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The rotation of white lupin (*Lupinus albus* L.) with metal-accumulating plant crops: A strategy to increase the benefits of soil phytoremediation

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ABSTRACT

Most of the plants employed to remove metals from contaminated soils are annuals and have a seed-to-seed life cycle of a few months, usually over spring and summer. Consequently, for most of the year, fields are not actively cleaned but are completely bare and subject to erosion by water and wind. The objective of this study was to evaluate the benefits of using *Lupinus albus* as a winter crop in a rotation sequence with a summer crop ideally selected for phytoextraction, such as industrial hemp. Lupin plants were grown in two alkaline soil plots (heavy metal-contaminated and uncontaminated) of approximately 400 m^2 each after the cultivation and harvest of industrial hemp. A smaller-scale parallel pot experiment was also performed to better understand the lupin behavior in increasing concentrations of Cd, Cu, Ni and Zn. White lupin grew well in alkaline conditions, covering the soil during the winter season. In few months plants were approximately 40–50 cm high in both control and contaminated plots. In fields where the bioavailable fraction of metals was low (less than 12%), plants showed a high tolerance to these contaminants. However, their growth was affected in some pot treatments in which the concentrations of assimilable Cu, Zn and Ni were higher, ranging from approximately 40–70% of the total concentrations. The lupin's ability to absorb heavy metals and translocate them to shoots was negligible with respect to the magnitude of contamination, suggesting that this plant is not suitable for extending the period of phytoextraction. However, it is entirely exploitable as green manure, avoiding the application of chemical amendments during phytoremediation. In addition, in polluted fields, white lupin cultivation increased the soil concentration of live bacteria and the bioavailable percentage of metals. On average live bacteria counts per gram of soil were $65 \times 10^6 \pm 18 \times 10^6$ and $99 \times 10^6 \pm 22 {*} 10^6$ before and after cultivation, respectively. The percentages of bioavailable Cu, Pb, Ni, Zn and Cr, which were 5.7 ± 0.7, 5.3 ± 1.7, 1.2 ± 0.1, 12 ± 1.5 and 0.1 ± 0.02%, respectively, before lupin growth, increased to 9.6 ± 1.6, 7 ± 2, 2 ± 0.3, 14 ± 1.5 and 0.1 ± 0.02% after lupin harvest.

On the whole, our results indicate that the winter cultivation of white lupin in sequence with a metal-accumulator summer crop can improve the recovery of soil quality during the phytoextraction period. It improves the safety of the area, limiting additional ecological and human health problems, and enhances soil health by avoiding the use of chemical amendments and by increasing the levels of viable microorganisms.

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1. Introduction

Phytoextraction may improve the quality of soil, mitigating the effects of metal contamination on soil resources. Nevertheless, although many studies have demonstrated the potential of this environmentally friendly technology, its employment in the field is still restricted, primarily due to the lack of truly effective plant species and the length of time required (Dickinson et al., 2009). In

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our previous studies (Citterio et al. 2003, 2005), we showed that industrial hemp (*Cannabis sativa* L.), a fast-growing and high-biomass-producing plant with multiple non-food uses, can accumulate consistent amounts of heavy metals, such as Cd and Ni, in its shoot. However, it is not a hyperaccumulator, and mainly for this reason, soil restoration with industrial hemp alone is too slow, both in terms of metal concentrations and of biological characteristics. One possibility for reducing the restoration time is the use of the entire year through crop rotation. *Lupinus albus* L. should represent a suitable crop to be cultivated in rotation with industrial hemp. In fact, the latter is a spring–summer crop, whereas some lupin varieties are winter season plants; they are cold tolerant and survive frosts up to −9° C. In addition, as a N_2-fixing legume, white lupin can also enhance soil fertility. Originating in the Mediterranean basin (Gladstones, 1998), it has been long used as a green manure crop in both vineyards and olive plantations in this area as well as in cotton cultivation in the southern USA. Currently, lupin is a minor crop in central Europe, while it is widely grown in the USA. It is also a traditional pulse crop cultivated in the Nile valley and in some parts of southeastern and southern Africa (Yeheyis et al., 2010). For its ability to grow in poor soils and in most areas of the world, lupin has already been considered as a potential candidate for soil phytoremediation; most available papers describe lupin as a suitable plant for phytostabilization, showing that it is not a hyperaccumulator for most of the metals tested (Reay and Waugh, 1981; Ximenez-Embun et al., 2002; Vazquez et al., 2006, 2009). However, lupin seems to accumulate consistent amounts of metals in root and shoot; this information is very important not only for evaluating phytoextraction but, particularly, in considering the use of lupin as a N_2-fixing organism for improving soil fertility by means of green manuring. This information is essential for choosing the best agricultural practice for ameliorating the general soil quality during the phytoremediation process.

Unfortunately, on the basis of the current literature, it is difficult to define the amount of metal absorbed and translocated to shoots by lupin, primarily because very few experiments studying a few metals have been performed directly in the field, and the extrapolation of data obtained from hydroponic systems is often unrealistic (Dickinson et al., 2009). Moreover, the available data from field experiments relates to acid soils (Vazquez et al., 2006), whereas many contaminated sites consist of neutral and alkaline substrates, on which white lupin may perform differently.

In this study, to determine the utility of growing *Lupinus albus* in crop rotation with industrial hemp or other annual summer crops, we grew white lupin in a heavy metal-contaminated site in the Po plain area (North Italy) after two consecutive cultivations (two successive years) of industrial hemp. The ability of white lupin to grow in metal-polluted and alkaline soils during winter season and its efficiency to absorb and accumulate heavy metals in root and shoot were evaluated. In addition, we considered the potential of this species as a cover crop, to improve soil fertility and general site quality, including the reduction of soil erosion. Finally, to better interpret our field results, we set up a pot experiment in laboratory-controlled conditions to assess the tolerance and accumulation capacity of white lupin with respect to single heavy metals in a neutral-alkaline substrate.

2. Materials and methods

2.1. Field experiment

The employment of white lupin as rotating crop succeeding industrial hemp in soil restoration was assessed in a 400 m^2 contaminated area in Ferrara Province (North Italy), near an industrial site where motor vehicles were dismantled and metal parts recovered. A non-contaminated area, adjacent to the contaminated soil, was used to grow lupin and was defined as the control plot.

In the study area, soil type was defined according to the regional soil map (Filippi and Sbarbati, 1994) and pedological observations. Natural soils are very deep, medium-textured to moderately fine-textured (silt loam or silty clay loam) along the profile and are very calcareous, slightly to moderately alkaline, and have good oxygen availability. On the basis of WRB classification (IUSS Working Group, 2007) these soils are Haplic Cambisols (Calcaric, Siltic).

Control and contaminated soils (0–20 cm layer) were analyzed between the second year of hemp cultivation and lupin sowing and between lupin growth and a new hemp sowing for the following features: organic carbon, pH, electrical conductivity, total carbonates, total nitrogen and bioavailable phosphorus concentration, total count of live bacteria and total and bioavailable concentrations of heavy metals. Methodologies are described in the online supplementary materials.

White lupin seeds (200 seeds m^{-2}) were planted in rows at the end of September, and plants were harvested at the end of March, before flowering, to allow the sowing of hemp. White lupin plants tolerated the Po plain winter climate blocking their development during the cold months; specifically, plants arrested their growth from November to mid-February, with a total period of active growth of about two months.

One week after sowing, the percentage of seedling field emergence with respect to the total number of sown seeds was estimated and, just after plant harvest, the plant growth was assessed by determining plant organ dry weight (DW). Shoots and roots from at least 60 plants per plot were separated, cut in small parts and placed in a dry cabinet at 40 °C until a constant weight was reached. Then, they were weighed and used for the quantification of metals with the same methodology used for soil analyses (Supplementary material).

2.2. Pot experiment

Three increasing concentrations of Cu (120, 600 and 1200 mg kg^{-1}), Zn (150, 1500 and 3000 mg kg^{-1}), Ni (120, 500 and 1000 mg kg^{-1}) and Cd (6, 45, and 90 mg kg^{-1}) were used in the pot experiment. These concentrations were chosen on the basis of the Italian legal limit values for contaminant contents in soils of green and industrial areas (D. M. 152/06). For the selection of Cd concentrations, the metal amounts found in polluted sites were also taken into account.

A 3% organic matter soil was prepared by mixing sterilized quartz sand (0.5 mm coarse grade) with autoclaved sowing potting compost (Compo Sana, Italy) with the following characteristics: organic carbon (C) = 48%, organic nitrogen (N) = 1.5%, pH: 6–7.

Copper sulfate, zinc sulfate, nickel chloride and cadmium sulfate solutions were used to contaminate soil for Cu, Zn, Ni and Cd treatments, respectively. For each metal treatment, three aliquots of soil were contaminated with the corresponding solution, appropriately diluted to obtain the above reported final metal concentrations. For each metal content, the contaminated soil was carefully mixed and then distributed into three pots. A total of 39 pots (0.25 m diameter and 0.20 m depth), 3 filled with the uncontaminated soil (control) and 36 filled with the contaminated soils, were prepared and used to germinate and grow *Lupinus albus* L. Before sowing, pH, total nitrogen content, bioavailable phosphorus and total and bioavailable metal concentrations were measured in 3 soil samples collected from each pot, applying the same methodologies used in the field experiment (Supplementary material).

Twenty-four seeds per pot were sown and left to germinate under controlled conditions (25 °C; 8 h dark/14 h light,

Table 1
Characteristics of polluted and control soils before and after lupin growth. Data are the mean ± standard deviation of 12 samples. The means with the same letter are not significantly different ($P > 0.05$).

	Before lupin sowing (after 2 cultivations of hemp)		After lupin harvest	
	Control plot	Polluted plot	Control plot	Polluted plot
pH-H_2O	8.0 ± 0.1 a	8.4 ± 0.1 b	8.1 ± 0.1 a	8.2 ± 0.1 a
EC (mS cm^{-1})	0.5 ± 0.1 a	0.7 ± 0.2 a	0.6 ± 0.1 a	0.8 ± 02 a
Organic carbon (g kg^{-1})	17 ± 0.2 a	16 ± 3 a	17 ± 0.5 a	18 ± 2 a
Total N (g kg^{-1})	1.7 ± 0.01 a	1.6 ± 0.3 a	1.7 ± 0.1 a	1.8 ± 0.2 a
Available P (mg kg^{-1})	11 ± 4 a	18 ± 5 a	11 ± 3 a	18 ± 4 a
Total carbonates (g kg^{-1})	136 ± 4 a	135 ± 2 a	134 ± 4 a	137 ± 6 a
Alive bacteria count per g (×10^6)	110 ± 30 a	65 ± 18 b	103 ± 38 a	99 ± 22 a
Cu concentration (mg kg^{-1})	25 ± 8 a	176 ± 21 b	24 ± 4 a	169 ± 14 b
Bioavailable Cu (mg kg^{-1})	1.8 ± 0.2 a	10.1 ± 0.3 b	2.2 ± 0.8 a	16 ± 2 c
Pb concentration (mg kg^{-1})	40 ± 4 a	156 ± 6 b	38 ± 11 a	154 ± 8 b
Bioavailable Pb (mg kg^{-1})	1.9 ± 0.1 a	8.3 ± 2.7 b	2.1 ± 0.3 a	10.8 ± 3.1 b
Ni concentration (mg kg^{-1})	60 ± 8 a	53 ± 6 a	55 ± 7 a	52 ± 5 a
Bioavailable Ni (mg kg^{-1})	0.70 ± 0.07 a	0.63 ± 0.01 a	0.96 ± 0.07 b	1.01 ± 0.15 b
Zn concentration (mg kg^{-1})	83 ± 15 a	459 ± 58 b	87 ± 20 a	422 ± 39 b
Bioavailable Zn (mg kg^{-1})	11 ± 1 a	54 ± 2 b	11 ± 1 a	61 ± 3 c
Cr concentration (mg kg^{-1})	73 ± 9 a	82 ± 9 a	66 ± 19 a	79 ± 13 a
Bioavailable Cr (mg kg^{-1})	0.03 ± 0.002 a	0.09 ± 0.01 b	0.02 ± 0.003 a	0.10 ± 0.01 b
Cd concentration (mg kg^{-1})	<LOD	<LOD	<LOD	<LOD
Bioavailable Cd (mg kg^{-1})	<LOD	<LOD	<LOD	<LOD

Cd LOD (limit of detection): 2 μg kg^{-1}.

150 μmol m^{-2} s^{-1}). After two weeks, the percentage of seed germination was assessed, and the number of plantlets was brought to 16 per pot by removing any excess plants. Plant growth (dry biomass) and heavy metal content in roots and shoots were determined for each treatment every 10 d for a total period of 55 d from seed germination. Three pools of plants from different pots for each metal concentration and for each stage were analyzed.

2.3. *Statistical analysis*

Data were statistically analyzed with the Statgraphics plus program for Windows (version 5.0, Manugistic, Maryland USA) as described in the online Supplementary material.

3. Results

3.1. *Behavior of white lupin in field experiments*

Lupin was sown in two plots, control and contaminated, after two cultivations of industrial hemp and the harvest of the whole plants (roots and shoots). The two successive cultivations of hemp greatly increased the biological and chemical quality of the contaminated soil (data not shown), leading to the soil characteristics assessed just before lupin sowing and reported in Table 1. The total mean concentrations of Zn (458 mg kg^{-1}), Cu (176 mg kg^{-1}) and Pb (156 mg kg^{-1}) exceeded the maximum concentrations permitted by Italian legislation for green and residential areas (D. M. 152/06). Additional metals (Cr and Ni) were present in the soil, but their mean concentrations were under the legal limits.

Lupin seeds were sown in control and contaminated soils at the end of September. Within two weeks, most of the seeds germinated, generating small plantlets; no significant difference in the number of growing seedlings was observed between the two plots ($P < 0.05$). On average, plants reached a height of approximately 20 cm before entering the quiescent status that allowed them to withstand the winter season. In February, their vegetative growth resumed, and after two months, they were approximately 40–50 cm high in both control and contaminated plots. White lupin, which is usually adapted to moderately acidic or neutral soils, demonstrated the ability to grow in alkaline conditions and showed a high tolerance to metal contamination; however, concerning tolerance, it must be noted that the bioavailable fraction of metal measured in both the plots was low, consistent with the high pH of the soil (Table 1).

At 6 months after sowing, lupin plants were harvested, and the amounts of metals accumulated in their roots and shoots were measured. Table 2a shows that the mean amounts of metals extracted by white lupin from control and polluted soils were relatively low compared to the values found in hyperaccumulator plants (Reeves and Baker, 2000). In our field experiment, on average, approximately 10, 7, 3, 72 and 7 mg plant^{-1} of Cu, Pb, Ni, Zn and Cr, respectively, were extracted by lupin from polluted soil, and lower amounts were found in control plants (Table 2b). Accumulation Factors (AF), defined as the ratio of the mean plant metal concentration to the mean total metal concentration in the soil, were also calculated and reported in Table S1. It can be observed that although AF are low compared to the values obtained for hyperaccumulator plants, a large proportion of the absorbed metals was translocated from root to shoot. Considering the dry weight of lupin root and shoot, it was calculated that about 70% of each metal absorbed was found in the aboveground biomass of lupin plants grown in the polluted plot (Table 2b and Table S1). Nevertheless, given the low biomass reached by lupin plants during the considered period, the total quantities of metals accumulated in shoots were negligible (Table 2b). For instance, on the basis of our data, it can be estimated that the total amount of Cu absorbed by lupin plants per m^2 was approximately 0.0015 g, an irrelevant quantity with respect to the bioavailable amount of Cu present in the superficial layer of soil (0–20 cm). Thus, both shoots and roots of white lupin could be used as green manure. Use of lupin as green manure should represent a good management practice to increase the nitrogen and organic matter content of soil because the cultivation of *L. albus* alone did not significantly increase these important soil components (Table 1). However, the lupin cultivation

Table 2
Heavy metal distribution in the roots and shoots of plants grown in control and polluted plots. (A) The mean concentration (mg kg^{-1}) ± standard deviation of heavy metals in the roots and shoots of 60 plants per plot. (B) The mean total content (μg plant^{-1}) of metals in the roots and shoots as assessed on the basis of the measured root and shoot dry weight and metal concentration. (C) The mean percentage of metals translocated from roots to shoots. The percentage of translocation was calculated by dividing the mean amount of metal found in shoot (mean μg in shoot) by the mean total amount of metal absorbed (mean μg in shoot + mean μg in root).

(A)

		Control plot	Polluted plot
Cu (mg kg^{-1})	Root	15 ± 3 a	28 ± 7 a
	Shoot	7 ± 1 b	16 ± 5 b
Pb (mg kg^{-1})	Root	6 ± 1 a	22 ± 19 a
	Shoot	0.5 ± 0.1 b	10 ± 0.3 b
Ni (mg kg^{-1})	Root	8 ± 0.6 a	12 ± 2 a
	Shoot	1.6 ± 0.2 b	5 ± 2 b
Zn (mg kg^{-1})	Root	67 ± 17 a	158 ± 95 a
	Shoot	36 ± 3 b	118 ± 37 b
Cr (mg kg^{-1})	Root	17 ± 1 a	21 ± 5 a
	Shoot	3 ± 1 b	11 ± 5 b
Cd (mg kg^{-1})	Root	<LOD	<LOD
	Shoot	<LOD	<LOD

(B)

		Control plot	Polluted plot
Cu (μg plant^{-1})	Root	1.3 ± 0.3 a	2.3 ± 0.5 a
	Shoot	3.1 ± 1.0 b	7.5 ± 1.9 b
Pb (μg plant^{-1})	Root	0.5 ± 0.02 a	2.4 ± 0,9 a
	Shoot	0.2 ± 0.1 b	4.9 ± 1.9 b
Ni (μg plant^{-1})	Root	0.7 ± 0.03 a	1.1 ± 0.3 a
	Shoot	0.7 ± 0.07 a	2.2 ± 0.8 b
Zn (μg plant^{-1})	Root	5.9 ± 1.1 a	16.1 ± 3.5 a
	Shoot	15.3 ± 2.6 b	56.2 ± 11.8 b
Cr (μg plant^{-1})	Root	1.5 ± 0.05 a	1.9 ± 0.6 a
	Shoot	1.2 ± 0.3 b	5.5 ± 2.0 b
Cd (μg plant^{-1})	Root	<0.03	<0.03
	Shoot	<0.03	<0.03

(C)

		Translocation (%)	
		Control plot	Polluted plot
Cu (μg plant^{-1})	Root	70	76
	Shoot		
Pb (μg plant^{-1})	Root	32	67[a]
	Shoot		
Ni (μg plant^{-1})	Root	49	67[a]
	Shoot		
Zn (μg plant^{-1})	Root	72	78
	Shoot		
Cr (μg plant^{-1})	Root		
	Shoot		
Cd (μg plant^{-1})	Root	78	74
	Shoot		

Different letters represent significant difference between roots and shoots for each metal inside the contaminated or control plot ($P < 0.05$).
Cd LOD (limit of detection): 2 μg kg^{-1}.
[a] Translocation percentage: significant difference compared to the control.

significantly promoted bacterial growth in the polluted soil, where the number of live bacteria counted before lupin sowing was statistically lower than in the control plot (Table 1). This growth promotion was not observed in the control plot, most likely due to the absence of a metal stress and to an already existing equilibrium in the microbial community.

Except for Cr, the bioavailable metal fraction was also increased by lupin cultivation in both the plots. Specifically, in contaminated soil, the mean percentages of bioavailable metals before lupin growth were 5.7 ± 0.7, 5.3 ± 1.7, 1.2 ± 0.1, 12 ± 1.5 and 0.1 ± 0.02% for Cu, Pb, Ni, Zn and Cr, respectively; these percentages were 9.6 ± 1.6, 7 ± 2, 2 ± 0.3, 14 ± 1.5 and 0.1 ± 0.02% after lupin growth.

Table 3
General proprieties of the soils used in the pot experiment. Data are the means of 9 samples (3 from each pot) for each treatment ± standard deviation.

Treatments	pH-H_2O	Bioavailable metal concentration (mg kg^{-1})	N (%)	Assimilable P (mg kg^{-1})
Control	7.6	n.d.	0.04 ± 0.002	6.3 ± 0.6
Cd 6	7.6	3.5 ± 0.1	0.05 ± 0.001	7.4 ± 1.2
Cd 45	7.6	30 ± 5	0.04 ± 0.002	6.5 ± 0.3
Cd 90	7.6	58 ± 7	0.03 ± 0.001	7.1 ± 0.9
Cu 120	6.9	75 ± 5	0.04 ± 0.004	6.7 ± 0. 1
Cu 600	7.3	276 ± 4	0.04 ± 0.002	6.6 ± 0.2
Cu 1200	7.5	852 ± 132	0.05 ± 0.003	5.8 ± 0.1
Ni 120	7.4	72 ± 1	0.04 ± 0.001	7.2 ± 0.3
Ni 500	7.5	226 ± 4	0.04 ± 0.001	5.9 ± 0.3
Ni 1000	7.7	440 ± 34	0.04 ± 0.003	6.4 ± 0.8
Zn 150	6.4	81 ± 1	0.05 ± 0.002	6.6 ± 0.5
Zn 1500	6.4	877 ± 40	0.04 ± 0.001	7.0 ± 0.3
Zn 3000	7.3	1330 ± 174	0.04 ± 0.002	6.8 ± 0.3

3.2. *Performance of white lupin in pot trials*

Table 3 reports the characteristics of the artificially contaminated soils used to grow *L. albus* plants. Although the soils were allowed to stabilize for three months after contamination with heavy metals, at the start of lupin culture, the amounts of soil bioavailable metals were consistent, ranging from approximately 40–70% of the total concentrations and, as expected on the basis of soil composition, were much higher than those measured in Ferrara's control and contaminated soils.

The metal tolerance of white lupin was assessed by evaluating the effect of increasing concentrations of Cd, Cu, Ni and Zn on seed germination and plant development (Fig. 1). Cd was the only metal that did not affect lupin germination and growth. Cu did not affect seed germination, but, at the two higher concentrations tested (600 and 1200 mg kg^{-1}), plants arrested their development approximately 30 d after germination. For Ni, the lower concentration (120 mg kg^{-1}) did not affect either germination or plant development, but the intermediate concentration (500 mg kg^{-1}) allowed the germination of most seeds but not the development of plants; the higher concentration (1000 mg kg^{-1}) completely prevented the germination of seeds. The same total inhibition effect was observed for the two higher Zn concentrations (1500 and 3000 mg kg^{-1}), whereas the lower concentration (150 mg kg^{-1}) partially affected seed germination and significantly slowed the plant growth during the last period of development.

During the experiment, every 10 d, three pools of plants for each metal concentration were harvested and used for the assessment of metal accumulation in root and shoot.

In roots, the concentration of metals rose with the increases of both soil metal concentration and plant development (Fig. 2); in shoots, the concentration of metals increased in parallel with the increasing of metal concentration in soil, but during plant growth, only the concentrations of Cd and Zn significantly increased, whereas those of Cu and Ni remained constant or even decreased (Fig. 2). However, all the metals tested were principally accumulated in the root, irrespective of their concentration in the soil and of the duration of plant growth. For instance, the shoot and root mean concentrations of Cu found in plants exposed for two months to the highest concentration of this metal were approximately 61 and 1711 mg kg^{-1}, respectively. Similarly, for the highest soil Cd concentration, 13 and 1606 mg kg^{-1} of this metal were found in plant shoots and roots, respectively (Fig. 2).

A better evaluation of the absorption and translocation capacity of *L. albus* was obtained through the analysis of the total amount (micrograms) of metals accumulated in roots and shoots and their

A	CTR	Cd 6	Cd 45	Cd 90	Cu 120	Cu 600	Cu 1200	Ni 120	Ni 500	Ni 1000	Zn 150	Zn 1500	Zn 3000
Germination (%)	85 ± 19	94 ± 20	85 ± 17	98 ± 21	92 ± 16	94 ± 14	88 ± 10	90 ± 15	65 ± 20*	0 *	67 ± 18*	0 *	0 *

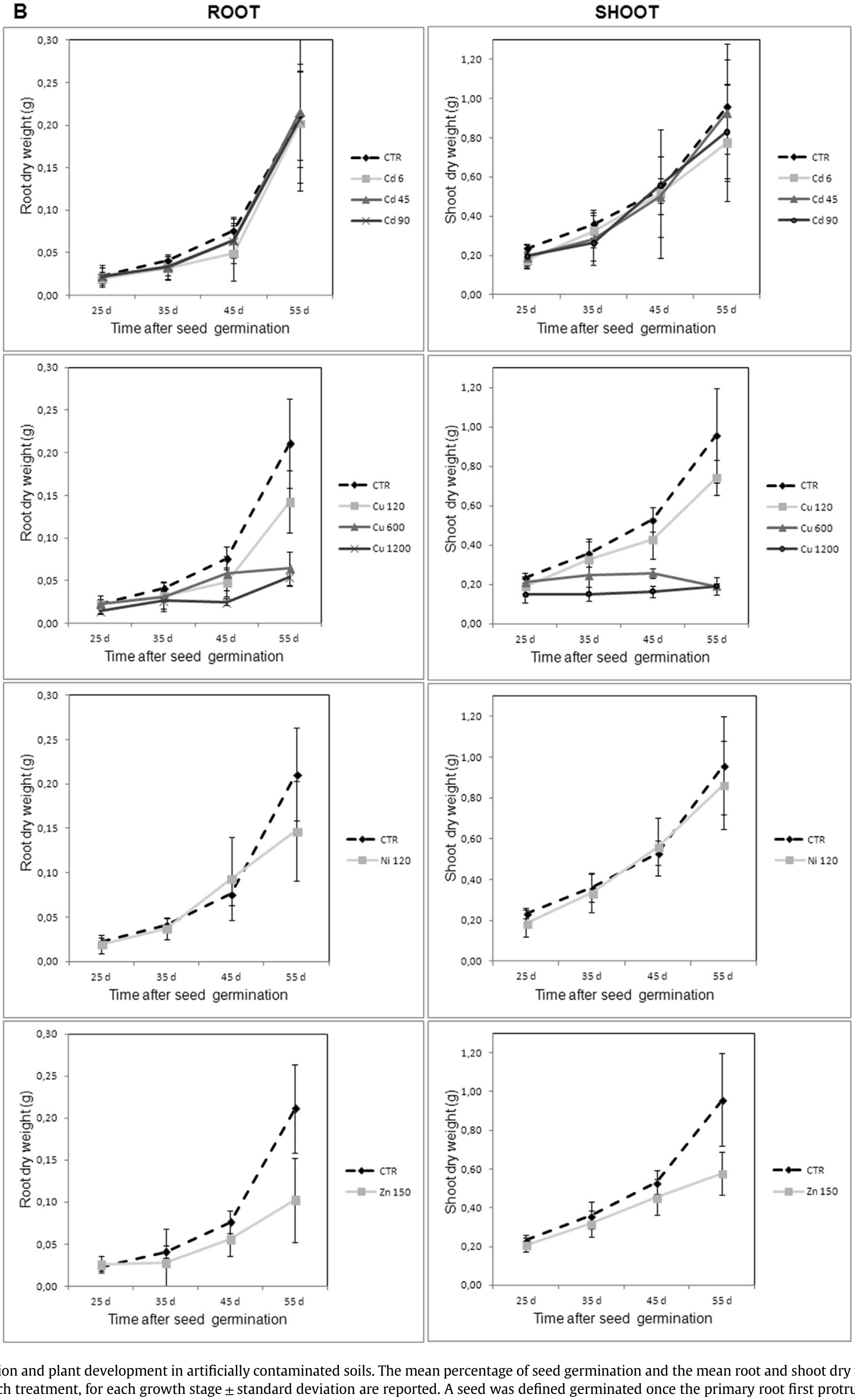

Fig. 1. Seed germination and plant development in artificially contaminated soils. The mean percentage of seed germination and the mean root and shoot dry weight of 12 plants (4 from each pot) for each treatment, for each growth stage ± standard deviation are reported. A seed was defined germinated once the primary root first protruded. The percentages reported were calculated on a total of 72 seeds (24 per pot). *Statistically different from the control.

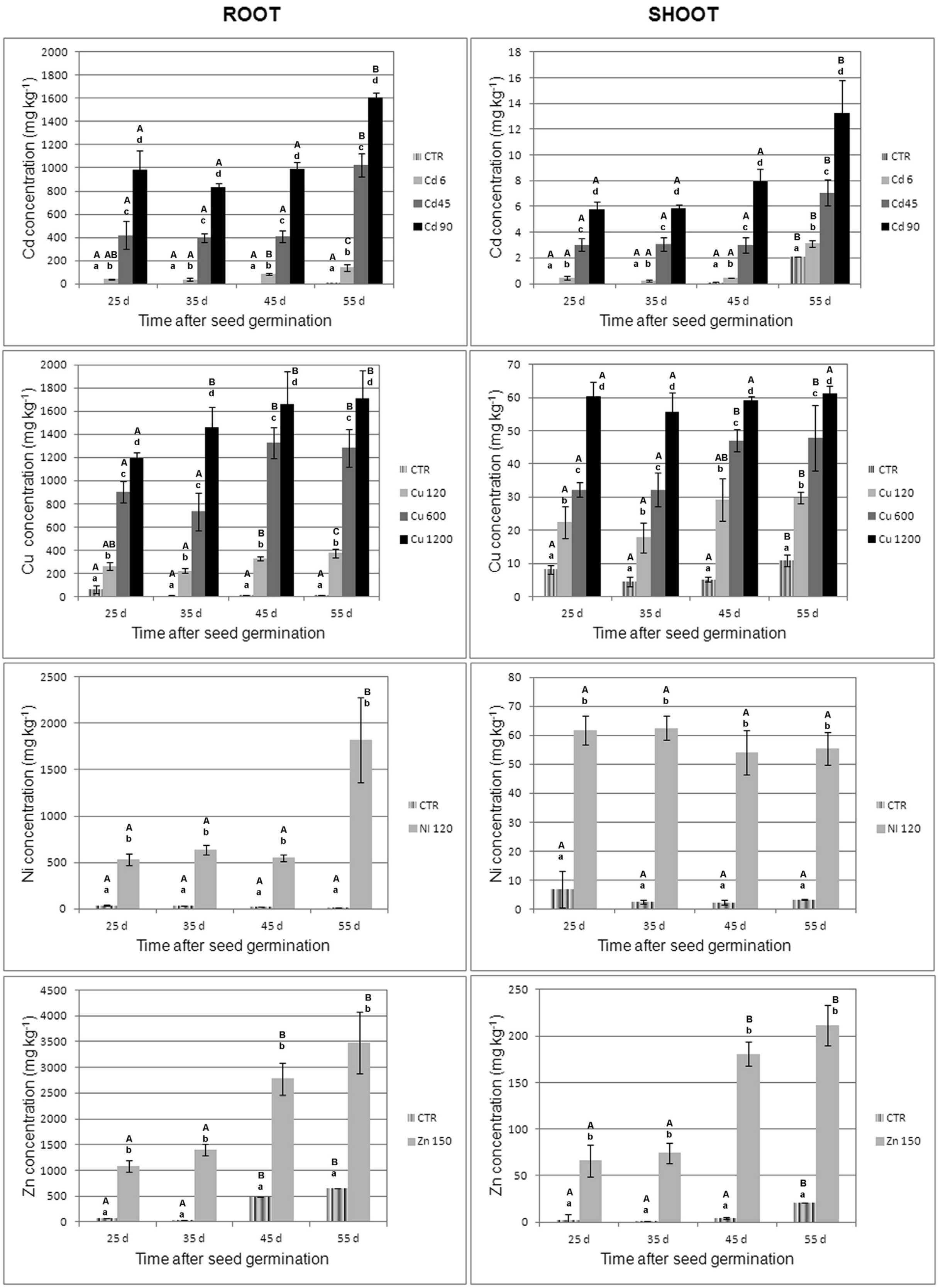

Fig. 2. Shoot and root metal concentrations at successive stages of plant development in pots. Data are the means of 12 plants for each metal concentration and for each stage. For Ni and Zn only data about the lowest concentrations are shown because plants died in Ni 500, Ni 1000, Zn 1500 and Zn 3000 soils. Different uppercase letters represent significant differences during development within a given concentration ($P < 0.05$); Different lowercase letters represent significant differences across concentrations at single stages.

percentage of translocation during development, calculated on the basis of the dry weight of the plants at different growth stages (Table S2). Specifically, the percentage of tranlocation was calculated by dividing the mean amount of metal found in shoot (mean μg in shoot) by the mean total amount of metal absorbed (mean μg in shoot + mean μg in root). The mean μg in shoot or in root was obtained by multiplying the mean concentration of metal measured by AAS in shoot or root by the correspondent organ dry weight.

The percentage of metals translocated from root to shoot was higher at lower metal concentrations in soil. During plant growth, the percentage generally remained constant or decreased. On average, after 55 d, the percentage of metal translocated to shoot with respect to the total metal accumulated in plant was 5, 17, 15 and 25% for Cd, Cu, Ni and Zn, respectively. However, given the low percentage of translocation and the low biomass reached by lupin plants after two months of continuous growth, the mean amounts of metals accumulated in lupin shoots were very low, reaching a maximum of approximately 11, 22, 48 and 122 μg/healthy plant for Cd, Cu, Ni and Zn, respectively. These maximum mean values were found in plant grown under Cd 90, Cu 120, Ni 120 and Zn 150 treatments, which did not affect plant development. On the contrary, plants exposed to higher concentrations of metals grew to reduced final sizes and accumulated smaller amounts of metal in their shoots. For instance, plants grown in soil containing 600 and 1200 mg kg^{-1} of Cu accumulated a final amount of metal (approximately 9 and 12 μg $plant^{-1}$, respectively) in their shoots, which was lower than that of plants grown in soil contaminated with 120 mg kg^{-1} of Cu (22 μg $plant^{-1}$).

4. Discussion

The accumulation of heavy metal in soils is of global concerns because heavy metals are persistent and cause bioaccumulation and biomagnification, detrimental effects on the plant–soil ecosystem, food-safety issues and potential health risks (Anjum et al., 2012). The application of phytoremediation technologies, as an alternative to expensive conventional chemical and physical methods, represents a great low-cost opportunity to reclaim contaminated soils, maintaining or even improving their biological characteristics. Nevertheless, economically sustainable phytoremediation requires further study, mainly aimed at increasing its efficiency. One of the drawbacks in phytoextraction technology regards the plants employed for metal extraction from soil, which are annuals and show a seed-to-seed life cycle occurring over a few months, usually in spring and summer (Sarma, 2011). Consequently, for most of the year, fields are not actively cleaned but are completely bare and subject to erosion by water and wind. This work investigated the benefit of using *L. albus* as a winter cover crop in a rotation sequence with a summer crop selected for phytoextraction. We showed that white lupin germinated and grew in an alkaline soil contaminated with heavy metals that had been partially restored by the application of continuous phytoextraction technology using industrial hemp during the two previous summer seasons. In those field conditions, where the fraction of bioavailable metals was very low, white lupin showed a high tolerance to heavy metals and absorbed and translocated a negligible amount of metals to its shoot. The scarce accumulation of heavy metals in white lupin shoot was also observed by Vazquez et al. (2006) during a field experiment performed in a region of Spain affected by mine sludge contaminated with As and Cd. In addition, previous studies performed in pots or hydroponic cultures also showed the retention of most metals in lupin roots (Ximenez-Embun et al., 2002; Pastor et al., 2003; Page et al., 2006; Esteban et al., 2008). Nevertheless, Page et al. (2006) noted that, although most of the metals were retained in root, their translocation and redistribution to younger leaves were dependent on the type of metal; for instance, in their hydroponic system, Cd and Co were nearly completely retained in root, whereas large quantities of Zn and Ni were accumulated in shoot via xylem. In our field experience, the percentage of translocation was almost equivalent for all of the metals present in the soil. This percentage was consistent at approximately 70% of the accumulated metals, but given the low amount of metals extracted by lupin plant, the quantity of metal translocated was a negligible fraction of the total bioavailable metal concentrations in the soil. To determine whether this high translocation percentage remained the same when the concentration of bioavailable metals in soil increased, we analyzed the data that we obtained through a pot trial performed in parallel with the field experiment. We found that the percentage of translocation decreased with the increase of assimilable metal concentrations in the soil, independent of the type of metal tested, and that the amounts of metal detected in the shoots were thus always negligible compared to the metal concentrations in the soil. Thus, during a continuous phytoextraction with summer crops, it can be supposed that the winter cultivation of lupin would not be useful to extend the period of active phytoextraction, but it would be useful for improving the soil quality by using the entire plants as green manure. In addition, our field experiment showed further benefits from the use of lupin as a rotation crop during phytoremediation, related to the increase in viable soil microorganisms and metal bioavailability in contaminated soil. Both of these actions could be related to the bacterial activity and to the release of metabolites into the soil from lupin roots and could have a positive effect on the metal extraction made by the successive summer crop (Hinsinger et al., 2003; Martínez-Alcalá et al., 2009). However, further studies are needed to clarify the mechanisms on the basis of these observations and their specific effects on the phytoextraction process. Finally, in evaluating the opportunity of exploiting white lupin as a rotation crop, we should consider that it also provides suitable cover crop and slows wind and water erosion. Preventing erosion is of primary importance in contaminated sites where the diffusion of pollutant-containing soil particles is very dangerous for residential populations and the environment.

In sum, we believe that the rotation of white lupin with a crop suitable for phytoextraction can improve the recovery of soil quality during phytoremediation. White lupin combines the reduction of metal concentration with the safety of the area by limiting additional ecological and human health problems and enhances soil health by avoiding the use of chemical amendments and by improving microbial soil diversity.

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Appendix A. Supplementary material

Supplementary materials associated with this article can be found online at http://dx.doi.org/10.1016/j.jenvman.2014.06.001.

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