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Phytoremediation in lead-contaminated soils of a mining area in SE Spain: role of a chelating agent and a growth promoter in lead uptake by *Medicago sativa*

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Background

• Environmental **lead contamination** via anthropogenic sources due to increased industrialization has resulted in serious problems in the food chain and, consequently the health of organisms, including man (Khairiah et al., 2002; Antunes et al., 2003; Jamal et al., 2006; Opeolu BO, 2006).

• In polluted areas, concentrations of 100 to 1000 times that of the normal level in soil can be found (Davies, 1995; Adriano, 1986; Peterson, 1978). Sources of Pb contamination in soils are commonly tailings from the mining of lead ores (Lapèrche V, 2000).

• **Phytoremediation** is a technology that uses specially selected metal-accumulating plants to remediate soil contaminated with heavy metals and radionuclides. It offers an attractive and economical alternative to currently practiced soil removal and burial methods (Blaylock J, 2000), which are environmentally invasive and expensive (Van der Lelie et al., 2001).

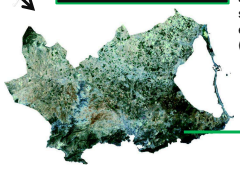
• ***Medicago sativa*** (alfalfa) has been studied for lead uptake from **water** with the addition of different auxiliary agents for metal uptake, including the chelating agent ethylenediaminetetraacetic acid (EDTA) and the growth promoting auxin indole-3-acetic acid (IAA) (Athalye et al., 1995; Jarvis and Leung, 2001; Piechalak et al., 2003; López M, 2005 Pasternak et al., 2003, López M, 2007).

• IAA and EDTA have been found to produce a synergistic effect dramatically increasing Pb movement/translocation from roots to leaves (2800% more lead in leaves than without these agents) (López M et al., 2005).

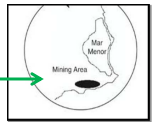
• Phytostabilisation – which uses plants to reduce contaminants' bioavailability in soils - has been done in the study zone.

Study Zone

The mining district Cartagena-La Unión is located at the Easter end of the region of Murcia (SE Spain) and its area is slightly over 50 km².



From 1992 heavy metals and trace elements have been released and dispersed following the cessation of the mining activity and the abandonment of the sterile swamps, which are easily eroded by wind and water (Moreno J *et al.*, 2009).



An important agricultural activity is developed in the surrounding area of this historical mining district (Belmonte F *et al.*, 2010) and shows an average lead concentration of 1210 ppm, which exceeds the normal range considered by following reference values established by Bowie y Thornton (1985) and maximal admitted by Spanish Legislation.

Objective and Hypotheses

The **aims** of the present study are to:

• Evaluate the effect of IAA combined with EDTA to enhance Pb uptake ability of alfalfa plants in artificially and original contaminated **soil** samples

• Determine if this improvement could be considered as a first step for phytoextraction's application in the mining area of Sierra Minera – La Unión (Murcia, SE Spain), as a method of progressive replacement of phytoestabilization.

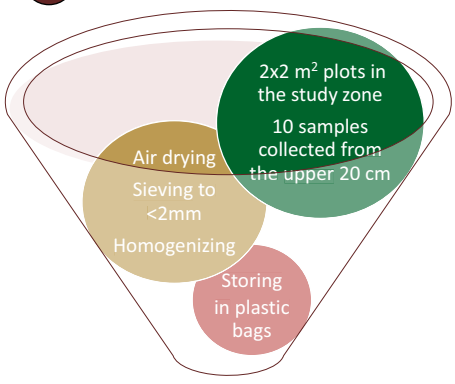
The initial **hypotheses** are:

• Alfalfa plant's ability to uptake lead from soil will be enhanced with the addition of EDTA and its combination with IAA in the soil treatments.

• Applying **phytoextraction** instead of mantaining phytostabilization will be a viable option once EDTA and IAA are applied in adequate proportion.

Methodology

1 PLOT SAMPLING



Laboratory Analyses
Electrical Conductivity (EC)
pH
Particle size distribution
Equivalent Calcium Carbonate
Total Organic Carbon (OC)
Total Nitrogen (TN)
Total As, Cd, Cu, Mn, Pb, Zn

2 TREATMENTS

Artificially contaminated soil
$Pb(NO_3)_2$
Pb0 → 0 ppm
Pb1 → 500 ppm
Pb2 → 1500 ppm
80d

Presoaking: ethanol 100%; 5'
Stirring: NaClO 4%; 30'
Washing: sterilized water
Soaking: antimycotic solution
Nursery: 21d, 18-24ºC
7 seedlings per pot; 10d; room TºC

Original soil
Taken from the upper 20 cm
Air drying: 25-30ºC
Sieving < 2mm
Bulk density: 1,35g/cm³

8 TREATMENTS IN PLANTPOTS

· CONTROL → NO EDTA/NO IAA
· NO EDTA
  - 10 µM IAA
  - 100 µM IAA
  - 200 µM IAA
· EDTA
  - NO IAA
  - 10 µM IAA
  - 100 µM IAA
  - 200 µM IAA

3 EXPERIMENTAL DESIGN

15 plants /treatment randomly selected
Washing: HNO_3, 0.01M
Rinsing: deionized water

Separation: roots, stems and leaves
Drying: Oven, 60ºC, 3d
Digestion: CEM Microwave Oven, 5mL
pure HNO_3 diluted to 15mL

Laboratory Analyses
Electrical Conductivity (EC)
pH
Particle size distribution
Equivalent Calcium Carbonate
Total Organic Carbon (OC)
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Total As, Cd, Cu, Mn, Pb, Zn

Expected Outcomes

• This study will analyze the possibilities of extrapolating alfalfa's efficiency in binding considerable amounts of lead from aqueous solution to real field conditions. That means taking another step towards the use of **economical and environmental friendly techniques to decontaminate metal-polluted soils.**

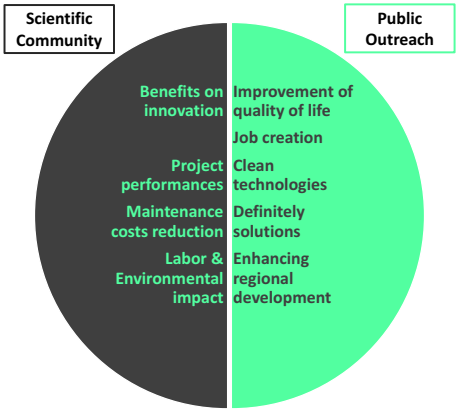
• Alfalfa is an excellent candidate to the study zone due to its tolerance to a variety of climates and moderate salinity, its high biomass and its resistance to drought.

• The combination of EDTA and IAA will allow the removal of a dangerous contaminant from soils surrounding agricultural areas, thus **ensuring human an environment's health security.**

• The possibility of a greater increment of Pb translocation from roots to leaves is given by increasing IAA's concentration in the last treatment of this experiment (200 µM IAA).

• Optimal results are expected, since an increase of **2800%** in leaves is reported in the treatment EDTA/100 µM IAA compared to those treated with Pb alone, and by about **600%** compared to those treated with Pb/EDTA.

Dissemination Plan



Belmonte F, Romero A, Alonso F, Moreno J (2010). *Contaminación ambiental por estériles Mineros en un espacio turístico en Desarrollo, la sierra minera de Cartagena-la unión (sureste de españa)*. Cuadernos de Turismo, nº 25; pp. 11-24; Belmonte F, Romero A, Alonso F, Moreno J, Rojo S (2010). *Afección de suelos agrícolas por metales pesados en áreas limítrofas a explotaciones mineras del sureste de España*. Papeles de Geografía, 51-52, pp. 45-54, ISSN: 0213-1781; Opeolu BO, Bamgbose O, Arowolo TA, Kadiri SI (2006). *Phytoremediation of Lead-contaminated soil using Amaranthus cruentus*. FAMAN J. 8(1): 58-63; Davies (1995); Adriano (1986); Peterson (1978); Lapèrche V (2000). *Immobilization of Lead by In Situ Formation of Lead phosphates in Soils*; in Skandar IK (2001). *Environmental Restoration of Metals-Contaminated Soils*. ISBN 1-56670-457-X.; Blaylock J, *Field Demonstrations of Phytoremediation of Lead-Contaminated Soils*. In Terry M, Bufuelos G (1999). *Phytoremediation of Contaminated Soil and Water*. ISBN: 1-56670-450-2.; Van Der Lelie D, Schwitzguébel JP, Glass D, Vangrosbeld J, Baker A, 2001. *Assessing Phytoremediation's Progress*. Environmental Science and Technology. November 1, 2001. pp: 441-463.; López M, 2007. *Evaluation of the effect of EDTA and Plant growth promoters on Lead uptake, translocation and oxidative response in Medicago sativa*. Center for Environmental Resource Management.; López M, 2005. *Enhancement of lead uptake by alfalfa (Medicago sativa) using EDTA and a plant growth promoter*. Chemosphere 61 (2005) 595–598.; Rodríguez JC (2006). *Capacidad de seis especies vegetales para acumular plomo en suelos contaminados*. Revista Fitotecnia Mexicana, Julio-Septiembre. Vol. 29, nº 003. pp: 239-245.; Chulsung K *et al.*, 2003. *Factors affecting EDTA extraction of Lead from Lead-Contaminated Soils*. Chemosphere 51. pp: 845-853.