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Metal Availability and Chemical Properties in the Rhizosphere of *Lupinus albus* L. Growing in a High-Metal Calcareous Soil

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Abstract Chemical processes in the rhizosphere play a major role in the availability of metals to plants. The objective of this study was to assess the potential of white lupin (*Lupinus albus* L.) for the phytoimmobilisation of heavy metals in a calcareous soil with high levels of Zn and Pb (2,058 and 2,947 μg g^{-1}, respectively) by evaluating the chemical changes in the rhizosphere, relative to bulk soil, which modify the solubility of heavy metals. Plants were cultivated for 74 days in specially designed pots (rhizopots) in which rhizosphere was sampled easily under controlled conditions. White lupin accumulated high concentrations of Mn in the shoots (average of 4,960 μg g^{-1}), well above the normal concentration in plants (300 μg g^{-1}). But the metal concentrations found in shoots were not at toxic levels. Rhizosphere soil showed a significantly greater redox potential (245 mV) and water-soluble organic carbon content (34.6 μg C g^{-1}) than bulk soil (227 mV; 27.6 μg C g^{-1}). Root activity decreased EDTA-extractable Pb, Zn and Fe and promoted their precipitation as insoluble compounds in the residual fraction (acid digestion), hardly available to plants. These results indicate the suitability of this annual N_2-fixing species for the initial phytoimmobilisation of heavy metals in contaminated soils.

Keywords Heavy metals · *Lupinus albus* · Phytoimmobilisation · Rhizosphere · Soil contamination

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1 Introduction

Soil pollution by heavy metals is a worldwide problem arising mainly from anthropogenic sources such as mining, industry and agriculture. Soil pollution exists where, due to human activities, a substance is present at a concentration above the natural (background) level and has a negative impact on some or all the constituents of the environment (Knox et al. 1999). Due to the mining activity carried out in the "Sierra Minera" of La Unión (Murcia, Spain) since Roman times, an area of 216 ha has been affected by the accumulation of metalliferous mine waste, meaning an environmental risk for the population and the ecosystem of the area. This previous mining activity still affects the nearby agricultural soils (Clemente et al. 2007) due to the transport of fine particles by the wind.

Phytoremediation is a technology that uses the combination of plants, soil amendments and agro-

nomical techniques for in situ treatment of contaminated soils, sediments and water. Salt et al. (1998) defined phytoremediation as the use of green plants to remove pollutants from the environment or render them harmless. Specifically, phytoimmobilisation refers to the use of plant roots to retain the contaminants in the rhizosphere through their immobilisation in the soil (Wenzel et al. 1999).

The term *rhizosphere* designates the zone of enhanced microbial abundance in the soil surrounding plant roots (Hinsinger et al. 2003). Root-induced changes in soil biochemical, physical and chemical properties of the rhizosphere can considerably affect solubility and availability of mineral nutrients (Hinsinger et al. 2003; Marschner 1983; Tao et al. 2003). Soil-plant interactions in the rhizosphere determine metal speciation, transformation, uptake and accumulation in plants, this determining overall metal bioavailability (Wenzel et al. 1999). Metal availability to plants also depends on the capacity of different plant species to mobilise soil metals via a wide range of rhizosphere process: acidification, redox processes, exudation of organic compounds, complexation and desorption of metals from the soil exchange complex (Marschner et al. 1987). Numerous organic anions released in the rhizosphere by plant roots have been reported to play a role in the mobilisation or immobilisation of mineral nutrients or undesirable elements due to their complexing properties (Jones and Darrah 1994).

Much research work has been focused on the rhizosphere to address root-induced processes concerning metal bioavailability. Puschenreiter et al. (2005) suggest that the exudation of organic compounds from roots of the Ni hyperaccumulator *Thlaspi goesingense* affects the different fractions of Ni in soil, except the adsorbed fractions, through interactions with the soil solid fraction, facilitating Ni uptake. Tao et al. (2003) showed changes in Cu speciation in the rhizosphere of maize resulting from root-induced decreases in redox potential and increases in pH, dissolved organic carbon and microbial activities, all favouring a transformation of copper from less available to more available fractions in the rhizosphere. Loosemore et al. (2004) found that the uptake of Zn by tobacco plants was limited by the concentration of exchangeable Zn in the rhizosphere soil, which was pH-dependent. Therefore, the initial soil pH and the ability of plant roots to change pH in the rhizosphere were of prime importance for predicting the exchangeable Zn and therefore Zn bioavailability.

White lupin (*Lupinus albus* L.) is a leguminous plant that is adapted to environments of low pH (Huyghe 1997), low nutrients availability (Marschner et al. 1987), high salinity, excess nitrate (Hernández et al. 1999) and high soil metal content (Pastor et al. 2003; Ximénez-Embún et al. 2002; Zornoza et al. 2002). All these attributes make this species an excellent candidate for the phytoremediation of polluted soils, which often combine these adverse conditions for plant growth. In addition, white lupin develops cluster roots to improve the acquisition of nutrients, particularly P (Johnson et al. 1996). Chemical changes in the rhizosphere of cluster roots include soil acidification, reduction of Fe III and Mn IV, release of organic acids and phenolic compounds and precipitation of salts in calcareous soils (Dinkelaker et al. 1989; Jung et al. 2003; Marschner et al. 1989). Sas et al. (2001) showed some evidence of cluster roots containing higher concentrations of Mg, Ca, N, Fe and Mn, indicating higher efficiency in the absorption of these ions by cluster roots relative to non-cluster roots. In a field experiment in a contaminated acidic soil, Vázquez et al. (2006) found that white lupin accumulated Cd and As in the roots, and they highlighted the usefulness of this species for phytostabilisation due to its beneficial effects on soil properties, particularly an increase in soil pH and decreased Cd and As solubility.

We wished to extend our knowledge of the influence of white lupin plants on soil heavy metals to include non-acidic soils. Accordingly, in this work, we have studied how the chemical properties of the rhizosphere of white lupin affect metal speciation, and thus availability in a calcareous soil with high Zn and Pb concentrations, with the objective of confirming a potential role in phytoremediation for this species.

2 Materials and Methods

2.1 Soil Characteristics

Soil was collected from an agricultural area (37°38′45″ N, 00°50′50″ W) situated 2 km north of the nearest former Pb–Zn mining site of the "Sierra Minera", La Unión (Murcia, SE Spain). The soil was collected from the top 20 cm, air-dried for 5–6 days and sieved to

<2 mm prior to analysis (Section 2.3). The soil was a calcareous sandy loam, classified as Xeric Torriorthent, with 15% $CaCO_3$, pH 7.8 (H_2O), 0.6% organic matter, 391 (meq H^+ kg^{-1})/0.5 pH buffer capacity, 2.4 μg g^{-1} available P and total metal concentrations of (μg g^{-1}): 2,058 Zn, 2,947 Pb, 24.1 Ni, 29.6 Cu and 3 Cd. The soil total concentrations of Pb and Zn exceed greatly the European Union maximum permitted levels for agricultural soil (300 μg g^{-1} for both Pb and Zn at soil pH 7; Council of the European Communities 1986).

2.2 Experimental Design

The rhizopots (Fig. 1) were designed on the basis of those described by Tao et al. (2003) and consisted of two compartments, a 7.5-cm diameter pot and a polyvinyl chloride cylinder placed on the top of the pot and separated by a nylon monofilament gauze with a 200-μm pore size (Sefar Inc., Switzerland). The upper compartment holds the soil (170 g) where plant roots grow (Fig. 1), and the lower part was filled with the bulk soil (120 g). Plant roots in the upper part were unable to penetrate the mesh, so when a root mat was developed at the bottom of the cylinder, a 2-mm-thick layer of soil was placed between the two compartments, separated from the bulk soil by a second nylon gauze; this was considered the rhizosphere soil.

Seeds of white lupin (*L. albus* L. cv. Marta) were surface-sterilised with 10% HClO for 30 min, washed three times with distilled water and then germinated in a plastic container, on filter paper moistened with 0.5 mM $Ca(SO_4)_2$, in an incubator at 28°C for 3 days. Two seedlings were planted in each rhizopot and were maintained in a growth chamber with a light/dark regime of 14/10 h, temperature of 23/18°C (day/night) and relative humidity of 50/70%. Plants were watered with deionised water from the base of the rhizopots using a dripping tray. Fertiliser was not applied.

Fig. 1 The rhizopot used in the experiment. Rhizosphere soil was considered the 2-mm layer of soil placed between two nylon meshes separating the upper cylinder and the lower pot

Three replicates were run, made from a total of 60 rhizopots distributed in three trays: 20 rhizopots were pooled to give one replicate. After 57 days, when a complete development of the root system filled the bottom of the upper part, the 2-mm layer of soil was added. Fourteen days later (74 days of growth), the rhizosphere soil (from 20 rhizopots growing in the same tray) was sampled, as was the bulk soil from the bottom part of the rhizopot. Plants were harvested (20 per replicate), separating roots from the shoot. Shoots were washed with distilled water and roots were first washed with tap water, then with distilled water under sonication (7.5 min) to remove soil particles and finally with 0.1 mM $SrCl_2$ for 30 s to remove adsorbed metals on the root surface (Lutts et al. 2004; Walker et al. 2007). Fresh and dry (70°C) weights of roots and shoots were determined before being ground for analysis. Only roots growing at the bottom of the upper part of the rhizopot, in contact with the rhizosphere soil, were used for chemical analysis, since they were easy to clean as very few soil particles adhered to them.

2.3 Analytical Methods

Plant and soil pseudo-total heavy metals (Cu, Fe, Mn, Pb, Zn) were determined by flame atomic absorption spectrometry (AAS) in a UNICAM 969 atomic absorption spectrometer (Thermo Elemental, UK) after nitric-perchloric acid (2:1) digestion to a maximum of 210°C for at least 2 h (Abrisqueta and Romero 1969). Sequential extraction of soil metals (McGrath and Cegarra 1992) had the following steps: 0.1 M $CaCl_2$ for 16 h (1:10, *w*/*v*), metals in soil solution and in exchangeable forms; 0.5 M NaOH (1:10, *w*/*v*) for 16 h followed by aqua regia digestion of the extract, metals associated with organic matter (OM); 0.05 M Na_2H_2EDTA (1:10, *w*/*v*) for 1 h, metals mainly in the carbonate fraction; acid digestion with aqua regia, residual metals.

Soil electrical conductivity was measured in a 1:5 water suspension (*w*/*v*). The pH and the redox

potential (E_h) were determined in saturated soil pastes. The total N (N_T) and organic C (TOC) concentrations of the soil and the plant total N were measured with a EuroVector automatic microanalyser (Eurovector, Milan, Italy). The OM content of the soil was determined by multiplying TOC by 1.72. Soil available P was determined colorimetrically after extraction with 0.5 M $NaHCO_3$ at pH 8.5. Soil microbial biomass C (fumigation-extraction procedure, Vance et al. 1987) and water-soluble organic carbon was determined, after a 1:10 water extraction (*w*/*v*) for 2 h, in a TOC/Skalar analyser. Biomass N, calculated from the difference between ninhydrin-reactive N in fumigated and non-fumigated soil extracts, was also determined (Joergensen and Brookes 1990). Total P in plants was determined colorimetrically as molybdovanadate phosphoric acid after nitric-perchloric acid digestion (210°C, 2 h). Inorganic anions (Cl^-, NO_3^-, $H_2PO_4^-$, SO_4^{2-}) were determined in the plants by ion chromatography after water extraction (1:200, *w*/*v*). Chemical analyses were done at least in duplicate. All metal concentrations, adjusted to values for oven-dried (12 h at 105°C) soil, were determined by flame AAS in a UNICAM AA spectrometer.

2.4 Statistical Analysis

All data (n=3) from the experiment were analysed by one-way analysis of variance (ANOVA) using SPSS version 14.0 software. Differences between means were determined using Tukey's test at a probability level of $P<0.05$.

3 Results and Discussion

3.1 Plant Composition

The growth of white lupin was evaluated by the fresh and dry weights per pot (two plants) of the aerial plant parts (5.273±0.133 and 1.069±0.013 g, respectively) and for the roots (2.585±0.137 and 0.438±0.019 g, respectively). Although previous results indicated that high soil pH affects negatively the growth of this species (Pastor et al. 2003; Castaldi et al. 2005), in the present experiment with a soil pH of 7.8, the plants were developed adequately, although they showed some visual symptoms of chlorosis at harvest. However, the concentrations of Fe found in this experiment for aerial part and roots (146±95; 2117±630 μg g^{-1}, respectively) were similar to those found by Kerley (2000) (80–150 μg g^{-1}) and Shane et al. (2008) (80–350 μg g^{-1}) in non-deficient lupin shoots, with higher values in roots than in shoots (Fig. 2a). Iron deficiency is usually the major cause of lime-induced chlorosis through uptake inhibition by HCO_3^- (Chaney et al. 1992). Organic acids such as citrate and malate are released by proteoid roots to mobilise Fe in the rhizosphere to avoid Fe deficiency (Jones 1998).

The concentrations of Mn were higher in shoots than in roots (Fig. 2a). The transport from root to shoot was therefore not inhibited, allowing the accumulation of very high concentrations in leaves, more than 5,000 μg g^{-1}. Although the Mn deficiency level for most plants ranges from 10 to 25 μg g^{-1} (dry weight, dw), the toxic tissue concentration of Mn is more variable depending on both plant species and

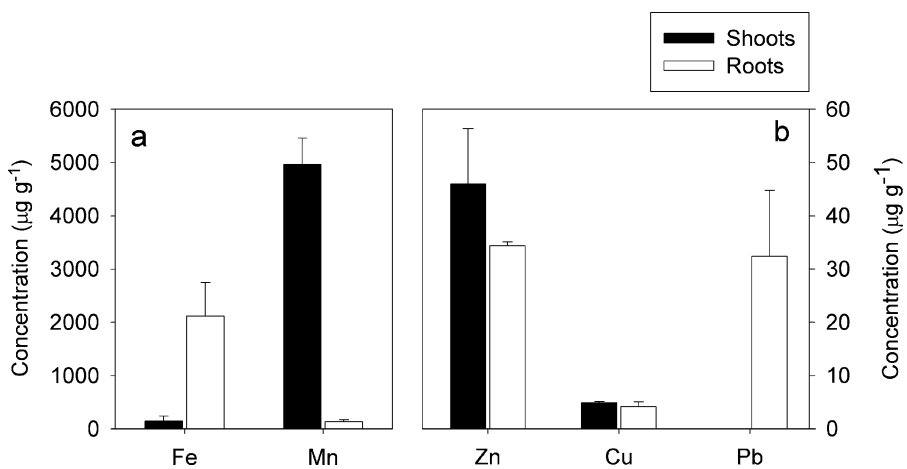

Fig. 2 Heavy metal concentrations in shoots and roots of white lupin (μg g^{-1}): **a** Fe and Mn, **b** Zn, Cu and Pb (n=3). ***$P<0.001$, **$P<0.01$, *$P<0.05$

soil factors (Kabata-Pendias 2001; Marschner 1995). White lupin shoots have been found to accumulate up to 6,100 μg g^{-1} dry weight (Kerley 2000) without showing any chlorosis symptoms. According to Page et al. (2006), Mn is transported in white lupin from the main root to the oldest leaves where it is accumulated. That distribution suggests that Mn is rapidly released from the roots into the xylem and reaches photosynthetically active leaves via the transpiration stream; no redistribution of Mn to other leaves was observed by Page et al. (2006). The slight visual symptoms of chlorosis found in our experiment could be due to a low Fe/Mn ratio in shoots (0.032). A reduced Fe/Mn ratio was associated with inhibited growth of *Brasicca juncea* plants (Walker et al. 2003), and Poschenrieder and Barceló (1981) found that the appearance of Fe deficiency symptoms in *Phaseolus vulgaris* was induced by high Mn levels, without any effect on Fe concentration, and that Fe/Mn ratio <0.1.

The concentration of Zn was slightly higher in shoots (46±10 μg g^{-1}) than in roots (34±1 μg g^{-1}; Fig. 2b), although the values are within the range for normal plant concentrations (20–150 μg g^{-1}; Macnicol and Beckett 1985; Kabata-Pendias 2001). Similar values were found in white lupin shoots by Kerley (2000) (40–70 μg g^{-1}) with no apparent detrimental effects. Contrastingly, other authors have found that Zn taken up by this species is accumulated mainly in the roots (Castaldi et al. 2005; Zornoza et al. 2002). The concentrations of Cu in roots were not statistically different from those in shoots (Fig. 2b), in agreement with previous results (Sas et al. 2001). The concentrations can be considered sufficient for plant growth (5–30 μg g^{-1}), below toxic levels (Kabata-Pendias 2001). Lead was accumulated in roots (Fig. 2b), typical behaviour of excluder species (Castaldi et al. 2005; Przymusiński et al. 2001; Zornoza et al. 2002), which restrict uptake and transport of elements between the root and shoot, maintaining low shoot levels over a wide range of external concentrations (Baker 1981). The concentration of Pb in shoots was below the detection limit (<0.5 μg g^{-1}), reflecting the low mobility of Pb in soils and plants (Kabata-Pendias 2001).

White lupin is a leguminous species with the ability to fix atmospheric N_2 (Page et al. 2006), and this fixation can be affected by environmental factors such as heavy metals in the soil (Castro and Ferreira 2006). Similar N concentrations (Table 1) were found by Dinkelaker et al. (1989) in shoots of lupin plants grown in a sterilised calcareous soil fertilised with NO_3–N (13.2 mg g^{-1}) and by Sas et al. (2001) in roots (17–18 mg g^{-1}). Root nodules associated with N_2-fixing bacteria were not found in our plants. However, the tissue N levels suggest that the decrease or absence of N_2 fixation did not result in tissue N deficiency. Phosphorus concentration in shoots was low compared to results found in non-contaminated soils by Kerley (2000) and Pastor et al. (2003), which ranged from 1 to 2.6 mg g^{-1} in an alkaline and an acid soil, respectively. The concentration of available P in the soil of the present experiment (2.4 μg g^{-1}) was lower than the values found by Dinkelaker et al. (1989) (18 μg g^{-1}). In general, the P requirement for optimal plant growth is in the range of 3–5 mg g^{-1} of dry matter (Marschner 1995). However, in an experiment with a non-contaminated calcareous soil (20% $CaCO_3$), Dinkelaker et al. (1989) found low levels of P in shoots (0.7 mg g^{-1}) and root (0.4 mg g^{-1}) of white lupin, close to those found in the present experiment (Table 1). In that experiment, a large amount (23% of total plant dry weight) of citrate was excreted into the rhizosphere by proteoid roots as response to P deficiency, but precipitation of calcium citrate was observed and plants could not take up enough P. The low P concentrations that were found in the plants of the present experiment (Table 1) could be due to the highly calcareous nature of the soil, leading to low P availability and thus stimulating citrate exudation by the roots. However, a white

Table 1 Nutrient (mg g^{-1} dry weight) and anion (μg g^{-1} dry weight) concentrations in the plants (n=3)

	Shoots	Roots	ANOVA
N	14±1.3	11±0.2	*
P	0.63±0.01	0.41±0.07	*
K^+	10.2±0.5	6.1±0.1	***
Ca^{2+}	11.7±1.5	12.8±3.2	NS
Mg^{2+}	1.5±0.0	3.8±1.0	*
Na^+	1.1±0.0	3.8±0.6	*
Cl^-	1143±9	3536±138	***
NO_3^-	202±45	105±9.7	*
$H_2PO_4^-$	1380±64	605±7.3	***
SO_4^{2-}	2726±318	1547±62	**

NS not significant

***P<0.001, **P<0.01, *P<0.05

precipitate of calcium citrate was not visible around the cluster roots.

The Mg and K requirements for optimal plant growth are within the range 1.5–3.5 mg g^{-1} and 20–50 mg g^{-1} in the dry matter of the vegetative parts, respectively (Marschner 1995). Although lower values occurred for K concentration in our plants (Table 1), the results were similar to those found by Kerley (2000) in shoots of white lupin grown in neutral and limed soil (11.9–12.4 mg g^{-1}), which were sufficient for this plant species. Calcium is one of the few elements to show an increased shoot concentration when plants are grown in limed or calcareous soil (Tyler and Olsson 2001). The concentrations were in the range found by Kerley (2000) in shoots: from 9.1 mg g^{-1} in neutral soil to 15.9 mg g^{-1} in a limed soil. The "normal" calcium content of plants varies between 1 and >50 mg g^{-1} of dry weight depending on the growing conditions, plant species and plant organ (Marschner 1995). Dinkelaker et al. (1989) observed that the precipitation of Ca by citrate secreted into the rhizosphere by cluster roots in a calcareous soil could restrict Ca uptake by the roots. The Ca/Mg ratio was 7.8 in the aerial part; high values were also found for this ratio by Pastor et al. (2003) in an alkaline soil. All these factors indicate that in our experiment, the plant nutrition was correct, with respect to the main nutrients.

Taking into account the dry weight and the inorganic cations and anions concentrations found in shoots and roots (Table 1), the cation-anion balance per plant was calculated. This showed an excess of cations (71.5±7.8 me/100 g, dw) with respect to inorganic anions (6.6±0.1 me/100 g, dw), leading to 64.9± 7.9 me/100 g (dw) cation excess which may be balanced by the presence of organic anions in the plant tissue (Sas et al. 2001). The difference in cations-anions was higher than that found by Dinkelaker et al. (1989) in a calcareous soil (44 me/100 g, dw). Differences in cation/anion uptake by roots have to be compensated for stoichiometric changes through the release of H^+ and OH^- or HCO_3^- from the roots, which can lead to changes in soil pH at the rhizosphere (Marschner 1983). In *L. albus*, the H^+ release as a response to excess cation uptake (Tang et al. 1997) results in acidification of the rhizosphere (Haynes 1990).

3.2 Chemical Properties of the Bulk Soil and Rhizosphere

The pH value in the rhizosphere was not statistically different from that of the bulk soil (Table 2). Although the cation/anion balance indicated a release of H^+ in the rhizosphere, acidification was not observed. This could be due to the high buffer capacity of this calcareous soil (391 meq H^+ kg^{-1}/0.5 pH; De la Fuente et al. 2008). Considering the proportion of dry weight of roots (0.438±0.019 g) in contact with the rhizosphere soil (5 g), the cation/anion balance and the buffer capacity of the soil, the protons released would not have been sufficient to reduce the soil pH of the rhizosphere (Jones and Darrah 1994).

Compared to the bulk soil, small but significant increases were detected for E_h in the rhizosphere (Table 2), which indicated more oxidant conditions in the rhizosphere than in the bulk soil. Jung et al. (2003) found that the redox potential of the nutrient solution increased during the 35 days of experiment in which white lupin plants were grown in the presence of Cu: under Mn- and Fe-deficient conditions, lupin root exudates increased the reductive potential for the chemical mobilisation of nutrients in the rhizosphere. But, in the present case, as there is an excess of these elements, maybe increasing the

Table 2 Soil properties in rhizosphere and bulk soil (n=3): pH, redox potential (E_h) water-soluble organic C, biomass C (B_C) and biomass ninhydrin reactive N (B_{NIN})

	pH	E_h (mV)	Water-soluble Org C (μg C g^{-1})	B_C (μg C g^{-1})	B_{NIN} (μg N g^{-1})
Rhizosphere	7.93±0.03	245±4	34.6±3.22	102±18.2	6.3±1.61
Bulk soil	7.86±0.05	227±6	27.6±2.68	86±13.7	5.5±0.40
ANOVA	N.S.	*	*	N.S.	N.S.

NS not significant

*P<0.05

oxidative nature of the conditions favoured metal immobilisation in order to avoid toxicity.

Water-soluble organic C was significantly higher in the rhizosphere than in the bulk soil, reflecting the activity of the roots (Table 2). This can be a consequence of the great amount of organic compounds released into the rhizosphere by diverse root exudates (Dinkelaker et al. 1989; Jung et al. 2003; Neumann et al. 1999; Shu et al. 2005) together with respired CO_2 (Mengel and Kirkby 2001). Roots of white lupin release several organic compounds, such as citric and malic acids, isoflavonoids and polyuronic acids (Dinkelaker et al. 1989; Neumann et al. 2000; Weisskopf et al. 2006). Soluble and high-molecular-weight phenols were exuded by lupin roots in the presence of high levels of Cu in a nutrient solution, which could chelate Cu, thus restricting Cu toxicity to plants (Jung et al. 2003).

Microbial biomass C and ninhydrin N in the rhizosphere were higher than in the bulk soil, but the values were not statistically different (Table 2). Increased microbial biomass can be found in the rhizosphere due to the exudation of organic compounds from the roots (Tao et al. 2003; Wenzel et al. 2001), which serves as a C source for the microorganisms to obtain energy.

The sequential extraction of soil heavy metals showed very low concentrations in soluble and exchangeable forms ($CaCl_2$-extractable) due to the calcareous character of the soil (Fig. 3). The values were always <0.05 μg g^{-1} for Fe, Mn and Pb, 0.34±0.06 and 0.18±0.09 μg g^{-1} for Zn, and 0.30±0.16 and 0.36±0.07 μg g^{-1} for Cu in rhizosphere and bulk soil, respectively. No differences were found between metal concentrations in this fraction between the bulk and rhizospheric soils. This soil has a high pH (≈8) value which makes the metals hardly soluble: De la Fuente et al. (2008) did not find an increase in metal solubility ($CaCl_2$ extraction) after decreasing soil pH by 0.5 units. The increase in water-soluble organic C in the rhizosphere through root exudation would be expected to increase the soluble organo-metallic chelates (Mench and Martin 1990). However, soluble and exchangeable metals ($CaCl_2$-extractable) did not increase in the rhizosphere. The organic compounds exuded from roots interact with soil components in several ways (Jones 1998): (a) they can be quickly degraded in soil; (b) they can become rapidly and readily sorbed to the soil solid phase (clay fraction and, especially, Fe oxides) and (c) root-derived organic acids can alter the molecular weight of humic acids (Canellas et al. 2008). Therefore, soluble organic compounds released from roots, which are not degraded, could be fixed in the soil clay fraction and Fe oxides, avoiding their solubilisation in water and thus making necessary an extraction with a stronger reagent, such as NaOH, able to disperse the organic matter and carry the adsorbed/complexed metals into suspension (Beckett 1989). In fact, the concentrations of NaOH-extractable Cu (Fig. 3d) and Pb (Fig. 3e) were higher in the rhizosphere than in the bulk soil. These elements can form chelates with soil organic matter, especially humic substances (fulvic and humic acids; Piccolo 2001). Jung et al. (2003) concluded that white lupin releases polyphenolic compounds as a response to Cu-imposed physiological stress, and they proposed that the complexation of Cu^{2+} ions in the rhizosphere by phenolic compounds could alleviate Cu toxicity.

The concentration of EDTA-extractable Cu was decreased in the rhizosphere of white lupin compared to the bulk soil (Fig. 3). This decrease was parallel to an increase in the organic fraction (NaOH-extractable Cu) in the rhizosphere. Metals bound to carbonates could, under certain circumstances (e.g. soil acidification), become available, and for micronutrients such as Cu, the uptake by plants can lead to a depletion in the carbonate fraction (Tao et al. 2003). The high decrease in the EDTA-extractable fraction for Fe, Zn and Pb (Fig. 3) cannot have been due only to the plant uptake (Fig. 2), so these metals should have been immobilised in the rhizosphere soil as a residual, non-extractable fraction. Besides its strong dependency on pH, the solubility of iron oxihydroxides and iron oxides is very much dependent on the redox conditions (McBride 1987). In the rhizosphere, the redox potential increased, so the conditions were more oxidant than in the bulk soil. The increase in redox potential in the rhizosphere could have led to a subsequent immobilisation of ferrous Fe by oxidation and precipitation as iron oxyhydroxide, decreasing the EDTA-extractable fraction. Such precipitation can minimise the uptake of potentially toxic metals. It is well known that metals are generally less soluble in oxidised conditions (Kabata-Pendias 2001). The reduction in the extractable fraction of Zn and Pb may be due to their fixation in Fe oxides through sorption and particularly co-precipitation processes

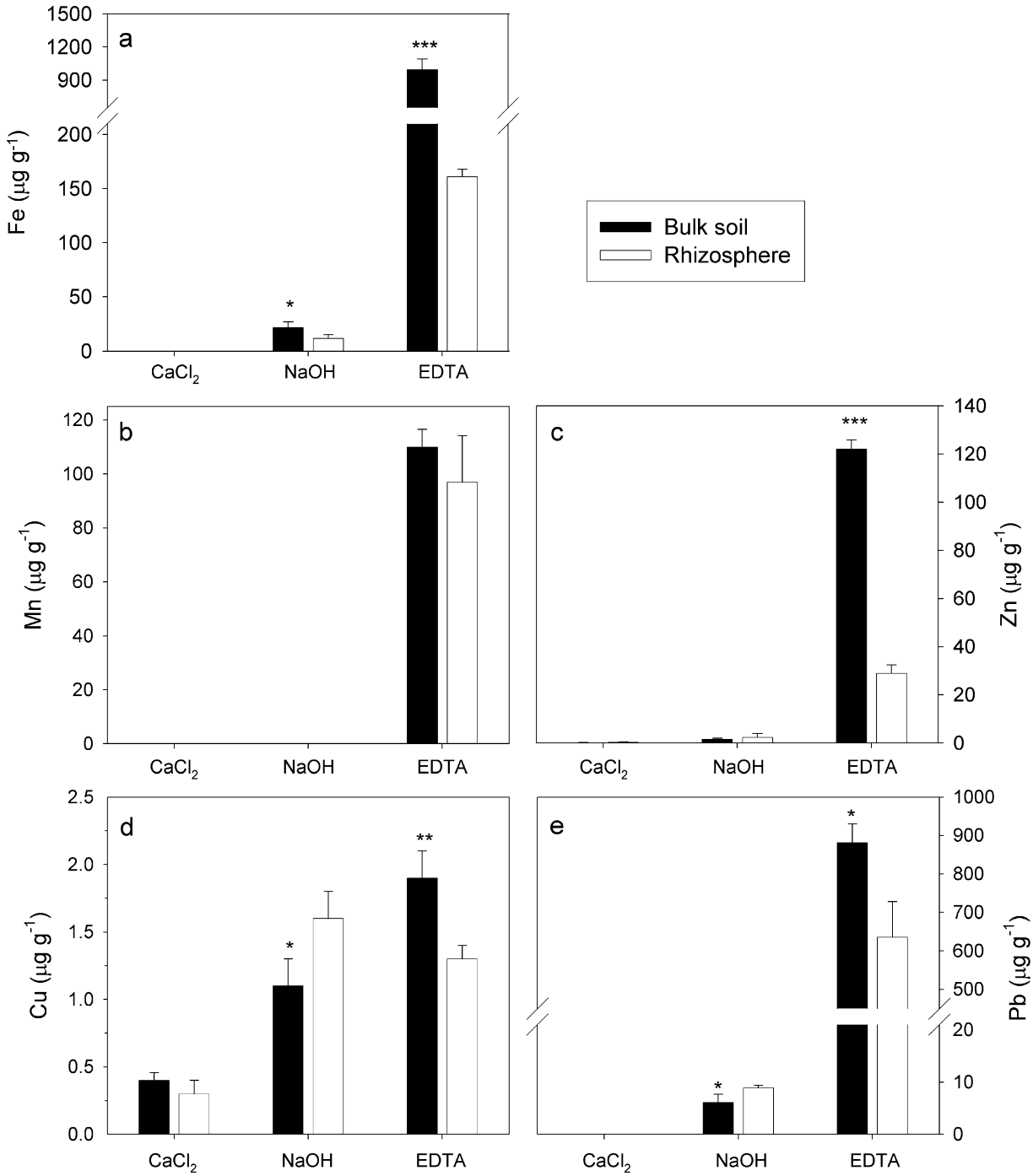

Fig. 3 Heavy metal fractionation by sequential extraction of Fe (**a**), Mn (**b**), Zn (**c**), Cu (**d**) and Pb (**e**) in the bulk soil and rhizosphere of white lupin (n=3). $CaCl_2$: Metals in soil solution and in exchangeable forms. NaOH: Metals associated with OM. EDTA: Metals mainly in the carbonate fraction

(Ross 1994), thus becoming hardly extractable with EDTA. The major benefit of root-induced oxidation for the plants is the detoxification of the root environment through a decrease in the concentration of ferrous Fe (Marschner 1995).

No significant changes were detected in Mn fractions between the rhizosphere and the bulk soil. The concentrations of the $CaCl_2$- and NaOH-extractable fractions were negligible (<0.5 μg g^{-1}), with detectable values only in the EDTA-extractable fraction. Similar results were found by De la Fuente et al. (2008) in the same soil during an incubation experiment under aerobic conditions using sulphur as an acidifying agent.

Dinkelaker et al. (1989) found an increase in the amount of available micronutrients, such as Fe, Mn and Zn, in the rhizosphere soil sampled near proteoid roots of white lupin, which were also shown to be the root zones responsible for intense excretion of citrate. These effects were a response to P deficiency of white lupin in that calcareous soil. In the present experiment, the metal availability was decreased in the rhizosphere in order to avoid toxic effects.

4 Conclusions

White lupin behaved as a Mn accumulator with higher concentrations in shoots than in roots, and as an excluder of Pb, which accumulated in roots, thus preventing its transport to shoots. The main chemical changes in the rhizosphere with respect to the bulk soil were the increase of both redox potential and organic C. The release of H^+ was not sufficient to decrease the soil pH in the rhizosphere of the well-buffered calcareous soil. The organic compounds released by the roots increased the OM-bound (NaOH-extractable) Cu and Pb in the rhizosphere due to the formation of stable chelates. But white lupin promoted the precipitation of Pb, Zn and Fe as insoluble compounds in the residual soil fraction, hardly available to plants in the rhizosphere, as a mechanism of metal tolerance, which may be due to the more oxidant conditions that occurred in the rhizosphere.

White lupin has shown an ability to fix heavy metals in the soil rhizosphere; this, together with improvement of the soil chemical properties due to its ability to incorporate atmospheric N_2 even in contaminated soils, makes it useful for in situ phytoimmobilisation of heavy metals in polluted soil. But as it is an annual crop, the fate of the contaminants accumulated in the plants, after harvesting and the subsequent degradation of the plant tissue remaining in/on the soil, should be taken into account for estimating the extent and efficiency of the metal phytoimmobilisation.

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