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Effect of *Lupinus albus* L. root activities on As and Cu mobility after addition of iron-based soil amendments

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HIGHLIGHTS

- Addition of iron sulfate and liming products reduced As solubility in bulk soil.
- Slight As solubilisation was observed in the white lupin rhizosphere.
- Despite As stabilisation in the amended soils, no effect on plant As uptake was found.
- Arsenic dynamics in the rhizosphere seemed to be related to iron oxides dissolution.

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ABSTRACT

Arsenic and Cu mobility was investigated in the rhizosphere of *Lupinus albus* L. grown in an iron-amended contaminated soil. White lupin was grown in rhizobags in contaminated soil either left untreated or amended with iron sulphate plus lime (Fe + lime) or biochar (Fe + BC). Porewater was monitored in rhizosphere and bulk soil throughout the experiment and the extractable fraction of several elements and As and Cu plant uptake was analysed after 48 days. The distribution of As, Cu, P and Fe in the lupin rhizosphere was evaluated with chemical images obtained by laser ablation-ICP-MS analysis of diffusive gradients in thin films (DGT) gels. The treatments effectively reduced the soluble and extractable As and Cu fractions in the bulk soil, but they did not affect plant uptake. In all cases, soluble As was slightly enhanced in the rhizosphere. This difference was more pronounced in the Fe + lime-treated rhizosphere soil, where an increase of pH as well as extractable As and Fe concentrations were also observed. Chemical imaging of the lupin rhizosphere also showed slightly higher As- and Fe-DGT fluxes around lupin roots grown in the non-amended soil. Our findings indicate As and Fe co-solubilisation by lupin root exudates, likely as a response to P deficiency. Arsenic mobilisation occurred only in the rhizosphere and was not decreased by the amendments.

1. Introduction

Arsenic has been identified by the WHO as one of the pollutants of major public health concern (WHO, 2010). Although it is naturally present in soils, anthropogenic activities and inappropriate waste management have led to soil and groundwater contamination. Gentle remediation strategies, such as aided phytostabilisation may mitigate this problem by controlling trace element mobility and creating a dense vegetal cover (Alvarenga et al., 2009). Iron oxides have been proposed as appropriate amendments for As-impacted soils, as they generally stabilise As in soil (Hartley et al., 2004; Kumpiene et al., 2006, 2012). The addition of carbonaceous materials, such as biochar, has shown contrasting results regarding As mobility, but great capacity to immobilise metals (such as Cd, Cu, Pb and Zn) and improve soil quality (e.g. fertility and water holding capacity) (Beesley et al., 2014; Beesley and Marmiroli, 2011; Forján et al., 2016).

Mitigating contaminant transfer to the food chain is a priority objective of any remediation strategy and the behaviour of

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contaminants in the rhizosphere, especially of crop plants, should be evaluated. Plants may induce changes in their rhizosphere for nutrient mobilisation by processes such as acidification/alkalinisation, exudation of organic acids and other complexing compounds as well as variations of the redox potential (Hinsinger, 2001), all of which substantially alter element mobility in the rhizosphere (Tao et al., 2003; Puschenreiter et al., 2005; Gonzaga et al., 2006; Obeidy et al., 2016). Although rhizosphere processes are likely to be important in controlling the success of phytoremediation treatments, research on aided phytostabilisation strategies often focused on the efficiency of immobilising agents in bulk soil, without considering the fate of As in the rhizosphere.

Lupinus albus L. (white lupin) can be used in phytostabilisation of metal(iod) contaminated soils due to its metal-excluding behaviour and its tolerance to highly polluted and acidic soils (Vazquez et al., 2006; Martínez-Alcalá et al., 2010, 2012). White lupin is highly effective in enhancing phosphorus solubility and availability by releasing large amounts of organic anions, such as citrate and malate, into the rhizosphere, that can desorb P or solubilise sparingly soluble P compounds (Dinkelaker et al., 1989; Neumann, 2000). Due to the similar chemical behaviour of phosphate and arsenate in soils (Adriano, 2001), simultaneous solubilisation of P and As in the rhizosphere of *L. albus* can be expected.

This work aimed at evaluating the influence of *L. albus* roots on changes in As dynamics in a contaminated soil and its interaction with iron-based amendments. Since the tested soil is also contaminated with Cu, its dynamics were also investigated. We monitored changes in soluble and extractable fractions in bulk and rhizosphere soil and evaluated the distribution of labile elements in the rhizosphere by chemical imaging using diffusive gradients in thin films (DGT) sampling combined with spatially resolved analysis by laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS).

2. Materials and methods

2.1. Experimental design

Contaminated waste material was collected from spoil heaps in the surroundings of an old smelting factory located in the north of Madrid (Spain), which contains large amounts of arsenopyrite and scorodite (Recio-Vazquez et al., 2010). The contaminated material (sieved at <2 mm to facilitate mixing) was mixed with an uncontaminated soil collected from a close-by area (<4 mm) in a ratio 1:19 (contaminated:uncontaminated) to obtain a homogenous composite soil. The sandy-loam composite soil was slightly acidic (pH 5.4) and had high concentrations of As and Cu (2258 ± 625 and 157 ± 33 mg kg^{-1}, respectively). This *composite soil* will be referred to as *soil* henceforth to ease reading. The materials used in the experiment as amendments were commercially available $FeSO_4 \cdot 7H_2O$ and $CaCO_3$ (Panreac, Barcelona, Spain) and biochar produced by pyrolysis of holm oak chips at 600 °C (Moreno-Jiménez et al., 2016). Some characteristics of soil and biochar are shown in Table S-1 (supplementary material). The soil was mixed with the corresponding amount of amendments, calculated on a dry soil weight basis. The mixtures were manually homogenised, resulting in the following treatments: (1) Control, consisting of the non-amended soil; (2) $FeSO_4$ (1%) + $CaCO_3$ (0.37%) (Fe + lime) and (3) $FeSO_4$ (1%) + $CaCO_3$ (0.15%) + biochar (3%) (Fe + BC). Each treatment was replicated 4 times. The amount of $CaCO_3$ added to Fe + BC was calculated to equal the amount of $CaCO_3$ added to Fe + lime, considering the total carbonate content of biochar. Soils were moistened to 70% of the water holding capacity (WHC) and left to equilibrate for 15 days.

A rhizobag system was used to differentiate between rhizosphere and bulk soil. The rhizobags consisted of a 13 × 6.5 cm (height × diameter) cylindrical methacrylate structure covered with a nylon mesh with 30 μm pore size. Each rhizobag was placed in the centre of a plastic pot and was filled with 600 g of soil; the rest of the pot was filled with the same soil up to a total of 3.5 kg. Rhizon samplers (Rhizosphere Research Products, Wageningen, Netherlands), consisting of a porous polymer tube, were used to obtain soil porewater from rhizosphere and bulk compartments by applying suction with a syringe. Rhizon samplers of 5 and 10 cm length were inserted inside and outside each rhizobag, respectively, at an inclination of 45°. One 7-day-old seedling of *L. albus* (cv. Marta), pre-grown in peat, was placed in each rhizobag. Plants were grown under controlled conditions (day/night: 13/11 h, 40/60% humidity and a photon flux density of 520 μmol m^{-2} s^{-1}) for 48 days. All soils were kept at 70% WHC during the experiment by weighing. Soil porewater was sampled every 2 weeks after planting by applying suction to the rhizon samplers for 24 h, pH was immediately measured and samples were filtered through 0.45 μm syringe filters subsequently (Teknokroma, Barcelona, Spain).

At harvest, rhizobags were separated from the pots for collecting rhizosphere soil. The remaining soil in the pots was considered bulk soil. The nylon mesh that covered the rhizobags was removed, and the rhizosphere soil was separated from roots by gentle shaking. Both rhizosphere and bulk soil were homogenised and air-dried. Plants were separated into shoots and roots and fresh weights were recorded. Roots were washed with tap and deionised water and sonicated for 3 min to remove soil particles. Then shoots and roots were frozen at −80 °C. Plant material was ground with liquid N_2 using a mortar and pestle and subsamples were dried at 65 °C for 3 days.

2.2. Plant and soil analysis

Ground and dried plant material (0.2 g) was digested with 4 mL of HNO_3 (65% v/v) and 1 mL of H_2O_2 (30% v/v) in an autoclave under a pressure of 1.5 kg cm^{-2} for 30 min (Lozano-Rodríguez et al., 1995), made up to 10 mL with ultrapure water (type I reagent grade, ρ = 18.2 MΩ cm^{-1}) and analysed by ICP-MS (Elan 9000 DRCe, PerkinElmer).

The non-specifically adsorbed element fraction in bulk and rhizosphere soils was determined by extraction with 0.1 M $(NH_4)_2SO_4$ (1:10 w:v, shaken at 140 rpm in a horizontal shaker for 4 h) (Wenzel et al., 2001; Vázquez et al., 2008). Arsenic was analysed in these extracts by hydride generation-atomic fluorescence spectroscopy (HG-AFS) and Cu and Fe by atomic absorption spectroscopy (AAS, AAnalyst 800 Perkin Elmer). P-Olsen was measured in 1:20 (w:v) $NaHCO_3$-extracts of bulk and rhizosphere soil by ICP-OES (ICAP 6500 DUO, Thermo Scientific). The concentration of As, Cu, Fe and P in soil porewater samples was measured by ICP-MS (Elan 9000 DRCe, PerkinElmer) and dissolved organic carbon (DOC) was analysed with a TOC analyser (Shimadzu TOC-V CSH). For quality assurance, an appropriate number of blank samples were included in sample analyses and quality control standards were analysed through the measurement.

2.3. Rhizotron experiment and rhizosphere chemical imaging

A rhizotron experiment was set up in order to evaluate the distribution of elements in the rhizosphere of white lupin grown in the non-amended soil. The rhizotrons, consisting of flat perspex boxes with inner dimensions of 40 × 10 × 1.5 cm and a front plate that could be removed, were carefully filled with sieved (<2 mm) contaminated composite soil (1:9 contaminated:uncontaminated) in order to obtain a very fine, flat soil surface, which was covered with a piece of 10 μm thick polycarbonate filter membrane (pore