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Efficiency of a phytoimmobilisation strategy for heavy metal contaminated soils using white lupin

I. Martínez-Alcalá, R. Clemente *, M.P. Bernal

Departamento de Suelos y Agua y Manejo de Residuos Orgánicos, Centro de Edafología y Biología Aplicada del Segura, CSIC, Campus Universitario de Espinardo, 30100 Murcia, Spain

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ABSTRACT

White lupin (*Lupinus albus* L.) has been shown to be a useful plant species for phytoimmobilisation of heavy metal contaminated soils. However, since it is an annual species, the heavy metals taken up by the plant and stored in roots can be incorporated back into the soil during root degradation, after plants have been harvested. In this work the efficiency of metal immobilisation by roots of white lupin has been studied in three metal polluted soils (calcareous, neutral and acid) after collection of the aerial part of the plants through an incubation experiment using the intact roots colonised soil. The pattern of C mineralisation in the soil allowed the estimation of the soil microbial activity and the degradation of the root tissues that remained in the soil. The proportion of root tissue degraded in soil was from 47% to 61%, the highest value being found in the calcareous soil. Heavy metal amounts released into the soil after root degradation were very low in comparison with the total soil metal concentration. Soluble metal concentrations in the calcareous and neutral soils were not affected by root degradation, and in the acid soil, soil conditions had a greater effect on heavy metals fractionation than root degradation. These results confirm the feasibility of the use of *L. albus* for metal phytoimmobilisation techniques.

1. Introduction

"Green" technologies such as phytoimmobilisation are currently being developed for the remediation of metal polluted soils. This technique refers to the use of plant roots to retain the contaminants in the soil through their immobilisation in the rhizosphere (Wenzel et al., 1999). The use of such plants is useful to recover soil biological activity and to enhance the organic matter cycle in the soil. Phytoimmobilisation may hold the metals in the soil in non-available forms, reducing metals leaching to groundwater, erosion of soil material and contamination of surface waters. Several authors have suggested white lupin (*Lupinus albus*) as a good candidate for phytoimmobilisation of heavy metals in contaminated soils (Castaldi et al., 2005; Martínez-Alcalá et al., 2009; Page et al., 2006; Vázquez et al., 2006; Zornoza et al., 2002). This plant species shows characteristics of especial interest, like N_2-fixation and the adaptability to acid soils poor in nutrients (Carpena et al., 2003; Dinkelaker et al., 1989), to excess of nitrate and lime, and to high salinity and heavy metal concentrations (Hernández et al., 1999; Pastor et al., 2003; Ximénez-Embún et al., 2002; Zornoza et al., 2002).

White lupin takes up Pb, Cd, Zn and As from the soil solution accumulating them mainly in the roots (Castaldi et al., 2005; Martínez-Alcalá et al., 2009; Page et al., 2006; Vázquez et al., 2006; Zornoza et al., 2002). Page et al. (2006), in an experiment using radioactive heavy metals in nutrient solution, found that ^{109}Cd (75%), ^{57}Co (55%) and ^{65}Zn (33%) were mostly retained in the main root, and only 6.5% of total ^{54}Mn was found in the roots. Zornoza et al. (2002) demonstrated that lupin roots have a high Cd retention capacity in cell walls and trapped by thiol groups. Vázquez et al. (2006) reported the ability of white lupin for As and Cd phytoimmobilisation in the soil, decreasing their soluble fractions in soil due mainly to accumulation in plant tissues together with an increase in soil pH. According to Dessureault-Rompré et al. (2008), total dissolved metal concentrations in the rhizosphere of white lupin increased sharply during the exudative burst of citrate and decreased again after the cessation of citrate release. Martínez-Alcalá et al. (2009) found that the solubility of heavy metals decreases in the rhizosphere of lupin plants with respect to the bulk soil, this fact being associated to differing redox conditions and the interaction of exudated organic compounds with metals and soil components.

The rhizosphere is the narrow region of soil that is directly influenced by root secretions and associated soil microorganisms. It is full of bacteria that feed on sloughed-off plant cells (rhizodeposition) and proteins and sugars released by roots. Therefore, much of the nutrient cycling and disease suppression needed by plants occurs immediately next to roots. In this environment the heavy metals are a very important factor which influences and changes all other. Heavy metals

* Corresponding author at: Departamento de Suelos y Agua y Manejo de Residuos Orgánicos, Centro de Edafología y Biología Aplicada del Segura, CSIC, Campus Universitario de Espinardo, PO Box 164, 30100 Murcia, Spain. Tel.: +34 968396385; fax: +34 968396213.
E-mail address: rclemente@cebas.csic.es (R. Clemente).

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can also limit or even inhibit plant and microorganism's growth and development due to their toxicity.

Lupin is an annual crop, so the contaminants accumulated in roots could be discharged into the soil after the culture cycle, when roots decomposition occurs. Then the efficiency of white lupin for soil metal immobilisation should consider the dynamics of metals after plant cropping. An attempt to identify the re-mobilisation of metals in soil after degradation of plant roots was made by Vázquez et al. (2008) and Moreno-Jiménez et al. (2009) using artificial mixtures of soils and metal-loaded roots from plants grown in nutrient solution.

The aim of this work was to determine the efficiency of metal immobilisation by roots of white lupin grown in metal contaminated soils, through an incubation experiment with a realistic soil to plant roots proportion, where the degradation of plant roots in the soil and the release of metals to soil where determined.

2. Materials and methods

2.1. Soil characteristics

Three soils with different characteristics were selected: a calcareous soil (C) from an agricultural area situated 2 km north of the nearest former Pb–Zn mining site of the "Sierra Minera" (37º38′45″ N, 00º50′50″W), La Unión (Murcia, SE Spain). It is a sandy loam soil (Table 1) classified as Xeric Torriorthent, with 15% $CaCO_3$ and very low in organic matter. Soil total concentration of Pb and Zn exceeded greatly average concentrations of these metals in soils (27 and 70 μg g^{-1}, respectively; Kabata-Pendias, 2011).

The other two soils were collected from the area called "El Vicario" placed near Sanlúcar la Mayor (37º26′21″N, 06º13′00″W), Seville (Spain), 10 km downstream from the Aznalcóllar mine, which underwent a pyritic toxic spill in 1996 (Bernal et al., 2007). Both soils are non-calcareous loam classified as Typic Xerofluvent, with $CaCO_3$ content lower than 0.5%. One of them is acid (A) with pH 4.2, while the other is neutral (N) with pH 6.8, having similar total metal concentrations but different metal solubility (Table 1). Both soils had total Zn, Pb, Cu and Cd concentrations above average concentrations of these metals in soils (70, 27, 39 and 0.41 μg g^{-1}, respectively; Kabata-Pendias, 2011). The soils were collected from the top 20 cm, air-dried for 5–6 days and sieved to <2 mm prior to analysis.

A rhizopot system was designed (Martínez-Alcalá et al., 2009) and consisted of two compartments, a 7.5 cm diameter pot and a PVC cylinder of the same diameter (5 cm depth) 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 (140 g for the calcareous soil and 170 g for the non-calcareous soils) where plants grow, and the lower part was filled with the soil without contact with roots.

Table 1
Characteristics of the soils used in the experiment: Calcareous (C), Acid (A), and Neutral (N). EC: Electrical conductivity; TOC: Total organic-C.

Parameters	C	A	N
pH	7.8	4.2	6.8
EC (dS m^{-1})	0.29	2.71	2.22
OM (%)	0.7	1.9	1.9
TOC (g kg^{-1})	4.4	11.1	11.3
N_T (g kg^{-1})	0.6	1.3	1.3
Available-P (μg g^{-1})	2.4	34.9	38.3
Buffer capacity (me kg^{-1} pH^{-1})	782	49.6	63.8
Clay (%)	39.2	19.7	19.7
Silt (%)	27.4	34.3	34.3
Sand (%)	33.4	46.0	46.0
Fe (g kg^{-1})[a]	112 (<0.5)	44.4 (<0.5)	40.2 (<0.5)
Mn (μg g^{-1})[a]	5000 (<0.5)	798 (230)	906 (24.5)
Cu (μg g^{-1})[a]	29.6 (<0.5)	135 (4.75)	122 (<0.5)
Zn (μg g^{-1})[a]	2058 (<0.5)	364 (138)	400 (1.91)
Cd (μg g^{-1})[a]	3.00 (<0.5)	3.80 (0.63)	4.20 (<0.5)
Pb (μg g^{-1})[a]	2947 (<0.2)	291 (<0.2)	210 (<0.2)

[a] $CaCl_2$-extractable metal concentration (μg g^{-1}) in brackets.

2.2. Plant material

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 $CaSO_4$, in an incubator at 28 °C for three days. Two seedlings were planted in the upper part of each rhizopot and were kept in a growth chamber with a light/dark regime of 14/10 h, temperature of 23/18 °C and relative humidity of 50/70% (day/night). Plants were watered with deionised water from the base of the rhizopots using a dripping tray. Fertiliser was not applied.

Three replicates were run for each soil, each one consisting of 20 rhizopots: 4 of them were used in the incubation experiment and the other 16 were used for roots isolation and chemical analysis. After 74 days of growth, the aerial part of the plants was harvested; intact soil from the upper (soils with roots) and lower (soils without roots) compartments was used in the incubation experiment (4 samples per replicated soil). Roots were separated from the soil in the rest of the rhizopots, 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. Fresh and dry (70 °C) weights of roots were determined before being ground for chemical analysis.

2.3. Incubation experiment

The soils without roots from the lower parts of the rhizopots (C–R, A–R, N–R) and the soils with roots from the upper compartment (C + R, A + R, N + R) were placed unaltered in separated 500 ml incubation vessels. Deionised water was added to the soil samples in order to bring their moisture content to 60% of their water-holding capacity. The incubation vessels were closed, but to maintain adequate oxygen levels they were opened for several minutes every two days during the first week, and then weekly. The incubations were performed in the dark in a temperature-controlled incubator at 26 °C, for 56 days. The CO_2 evolved was trapped into a beaker containing a NaOH solution with the concentration and volumes required to retain all the CO_2 produced from the soil, which was placed on top of the soil in the incubation vessels. After two days during the first week and then weekly to 56 days, the CO_2 evolved was measured by titration of the NaOH solution with HCl in an excess of $BaCl_2$. The amount of CO_2–C evolved from the roots was determined by subtracting the amount produced by the soils without roots from that produced by the soil + root samples, and expressed as a percentage of total organic carbon (TOC) of the roots. Empty vessels were used as blank. After 56 days of incubation, the soils were analysed for pH and heavy metals sequential extraction.

2.4. Analytical methods

Plant roots and soil pseudo-total heavy metal (Cu, Fe, Mn, Pb and Zn) concentrations 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. The total-N and total organic-C (TOC) concentrations of roots and soils were measured in a Eurovector automatic microanalyser. The soil pH was determined in H_2O for saturated soil pastes. Sequential extraction of soil metals (McGrath and Cegarra, 1992) had the following steps: 0.1 M $CaCl_2$ (1:10 w/v), metals in soil solution and in exchangeable forms; 0.5 M NaOH (1:10, w/v), metals associated with organic matter (OM); 0.05 M Na_2H_2EDTA (1:10, w/v), metals mainly in the carbonate fraction; acid digestion with aqua regia,

residual metals. All metal concentrations were adjusted to values for oven-dried (12 h at 105 °C) soil. General soil analyses (electrical conductivity, redox potential, and organic matter) were carried out according to the methods described by Martínez-Alcalá et al. (2009). Chemical analyses were done at least in duplicate.

2.5. Statistical analysis

All data were analysed by one-way ANOVA using SPSS version 17.0 software. Differences between means were determined using Tukey's test at a probability level of $P<0.05$. Data concerning CO_2–C evolution from the soils were fitted to a first-order kinetic function by a non-linear least-square procedure (Marquard–Levenberg algorithm), using the SigmaPlot computer programme. The model is expressed according to the following equation: $C_m = C_0(1-e^{-kt})$; where C_m is the mineralised-C ($\mu g\, g^{-1}$) at time t (day), C_0 the potentially mineralisable-C ($\mu g\, g^{-1}$) and k the rate constant (day^{-1}). For statistical significance of curve fitting, the residual mean square (RMS) and F-values were calculated.

3. Results and discussion

3.1. Mineralisation dynamics of plant roots

Plant production, determined as shoot dry weight, was the highest in the calcareous soil ($1.07 \pm 0.01\ g\, pot^{-1}$) and lowest in the acid soil ($0.38 \pm 0.04\ g\, pot^{-1}$), it being $0.61 \pm 0.01\ g\, pot^{-1}$ in the neutral soil. Root density was different in each soil, according to the characteristics and metal content of the soil (2.04, 1.68 and 0.54 g root kg^{-1} soil for calcareous, neutral and acid soil, respectively).

Carbon mineralisation in the three soils having lupin roots was always higher than in the corresponding soils without roots, indicating the degradation of plant root tissues that remained in the soil after harvesting (Fig. 1). It has been demonstrated that the addition of foreign carbon sources to the soils involves an increase in their microbial activity (Clemente and Bernal, 2006; Clemente et al., 2006). The daily CO_2 production increased, by the presence of roots, to a greater extent in the calcareous soil (C + R). The acid soil had low CO_2–C emission reflecting the inadequate pH value for an appropriate development of the soil microbial biomass, together with high heavy metal concentrations in this soil, which may decrease the microbial population and its activity (McGrath, 1994; Pérez de Mora et al., 2006). In addition, the acid soil had the lowest amount of roots remaining after harvesting, as a result of the scarce plant growth in this soil, another indicator of metal toxicity. The neutral soil was able to hold a high microbial activity during the first days, and then the activity decreased until the end of the experiment; the difference between CO_2–C evolved from N + R and N – R was smaller than between the equivalent rooted and non rooted calcareous soil samples. The three soils without roots led to low CO_2–C production rates, showing low organic-C mineralisation.

The cumulative CO_2–C produced indicates the degree of mineralisation of the organic-C in the soil. In the present experiment, the values decreased in the order: C<N<A for both −R and +R soils (Table 2; Fig. 2). In all the soils, accumulative CO_2–C production was significantly higher in soil with roots than in soils without roots. Plant roots remaining in the soil acted as a source of energy for the soil microbial population, and the microbial respiration increased significantly, indicating a strong degradation of the lupin roots.

Soil C mineralisation usually follows a first-order kinetic function, in which the mineralisation rate depends on the amount of degradable organic-C present in the soil. In this situation, the mineralisation rate is a function of the potential mineralisable carbon (C_0) in the soil. This was the case in all the soils studied here, except in the non rooted calcareous one that followed a zero-order kinetic model (Table 2). In both acid and neutral soils, C_0 concentrations in the soils without roots were greater than those in the soils with roots (Table 2), although these differences were not statistically significant ($P>0.05$). But the rate constants (k) were lower in the soils without roots than in soils with roots. These results indicated that the presence of roots in the soil did not increase the degradable fraction of organic-C in these soils, and that the organic-C from roots was not more easily degraded than the soil organic-C. However, the presence of plant roots enhanced soil microbial activity, producing a faster degradation of the organic-C than in soils without plant roots.

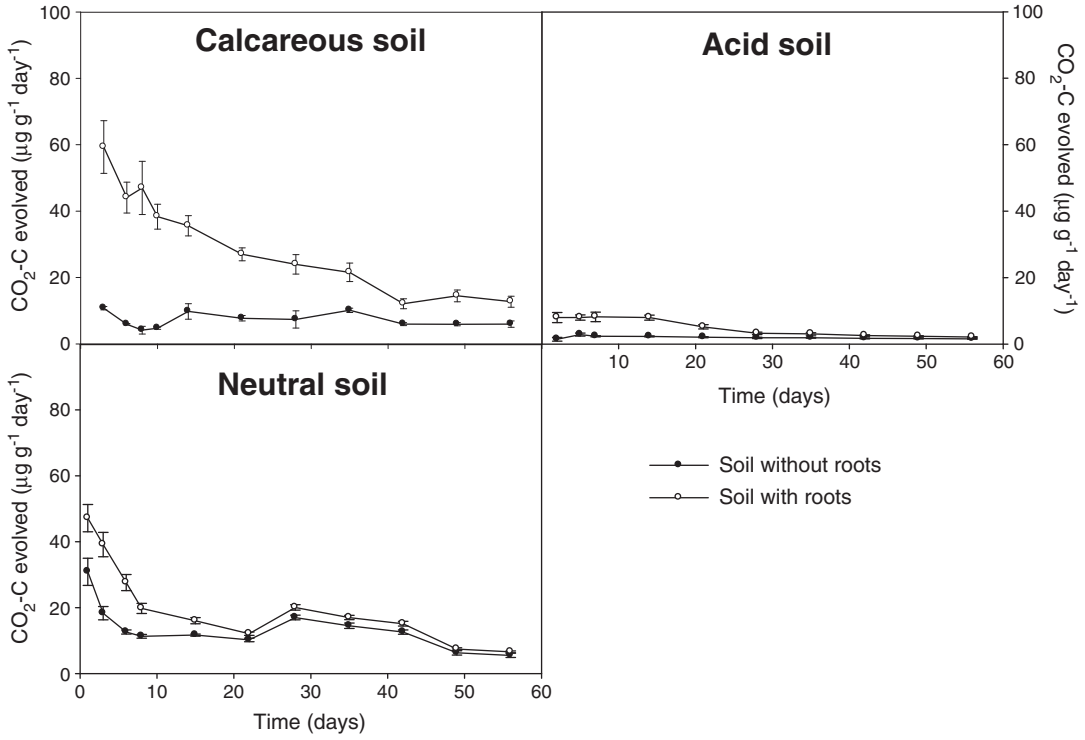

Fig. 1. CO_2–C evolution rate ($\mu g\, g^{-1}\, day^{-1}$) during the incubation of soils with and without (control) roots.

Table 2
Organic-C mineralisation in soil after 56 days (C_m), and parameters of the first-order kinetic model $C_m = C_0(1-e^{-kt})$: potentially mineralisable-C (C_0), rate constant (k) and statistical parameters of the curve fitting (residual mean square and F-ANOVA) (mean ± se). (C: calcareous, A: acid, N: neutral; −R, +R, without and with roots, respectively).

Soil	C_m (μg C g^{-1})	C_0 (μg C g^{-1})	k (day^{-1})	RMS	F
C − R[a]	412 ± 25.4	Results fit to a zero-order kinetic model (see footnote)			
C + R	1425 ± 93.9	1580 ± 35.3	0.038 ± 0.002	565	3625***
A − R	108 ± 2.81	311 ± 25.2	0.008 ± 0.001	0.64	19311***
A + R	243 ± 19.4	280 ± 9.51	0.035 ± 0.002	25.1	2443***
N − R	655 ± 24.0	1510 ± 329	0.011 ± 0.003	409	1311***
N + R	874 ± 38.4	1201 ± 114	0.024 ± 0.004	1.03	810***

*** Significant at probability level $P<0.001$.
[a] C − R followed a zero-order kinetic model: $C_m = A + Bt$; A = 7.458 ± 1.8965 μg C g^{-1}; B = 7.3217 ± 0.2082 μg C g^{-1} day^{-1}; $r^2 = 0.9912$.

Mayer et al. (2004) in an incubation experiment using crop residues (stems, leaves and roots) of pea, bean and lupin in a non-polluted soil, showed a great increase of organic-C mineralisation after their addition to soil. Vázquez et al. (2008) incubated ground polluted roots of white lupin in neutral and acid soils (2 g of roots per 100 g of soils) after plant growth in nutrient solution. The amounts of CO_2–C evolved (1757 ± 32, 2434 ± 30 and 1940 ± 13 μg C g^{-1} in a non-contaminated control, and a neutral and an acid contaminated soil, respectively) were much higher than the results found in this experiment, except for the calcareous soil. The fact that a higher ratio of lupin roots was used by Vazquez et al. (2008), that the plants were grown in nutrient solution, and that the roots were ground before adding them to the soil could be responsible for the higher degradation found by these authors. When plants are grown in soil as in the present experiment, they can reach different proportions of major constituents of plant tissue (lignin, cellulose and hemicellulose) to those in plants grown in nutrient solution. Such polymers have different (lower) degradability in the soil (Kirchmann and Bergqvist, 1989). In addition, in the present experiment the roots were not manipulated, as the plants were grown in the soil and intact soil cores with the roots were used for incubation. The 'artificial' addition of grounded metal-rich roots to soil may not reflect the real mineralisation rate of intact roots that remain in the soil after plant harvest.

At the end of the incubation (56 days), the mineralised C from the roots (calculated as the difference of CO_2–C evolved from soil with roots and soil without roots) followed the sequence (μg C g^{-1}): calcareous (1013) > neutral (219) > acid (135), parallel to the root density. The acid soil had the lowest C mineralisation, indicating again that pH and high soluble metal concentrations in this soil may be toxic not only for soil microorganisms, but also to the plants that scarcely developed their root system. Then the percentage of root tissue degraded in soils C, A and N was calculated to be 61% ≥ 58% > 47% of the organic-C provided by the roots, respectively. In an experiment using heavy metal polluted roots of Mediterranean shrubs in a heavy metal polluted soil, Moreno-Jiménez et al. (2009) found C mineralisation percentages of 13–40%, depending on the plant species. Mayer et al. (2004) reported that the percentage of mineralised-C from crop residue was 45% for pea, 48% for faba bean and 51% for lupin after 168 days of incubation. Similar percentages of degradation to those found in the present experiment have been reported for plant tissue of pea, cowpea and white clover (52, 50 and 58%, respectively) in non-polluted soils (Franzluebbers et al., 1994; Jensen, 1994). Scheid et al. (2009) found that the rate of decomposition of leaf litter from alder and poplar was not affected by metal pollution of the soil. They also concluded that leaf litter can act as a temporary pool for retention of soil metals.

3.2. Effects of root degradation on heavy metals fractionation

The pH values of the soils at the end of the incubation were similar to the initial ones (data not shown), and only a slight (but significant) lower value was found in A + R than in A − R (3.8 and 4.2, respectively). Heavy metal (Mn, Zn, Cu and Cd) concentrations in roots (Table 3) were clearly the highest for plants grown in the acid soil, indicating the greatest metal availability in this soil, despite it was the calcareous soil that had the highest total concentrations (Table 1).

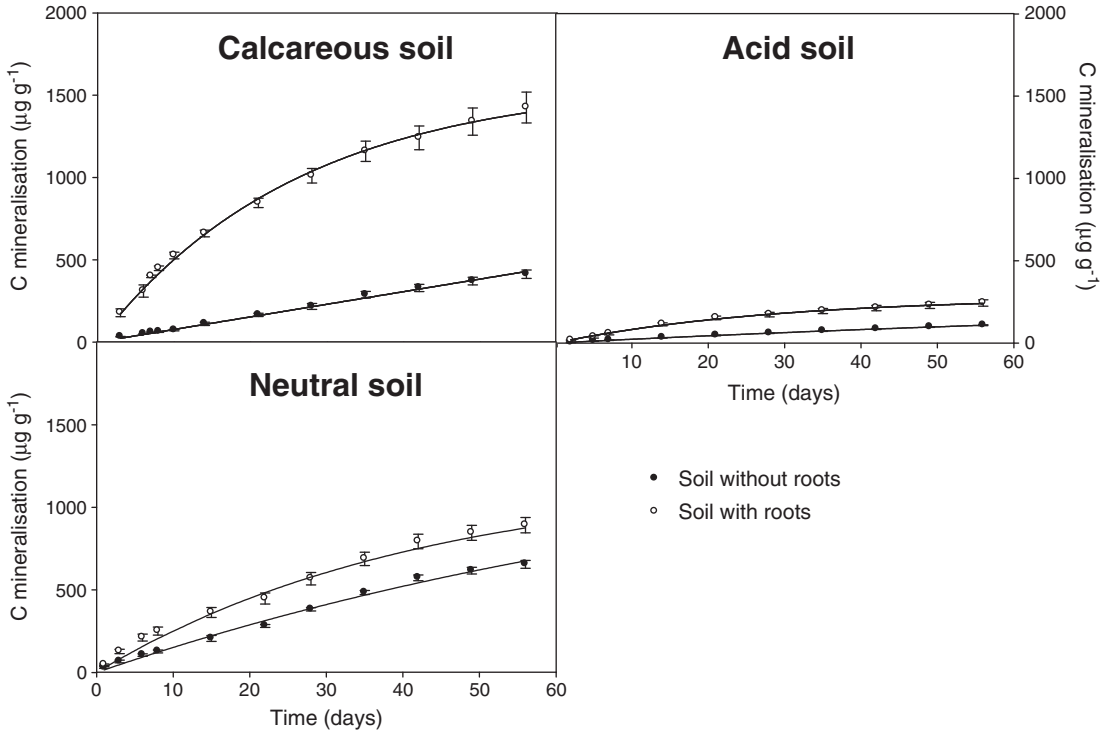

Fig. 2. Cumulative organic-C mineralisation (μg g^{-1}) in the soils with and without (control) roots. Dots are experimental results and lines are the fitting curve (Table 2).

No significantly different values were obtained for Fe concentrations in roots grown in the acid and in the calcareous soils.

Considering the total concentrations of heavy metals in lupin roots (Table 3), the amount of soil and roots used in the incubation experiment, and the percentage of mineralised lupin roots after 56 days of incubation, the estimated input of pollutants from degraded roots to soils was calculated (Table 4). The roots of the plants grown in the acid soil provided the highest amounts of Zn and Pb to the soil, despite the low root biomass in these samples. The estimated inputs of heavy metals into the soils by the degradation of lupin roots were lower than the ones estimated by Vázquez et al. (2008) for Cu (0.89 μg g^{-1}) and Zn (5.88 μg g^{-1}), but similar to those reported by Moreno-Jiménez et al. (2009) for Cu from *Arbustus unedo* roots (0.02 μg g^{-1}) and for Zn from *A. unedo* and *Retama sphaerocarpa* roots (0.04 and 0.09 μg g^{-1}, respectively), both of them in contaminated soils.

A sequential extraction of heavy metals was performed in the initial soils (before the incubation) and in the soils with and without roots at the end of the incubation. The amounts of heavy metals released from root degradation (Table 4) were too low for provoking a significant change in the soluble ($CaCl_2$-extractable) metal fraction of the soil. The initial acid soil (A) had high soluble and exchangeable Zn and Mn concentrations ($CaCl_2$-extractable, Table 1). But Zn concentrations in the soluble fraction of the rooted acid soil (A + R) were significantly higher than in the non-rooted (A–R) or initial (A) soils (Table 5), which suggests a solubilising effect of the presence of the roots on this metal. In the case of Mn, its concentrations in A + R soil were also higher than in A − R soil, but both lower than in the initial soil (Table 5). Therefore, this could not be due to the Mn released to soil by root degradation, but the fast degradation rate of the organic matter from roots could have affected the redox conditions of the soil towards less oxidant conditions, this being one of the main factors controlling Mn solubility in soils (Ross, 1994). In addition, the pots were watered from the bottom, so certain movement of soluble salts up and down the compartments of the pots could have occurred during plant transpiration, capillary action and diffusion. In fact, the electrical conductivity of A–R (bottom compartment) decreased to 0.16 dS m^{-1} from 2.71 dS m^{-1} of the initial soil (Table 1). Then changes in $CaCl_2$-extractable metal fractions cannot be considered exclusively as a plant effect, but also to the movement of soluble salts during plant growth.

In the neutral soil, $CaCl_2$-extractable Zn and Mn concentrations decreased in N − R and N + R with respect to the initial soil (Table 5). This could be due to a salt displacement in the case of N − R, as the electrical conductivity decreased from 2.22 dS m^{-1} in N to 0.44 dS m^{-1} in N − R, but not in the case of N + R, which EC was 2.27 dS m^{-1} at the end of the experiment, showing a certain immobilisation in roots tissue. Boucher et al. (2005) concluded that the most soluble fraction of Zn located in the leaves of *Arabidopsis halleri* was released to the soil, but they were fast adsorbed on soil reactive surfaces, which led to an immobilisation of the metals, limiting the possible toxic effects towards degradation. The NaOH- and, especially, EDTA-extractable Mn concentrations were significantly higher for N + R than for the original soil (Table 5), showing a displacement of Mn from the $CaCl_2$-extractable fraction to less-soluble fractions during plant growth and root degradation.

The EDTA-extractable Cu concentrations were higher in N − R than in N + R after the incubation and than in the initial soil, this showing again certain immobilisation of the metal by roots tissue. On the other hand, during organic-C mineralisation in soil Cu chelated by organic compounds (extracted with NaOH) could have been shifted to EDTA-extractable forms (Moreno-Jiménez et al., 2009), reducing Cu solubility, although no significant differences were found between NaOH fractions of the neutral soils (Table 5). In the calcareous soil, EDTA-extractable Mn concentrations were greater in C + R (70.1 μg g^{-1}), than in C − R (38.9 μg g^{-1}) at the end of the incubation, but both decreased with respect to the initial soil C. In the acid soil, Cu and Zn EDTA-extractable concentrations were significantly higher in rooted soils (A + R) than in non rooted (A − R) or initial soils, suggesting again that the presence of the roots in the soil somehow slightly increased the solubility of these metals in this soil (Table 5).

Table 3
Heavy metal concentrations in white lupin roots grown in the different soils (calcareous: C, acid: A, and neutral: N) (μg g^{-1}).

Soils	Fe	Mn	Zn[a]	Cu	Pb	Cd
C	2117 ± 630ab	134 ± 35.4b	33.5 ± 0.73c	4.15 ± 0.92c	32.5 ± 12.4	0.28 ± 0.18b
A	3503 ± 169a	352 ± 17.6a	4117 ± 440a	254 ± 1.76a	24.9 ± 6.57	30.1 ± 1.17a
N	709 ± 90.5b	19.2 ± 1.15c	103 ± 0.58b	17.3 ± 0.82b	7.08 ± 0.66	1.55 ± 0.08b
ANOVA	**	***	***	***	N.S.	***

***, **: significant at $P<0.001$, 0.01, respectively. N.S.: not significant. Values denoted by the same letter in a column do not differ significantly according to Tukey's test at $P<0.05$.

[a] ANOVA was run in the log transform data for data normalisation.

Table 4
Heavy metals released to the soil by mineralisation of the roots after 59 days of incubation (μg g^{-1} soil). (C: calcareous, A: acid, N: neutral).

Soil	Fe	Mn	Zn	Pb	Cu
C	2.98 ± 0.08a	0.18 ± 0.01a	0.04 ± 0.00b	0.04 ± 0.01b	0.01 ± 0.00
A	1.10 ± 0.05b	0.11 ± 0.01b	1.30 ± 0.14a	0.08 ± 0.00a	0.01 ± 0.00
N	0.64 ± 0.06c	0.02 ± 0.00c	0.09 ± 0.00b	0.02 ± 0.00b	0.01 ± 0.00
ANOVA	***	***	***	**	N.S.

***, **: significant at $P<0.001$, 0.01, respectively. N.S.: not significant. Values denoted by the same letter in a column do not differ significantly according to Tukey's test at $P<0.05$. Cd < 0.002 (μg g^{-1} soil), except for A (0.01 μg g^{-1} soil).

4. Conclusions

Plant roots increased soil microbial activity when compared with soils without roots, enhancing soil respiration. Roots degradation in the soil after harvesting was high (47–61%) and was not affected by the degree of metal contamination in the soil, but root growth was clearly reduced in the acid metal contaminated soil, as occurred for plant growth, indicating phytotoxicity.

The amount of heavy metals released to the soil due to the mineralisation of the roots was very low in comparison to total and even available soil metal concentrations. Although plant roots grown in the acid soil reached higher Cd, Cu and Zn concentrations than the total soil, the actual root biomass was so low that only significant amounts of Zn were released into the soil solution.

Soluble and exchangeable metal fractions of the soils were not affected by root degradation, and in the acid soil with high Zn and Mn solubility, the plant growing conditions were more relevant than root degradation for changing heavy metal fractionation. In the neutral soil a re-distribution of different soil fractionations of Cu, Zn and Mn occurred during white lupin growth and after harvest due to root degradation, which reduced metal solubility and favoured metal fixation in less-soluble forms.

Although this experiment was carried out under controlled temperature and humidity conditions that optimise roots mineralisation, the results obtained in this work could reliably reflect the behaviour of *L. albus* roots under field conditions. The use of this plant species for phytoimmobilisation of heavy metal polluted soils can be confirmed as a good option, since the degradation of its roots does not

Table 5
Heavy metal fractionation by sequential extraction ($\mu g\ g^{-1}$) in the original soils (C: calcareous, A: acid, N: neutral) and the soils at the end of the experiment without roots (−R) and with roots (+R).

	pH	Fe			Cu			Zn			Mn			Pb		
Soils		$CaCl_2$	NaOH	EDTA	$CaCl_2$	NaOH	EDTA	$CaCl_2$	NaOH	EDTA	$CaCl_2$	NaOH	EDTA	$CaCl_2$	NaOH	EDTA
C	7.81c	<0.5	13.1b	364	<0.5	2.75a	1.75	<0.5	2.95	57.5	<0.5	<0.5	208a	<0.2	13.1	554b
C−R	8.04a	<0.5	34.5a	417	<0.5	1.34b	1.87	<0.5	2.78	49.9	<0.5	0.32	38.9c	<0.2	13.6	670a
C+R	7.99b	<0.5	28.1ab	403	<0.5	1.41b	1.83	<0.5	2.40	44.3	<0.5	0.35	70.1b	<0.2	14.2	646a
ANOVA	***	–	*	NS	–	**	NS	–	NS	NS	–	NS	**	–	NS	*
A	4.20a	<0.5	140b	2484a	4.75	27.9	26.5b	139b	<0.5	33.5b	230a	<0.5	73.8	<0.2	1.24b	40.9
A−R	4.18a	<0.5	349a	1867c	4.53	26.4	26.2b	31.2c	3.06	25.1c	43.7b	1.56	84.6	<0.2	2.33a	42.1
A+R	3.81b	<0.5	189b	2132b	5.59	28.5	31.3a	181a	<0.5	48.7a	198a	<0.5	97.0	<0.2	1.25b	37.8
ANOVA	***	–	**	**	NS	NS	**	***	–	***	**	–	NS	–	*	NS
N	6.82b	<0.5	97.5	1436b	<0.5	21.3	30.4c	1.92a	12.7	91.5	24.6	1.07b	255b	<0.2	<0.2	24.4
N−R	6.95a	<0.5	131	1844a	<0.5	18.4	39.1a	0.96b	14.0	110	<0.5	2.84a	188b	<0.2	1.16	25.7
N+R	6.84b	<0.5	108	1840a	<0.5	17.1	34.2b	1.23b	15.2	107	<0.5	2.45a	344a	<0.2	1.00	26.3
ANOVA	**	–	NS	**	–	NS	**	**	NS	NS	–	*	**	–	NS	NS

***, **, *: significant at $P<0.001$, 0.01, 0.05, respectively. N.S.: not significant. Values denoted by the same letter in a column for each soil do not differ significantly according to Tukey's test at $P<0.05$.

raise heavy metal solubility in the soil to levels of concern, and even an overall reduction in the solubility of some metals is achieved.

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