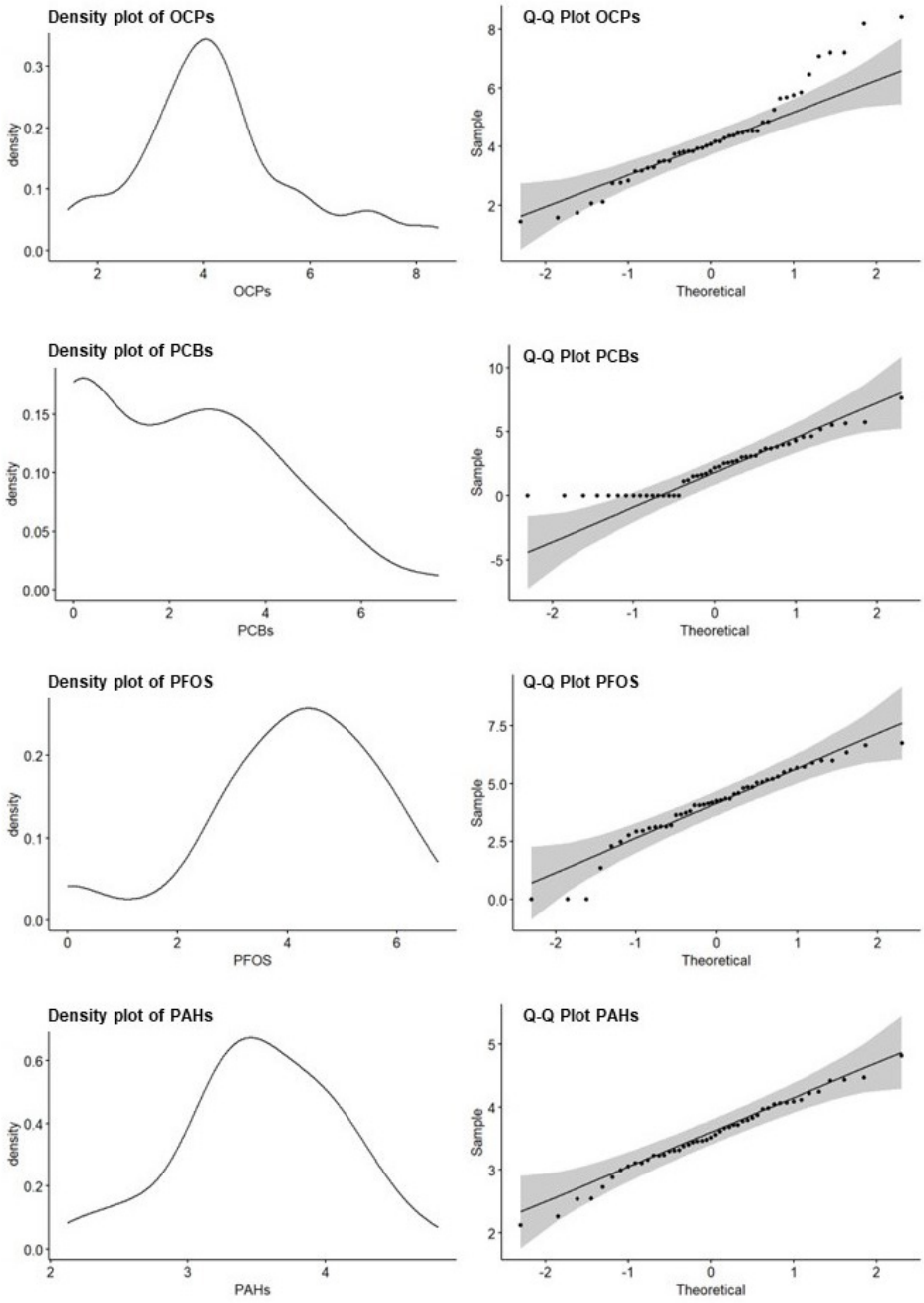

Figure S1. Normality plots for the sum of the log x+1 concentrations of the chemical families.

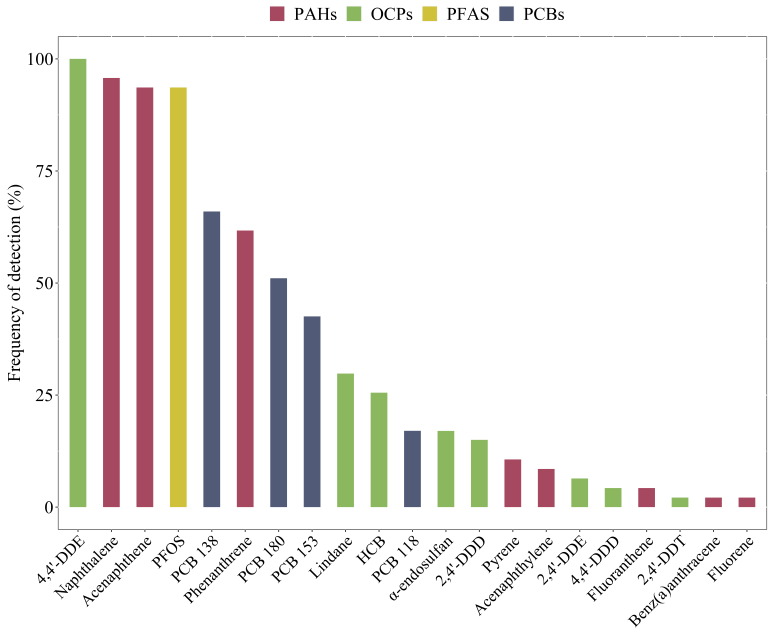

Figure S2. Frequency of detection (%) of the 21 compounds detected in the 47 analysed owl livers.

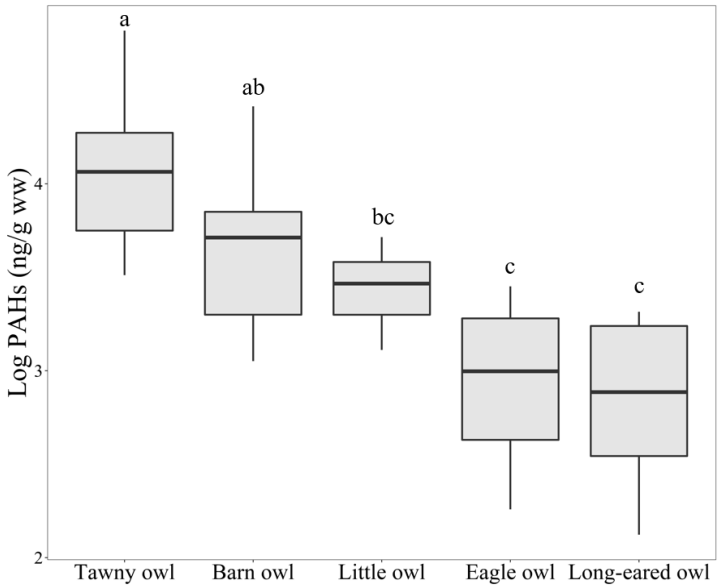

Figure S3. ∑PAHs concentrations in analysed road-killed owl species. Different letters indicate statistically significant differences (P≤0.05).

4. GENERAL DISCUSSION

The main purpose of this Thesis was to assess the impact of contaminants on biodiversity, focusing on areas relevant to birds' conservation and using birds themselves as sentinel species. The discussion section follows the order of the Thesis structure. The first part englobes the monitoring studies based on the analysis of contaminants in water, soil, and sediments in IBAs, related to objectives 1, 2 and 3. In the second part, the different biomonitoring approaches used to work with birds and the main findings are discussed, regarding objectives 4 and 5. Finally, an overview of the utility of birds as sentinel species to address chemical pollution is provided.

4.1. Part I: Contaminants in Important Bird and Biodiversity Areas

This Thesis quantified the threat of chemical contamination in IBAs, which are identified as key sites for biodiversity conservation. The reported data provided a first picture of the presence and distribution of organic micropollutants of environmental relevance in water, soil, and sediment. The following section encompasses the discussion of the IBA monitoring programme (chapters I to IV), with specific attention to the design of the sampling campaigns, the methodology approaches used, and the main findings reported in each studied compartment.

For water, a total of 59 organic micropollutants from 5 different chemical families were analysed, which allowed the characterization of the main water pollution threats in IBAs water bodies. Chemical families studied included high-volume consumption pharmaceuticals or with low degradability (Gómez-Canela et al., 2019), pesticides extensively used according to the Ministry of Agriculture (https://www.mapa.gob.es), OPEs and benzophenone, selected for their high-volume production and wide distribution in surface waters (Cristale et al., 2013), as well as PFASs due to their high mobility and persistence in the aquatic environment.

For soils and sediments, 52 compounds were studied including PAHs, OCPs, PCBs, plasticizers, and current-use pesticides. The selection was made to include both legacy contaminants listed in the Stockholm Convention and emerging contaminants related to plastic waste and current-use pesticides.

4.1.2. Sampling strategy

140 IBAs were selected as a representative portion of the total 469 IBAs defined in Spain. The selected areas were distributed throughout the country, encompassing the main habitats: aquatic inland, agricultural, Atlantic forest, riparian forest, Mediterranean forest, coastal areas, and rocky mountain areas. In the selection of the study areas, it was also considered the presence

of water, and the balance between sampling feasibility (possibility of reaching the sampling site) and logistics to send the samples to IDAEA-CSIC in Barcelona for chemical analysis.

The study areas presented a great variability among them, for example regarding their location, altitude, habitat, protection status, and degree of anthropogenic pressures. The heterogeneity of the studied IBAs can be observed in Figure 4.1. The locations included pristine areas with non-evident presence of contamination (Figure 4.1.A), and also sites with evident anthropogenic pressures, such as illegal dumping (Figure 4.1.B), and agricultural areas with regular application of pesticides (Figure 4.1.C), which can be easily identified as a potential source of contamination.

Figure 4.1. Examples of heterogeneity of IBAs. A- Lack of evident anthropogenic pressures in IBA 148 Ebro Delta (Catalunya); B- illegal landfill in IBA 015 Sierras de Gistreo y Coto (Leon); C- Application of pesticides in IBA 095-Gallocanta (Teruel, Zaragoza, Guadalajara).

A uniform sampling methodology was defined to ensure comparability among IBAs samples. To achieve this, the same sampling criteria was applied in all territories, which consisted in the collection of 3 samples in each IBAs to include a contamination gradient. The first sampling point was selected in the most impacted location within the IBA boundaries, considering the anthropogenic pressures that could be potential sources of contamination, such as WWTP discharges, agricultural areas, sites near urban areas, or locations with a high affluence of tourism. The second point was established at approximately 500 m downstream from point 1 to obtain a sample with an intermediate pressure, and the third sampling point was collected upstream from point 1, in a pristine location without evident anthropogenic impact and without the influence of point 1. This third sampling point tried to get water samples in river or stream sources to be used as negative controls, but this was not always the case. Figure 4.2 shows an overview of the sampling strategy used.

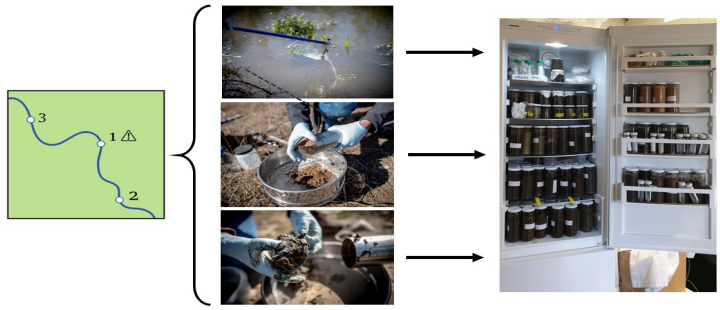

Figure 4.2. Collection of water, soil and sediment samples following the gradient of contamination, and further storage in the laboratory at 4ºC before analysis.

The IBAs' sampling was performed only once, providing a one-shot picture of the contamination status in IBAs. It is important to consider that the input of contaminants can change among seasons, especially in water as it is a constant changing matrix. For instance, the presence of pesticides in waterbodies is influenced by the seasonality of their application in crops (Chow et al., 2020). This means that to have a more complete picture of the pollution status in waters from IBAs, the samplings should be performed by collecting samples at least twice a year (winter/summer) or preferably each season. This Thesis has set the protocols and procedures to implement long-term contaminant monitoring in IBAs. Also, sampling in different years allows the evaluation of temporal trends and assess the efficiency of mitigation actions. While grab water is the most commonly used sampling technique, due to its easy and economic handling (Sousa et al., 2018), other methodologies based on passive samplers could improve the representativeness of the study, as these devices provide time-integrated concentrations on various contaminants in water. However, their use for the broad contaminant characterization in waterbodies is rarely performed as many passive samplers are not sufficiently validated for large field application (Taylor et al., 2020).

4.1.3. Analytical methodology

Considering the large scope of the IBAs monitoring program and the sampling effort that allowed the collection of valuable samples, the extraction and analysis methods were optimized to include compounds that were relevant in each matrix. The methodology aimed to develop a single generic extraction to retain multiple contaminants of different chemical families efficiently, while minimizing the economic and time costs. This approach is important for conducting chemical analyses with fewer materials and solvents, ultimately reducing the environmental impact of laboratory analysis (López-Lorente et al., 2022). To ensure the quality of our results, we established a coordinated

effort between sample collection and the laboratory analysis, so that once samples were sent by courier to the Institute of Environmental Assessment and Water Research (IDAEA), they could be stored in proper conditions. Extraction of water was done within 1 to 3 days after collection to avoid the degradation of the most labile compounds such as pharmaceuticals, lifestyle compounds and polar pesticides (Fedorova et al., 2014; Llorca et al., 2014). Waters were extracted without a previous filtration step to provide the total concentration of contaminants considering the dissolved and particulate phase. Only in the case that there was gross material in suspension (leaves, daphnia, or flocks of suspended matter), the water was decanted prior to extraction. The extraction of water was performed using a SPE extraction using Oasis HLB cartridges with methanol and water as conditioning solvents and methanol and acetone as eluting solvents. Samples were analysed by three different LC/MS-MS methods: one to analyse pharmaceuticals and lifestyle compounds, another to analyse pesticides and OPEs and a third one to analyse PFASs.

In the case of soil and sediment analysis, the methodology involved a longer pre-treatment, consisting of drying or freeze drying the samples followed by sieving them through 500 µm and 125 µm meshes. This pre-treatment had the dual purpose of removing moisture of the matrix and obtaining a homogenous fraction of the sample, which is necessary for an efficient extraction of the target compounds and for comparability among study sites. This was a tedious and time-consuming step that limited the capacity to analyse all three sampling points from each IBAs. This is why in chapters III and IV, the analysis of contaminants in soils and sediments was based only on sampling point number 1, which is representative of the most impacted area inside the IBA. However, it must be noted that contaminants in soils and sediments are associated either with diffuse sources of pollution, or past release of POPs to the environment, which do not always match the current anthropogenic pressures. Wang et al. (2023) assessed the different patterns of distribution of OCPs and current-use pesticides and reported that the presence of legacy compounds in soils was influenced by their application in the past but also by their distribution, re-emissions, long-transport ranges, and retention by TOC. Therefore, the gradient of contamination based on actual anthropogenic pressures may not be relevant to characterize past pollution episodes.

In soils and sediment also a generic extraction method was used to analyse 52 contaminants in a single extraction, followed by a clean-up step and the determination of the target compounds was achieved by GC/MS-MS using a multiresidue method.

Overall, 411 water samples were analysed for 59 organic micropollutants, and 140 soil and 140 sediment samples were analysed for 52 other

contaminants. This has provided a total of 38,809 data values about the presence of environmental contaminants in IBAs. This data served to determine contamination patterns in each IBA, map pollution hotspots and identify the most concerning compounds.

4.1.4. Assessing the occurrence, sources, and distribution of contaminants in IBAs

Organic micropollutants were detected in all studied IBAs. The distribution of contaminants was not homogeneous among sampling areas, and different concentrations were found in water, soils, and sediment samples. In addition to providing data on the occurrence of organic contaminants in IBAs, another main objective of this Thesis was to identify potential sources and patterns of contamination among IBAs. This knowledge is essential for the proper management of natural areas and the implementation of conservation actions against chemical pollution. To assess potential sources of contamination, field data and spatial information derived by GIS were integrated and processed to identify the main drivers of pollution in each IBA.

Spatial information was collected considering a buffer area around the sampling points to ensure uniformity among the data collected in all IBAs, regardless of their size. This approach also allowed us to work at an optimal scale to identify relevant sources of contamination associated with the detected contaminants. The size of the buffer area varied between the water monitoring (chapter II) and the soil study (chapter III), driven by the specific target compounds analysed in each matrix. For water analysis, an optimum buffer area of 1 km around the sampling points was selected through a preliminary multiscaling approach. Buffer areas smaller than 1 km were not representative of the anthropogenic pressures affecting the sampled area. Contrarywise, buffer areas higher than 1 km were considered too large, given that water is a changing medium, and the chemical pollution is more likely to be associated to close contaminant inputs. In the case of soils (chapter III), where the target compounds were related with historical and diffuse sources of pollution, a larger buffer area of 10 km was considered. This decision was supported by other contaminant deposition studies that used similar scales (Hu et al., 2021; Rossini et al., 2005).

One of the most important spatial characterizations is land-use data, which classifies the use of the territory. Land-use change has been identified as one of the major drivers of biodiversity loss as it has a direct impact on the species through the modification or destruction of their habitat (Powers and Jetz, 2019), and have strong links to other important drivers as climate change and pollution (Oliver and Morecroft, 2014). A clear example of the impact of land-use on chemical pollution is the relationship between agricultural landscapes

and the occurrence of pesticides which has been widely recognized (Pascual Aguilar et al., 2017; Szöcs et al., 2017). In fact the analysis of agricultural landscape using GIS and remote sensing methods can be used to estimate the pesticide exposure (VoPham et al., 2015). In this Thesis, the land-use information was obtained from Corine Land Cover inventory in Europe from the Copernicus database (Copernicus, 2023). Corine Land Cover provides a vectorial layer with qualitative information of land cover categories (e.g., agricultural area, artificial surface, forest and semi-natural areas, wetlands, and waterbodies). This categorical information was transformed in quantitative data by calculating the percentage of surface occupied by each category in the buffer area around the sampling points. The selection of the spatial data was different when analysing water and soils due to the multiple anthropogenic activities.

To assess the sources of water pollution, a very important feature to consider is the presence of WWTPs discharges as are known to be related with high concentrations of organic micropollutants such as pharmaceuticals, personal care products and pesticides into surface waters (Adeleye et al., 2022; Lopez-Herguedas et al., 2022). Also, the proximity to other urban elements (buildings, roads) is associated to urban runoff, which ultimately discharges into surface waters (Müller et al., 2020). The relation between the contaminants detected in surface waters and the spatial information around the sampling points were analysed by censored regression model (Tobbit analysis). A main finding in this Thesis is the identification of agricultural areas, WWTPs discharges, and artificial surface as sources of organic micropollutants in surface waters from IBAs. The agricultural surface was found to be related with significant higher concentrations of all chemical families analysed in water as pesticides but also pharmaceuticals, lifestyle compounds, OPEs, and PFASs. This finding is expected as agricultural surface was the most common anthropogenic pressure found in IBAs. Agricultural practices involve not only the application of pesticides, but also are related to the release of other organic micropollutants. For instance, the use of reclaimed water and WWTP sewage sludge in agricultural fields is a common practice in Spain which has raised concern in the recent years due to the release of organic micropollutants such as pharmaceuticals to agricultural soils (Mejías et al., 2021). The organic compounds present in soils can leach to ground waters. McEachran et al., (2016) reported that pharmaceuticals present in wastewater applied in forestal soils resulted in concentrations up to 10 ng/L in surface waters. The presence of pharmaceuticals, lifestyle compounds and PFASs in the studied surface waters were also related to the presence of WWTPs discharges close to the sampling points. This is consistent with the existent knowledge of the WWTPs being sources of urban compounds into surface waters (Adeleye et al., 2022). The presence of artificial surface was found to be a significant factor associated with high concentrations of

lifestyle compounds and PFASs, which is consistent with the association of these compounds in urban environments (Ahmadireskety et al., 2022; Roder Green et al., 2014).

Another important factor to consider when discussing the status of contamination in IBAs is the degree of protection. Limiting or forbidding some activities, such as resource exploitation in natural areas, can have a positive impact on reducing the chemical pollution. The analysis of the contamination status between protected and non-protected areas can be a very important tool to assess the effectiveness of environmental protection policies to mitigate pollution. IBAs protection status was assessed as a variable regarding the water bodies contamination in chapter II. The designation of IBAs as Natura 2000 areas was not found to be a relevant characteristic to minimize contamination. This can be explained as the water contamination in protected areas can be influenced by the inputs of water from non-protected areas, as has been observed in pesticide monitoring in protected areas from Germany (Wolfram et al., 2023). Further research should be taken in this direction especially considering that the goal of protecting the 30% of the EU territory by 2030 could not be effective to restore biodiversity if it is not accompanied by mitigation measures against chemical pollution.

The spatial information used in the soil monitoring study (chapter III), apart from land-use information, included also potential sources of the target compounds such as number of industrial sites, incineration plants, density of roads, and other characteristics potentially affecting soils such as the burned areas in the previous 5 years. The source assessment in soil samples was more difficult to interpret as this was hampered by analysing only one sample for each IBA, and the presence of contamination was mostly related to diffuse sources. In the study, the organic carbon of soil could not be analysed, although it is an important property to consider as may influence on the retention of some compounds in soil such as PAHs (Nam et al., 2008) or PCBs (Di Guardo et al., 2020). In order to consider this variable, data of organic carbon content in IBAs were obtained from the Topsoil Soil Organic Carbon (LUCAS) database (de Brogniez et al., 2015). Although the use of this data must be taken with precaution, as they are estimations and not real measurements, it is an example of the powerful application of remote sensing data and GIS as complementary tools for a better comprehension of environmental monitoring data. The expanded database with the contaminant data and spatial data variables was processed by performing a Hierarchical Clustering on Principal Components (HCPC) analysis, which was used to obtain specific clusters according to contamination patterns within the studied IBAs. Two main groups were identified: one including IBAs affected by high concentrations of PAHs and another one affected by a higher occurrence of PCBs compared to other areas. The occurrence of PCBs was found in IBAs with a higher artificial surface related to the proximity to large

cities. This is in line with previous studies that linked high PCB contents in top soils to past industrial development in major cities and diffuse pollution (Cachada et al., 2009).

For sediment samples (chapter IV) the sources of contamination were not assessed through spatial analysis due to the unclear factors affecting sediment quality. However, GIS data was used to represent the contamination distribution patterns in maps, enabling identifying the most polluted areas. This has resulted in a useful tool to communicate the IBAs contamination status and identify potential impacted areas.

4.1.5. Identifying the most concerning compounds

Often information on the concentrations of contaminants in a specific area is meaningless unless information on the potential risks is evaluated. This is especially important for non-legislated contaminants, which do not have an established EQS according to the legislation.

The most common approach to determine the chemicals' risk in the environment is to calculate RQs, obtained as the ratios of the measured environmental concentrations and the PNEC values of each compound. Therefore, the environmental risk assessment is depending on the availability of PNEC values to compare the detected concentrations with. For water risk assessment, the PNEC values were obtained from the Norman Database (NORMAN, 2022), which provides a list of the lowest PNEC calculations available for freshwaters. There are a higher number of toxicological studies providing data to calculate PNEC based on freshwaters organism than in sediment or soils organism. The understanding of chemical stressors on soil organisms is still very limited. This is because the studies based on soil toxicity are mostly performed in limited number of animals and for a few chemical groups, mostly metals (Beaumelle et al., 2021). For this reason, freshwater PNEC are often used to extrapolate PNECs in sediments and soils. The used soil PNECs, despite being an approximation used by ECHA (European Commission, 2003), have a higher level of uncertainty compared to toxicological data available for water. Toxicological testing based on soil organisms is needed to enhance the comprehension of the impact of soils contamination on biodiversity.

The common criteria for interpreting RQ values in risk assessment studies is by establishing different levels of risk: low (0.01 to 0.1), medium (0.1 to 1), and high-risk (RQs>1). If the RQs exceed 1, it is indicative that the compounds are detected at concentrations higher than those established as safe for the environment, and therefore, adverse effects are expected. The characterization of the risk based only with RQs present some limitations as low RQs can result from excessively high PNEC values due to the lack

of experimental studies. To prevent the underestimation of the chemical risk, other characteristics must also be taken into account, such as the detection frequency, or high concentrations of the target compounds in the environment, and have been discussed in chapters II, III, and IV.

Taken together, the variables of detection frequency, total concentrations, and RQs for the target compounds in each matrix are represented in Principal Component Analyses (PCAs) for water (Figure 4.3), soils (Figure 4.4), and sediment (Figure 4.5). The PCAs and the subsequent discussion, aim to highlight the compounds identified as of higher concern in IBAs considering each matrix.

In the analysis of water in chapter II, the most common chemicals detected were pharmaceuticals, lifestyle compounds and OPEs which presented a frequency of detection higher than pesticides and PFASs, that were detected below 25% of the samples. The PCA shown in Figure 4.3 groups compounds detected in water that are of concern due to either their high-risk concentrations (RQs >1) in IBAs, or because of their high occurrence and concentrations. The compounds that are highlighted for their high-risk concentrations for aquatic organisms are distributed in the bottom left quadrant (Figure 4.3). The compound that was found in a higher number of samples at high-risk concentrations was the organophosphate pesticide chlorpyrifos, which surpassed the PNEC value of 0.46 ng/L in 35 IBAs. Chlorpyrifos has been detected at high-risk concentrations in previous monitoring studies in freshwaters form Spain, as in the Tagus river (Arenas-Sánchez et al., 2019) and in the Ebro delta (Ccanccapa et al., 2016). The use of chlorpyrifos was banned in EU in 2020 due to its high toxicity (European Commission, 2020). It must be considered that most of the samples were collected when this pesticide was still in use. Therefore, the detection of high-risk concentrations is expected to be solved thanks to the new regulation. Our results support the necessity of its restriction to minimize the impact of pesticides in water. Following, the antidepressant venlafaxine was also detected as a concerning compound as surpassed the PNEC value of 38 ng/L for freshwaters in 18 IBAs. Our results are in accordance with recent monitoring studies, where venlafaxine has been identified as priority compound to address in freshwaters from Sweden (Figuière et al., 2022), and also in the water of Antarctica (Postigo et al., 2023), reinforcing the need to monitor its occurrence in the aquatic ecosystems. In 2022, the most recent European Watch List (European Commission, 2022) included venlafaxine as a compound to be monitored in surface waters, prior to its potential future inclusion in the European Water Framework Directive. Another concerning compound for its toxicity in freshwaters from IBAs was PFOS, as it was detected in 17 IBAs above the PNEC value of 0.65 ng/L. The European Water Framework (Directive 2013/39/EU) also lists PFOS, with a Maximum Allowable Concentration - Environmental Quality Standard (MAC-EQS) of 36000 ng/L. This threshold was not surpassed in any sample. The

toxicity of PFOS to aquatic organism has been described for microorganisms, plankton, and invertebrates, also it is of great concern for freshwater due to their accumulation through the food web (Ma et al., 2022). Another concerning compound was the NSAID diclofenac detected in 11 IBAs above the PNEC of 50 ng/L.

The compounds detected at very high detection frequency and high concentrations are grouped in the upper left quadrant (Figure 4.3). Caffeine was the compound detected at the highest number of samples (73% detection frequency, at concentrations from 0.9 to 41,083 ng/L), related to artificial surface and WWTPs discharges, which support its use as a marker of urban activity (Buerge et al., 2003; Spence, 2015). The PNEC value of caffeine is 1200 ng/L. It has been described as a neurotoxic agent for freshwater organisms (Aguirre-Martínez et al., 2018; Aguirre-Martínez et al., 2016), and produces oxidative stress (Cruz et al., 2016), and reproduction and development alterations (Li et al., 2012). Another ubiquitous compound in surface waters was the anti-hypertensive valsartan, detected in 43% of the IBAs at concentrations ranging from 2 to 22473 ng/L. Valsartan is one of the most consumed pharmaceuticals (Gómez-Canela et al., 2019) and presents low metabolization in the body after consumption (Siddiqui et al., 2011). These two key factors together explain its high occurrence in surface waters. Valsartan has been identified as one of the most predominant pharmaceuticals in surface waters from Spain and Italy (Bade et al., 2015), and Denmark (Nanusha et al., 2022). Valsartan becomes then an abundant compound in IBAs waters due to its occurrence and concentrations which are indicative of its constant release to surface waters, however it was not found at high-risk levels in any of the studied IBAs, considering a PNEC value of 560000 ng/L. Valsartan and caffeine are persistent and mobile compounds and can leach to groundwaters (McEachran et al., 2016). Other pharmaceuticals were also found at high detection frequencies in freshwaters, such as carbamazepine (38% detection frequency, ranging from 1 to 698 ng/L), tramadol (32% detection frequency, ranging from 3 to 1714 ng/L), and sulfamethoxazole (27% detection frequency, ranging from 1.2 to 3045 ng/L). Benzophenone was found in 27% of the IBAs at concentrations ranging from 0.5 to 10266 ng/L. The presence of benzophenone is associated to its use in UV-filters, which have been found widespread in rivers worldwide (Ramos et al., 2015). The concentrations of benzophenone, despite being high, did not exceed the PNEC values in any samples. However, due to the detected high concentrations, its potential risk in surface waters should be further assessed.

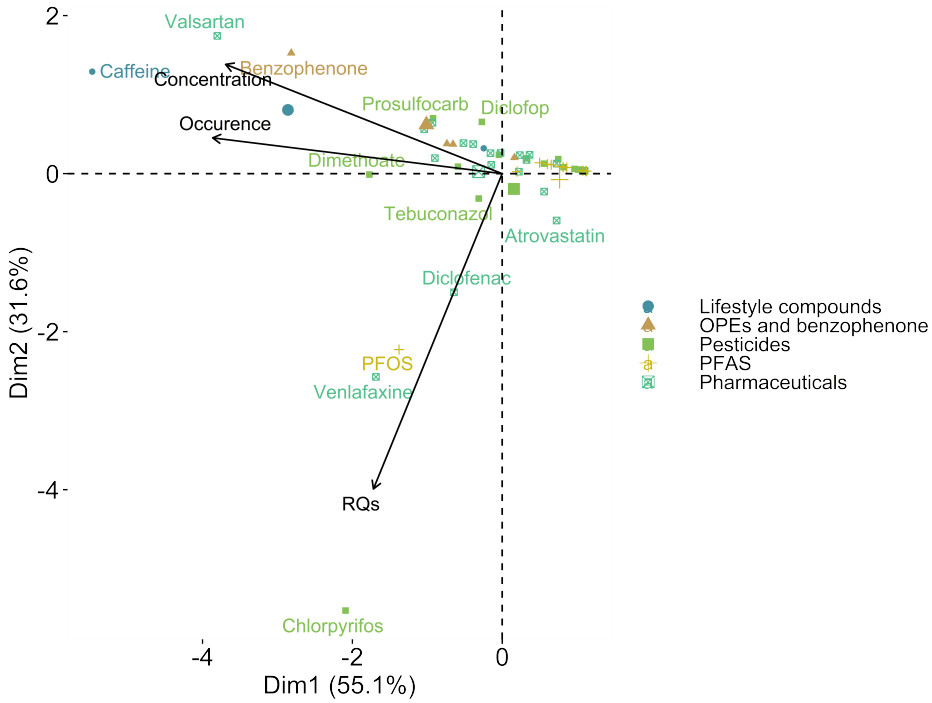

Figure 4.3. PCA for the concerning compounds identified in water samples from IBAs.

In the study of soils in chapter III, the most frequently detected chemical groups were OCPs and PAHs detected in 87% and 69% of the samples, respectively. PCBs, OPPs, OPEs and plasticizers were detected below 50% of the samples. Plasticizers were the compounds detected at the highest concentrations (5.50 to 7026 ng/g). The PCA shown in Figure 4.4, groups the compounds in soils that are of concern for its high occurrence and high-risk concentrations, or due to their high concentrations. The compounds that were more frequently detected and found at high-risk concentrations are distributed in the low right quadrant (Figure 4.4). The OCP 4,4'-DDE was the most ubiquitous, detected in 76% of the soils samples, evidencing its long persistence in the environment even after its total prohibition in Spain since 1994 (BOE, 1994). The concentrations of 4,4'-DDE surpassed the estimated PNEC value for soils of 3.56 ng/g in 21 IBAs, and this indicates that soils from natural areas are still impacted by the historical use of DDT. The prevalence of 4,4'-DDE has been also reported in soils from Portugal, where it was associated with the historical use of DDTs in vineyards and the study highlighted the role of soils as sources of 4,4'-DDE to aquatic ecosystems (Patinha et al., 2018). PAHs were also found predominant in soils as were detected in 69% of the samples and were the chemical group with a higher number of high-risk concentrations (Figure 4.4). Our results align with established knowledge that

soils act as sinks for OCPs and PAHs, as was evidenced by their high detection frequency in this compartment.

The compounds grouped in the upper right quadrant correspond to those found at high concentration levels (Figure 4.4). This group includes nonylphenol detected at concentrations from 1247 to 3488 ng/g, and phthalates DEP ranging from 534 to 5475 ng/g, DiBP from 176 to 2921 ng/g and DEHP from 1223 to 4073 ng/g. Despite the high concentrations found, the PNEC values were exceeded in a low number of samples (from 0 to 16, depending on the compound). Due to the lack of experimental toxicological data for these compounds in soils organisms, the risk of NP and phthalates in soils could be underestimated.

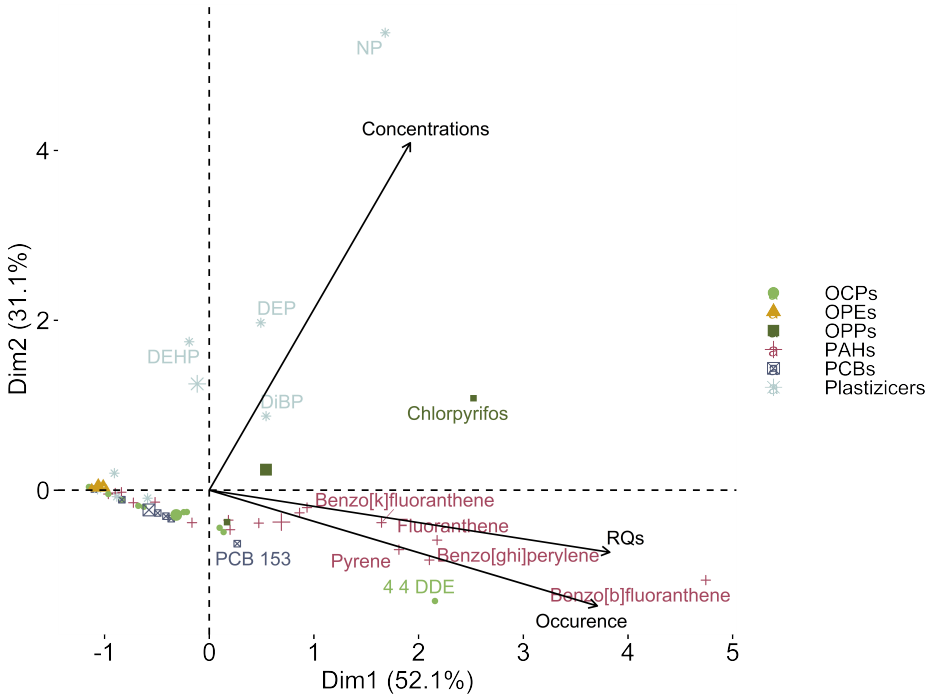

Figure 4.4. PCA for the concerning compounds identified in soils samples from IBAs.

In the analysis of sediment in chapter IV, a similar pattern of contamination as soils was observed. The PCA (Figure 4.5), separated those compounds found at high detection frequencies and at high-risk concentrations, from those found only at high concentrations. PAHs, were the most predominant compounds, detected in 87% of the IBAs. In addition, the HMW-PAHs benzo[b] fluoranthene and benzo[k]fluoranthene were at high-risk concentrations in 50 and 51% of the analysed samples, respectively. In sediments, 4,4'-DDE was detected in 61% of the sediment samples, from which in 26 IBAs surpassed the PNEC value of 1.31 ng/g, extracted from the Norman Database (NORMAN,

2022). The bottom left quadrant in Figure 4.5 grouped plasticizers which were detected at high concentrations, especially NP (from 75.5 to 20708 ng/g) and DEP (from 584 to 5584 ng/g).

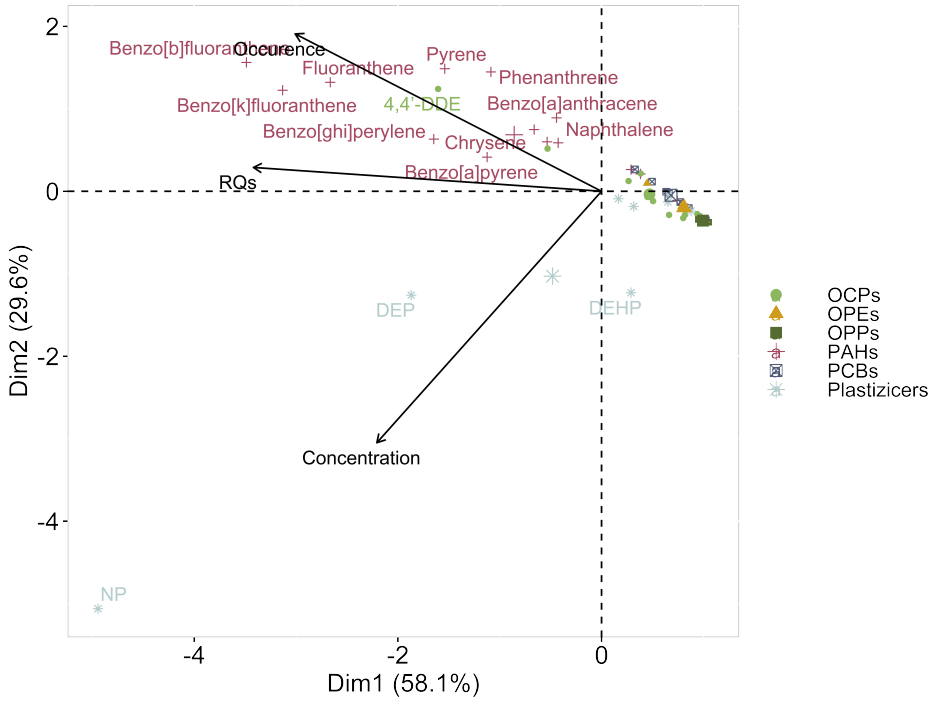

Figure 4.5. PCA for the concerning compounds identified in sediment samples from IBAs.

The Environmental Risk Assessment performed in IBAs was based on individual compounds. However, this Thesis has evidenced that IBAs are affected by chemical mixtures occurring in the natural environment through a great variety of sources. When contaminants occur in complex mixtures their modes of action can be additive, meaning that compounds that interact with the same biological receptors or metabolic pathways in organisms produce effects by summing their concentrations, this is known as "concentration addition" effect (Backhaus and Faust, 2012). As a result, even if a chemical is found below their toxicity threshold, it contributes to the whole toxicity of the chemical mixture (Kortenkamp and Faust, 2018). In chapter II, the chemical mixtures toxicity was assessed by summing all calculated risk quotients and identifying those areas with a higher sum of risk. Innovative strategies for dealing with chemical mixture effects in environmental regulations are needed for a better protection of human and ecosystem health (Escher et al., 2020).

4.1.5. IBAs impacted by chemical pollution

Different contamination status was observed across IBAs. The total concentrations for each matrix give an overview of the degree of contamination levels detected in each area, classified from very low to very high concentrations. The maps below highlight the most impacted areas considering water (Figure 4.6), soil (Figure 4.7) and sediments (Figure 4.8). In the following section, the most vulnerable IBAs that can suffer a high impact of contamination according to the results from chapters II, III, IV are detailed.

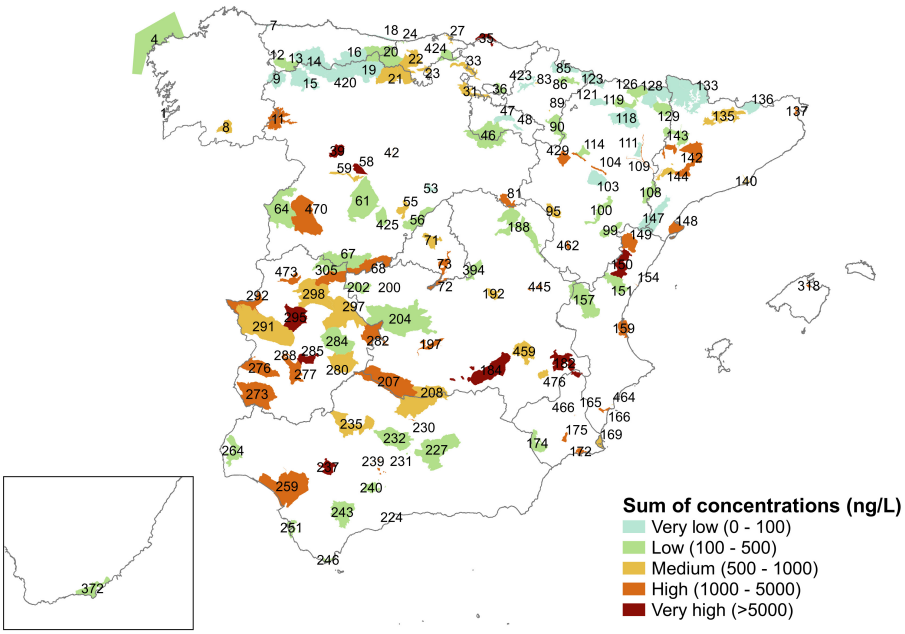

Figure 4.6. ∑Concentrations (ng/L) of water contaminants, classified in very low (0-100 ng/L), low (100-500 ng/L), medium (500-1000 ng/L), high (1000-5000 ng/L), and very high (>5000 ng/L) in the 140 studied IBAs. Numbers indicates IBAs code. Bottom left quadrant shows Canary Islands.

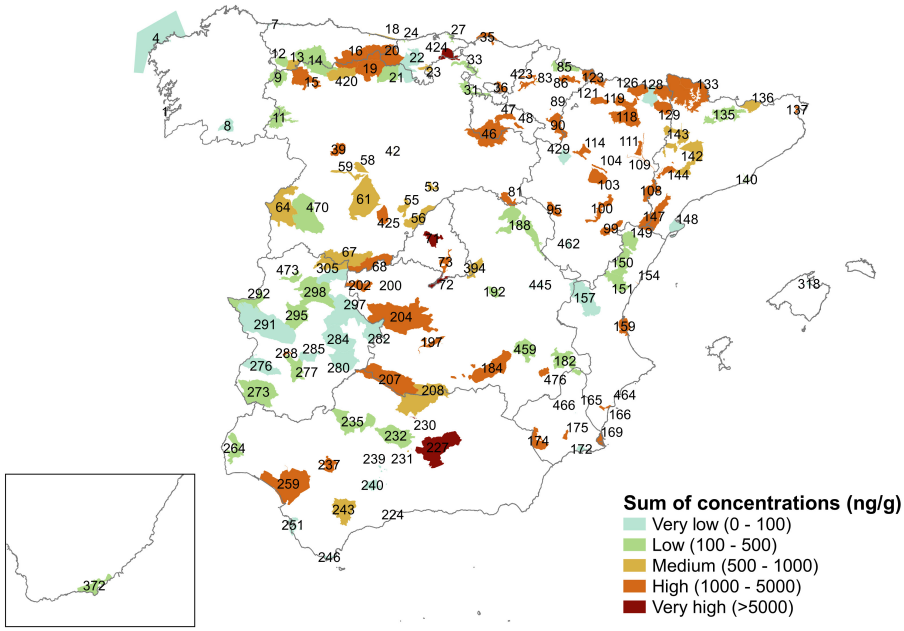

Figure 4.7. ∑Concentrations (ng/g) of soils contaminants classified in very low (0-100 ng/g), low (100-500 ng/g), medium (500-1000 ng/g), high (1000-5000 ng/g), and very high (>5000 ng/g) in the 140 studied IBAs. Numbers indicates IBAs code. Bottom left quadrant shows Canary Islands.

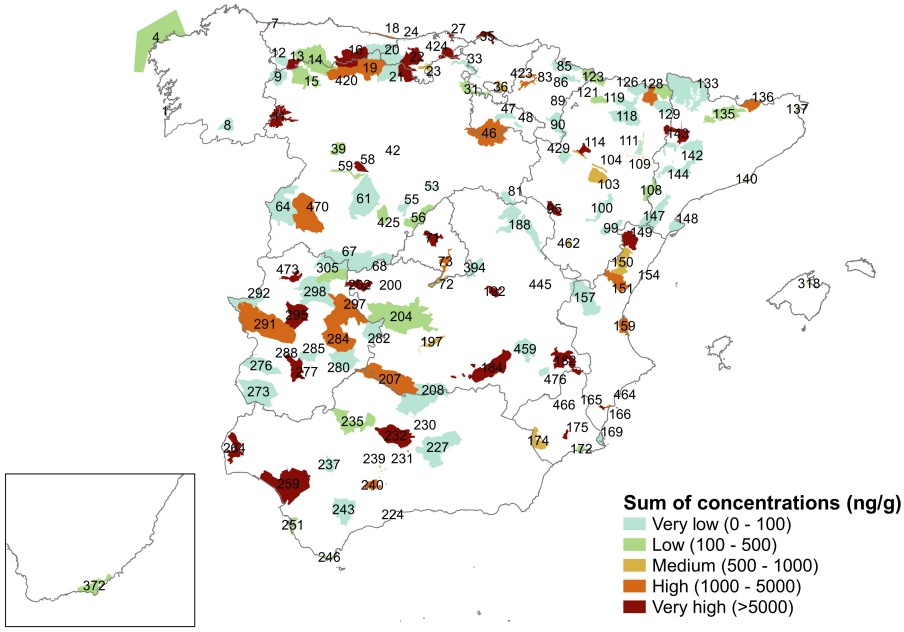

Figure 4.8. ∑Concentrations (ng/g) of sediment contaminants, classified in very low (0-100 ng/g), low (100-500 ng/g), medium (500-1000 ng/g), high (1000-5000 ng/g), and very high (>5000 ng/g) in the 140 studied IBAs. Numbers indicates IBAs code. Bottom left quadrant shows Canary Islands.

From the 140 IBAs included in the monitoring programme, 7 are classified as "IBAs in Danger" by BirdLife International, which are sites identified to present unfavourable conditions to maintain their ecological value (BirdLife International, 2022a). The level of contamination in these areas varied depending on the analysed compartments (Figure 4.6 to 4.8). Our results not only support their classification as "IBAs in Danger" but also stress out the importance to consider chemical pollution as a driver of their unfavourable conservation status, and the urgent need of a proper management of chemical pollution in these highly valuable natural areas. Following, the chemical pollution from the 7 "IBAs in Danger" is described:

- IBA 259 - Guadalquivir marshes (Huelva, Cadiz, Sevilla) is one of the largest wetlands of Europe of international significance for birds' conservation, especially for breeding, passage and wintering of waterbirds and passerines. Part of its surface is protected by Doñana National park. However, it is a very impacted area due to agricultural intensification, industrial pollution, tourism, and urban development (BirdLife International, 2023a). The levels of contamination of this site were high in water (Figure 4.6), where the pesticides chlorpyrifos and dimethoate were found at high-risk concentrations. The levels found in soils were also classified as high (Figure 4.7), with high-risk concentrations of the PAH pyrene, the pesticide chlorpyrifos and the plasticizer DiBP. In sediments, the concentrations were classified as very-high (Figure 4.8), with high-risk levels for the HMW-PAHs benzo[b] fluoranthene, benzo[k]fluoranthene, and benzo[ghi]perylene, and the plasticizers nonylphenol, DEP, and BPA.
- IBA 159 - Albufera de Valencia marshes (Valencia) is a coastal area with freshwater lagoon, used for waterbirds for breeding and wintering. It is a very impacted area associated to agricultural, urban, tourism, and industrial activities (BirdLife International, 2023b). The levels of contamination have been found to be high in all compartments analysed (Figure 4.6, 4.7, 4.8). In water, high-risk concentrations were found for PFOS, and the pesticides chlorpyrifos and tebuconazole. In soils high-risk concentrations were detected for PAHs (fluoranthene, pyrene, benzo[a] anthracene, benzo[b]fluoranthene, benzo[a]pyrene, indeno[1,2,3-cd] pyrene, and benzo[ghi]perylene), the pesticide chlorpyrifos, and the OCPs 4,4'-DDE, 4,4'-DDD and 2,4'-DDT. In sediment samples the risk was attributed to PAHs (fluoranthene, pyrene, chrysene, benzo[b] fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, indeno[1,2,3-cd] pyrene and benzo[ghi]perylene) and 4,4'-DDE.
- IBA 95 - Gallocanta lake (Teruel, Zaragoza, Guadalajara) is characterized by a brackish lake surrounded by cereals fields. It is an area of great importance in Europe for the passage for cranes (*Grus grus*) in their

migration, and for wintering of wildfowl and steppic birds (BirdLife International, 2023c). Very high total concentrations were found in water (Figure 4.6) and sediments (Figure 4.8). High-risk concentrations were only detected in sediment samples for PAHs (fluoranthene, benzo[b]fluoranthene, benzo[k]fluoranthene and benzo[a]pyrene), nonylphenol and DEP.

- IBA 295 - Plain between Cáceres and Trujillo-Aldea del Cano (Cáceres) is a Mediterranean area formed by dry-grasslands, garrigue and arable cultivations. It is an important site for steppic birds, as the Spanish imperial eagle (*Aquila adalberti*), and for the passage of black stork (*Ciconia nigra*) and cranes. It is an area impacted by agricultural intensification and urbanization (BirdLife International, 2023d). The level of contamination was very high in water (Figure 4.6) with high-risk concentrations for the pesticide chlorpyrifos and the pharmaceutical venlafaxine. In soils, the level of contamination was low (Figure 4.7), but with high-risk concentrations for PAHs (fluoranthene, pyrene, benzo[a] anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, indeno[1,2,3-cd]pyrene, and benzo[ghi]perylene), the plasticizer BPA, and PCBs 138 and 180. The level of contamination in sediments were classified as very high (Figure 4.8), with risk concentrations for PAHs (fluoranthene, benzo[b]fluoranthene, and benzo[k]fluoranthene), and the plasticizers nonylphenol, BPA and DEP.
- IBA 169 - Mar Menor coastal lagoon (Murcia) is an important site for the breeding and wintering of ducks, shorebirds, and seabirds. It is a very impacted area by urban development and tourism (BirdLife International, 2023e). The levels of contamination in water were classified as medium (Figure 4.6) and only high-risk concentrations were found for the pesticide chlorpyrifos. Soils also presented a medium contamination (Figure 4.7) with high-risk concentrations for the pesticide malathion. The contamination in sediments was classified as low (Figure 4.8) but with high-risk concentration for the OCP 4,4'-DDE.
- IBA 318 - Albufera de Mallorca and Albufereta de Pollença marshes (Illes Balears) is an area of wetlands and a large coastal lagoon connected to the sea. It is a very important site for the breeding, passage and wintering of waterbirds, raptors, and passerines. The main threat in the area is the urban development (BirdLife International, 2023f). The contamination of water was very high (Figure 4.6) with high-risk concentrations for the pesticides tebuconazole and chlorpyrifos. The level of contamination in soils was classified as very low (Figure 4.7), but with high-risk concentrations for PAHs (fluoranthene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene). In sediments the levels of

contaminations were low (Figure 4.8) but with high-risk concentrations the PAHs (fluoranthene, chrysene, benzo[b]fluoranthene, benzo[k] fluoranthene, benzo[a]pyrene, and benzo[ghi]perylene), and for the OCP 4,4'-DDE .

- IBA 148 - Ebro delta (Tarragona) is formed by shallow brackish lagoons salt marshes and lakes, dunes, and agricultural rice fields. It is a site of international importance for breeding, passage, and wintering of waterbirds. It is a very impacted area by urbanization, tourism, and chemicals used in agricultural fields (BirdLife International, 2022b). The levels of water pollution were high (Figure 4.6) with high-risk concentrations for the pesticide tebuconazole. In soils the contamination levels were very low (Figure 4.7), with high- risk concentrations only found for the PAHs benzo[b]fluoranthene and benzo[ghi]perylene. In sediments the contamination was also classified as very low (Figure 4.8), but with high-risk concentrations for the PAHs benzo[b]fluoranthene and benzo[k]fluoranthene.

It is also worth mentioning other IBAs that despite not being classified as "IBAs in Danger" by BirdLife International, may be considered as vulnerable to suffer habitat degradation due to also the significant chemical pollution detected. These are the following:

- IBA 35 – Urdaibai – Matxitxako (Vizcaya) is a coastal area with a deep estuary and brackish marshes. It is an important area for wintering shorebirds and eurasian spoonbill (*Platalea leucorodia*). The main threats affecting the area are tourism and human disturbance (BirdLife International, 2023g). The concentration levels found were very high in water (Figure 4.6), and high-risk compounds were caffeine, the pharmaceutical atrovastatin, the pesticides chlorpyrifos and dimethoate, and PFOS. The levels detected in soils were high (Figure 4.7), with high-risk concentrations for PAHs (fluoranthene, pyrene, benzo[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k] fluoranthene, benzo[a]pyrene, indeno[1,2,3-cd]pyrene, and benzo[ghi] perylene), and ∑HCHs. The concentrations in sediments were classified as very high (Figure 4.8), and high-risk levels were detected for PAHs (naphthalene, fluoranthene, pyrene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, indeno[1,2,3-cd]pyrene, and benzo[ghi]perylene), nonylphenol, and the plasticizers, DEP, DiBP, and BPA.
- IBA 184 – Campo de Montiel (Ciudad Real, Albacete) it is an important area for steppic birds and affected by agricultural intensification (BirdLife International, 2023h). The concentrations in water were classified as very high (Figure 4.6), and high-risk concentrations were found for caffeine, pharmaceuticals including venlafaxine, atorvastatin

and diclofenac, and the pesticides chlorpyrifos and dimethoate. In soils the concentrations found were high (Figure 4.7) but high-risk concentrations were only detected for the plasticizer DEHP. In sediments the concentrations were very high (Figure 4.8) and of risk for PAHs (naphthalene, fluoranthene, pyrene, benzo[a]anthracene, benzo[b] fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, indeno[1,2,3-cd] pyrene, and benzo[ghi]perylene), and plasticizers such as nonylphenol, DEP, BPA, and DEHP, and the OCPs 4,4'-DDE and ∑HCHs.

- IBA 58- Tordesillas-Mota del Marqués (Valladolid) is an agricultural area of importance for the breeding of steppic birds and raptors. It is occupied by plains of cereal production and also urban settlements (BirdLife International, 2023i). The concentrations found were very high in water (Figure 4.6), with high-risk concentrations for caffeine, the pharmaceuticals diclofenac and ibuprofen, and the pesticide chlorpyrifos. For soils samples the concentrations were medium (Figure 4.7), with high-risk levels only for the PAH benzo[b]fluoranthene and the pesticide chlorpyrifos. In sediments the levels were classified as very high (Figure 4.8), and high-risk concentrations were found for PAHs (fluoranthene, pyrene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, indeno[1,2,3-cd]pyrene and benzo[ghi]perylene), the pesticide chlorpyrifos, the OCPs 4,4'-DDE, 2,'4-DDE, 4,4'-DDD and ∑HCHs, and the plasticizers nonylphenol,.
- IBA 71 -El Pardo-Viñuelas (Madrid) it is a Mediterranean area very close to Madrid city, identified to be important for the breeding of a significant number of species. It is a threatened area by urban development and construction of roads (BirdLife International, 2023j). The level of concentrations was medium in water (Figure 4.6), with high-risk concentrations only found for PFOS. In soils, the levels were classified as very high (Figure 4.7), with high-risk concentrations for the PAHs fluoranthene and pyrene, the pesticide malathion, and plasticizers such as DBP, DiBP, DEP and BPA. For sediment samples the levels were very high (Figure 4.8), with high-risk concentrations identified for PAHs (naphthalene, fluoranthene, benzo[b]fluoranthene, benzo[k] fluoranthene, and benzo[a]pyrene), the OCPs 4,4'-DDE and 4,4'-DDD, and nonylphenol and DEP.

The IBAs described above are only a fraction of the many other natural areas that would benefit from urgent actions against chemical pollution. The management of chemical contamination should be addressed in all IBAs in order to prevent their further degradation and ensure the long term high ecological values.

4.2. Part II: Biomonitoring of contaminants in birds

The second part of the discussion focuses on the biomonitoring studies with birds. It includes the main considerations to use birds as sentinel species to stablish biomonitoring schemes (chapter V) regarding the objective 4. The chapters VI and VII addressed objective 5 involving the assessment of the exposure to organic contaminants of several chemical families in a waterbird species (flamingos) and in various nocturnal raptors (eagle owl, long eared owl, tawny owl, barn owl, and little owl).

4.2.1. Collection of bird's samples

Birds are known to be excellent sentinel species to monitor environmental contaminants. A prove of that is the great number of studies providing data about the exposure to contaminants in birds worldwide, that allows the comparison of compounds and concentrations among species and areas. Among birds, raptors are excellent bioindicators of chemical pollution due to their top position in the food web and their long lifespan, also they are well distributed around the globe. For these reasons, they have been pointed out to be the best candidates to stablish pan-European schemes to monitor contaminants. A prove of that is the great number of on-going programmes providing data related to their ecology and also to their contaminant exposure (Gómez-Ramírez et al., 2014; Vrezec et al., 2014). Biomonitoring using raptors has been the topic in European projects such as Eurapmon (www.eurapmon.net) and ERBFacility (www.erbfacility.eu).

As has been reviewed in chapter V, working with raptors, similar to other protected species, can be also challenging and some constraints in the sampling procedure must be addressed so that these valuable samples are suitable for chemical analysis. A total of 31 constraints related to the collection of raptor samples were identified in order to stablish European biomonitoring programmes based on raptors. These constraints included legal aspects, methodological limitations, difficulties related to the skills of field workers, and spatial coverage constraints related to species distribution or the lack of monitoring programmes to obtain samples. Chapter V addresses the identified constraints by providing feasible solutions, demonstrating the possibility of implementing long-term biomonitoring schemes based on raptors. For example, storage capacity and the skills to practice necropsies were identify as important limitations to obtain internal tissue samples for contaminant monitoring. These constraints are feasible to solve by providing best practice guidance on how to perform necropsies for contaminant monitoring, as developed in Espín et al. 2021. These constraints and others identified in chapter V were experienced in first person when conducting the biomonitoring studies with flamingos (chapter VI) and nocturnal raptors

(chapter VII). The advantages and difficulties of different bird sampling procedures and the use of different tissues to determine the exposure to a wide number of contaminants are detailed in the following sections.

4.2.2. Methodology used to assess contaminants in birds

The bird samples were analysed to determine all chemical families monitored in water, soils, and sediment, and included OCPs, PCBs, PAHs, PFASs, pharmaceuticals, polar pesticides and OPEs. Biomonitoring of such a large number of chemical families served as a first screening to determine the accumulation potential of both legacy and emerging contaminants to flamingos and five species of nocturnal raptors and goes beyond most studies performing biomonitoring studies where only a single or few chemical families are studied. To achieve this purpose, several extraction protocols had to be optimized to effectively recover all target contaminants in blood and liver. An important limitation was found for the analysis of blood from flamingos' chicks as a little amount of sample was available (<1 mL), and therefore the extraction protocol had to be miniaturized and refined to allow the analysis of contaminants from different chemical families. This limitation was not found when working with liver samples.

Both matrices were lyophilized prior to the extraction. The extraction of the target compounds in blood and liver samples was achieved following three different protocols: one to analyse OCPs, PCBs and PAHs based on the protocol used to extract soils and sediments and adapted from a previous protocol by Velázquez-Gómez et al. (2018), a second one to analyse PFASs based on a previous method (Colomer-Vidal et al. 2022), and the third extraction and analytical method protocol was developed in the frame of this Thesis for the analysis of pharmaceuticals, polar pesticides and OPEs. The three used analytical methods allow the identification of 91 target compounds in blood samples and 81 in liver samples. The fact that less compounds were analysed in liver is due to the low recoveries of some compounds in this matrix as detailed below. Figure 4.9 show an overview of the methodology used in biological matrices.

The development of the method for the analysis of pharmaceuticals, polar pesticides and OPEs was achieved first by optimizing the extraction and clean-up conditions. The solid-liquid extraction method was tested with different solvents: i) 1.5 mL of acetonitrile, and ii) 1.5 mL with acidified acetonitrile with 0.1% formic acid. Following, 30 mg Oasis HLB prime cartridges (1 cm^3 cartridge tube, Waters, USA) were used to purify the extract. The elution step was tested by i) a simple pass through without conditioning following the manufacturers indications; ii) a elution with acetonitrile and water (80:20), adapted from Gross et al., (2020) and iii) a elution with 100% acetonitrile.

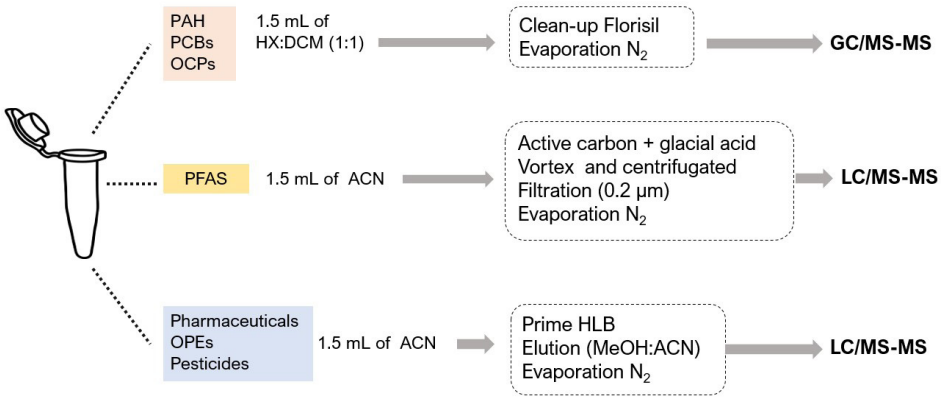

Figure 4.9. Overview of the methods used to analyse organic micropollutants in blood and liver from birds.

The final optimized method consisted of a solid liquid extraction with 1.5 mL of acetonitrile, followed by a clean-up with 30 g Oasis HLB prime cartridges using 1.5 mL of acetonitrile - water (80:20) as elution solvent.

Then to unify the 2 LC-MS/MS methods to determine 19 pharmaceuticals, 2 lifestyle compounds, 17 pesticides and 3 OPEs in water to a single more effective LC-MS/MS method, conditions were optimized using the mobile phases: (A) with 0.1% formic acid in water and (B) 0.1% formic acid in acetonitrile. This combination was selected as the compounds were properly ionized. Following, the gradient of elution was optimized to avoid the coelutions of the analytes. The developed method achieved recoveries between 70 and 130% for most of the compounds in blood samples and liver samples, with standard deviation (SD) lower than 20%. The instrumental limits of detection (IDL) ranged from 0.01 to 4.65 ng. The method limits of detection were between 0.01 and 15.3 ng/mL in blood, and 0.06 to 4.62 ng/g in liver. Overall, the optimized method for pharmaceuticals, pesticides and OPEs was used for the analysis of 37 target compounds in blood and 30 in liver samples. The number of target compounds in liver samples is slightly lower than in blood, due to the low recoveries for nicotine, metformin, alopurinol, levetiracetam, quetiapine, diclofop and pendimethalin. The same situation was found for the quantification of the three high-molecular PFASs (PFTeDA, PFDOA, PFHxDA), which were finally not included in the analysis of liver samples.

It has to be mentioned that plasticizers were excluded from the biomonitoring studies as there was a strong blank contribution at concentrations much higher than the spiking level used to calculate the extraction efficiency, and obviously, they could not be analysed. This problem has been described for phthalates, nonylphenol and benzophenone and

indicates that these compounds should be analysed in separate specific methods than minimized the external contamination (Guo and Kannan, 2012). Overall, the methods described in this Thesis allow the multiresidue analysis of both legacy and emerging contaminants in blood and liver samples. These methods present good quality parameters, allowing their use in bird biomonitoring studies.

4.2.3. Biomonitoring of contaminants in flamingos

In chapter VI, the exposure to organic micropollutants in flamingos' chicks from the Ebro delta natural park was assessed for the first time by performing an active monitoring using blood. The sampling was possible thanks to the collaboration with the staff from the natural park who annually organizes a ringing activity to gather information about the state of the breeding colony. Despite the Ebro delta breeding colony has been monitored since 2004, in 2019 it was the first time that blood of flamingos was extracted to assess their exposure to contaminants. In this study, legal and skills constraints were avoided as professional ringers and veterinarians were available to handle birds and collect the samples, and all permits were guaranteed when collaborating with a well-established monitoring activity organised by the natural park. This activity involves recording biometric measurements and ringing the chicks for their tracking. This is not an easy task, and it needs the collaboration of 200 volunteers including professional ringers and veterinarians. Flamingo's chicks are ringed when they are only two-months old and are still unable to fly. At this moment, they gather in what is known as the "nursery", under the care of a few adults. During the day the progenitors feed in surrounding fields, and by the end of the day they return to the nursery to feed their offspring. Considering these habits, the sampling was organized to capture around 400-500 chicks out of the roughly 2000 chicks present in the breeding colony in 2019. The sampling took place in 4th of August of 2019 starting at 4 am in the morning. At night to avoid being seen by the birds, a group of 200 volunteers surrounded the nursery containing the chick's colony (and some adults). At dawn, when flamingos spotted the humans, they instinctively moved away in the opposite direction, inadvertently directing a portion of the group into an enclosure. Once the chicks were inside the enclosure, they were easily and safely captured to be ringed and measured. During the process, 50 flamingos were set aside to extract blood samples from the tarsal veins by veterinarians. Samples were kept in eppendorfs tubes and recorded with the ring code of each individual. Images of the whole sampling process are shown in Figure 4.10.

Figure 4.10. Images from the sampling process and collection of blood of flamingo's chicks in the Ebro delta.

Flamingos' chicks were found to be exposed to environmental contaminants from a very young age. As all individuals were from the same age and location, a very similar pattern of contamination was observed among individuals, where PFASs were the most ubiquitous compounds detected in all individuals, followed by OCPs (92%), PAHs (90%), and PCBs (48%). Pharmaceuticals, in-used pesticides and OPEs were not detected in any sample. A clear main finding was the unexpectedly high concentrations of ∑PFASs ranging from 9.34 ng/mL to 576 ng/mL. The PFASs detected at the highest concentrations was PFOA ranging from 3.04 to 366 ng/mL, which are considered very high levels comparing to the reported concentrations in other bird species, as discussed in chapter VI. After the study performed in 2019, it was not possible to obtain samples in 2020 and 2021 due to the pandemic situation, which hampered the temporal trend analysis of the contaminant exposure in this Thesis. In 2022, the ringing activity was performed again allowing the analysis of blood for PFASs. Although the results are not reported in this Thesis, flamingos from 2022 presented similar levels than the ones reported in 2019 proving that the breeding colony is chronically exposed to PFASs. PFOA is a component of some pesticides formulations and firefighting foams (Glüge et al., 2020). The application of these products near the colony or in feeding

areas is a hypothesis that could explain the concentrations found. To explore the potential sources of PFASs, water, soil and sediment samples from the breeding colony and from flamingos feeding areas were collected in 2022 to identify the sources of exposure to PFOA and take actions to minimize this exposure. However, the results of the 2022 campaign are not yet available.

Another question that remains to be solved is the implication of the exposure of these contaminants to the health of flamingo's chicks. Sebastiano et al. (2023) reported the association between the exposure of PFASs and alterations in thyroid hormones, length of telomers, and body condition of gull's chicks. The concentrations related to these alterations are similar, and lower for PFOA, than the ones found in flamingos' chicks. The body indexes of individuals were compared to evaluate the relationships between exposure to contaminants and development, but no associations were found. It is worth noting that all individuals in the study exhibited a similar exposure to contaminants, including PFOA, PFOS, and PFHxS that were detected in all individuals. As a result, comparing body indexes among individuals may have been limited by this uniform exposure pattern. To address this limitation and explore potential differences in their development, future studies comparing the exposure of chicks from different years would enable to assess temporal changes in the exposure of contaminants and seek potential differences in their development.

Valuable contextual data such as biometric measurements and the ring information allows the possibility to track future movements of these birds, which is of interest for long-term monitoring studies. Flamingos wear large plastic rings that are easily readable from long distances, making it more feasible to gather data about their movements compared to other species with less legible rings. As a curiosity, the movement of a flamingo ringed in 2019 in the Ebro delta has been tracked over the past four years (Figure 4.11). This information may be of interest for example to assess sources of contamination, or changes in their movements related to habitat loss or health condition. Recently, Scridel et al. (2023) studied the role of body condition and sex in the migration of flamingo's juveniles. It would be of great interest to add in these studies the effect (or not) of the total exposure of contaminants in the movements of flamingos. The monitoring network of flamingos in the Mediterranean region include the ringing of this species since 1977 in several regions until now, including: Spain, France, Italy, Turkey, Algeria, and in other parts outside the Mediterranean region as Germany and The Netherlands (Flamingo atlas, 2023). Despite the ongoing monitoring programmes, this Thesis provided the first record of the organic contaminant's exposure in flamingos' chicks. Taking advantage of existing monitoring programmes is an opportunity to obtain samples for biomonitoring and understand the threat of chemical pollution in waterbirds from threatened Mediterranean wetlands. Moreover, joint studies including ringing for population control,

analysis of contaminants and obtention of contextual data allows extraction interdisciplinary information that allows a better understanding on the potential sources and impacts of chemical pollutants in birds, while at the same time minimizing animal disturbance. Such information would help in decision-making regarding the management of the Ebro delta natural area, which has been identified as an IBA in danger.

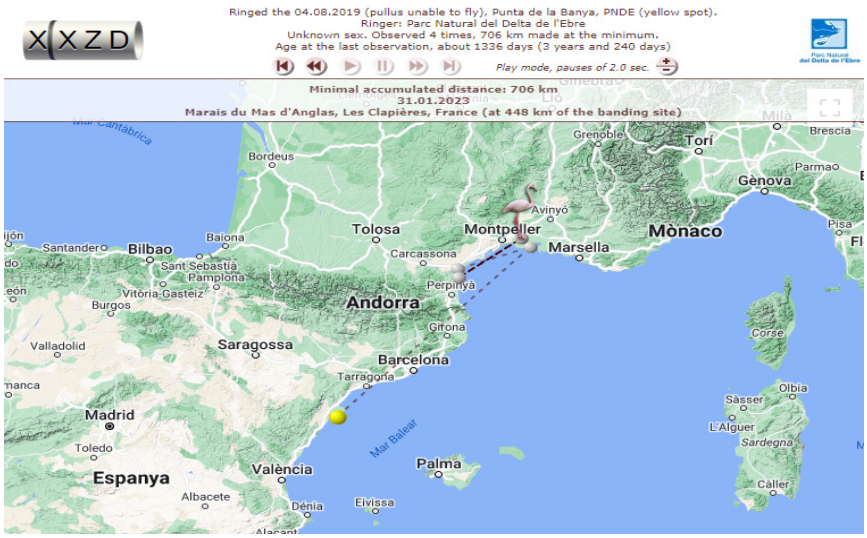

Figure 4.11. The journey record of one of the flamingo chicks analysed in 2019, from the Ebro delta colony to wetlands in Camargue (France).

4.2.4. Biomonitoring of contaminants in nocturnal raptors

In chapter VII, the exposure of contaminants in nocturnal raptors was assessed by performing a passive monitoring study using liver samples from road-killed owls collected during 9 years (2010-2019). The use of road-killed birds offers the opportunity to obtain valuable samples, such as internal tissues, without animal disturbance and legal restrictions. Therefore, some of the legal constraints identified in chapter V regarding the disturbance of protected species are minimised. Livers are collected performing the necropsies of dead birds. As the whole bird is available during the necropsy, other relevant data can be obtained such as the health condition of the bird, the moulting pattern of feathers, and biometric measurements. Moreover, an advantage in front of samples from alive bird is the availability of the different birds' organs and tissues which offer the possibility to perform multiple analysis and to determine the distribution of contaminants within the organism. There are already examples of successful monitoring schemes based on the collection of road-killed animals to monitor environmental contaminants. For instance, the "Cardiff University Otter Project" (https://www.cardiff.ac.uk/otter-project), collects dead otters (*Lutra lutra*) to obtain

samples for contaminant monitoring. The project allowed the determination of legacy compounds including DDTs, HCHs, PCBs and PBDEs (Pountney et al., 2015), and PFASs (O'Rourke et al., 2022) in this top predator. Similarly, the "Predatory Bird Monitoring Scheme" from the UK Centre for Ecology and Hydrology (CEH) (https://pbms.ceh.ac.uk/), uses the collection of road-killed birds of prey for contaminant monitoring which resulted in the study of the impact of OCPs in sparrowhawk (*Accipiter nisus*) (Heys et al., 2017), PBDEs in sparrowhawks (Crosse et al., 2013), and also anticoagulant rodenticides in tawny owls (Walker et al., 2008).

The opportunistic recollection of carcasses limited the capacity to control the number of individuals, the species, and the cohort groups in the study such as age or sex. Also, it must be considered that not all collected carcasses were used in the study, as only 47 livers were suitable for the chemical analysis were obtained from the 73 total necropsies, as the rest of carcasses presented livers too damaged or decomposed. To address the limitations arising from the relatively small sample size, future studies could benefit from collaborations with wildlife rehabilitations centers to collect and store in proper conditions a larger number of dead individuals.

The number of individuals for each species was biased towards the most frequently road-killed species in the area. Because of this, only 5 individuals of long-eared owls and 7 of eagle owls could be analysed, in front of 11 individuals for barn owl and 12 for tawny and little owl. Among all birds collected, 16 were juvenile, and 26 were adults, 7 individuals were not possible to be aged due to the unclear moulting pattern of the feathers. Considering all species, adults presented significantly higher mean concentrations of the bioacumulative compounds ∑OCPs, ∑PCBs and PFOS than juveniles. This result supports the accumulation of these compounds during lifetime.

OCPs were the compounds that showed the highest prevalence in liver samples as were detected in all individuals at high concentrations (from 3.24 to 4480 ng/g ww). The most predominant OCP was the metabolite 4,4'-DDE detected from 2.19 to 4475 ng/g ww. These findings are consistent with other recent biomonitoring studies with top-predators species that found 4,4'-DDE as the most predominant compound in birds samples (Ayele et al., 2022; Peris et al., 2023; Roque et al., 2022; Yohannes et al., 2017). This illustrates that, despite the existing restrictions, persistent legacy compounds are still a threat to wildlife.

The highest concentrations of 4,4'-DDE were found in two adult individuals of eagle owl with concentrations of 4475 ng/g ww (BB1, female) and 3577 ng/g ww (BB2, male) (Figure 4.12). According to the moulting pattern of the feathers, both individuals were at least older than 3 years, the male individual was classified as 5A+A, indicating the maximum possible age assessed by the moult feathers. As eagle owls begin to breed in the second year of calendar

(Martínez et al., 2002), these two individuals were probably sexually mature. The high exposure to 4,4'-DDE has been related to an impairment in the reproduction of bird species. For instance, 4,4'-DDE has been associated with the inhibition of carbonic anhydrase activity which affects the metabolism of calcium, leading to eggshell thinning and breakage of eggs in bird species (Beyer and Meador, 2011; Bitman et al., 1970; Lundholm, 1997). The eggshell thinning is not the only factor related to the reproductive impairment of 4,4'-DDE in birds. Exposure to 4,4'-DDE has been also related to alterations in the breeding behaviour of birds, such as a reduction in the chicks care (Fry, 1995; Hellou et al., 2013). The male individual (BB2) was found dead in May which is the period when eagle owls' chicks have already hatched and are fed by both parents. As raptors are monogamous species, the death of one individual at this moment represent a higher difficulty for the brood success. This is an example of how chemical pollution can directly or indirectly impair the species reproduction, potentially leading to negative impacts at population level, especially for more vulnerable species. The exposure of OCPs has been associated with the reproduction effects in several raptor species such as Spanish imperial eagle (*Aquila adalberti*) in Spain (Hernández et al., 2008), goshawk (*Aaccipiter gentilis*) in Germany (Scharenberg and Looft, 2004), ospray (*Pandion halietus*) in USA (Toschik et al., 2005), or in Californian condor (*Gymnogyps californianus*) (Bakker et al., 2023). This proves that chemical pollution in wildlife represents a real threat and needs to be addressed in species conservation programmes.

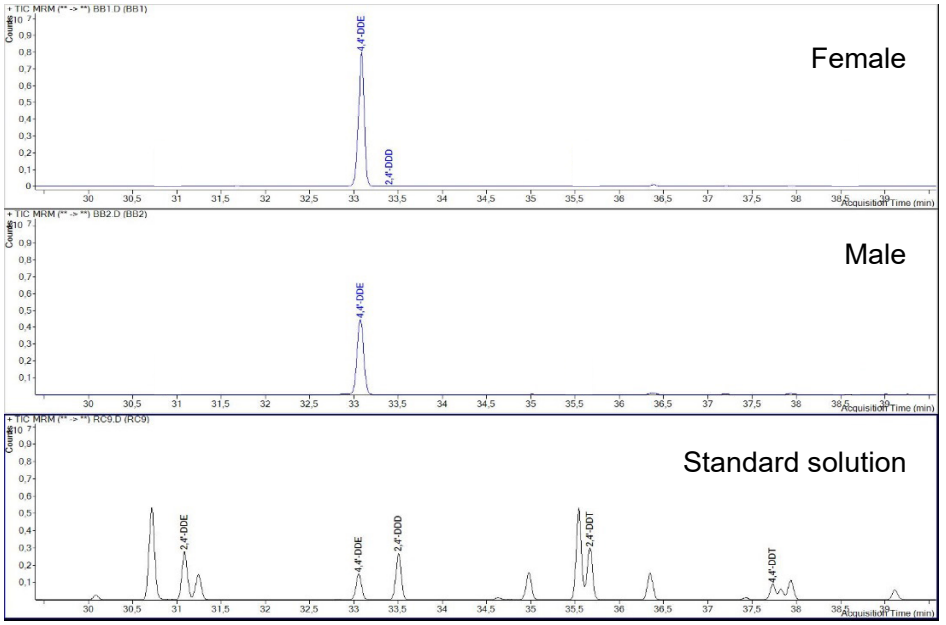

Figure 4.12. Chromatograms of DDTs in two liver samples of Eagle owl (*Bubo bubo*) and a standard at 0.6 ng/µL.

The patterns of contaminants exposure differed among nocturnal raptors. The differences were attributed to factors such as habitat and diet, that could be considered thanks to the information provided by ecological studies on the species in the area. For instance, the exposure of PAHs was more prevalent in tawny owl, barn owl, and little owl in contrast with the lower levels found in eagle owl and long-ear owl. The higher concentrations of PAHs in the first group were attributed to the higher use of urban areas and roadsides for hunting. This result raises the question of the impact of roads, beyond road fatalities, on the exposure of wildlife to pollutants. It is known that some birds of prey find roads and their surroundings as an easy source of food either by increased visibility or by feeding on the carcases of other roadkill animals (Hanmer and Robinson, 2021). The role of this food source in the exposure of PAHs is a question that could be of interest to address in further monitoring studies based on road killed birds.

4.3. Birds as allies to understand chemical pollution as a driver of biodiversity loss.

The impact of chemical pollution on birds is the main focus of the studies described in this Thesis. This topic was addressed through the quantification of contaminants in natural areas important for bird conservation and using bird species themselves as contaminant sentinel species. Our findings support the claim that chemical pollution is a persistent stressor in natural ecosystems, given the widespread presence of organic micropollutants in abiotic compartments (water, soil, and sediment), as well as in biological matrices (blood and liver of birds).

IBAs have been proved to be a good framework to assess the impact of chemical pollution in natural areas. They are sites well-defined by scientific criteria, and their ecological values are described and monitored by BirdLife International. This provides the opportunity to evaluate the role of chemical pollution on habitats conservation. The methodology presented in this Thesis could be applied to quantify the threat of chemical pollution in IBAs in future monitoring programmes. Given that IBAs have been identified worldwide, similar studies as the ones presented here could be implemented to assess the contamination status of IBAs in other countries. Moreover, as IBAs are used to designate legal protected areas, the monitoring studies within these sites offers the opportunity to evaluate the effectiveness of new regulations in the mitigation of chemical pollution.

The biomonitoring studies with birds have confirmed that chemical contamination is a threat to wildlife health, as organic micropollutants have been detected in all individuals of the species studied (flamingos and nocturnal raptors). The existing studies working with birds have been an important

advantage to obtain samples for the analysis through collaborations, but also to obtain contextual information about the species. The available information on diet, habitat and behaviour was an advantage when discussing the sources and patterns of contaminant exposure. The results of the biomonitoring studies also revealed high exposures to some compounds that were not noticed in other monitoring studies, as is the case of the high levels of PFASs found in flamingos. This underlines the importance of including contaminant analysis when conducting biodiversity conservation programmes.

The impact of the chemical pollution on biodiversity is a very complex issue as many factors are involved, and there are still serious gaps of knowledge about how to quantify this driver of biodiversity loss. Besides the direct harmful effects of chemicals on wildlife, contaminants are known to trigger indirect effects, such as changes in food resources or habitat quality. However, the relationship between chemicals and changes in populations trends are very difficult to be identified, as many other factors can influence the changes in the distribution of species. To achieve a better comprehension of this overwhelming problem, there is the need to collaborate between fields of expertise to obtain relevant data to quantify the presence of chemical pollution and their implication on biodiversity (Sigmund et al., 2023). In this Thesis, birds have been proven to be excellent allies to unify both fields of expertise. In addition, the popularity of birds serves as an excellent communication tool to explain the impacts of chemical pollution on natural areas and on biodiversity, which has the potential to reclaim more effective mitigation measures against chemical pollution.

4.4. References

Adeleye, A.S., Xue, J., Zhao, Y., Taylor, A.A., Zenobio, J.E., Sun, Y., Han, Z., Salawu, O.A., Zhu, Y., 2022. Abundance, fate, and effects of pharmaceuticals and personal care products in aquatic environments. J. Hazard. Mater. 424, 127284. https://doi.org/10.1016/j.jhazmat.2021.127284

Aguirre-Martínez, G. V, André, C., Gagné, F., Martín-Díaz, L.M., 2018. The effects of human drugs in *Corbicula fluminea*. Assessment of neurotoxicity, inflammation, gametogenic activity, and energy status. Ecotoxicol. Environ. Saf. 148, 652–663. https://doi.org/https://doi.org/10.1016/j.ecoenv.2017.09.042

Aguirre-Martínez, G. V, DelValls, T.A., Martín-Díaz, M.L., 2016. General stress, detoxification pathways, neurotoxicity and genotoxicity evaluated in *Ruditapes philippinarum* exposed to human pharmaceuticals. Ecotoxicol. Environ. Saf. 124, 18–31. https://doi.org/https://doi.org/10.1016/j.ecoenv.2015.09.031

Ahmadireskety, A., Da Silva, B.F., Robey, N.M., Douglas, T.E., Aufmuth, J., Solo-Gabriele, H.M., Yost, R.A., Townsend, T.G., Bowden, J.A., 2022. Per- and Polyfluoroalkyl Substances (PFAS) in Street Sweepings. Environ. Sci. Technol. 56, 6069–6077. https://doi.org/10.1021/acs.est.1c03766

Arenas-Sánchez, A., Rico, A., Rivas-Tabares, D., Blanco, A., Garcia-Doncel, P., Romero-Salas, A., Nozal, L., Vighi, M., 2019. Identification of contaminants of concern in the upper Tagus river basin (central Spain). Part 2: Spatio-temporal analysis and ecological risk assessment. Sci. Total Environ. 667, 222–233. https://doi.org/10.1016/J.SCITOTENV.2019.02.286

Ayele, S., Mamo, Y., Deribe, E., Eklo, O.M., 2022. Organochlorine pesticides and polychlorinated biphenyls in carnivorous waterbird species from Lake Ziway, Ethiopia. SN Appl. Sci. 4, 333. https://doi.org/10.1007/s42452-022-05215-5

Backhaus, T., Faust, M., 2012. Predictive environmental risk assessment of chemical mixtures: A conceptual framework. Environ. Sci. Technol. 46, 2564–2573. https://doi.org/10.1021/es2034125

Bade, R., Rousis, N.I., Bijlsma, L., Gracia-Lor, E., Castiglioni, S., Sancho, J. V, Hernandez, F., 2015. Screening of pharmaceuticals and illicit drugs in wastewater and surface waters of Spain and Italy by high resolution mass spectrometry using UHPLC-QTOF MS and LC-LTQ-Orbitrap MS. Anal. Bioanal. Chem. 407, 8979–8988. https://doi.org/10.1007/s00216-015-9063-x

Bakker, V.J., Finkelstein, M.E., Doak, D.F., Wolstenholme, R., Welch, A., Burnett, J., Punzalan, A., Brandt, J., Kirkland, S., Seal Faith, N.E., Lehnert, E.R., Sandhaus, E.A., 2023. Lead, trash, DDE, and young age of breeders linked to lower fertility in the first two decades of reintroduction for critically endangered California Condors in California. Ornithol. Appl. 125, duad022. https://doi.org/10.1093/ornithapp/duad022

Beaumelle, L., Thouvenot, L., Hines, J., Jochum, M., Eisenhauer, N., Phillips, H.R.P., 2021. Soil fauna diversity and chemical stressors: a review of knowledge gaps and roadmap for future research. Ecography (Cop.). 44, 845–859. https://doi.org/https://doi.org/10.1111/ecog.05627

Beyer, W.N., Meador, J.P., 2011. Environmental Contaminants in Biota: Interpreting Tissue Concentrations, 2nd ed. CRC Press, Boca Raton.

BirdLife International, 2023a. Important Bird Area factsheet: Guadalquivir marshes [WWW Document]. URL http://datazone.birdlife.org/site/factsheet/guadalquivir-marshes-iba-spain (accessed 8.30.23).

BirdLife International, 2023b. Important Bird Area factsheet: Albufera de Valencia marshes [WWW Document]. URL https://datazone.birdlife.org/site/factsheet/albufera-de-valencia-marshes-iba-spain (accessed 8.30.23).

BirdLife International, 2023c. Important Bird Area factsheet: Gallocanta lake. [WWW Document]. URL http://datazone.birdlife.org/site/factsheet/gallocanta-lake-iba-spain (accessed 8.30.23).

BirdLife International, 2023d. Important Bird Areas factsheet: Plain between Cáceres and Trujillo-Aldea del Cano [WWW Document]. URL https://datazone.birdlife.org/site/factsheet/plain-between-c%C3%A1ceres-and-trujillo-aldea-del-cano-iba-spain/text (accessed 8.30.23).

BirdLife International, 2023e. Important Bird Area factsheet: Mar Menor coastal lagoon [WWW Document]. URL http://datazone.birdlife.org/site/factsheet/mar-menor-coastal-lagoon-iba-spain (accessed 8.30.23).

BirdLife International, 2023f. Important Bird Area factsheet: Albufera de Mallorca and Albufereta de Pollença marshes [WWW Document]. URL http://datazone.birdlife.org/site/factsheet/albufera-de-mallorca-and-albufereta-de-pollença-marshes-iba-spain (accessed 8.30.23).

BirdLife International, 2023g. Important Bird Area factsheet: Urdaibai - Matxitxako [WWW Document]. URL http://datazone.birdlife.org/site/factsheet/urdaibai--matxitxako-iba-spain (accessed 8.30.23).

BirdLife International, 2023h. Important Bird Area factsheet: Campo de Montiel [WWW Document]. URL http://datazone.birdlife.org/site/factsheet/campo-de-montiel-iba-spain (accessed 8.30.23).

BirdLife International, 2023i. Important Bird Areas factsheet: Tordesillas-Mota del Marqués [WWW Document]. URL https://datazone.birdlife.org/site/factsheet/tordesillas-mota-del-marqu%C3%A9s-iba-spain (accessed 8.30.23).

BirdLife International, 2023j. Important Bird Area factsheet: El Pardo-Viñuelas [WWW Document]. URL https://datazone.birdlife.org/site/factsheet/el-pardo-vi%C3%B1uelas-iba-spain (accessed 8.30.23).

BirdLife International, 2022a. State of the World's Birds 2022: Insights and solutions for the biodiversity crisis. Cambridge, UK.

BirdLife International, 2022b. Important Bird Areas factsheet: Ebro delta [WWW Document]. URL https://datazone.birdlife.org/site/factsheet/ebro-delta-iba-spain (accessed 8.30.23).

Bitman, J., Cecil, H.C., Fries, G.F., 1970. DDT-Induced Inhibition of Avian Shell Gland Carbonic Anhydrase: A Mechanism for Thin Eggshells. Science (80-.). 168, 594–596. https://doi.org/10.1126/science.168.3931.594

BOE, 1994. Orden de 4 de febrero de 1994 por la que se prohíbe la comercialización y utilización de plaguicidas de uso ambiental que contienen determinados ingredientes activos peligrosos.

Buerge, I.J., Poiger, T., Müller, M.D., Buser, H.R., 2003. Caffeine, an anthropogenic marker for wastewater contamination of surface waters. Environ. Sci. Technol. 37, 691–700. https://doi.org/10.1021/es020125z

Cachada, A., Lopes, L. V, Hursthouse, A.S., Biasioli, M., Grčman, H., Otabbong, E., Davidson, C.M., Duarte, A.C., 2009. The variability of polychlorinated biphenyls levels in urban soils from five European cities. Environ. Pollut. 157, 511–518. https://doi.org/https://doi.org/10.1016/j.envpol.2008.09.002

Ccanccapa, A., Masiá, A., Navarro-Ortega, A., Picó, Y., Barceló, D., 2016. Pesticides in the Ebro River basin: Occurrence and risk assessment. Environ. Pollut. 211, 414–424. https://doi.org/10.1016/j.envpol.2015.12.059

Chow, R., Scheidegger, R., Doppler, T., Dietzel, A., Fenicia, F., Stamm, C., 2020. A review of long-term pesticide monitoring studies to assess surface water quality trends. Water Res. X 9, 100064. https://doi.org/10.1016/j.wroa.2020.100064

Colomer-Vidal, P., Bertolero, A., Alcaraz, C., Garreta-Lara, E., Santos, F.J., Lacorte, S., 2022. Distribution and ten-year temporal trends (2009–2018) of perfluoroalkyl substances in gull eggs from Spanish breeding colonies. Environ. Pollut. 293, 118555. https://doi.org/10.1016/J.ENVPOL.2021.118555

Copernicus, 2023. Copernicus Global Land Service [WWW Document]. URL https://land.copernicus.eu/

Cristale, J., Vázquez, A.G., Barata, C., Lacorte, S., 2013. Priority and emerging flame retardants in rivers : Occurrence in water and sediment, *Daphnia magna* toxicity and risk assessment. Environ. Int. 59, 232–243. https://doi.org/10.1016/j.envint.2013.06.011

Crosse, J.D., Shore, R.F., Jones, K.C., Pereira, M.G., 2013. Key factors affecting liver PBDE concentrations in sparrowhawks (*Accipiter nisus*). Environ. Pollut. 177, 171–176. https://doi.org/https://doi.org/10.1016/j.envpol.2013.02.006

Cruz, D., Almeida, Â., Calisto, V., Esteves, V.I., Schneider, R.J., Wrona, F.J., Soares, A.M.V.M., Figueira, E., Freitas, R., 2016. Caffeine impacts in the clam *Ruditapes philippinarum*: Alterations on energy reserves, metabolic activity and oxidative stress biomarkers. Chemosphere 160, 95–103. https://doi.org/https://doi.org/10.1016/j.chemosphere.2016.06.068

de Brogniez, D., Ballabio, C., Stevens, A., Jones, R.J.A., Montanarella, L., van Wesemael, B., 2015. A map of the topsoil organic carbon content of Europe generated by a generalized additive model. Eur. J. Soil Sci. 66, 121–134. https://doi.org/https://doi.org/10.1111/ejss.12193

Di Guardo, A., Raspa, G., Terzaghi, E., Vergani, L., Mapelli, F., Borin, S., Zanardini, E., Morosini, C., Anelli, S., Nastasio, P., Sale, V.M., Armiraglio, S., 2020. PCB vertical and horizontal movement in agricultural soils of a highly contaminated site: Role of soil properties, cultivation history and PCB physico-chemical parameters. Sci. Total Environ. 747, 141477. https://doi.org/https://doi.org/10.1016/j.scitotenv.2020.141477

Directive 2013/39/EU of the European Parliament and of the Council of 12 August 2013 amending Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy, 2013.

ECHA, 2022. European Chemicals Agency [WWW Document]. URL https://echa.europa.eu/ (accessed 2.8.22).

Escher, B.I., Stapleton, H.M., Schymanski, E.L., 2020. Tracking complex mixtures of chemicals in our changing environment. Science (80-.). 367, 388–392. https://doi.org/10.1126/science.aay6636

Espín, S., Andevski, J., Duke, G., Eulaers, I., Gómez-Ramírez, P., Hallgrimsson, G.T., Helander, B., Herzke, D., Jaspers, V.L.B., Krone, O., Lourenço, R., María-Mojica, P., Martínez-López, E., Mateo, R., Movalli, P., Sánchez-Virosta, P., Shore, R.F., Sonne, C., van den Brink, N.W., van Hattum, B., Vrezec, A., Wernham, C., García-Fernández, A.J., 2021. A schematic sampling protocol for contaminant monitoring in raptors. Ambio 50, 95–100. https://doi.org/10.1007/s13280-020-01341-9

European Commission, 2022. Commission Implementing Decision (EU)2022/1307 of 22 July 2022 establishing a watch list of substances for Union-wide monitoring in the field of water policy pursuant to Directive 2008/105/EC of the European Parliament and of the Council. Official Journal of the European Union.

European Commission, 2020. Commission implementing regulation (EU) 2020/18 of 10 January 2020 concerning the non-renewal of the approval of the active substance chlorpyrifos, in accordance with Regulation (EC) No 1107/2009. Off. J. Eur. Union L 7/14.

European Commission, 2003. Technical Guidance Document on Risk Assessment.

Fedorova, G., Golovko, O., Randak, T., Grabic, R., 2014. Storage effect on the analysis of pharmaceuticals and personal care products in wastewater. Chemosphere 111, 55–60. https://doi.org/https://doi.org/10.1016/j.chemosphere.2014.02.067

Figuière, R., Waara, S., Ahrens, L., Golovko, O., 2022. Risk-based screening for prioritisation of organic micropollutants in Swedish freshwater. J. Hazard. Mater. 429, 128302. https://doi.org/10.1016/j.jhazmat.2022.128302

Flamingo atlas, 2023. Greater flamingo (*Phoenicopterus roseus*) banding schemes in the Mediterranean from 1977 to 2022 [WWW Document]. URL http://www.flamingoatlas.org/dwld_bandsummaries.php

Fry, D.M., 1995. Reproductive effects in birds exposed to pesticides and industrial chemicals. Environ. Health Perspect. 103, 165–171. https://doi.org/10.1289/ehp.95103s7165

Glüge, J., Scheringer, M., Cousins, I.T., DeWitt, J.C., Goldenman, G., Herzke, D., Lohmann, R., Ng, C.A., Trier, X., Wang, Z., 2020. An overview of the uses of per-and polyfluoroalkyl substances (PFAS). Environ. Sci. Process. Impacts 22, 2345–2373.

Gómez-Canela, C., Pueyo, V., Barata, C., Lacorte, S., Marcé, R.M., 2019. Development of predicted environmental concentrations to prioritize the occurrence of pharmaceuticals in rivers from Catalonia. Sci. Total Environ. 666, 57–67. https://doi.org/10.1016/j.scitotenv.2019.02.078

Gómez-Ramírez, P., Shore, R.F., van den Brink, N.W., van Hattum, B., Bustnes, J.O., Duke, G., Fritsch, C., García-Fernández, A.J., Helander, B.O., Jaspers, V., Krone, O., Martínez-López, E., Mateo, R., Movalli, P., Sonne, C., 2014. An overview of existing raptor contaminant monitoring activities in Europe. Environ. Int. 67, 12–21. https://doi.org/10.1016/j.envint.2014.02.004

Gross, M.S., Bean, T.G., Hladik, M.L., Rattner, B.A., Kuivila, K.M., 2020. Uptake, Metabolism, and Elimination of Fungicides from Coated Wheat Seeds in Japanese Quail (*Coturnix japonica*). J. Agric. Food Chem. 68, 1514–1524. https://doi.org/10.1021/acs.jafc.9b05668

Guo, Y., Kannan, K., 2012. Challenges encountered in the analysis of phthalate esters in foodstuffs and other biological matrices. Anal. Bioanal. Chem. 404, 2539–2554. https://doi.org/10.1007/s00216-012-5999-2

Hanmer, H.J., Robinson, R.A., 2021. Incidence of road mortality in ringed raptors and owls: a spatial analysis. British Trust for Ornithology.

Hellou, J., Lebeuf, M., Rudi, M., 2013. Review on DDT and metabolites in birds and mammals of aquatic ecosystems. Environ. Rev. 21, 53–69. https://doi.org/10.1139/er-2012-0054

Hernández, M., González, L.M., Oria, J., Sánchez, R., Arroyo, B., 2008. Influence of contamination by organochlorine pesticides and polychlorinated biphenyls on the breeding of the Spanish Imperial Eagle (*Aquila adalberti*). Environ. Toxicol. Chem. 27, 433–441. https://doi.org/https://doi.org/10.1897/07-308R.1

Heys, K.A., Shore, R.F., Pereira, M.G., Martin, F.L., 2017. Levels of Organochlorine Pesticides Are Associated with Amyloid Aggregation in Apex Avian Brains. Environ. Sci. Technol. 51, 8672–8681. https://doi.org/10.1021/acs.est.7b00840

Hu, X.C., Ge, B., Ruyle, B.J., Sun, J., Sunderland, E.M., 2021. A Statistical Approach for Identifying Private Wells Susceptible to Perfluoroalkyl Substances (PFAS) Contamination. Environ. Sci. \& Technol. Lett. 8, 596–602. https://doi.org/10.1021/acs.estlett.1c00264

Kortenkamp, A., Faust, M., 2018. Regulate to reduce chemical mixture risk. Science (80-.). 361, 224–226. https://doi.org/10.1126/science.aat9219

Li, Z., Lu, G., Yang, X., Wang, C., 2012. Single and combined effects of selected pharmaceuticals at sublethal concentrations on multiple biomarkers in *Carassius auratus*. Ecotoxicology 21, 353–361. https://doi.org/10.1007/s10646-011-0796-9

Llorca, M., Gros, M., Rodríguez-Mozaz, S., Barceló, D., 2014. Sample preservation for the analysis of antibiotics in water. J. Chromatogr. A 1369, 43–51. https://doi.org/https://doi.org/10.1016/j.chroma.2014.09.089

Lopez-Herguedas, N., González-Gaya, B., Castelblanco-Boyacá, N., Rico, A., Etxebarria, N., Olivares, M., Prieto, A., Zuloaga, O., 2022. Characterization of the contamination fingerprint of wastewater treatment plant effluents in the Henares River Basin (central Spain) based on target and suspect screening analysis. Sci. Total Environ. 806. https://doi.org/10.1016/j.scitotenv.2021.151262

López-Lorente, Á.I., Pena-Pereira, F., Pedersen-Bjergaard, S., Zuin, V.G., Ozkan, S.A., Psillakis, E., 2022. The ten principles of green sample preparation. TrAC Trends Anal. Chem. 148, 116530. https://doi.org/https://doi.org/10.1016/j.trac.2022.116530

Lundholm, C.E., 1997. DDE-induced eggshell thinning in birds: Effects of p,p'-DDE on the calcium and prostaglandin metabolism of the eggshell gland. Comp. Biochem. Physiol. Part C Pharmacol. Toxicol. Endocrinol. 118, 113–128. https://doi.org/https://doi.org/10.1016/S0742-8413(97)00105-9

Ma, T., Ye, C., Wang, T., Li, X., Luo, Y., 2022. Toxicity of Per- and Polyfluoroalkyl Substances to Aquatic Invertebrates, Planktons, and Microorganisms. Int. J. Environ. Res. Public Health 19. https://doi.org/10.3390/ijerph192416729

Martínez, J.A., Zuberogoitia, I., Alonso, R., 2002. Rapaces nocturnas: guıa para la identificación de la edad y el sexo en las estrigiformes ibéricas. Monticola ediciones, Madrid (in Spanish).

McEachran, A.D., Shea, D., Bodnar, W., Nichols, E.G., 2016. Pharmaceutical occurrence in groundwater and surface waters in forests land-applied with municipal wastewater. Environ. Toxicol. Chem. 35, 898–905. https://doi.org/https://doi.org/10.1002/etc.3216

Mejías, C., Martín, J., Santos, J.L., Aparicio, I., Alonso, E., 2021. Occurrence of pharmaceuticals and their metabolites in sewage sludge and soil: A review on their distribution and environmental risk assessment. Trends Environ. Anal. Chem. 30, e00125. https://doi.org/https://doi.org/10.1016/j.teac.2021.e00125

Müller, A., Österlund, H., Marsalek, J., Viklander, M., 2020. The pollution conveyed by urban runoff: A review of sources. Sci. Total Environ. 709, 136125. https://doi.org/https://doi.org/10.1016/j.scitotenv.2019.136125

Nam, J.J., Thomas, G.O., Jaward, F.M., Steinnes, E., Gustafsson, O., Jones, K.C., 2008. PAHs in background soils from Western Europe: Influence of atmospheric deposition and soil organic matter. Chemosphere 70, 1596–1602. https://doi.org/https://doi.org/10.1016/j.chemosphere.2007.08.010

Nanusha, M.Y., Frøkjær, E.E., Liigand, J., Christensen, M.R., Hansen, H.R., Hansen, M., 2022. Unravelling the occurrence of trace contaminants in surface waters using semi-quantitative suspected non-target screening analyses. Environ. Pollut. 315, 120346. https://doi.org/https://doi.org/10.1016/j.envpol.2022.120346

NORMAN, 2022. NORMAN Ecotoxicology Database - Lowest PNECs [WWW Document]. URL https://www.norman-network.com/nds/ecotox/lowestPnecsIndex.php (accessed 3.16.22).

O'Rourke, E., Hynes, J., Losada, S., Barber, J.L., Pereira, M.G., Kean, E.F., Hailer, F., Chadwick, E.A., 2022. Anthropogenic Drivers of Variation in Concentrations of Perfluoroalkyl Substances in Otters (*Lutra lutra*) from England and Wales. Environ. Sci. Technol. 56, 1675–1687. https://doi.org/10.1021/acs.est.1c05410

Oliver, T.H., Morecroft, M.D., 2014. Interactions between climate change and land use change on biodiversity: attribution problems, risks, and opportunities. WIREs Clim. Chang. 5, 317–335. https://doi.org/https://doi.org/10.1002/wcc.271

Pascual Aguilar, J.A., Andreu, V., Campo, J., Picó, Y., Masiá, A., 2017. Pesticide occurrence in the waters of Júcar River, Spain from different farming landscapes. Sci. Total Environ. 607–608, 752–760. https://doi.org/10.1016/j.scitotenv.2017.06.176

Patinha, C., Durães, N., Dias, A.C., Pato, P., Fonseca, R., Janeiro, A., Barriga, F., Reis, A.P., Duarte, A., Ferreira da Silva, E., Sousa, A.J., Cachada, A., 2018. Long-term application of the organic and inorganic pesticides in vineyards: Environmental record of past use. Appl. Geochemistry 88, 226–238. https://doi.org/https://doi.org/10.1016/j.apgeochem.2017.05.014

Peris, A., Baos, R., Martínez, A., Sergio, F., Hiraldo, F., Eljarrat, E., 2023. Pesticide contamination of bird species from Doñana National Park (southwestern Spain): Temporal trends (1999–2021) and reproductive impacts. Environ. Pollut. 323, 121240. https://doi.org/https://doi.org/10.1016/j.envpol.2023.121240

Postigo, C., Moreno-Merino, L., López-García, E., López-Martínez, J., López de Alda, M., 2023. Human footprint on the water quality from the northern Antarctic Peninsula region. J. Hazard. Mater. 453, 131394. https://doi.org/https://doi.org/10.1016/j.jhazmat.2023.131394

Pountney, A., Filby, A.L., Thomas, G.O., Simpson, V.R., Chadwick, E.A., Stevens, J.R., Tyler, C.R., 2015. High liver content of polybrominated diphenyl ether (PBDE) in otters (*Lutra lutra*) from England and Wales. Chemosphere 118, 81–86. https://doi.org/https://doi.org/10.1016/j.chemosphere.2014.06.051

Powers, R.P., Jetz, W., 2019. Global habitat loss and extinction risk of terrestrial vertebrates under future land-use-change scenarios. Nat. Clim. Chang. 9, 323–329. https://doi.org/10.1038/s41558-019-0406-z

Ramos, S., Homem, V., Alves, A., Santos, L., 2015. Advances in analytical methods and occurrence of organic UV-filters in the environment — A review. Sci. Total Environ. 526, 278–311. https://doi.org/https://doi.org/10.1016/j.scitotenv.2015.04.055

Roder Green, A.L., Putschew, A., Nehls, T., 2014. Littered cigarette butts as a source of nicotine in urban waters. J. Hydrol. 519, 3466–3474. https://doi.org/10.1016/J.JHYDROL.2014.05.046

Roque, I., Lourenço, R., Marques, A., Martínez-López, E., Espín, S., Gómez-Ramirez, P., García-Fernández, A.J., Roulin, A., Rabaça, J.E., 2022. A First Record of Organochlorine Pesticides in Barn Owls (*Tyto alba*) from Portugal: Assessing Trends from Variation in Feather and Liver Concentrations. Bull. Environ. Contam. Toxicol. 109, 436–442. https://doi.org/10.1007/s00128-022-03576-6

Rossini, P., Guerzoni, S., Matteucci, G., Gattolin, M., Ferrari, G., Raccanelli, S., 2005. Atmospheric fall-out of POPs (PCDD-Fs, PCBs, HCB, PAHs) around the industrial district of Porto Marghera, Italy. Sci. Total Environ. 349, 190–200. https://doi.org/https://doi.org/10.1016/j.scitotenv.2005.01.011

Scharenberg, W., Looft, V., 2004. Reduction of Organochlorine Residues in Goshawk Eggs (*Accipiter gentilis*) from Northern Germany (1971–2002) and Increasing Eggshell Index. AMBIO A J. Hum. Environ. 33, 495–498. https://doi.org/10.1579/0044-7447-33.8.495

Scridel, D., Pirrello, S., Imperio, S., Cecere, J.G., Albanese, G., Andreotti, A., Arveda, G., Borghesi, F., La Gioia, G., Massa, L., Mengoni, C., Micheloni, P., Mucci, N., Nardelli, R., Nissardi, S., Volponi, S., Zucca, C., Serra, L., 2023. Weather, sex and body condition affect post-fledging migration behaviour of the greater flamingo *Phoenicopterus roseus*. Mov. Ecol. 11, 51. https://doi.org/10.1186/s40462-023-00409-x

Sebastiano, M., Jouanneau, W., Blévin, P., Angelier, F., Parenteau, C., Pallud, M., Ribout, C., Gernigon, J., Lemesle, J.C., Robin, F., Pardon, P., Budzinski, H., Labadie, P., Chastel, O., 2023. Physiological effects of PFAS exposure in

seabird chicks: A multi-species study of thyroid hormone triiodothyronine, body condition and telomere length in South Western France. Sci. Total Environ. 901, 165920. https://doi.org/https://doi.org/10.1016/j.scitotenv.2023.165920

Siddiqui, N., Husain, A., Chaudhry, L., Alam, M.S., Mitra, M., Bhasin, P.S., 2011. Pharmacological and pharmaceutical profile of valsartan: a review. J. Appl. Pharm. Sci. 12–19.

Sigmund, G., Ågerstrand, M., Antonelli, A., Backhaus, T., Brodin, T., Diamond, M.L., Erdelen, W.R., Evers, D.C., Hofmann, T., Hueffer, T., Lai, A., Torres, J.P.M., Mueller, L., Perrigo, A.L., Rillig, M.C., Schaeffer, A., Scheringer, M., Schirmer, K., Tlili, A., Soehl, A., Triebskorn, R., Vlahos, P., vom Berg, C., Wang, Z., Groh, K.J., 2023. Addressing chemical pollution in biodiversity research. Glob. Chang. Biol. 29, 3240–3255. https://doi.org/https://doi.org/10.1111/gcb.16689

Sousa, J.C.G., Ribeiro, A.R., Barbosa, M.O., Pereira, M.F.R., Silva, A.M.T., 2018. A review on environmental monitoring of water organic pollutants identified by EU guidelines. J. Hazard. Mater. 344, 146–162. https://doi.org/10.1016/j.jhazmat.2017.09.058

Spence, P.L., 2015. Using Caffeine as a Water Quality Indicator in the Ambient Monitoring Program for Third Fork Creek Watershed, Durham, North Carolina. Environ. Health Insights 9. https://doi.org/10.4137/EHI.S19588

Szöcs, E., Brinke, M., Karaoglan, B., Schäfer, R.B., 2017. Large Scale Risks from Agricultural Pesticides in Small Streams. Environ. Sci. Technol. 51, 7378–7385. https://doi.org/10.1021/acs.est.7b00933

Taylor, A.C., Fones, G.R., Mills, G.A., 2020. Trends in the use of passive sampling for monitoring polar pesticides in water. Trends Environ. Anal. Chem. 27, e00096. https://doi.org/https://doi.org/10.1016/j.teac.2020.e00096

Toschik, P.C., Rattner, B.A., McGowan, P.C., Christman, M.C., Carter, D.B., Hale, R.C., Matson, C.W., Ottinger, M.A., 2005. Effects of contaminant exposure on reproductive success of ospreys (*Pandion haliaetus*) nesting in delaware river and bay, USA. Environ. Toxicol. Chem. 24, 617–628. https://doi.org/https://doi.org/10.1897/04-141R.1

Velázquez-Gómez, M., Hurtado-Fernández, E., Lacorte, S., 2018. Comprehensive method for the analysis of multi-class organic micropollutants in indoor dust. Sci. Total Environ. 635, 1484–1494. https://doi.org/https://doi.org/10.1016/j.scitotenv.2018.04.186

VoPham, T., Wilson, J.P., Ruddell, D., Rashed, T., Brooks, M.M., Yuan, J.-M., Talbott, E.O., Chang, C.-C.H., Weissfeld, J.L., 2015. Linking pesticides and human health: A geographic information system (GIS) and Landsat remote sensing method to estimate agricultural pesticide exposure. Appl. Geogr. 62, 171–181. https://doi.org/https://doi.org/10.1016/j.apgeog.2015.04.009

Vrezec, A., Duke, G., Kovács, A., Saurola, P., Wernham, C., Burfield, I., Movalli, P., Bertoncelj, I., 2014. Overview of raptor monitoring activities in Europe. Acrocephalus 33, 145–157. https://doi.org/10.2478/v10100-012-0003-y

Walker, L.A., Turk, A., Long, S.M., Wienburg, C.L., Best, J., Shore, R.F., 2008. Second generation anticoagulant rodenticides in tawny owls (Strix aluco) from Great Britain. Sci. Total Environ. 392, 93–98. https://doi.org/https://doi.org/10.1016/j.scitotenv.2007.10.061

Wang, L., Zhang, Z.-F., Liu, L.-Y., Zhu, F.-J., Ma, W.-L., 2023. National-scale monitoring of historic used organochlorine pesticides (OCPs) and current used pesticides (CUPs) in Chinese surface soil: Old topic and new story. J. Hazard. Mater. 443, 130285. https://doi.org/https://doi.org/10.1016/j.jhazmat.2022.130285

Wolfram, J., Bub, S., Petschick, L.L., Schemmer, A., Stehle, S., Schulz, R., 2023. Pesticide occurrence in protected surface waters in nature conservation areas of Germany. Sci. Total Environ. 858, 160074. https://doi.org/https://doi.org/10.1016/j.scitotenv.2022.160074

Yohannes, Y.B., Ikenaka, Y., Nakayama, S.M.M., Mizukawa, H., Ishizuka, M., 2017. DDTs and other organochlorine pesticides in tissues of four bird species from the Rift Valley region, Ethiopia. Sci. Total Environ. 574, 1389–1395. https://doi.org/https://doi.org/10.1016/j.scitotenv.2016.08.056

5. CONCLUSIONS

Chemical pollution impacts Important Biodiversity Areas and represents an underestimated threat to biodiversity. Monitoring studies have been proven to be important to evaluate the contamination patterns and their geographical distribution in different habitats and address the potential harmful effects of pollutants to bird species.

From the monitoring program in IBAs, the main conclusions are derived:

- A sampling protocol was designed and implemented to determine the presence and distribution of organic micropollutants in water, soils, and sediment from 140 IBAs in Spain. This methodology represents a first step to establish future monitoring programs in areas of high ecological value.
- Multiresidue methods were developed, and quality parameters defined to determine pharmaceuticals, pesticides, OPEs and PFASs in water using LC-MS/MS.
- A multiresidue method was developed, and quality parameters defined to determine OCPs, PAHs, PCBs, OPPs, and plasticizers in soils and sediments using GC-MS/MS.
- The integration of chemical data with spatial data using GIS tools was a useful approach for the identification of sources and distribution patterns of contaminants in water, soils, and sediments.

From the water monitoring study, the main conclusions are derived:

- Freshwaters from IBAs are impacted by anthropogenic pollutants. Pharmaceuticals and lifestyle compounds were the most widespread type of contamination, while pesticides and PFASs showed a detection frequency below 25% of the samples.
- The highest concentrations of organic micropollutants in water have been related with the presence of agricultural surface, artificial surface and WWTPs discharges in IBAs.
- In the analysis of water, from the 140 IBAs analysed, 52 IBAs presented at least one compound at high-risk concentrations for freshwater organisms.
- Fifteen out of 59 target compounds have been found at levels posing a high-risk for freshwater organisms, being the insecticide chlorpyrifos, the antidepressant venlafaxine and perfluorooctanesulfonic acid (PFOS) the most concerning compounds.

In the soil monitoring study, the main conclusions are derived:

- Soils from IBAs are impacted by chemical pollutants. OCPs were the most ubiquitous compounds found in 87% of the samples at

concentrations ranging from 0.03 to 626 ng/g. PCBs were detected in 44% of the samples, but at low levels, ranging from 0.02 to 67.8 ng/g. PAHs were also ubiquitous compounds detected in 69% of the samples at concentrations ranging from 2.57 to 1909 ng/g. Plasticizers were found in 50% of the samples ranging from 5.50 to 7026 ng/g.

- The higher levels of PCBs and plasticizers in soils were found in sampling areas with influence of artificial surface. The specific sources of pollution were not identified in a great number of IBAs as they are impacted by diffuse pollution sources.
- From the 140 soils samples, 95 presented at least one compound exceeding the calculated PNEC values.

In the sediment monitoring study, the main conclusions are derived:

- Sediments from IBAs are impacted by legacy and emerging compounds. PAHs were identified as the most ubiquitous chemical family detected in 87% of the samples at concentrations from 1.24 to 2193 ng/g. Plasticizers were the chemical group found at the highest concentrations ranging from 18.5 to 30278 ng/g.
- IBAs receive the impact of multiple human activities affecting sediment quality. Half of the studied IBAs presented trash residues documented during field sampling.
- Further investigations are needed to evaluate the sources of contamination in sediment as they are affected by both direct and diffuse sources of pollution.

From the biomonitoring studies with birds, the main conclusions are derived:

- Birds of prey are excellent sentinel species to monitor contaminants, but some challenges must be considered on sample collection to stablish successful monitoring programs based on raptors.
- The main constraints identified to obtain raptor samples are related to the existing low number of long-term monitoring schemes to provide valuable samples and contextual data to understand and discuss the presence and potential impacts of contaminants in birds.
- Biomonitoring studies using blood and liver samples included the analysis of PFASs, pharmaceuticals, pesticides and OPEs by LC-MS/MS, and OCPs, PAHs, and PCBs by GC-MS/MS.
- A single extraction followed by a multiresidue LC-MS/MS method was optimized to simultaneously determine pharmaceuticals, OPEs and pesticides in blood and liver.

- The analysis of multiple organic micropollutants in flamingos blood evidenced the chemical exposure from a very young age.
- All flamingos' chicks from Ebro delta presented high concentrations of PFASs. PFOA was detected at unexpectedly high concentrations in flamingos and although the immediate cause remains uncertain, its presence might be associated either to the use of pesticides in rice cultivation or fire-fighting foams.
- PAHs naphthalene and pyrene presented a high occurrence (>60%). OCPs and PCBs were also ubiquitous but present at trace concentrations. The most prevalent halogenated compound was the metabolite 4,4'-DDE, detected in 90% of the individuals.
- The contaminant exposure in flamingos was not related to any alteration in the body condition of the chicks, but further studies are needed to address the sources of contamination and the implications of the detected concentrations.
- Road-killed birds are a valuable source of samples to monitor the exposure of contaminants in protected species such as nocturnal raptors. Information about their habitat and diet in the area were very valuable to understand the sources of the detected chemicals.
- Nocturnal raptors are top predators exposed to high concentrations of persistent compounds such as OCPs, PCBs and PFOS, with different patterns of exposure among species. The high PAHs concentrations has been related to the diet and foraging behaviour of some raptor species in roadsides, which could be a source of these contaminants.

This Thesis highlights the widespread threat of chemical pollution in important biodiversity areas. The contamination status of these natural areas implies the exposure of chemicals to wildlife, as has been observed in the biomonitoring studies using birds. Given the outcomes provided in this Thesis, chemical pollution is a factor that has to be considered in conservation strategies in for natural areas to halt the biodiversity loss crisis.