Root Exudates Increase Metal Accumulation in Mixed Cultures: Implications for Naturally Enhanced Phytoextraction

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Abstract Soluble root exudates collected from the barley grown in Fe deficient-nutrition solutions were added to soil to study their effects on metal solubility. The results showed that the addition of barley root exudates from the Fe deficient-nutrition solutions resulted in a 4.7-, 3.2-, 9.7-, 4.9- and 11.5-fold increase in the concentrations of soluble Cu, Pb, Zn, Cd and Fe, respectively, in comparison with the root exudates from the full-nutrition solutions. When peas were placed in a mixed culture with barley in pots, the concentrations of Cu, Pb, Zn, Cd and Fe in the shoots of the peas were 1.5-, 1.8-, 1.4-, 1.4- and 1.3 times higher than those grown in sole (single culture pots). It was hypothesized that the root exudates from barley in the mixed culture system played an important role in the process of solubilizing metals in soil and facilitating the uptake of metals by peas. Although the improved efficiency from the current experiments was relatively low, it may indicate a potential approach to the remediation of metal-contaminated soils in a naturally enhanced way.

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1 Introduction

The contamination of soils with heavy metals is a serious environmental problem that is threatening human health and ecosystems. Conventional cleanup technologies are usually associated with high operating costs and can cause damage to the normal properties of the soil (Holden 1989; Smith et al. 1995). In the past two decades, plant-based remediation technology (i.e., phytoremediation) has received increasing attention. The most often-mentioned forms of phytoremediation include the use of hyperaccumulator plants and/or chemically enhanced phytoextraction to remove metals from contaminated soils. Unfortunately, most hyperaccumulators grow slowly and produce low biomass, which greatly limit their application in the field (Mulligan et al. 2001; Puschenreiter et al. 2001). Chemically enhanced phytoextraction with the addition of some artificially produced chelates, such as EDTA (ethylenediaminetetraacetic acid), CDTA (trans -1, 2 -diaminocyclohexane -N, N, N', N'-tetraacetic acid), EGTA [ethyleneglycol -bis (ß -aminoethyl ether), N, N, N', N-tetraacetic acid], and EDDHA [etylenediamine-di (*o*-hydroxyphenylacetic acid)]) has been suggested an efficient method for the cleaning up of soils contaminated with heavy metals (Blaylock et al. 1997; Huang et al. 1997; Cooper et al. 1999; Wu et al. 1999; Shen et al. 2002; Luo et al., 2005 and 2006). However, the associated leaching of mobilized metals from the chemically assisted phytoremediation process will pose a potential risk to the ground water and surrounding environment

(Nowack 2002). Hence, it is necessary to explore some more environmental friendly and economical technologies to solve this potential problem.

Under nutrient-limiting conditions, plants can modify the rhizosphere to enhance the acquisition of nutrients (Marschner 1995). Rhizosphere acidification and the release of root exudates are two common mechanisms involved in the process. Root exudates can change the pH of the rhizosphere, provide ligands for metal complexation, and facilitate microbial activity, which in turn enhance the concentrations of soluble metals in soil (Tao et al. 2004). Root exudates from different plant species and cultivars may have different capacities for increasing the solubility of metals (Mench and Martin 1991; Zhao et al. 2001; Ma et al. 2003). Under the Fe deficient condition, which is typical in calcareous soils, graminaceous plants usually markedly increase the release of phytosiderophores to the rhizosphere. Based on a study for an intercropping system, Ma et al. (2003) concluded that the secretion of DMA (2’-deoxymugineic acid), the first product of phytosiderophore synthesis, from the roots of perennial grasses could be partly responsible for the converting of insoluble soil Fe to the forms available to fruit trees and for the “re-greening effect” observed in fruit trees grown on calcareous soils after the introduction of grasses in the tree rows. Phytosiderophores are capable of chelating not only Fe, but also Cu, Zn and Mn, thus mobilizing these metals from soils (Treeby et al. 1989). Mench and Martin (1991) observed that the uptake of Cd from soils by the three plant species of *Nicotiana tabacum*, *Nicotiana rustica* and *Zea mays* followed the same order as the extent of Cd extraction by their root exudates. In addition, root exudates are confined to the same small portion of the soil where roots are located. Therefore, the potential adverse result of the increased leaching of heavy metals by the application of artificial chelates used in the chemically enhanced phytoremediation can be expected to be negligible in soil.

Under some conditions, such as where the soil is Fe deficient, mixing the culture of graminaceous plants with other plants may enhance the uptake of metals (Marschner 1995; Ma et al. 2003). Therefore, this approach will provide one meaningful alternative in the remediation of low-level contaminated soils with regard to operating costs and possible risks to the environment. Graminaceous plant of barley (*Hordeum vulgare* L. cv. Jian 4) and dicotyledon plant of peas (*Pisum sativum* L. cv. Qinxuan No. 2) were used in the present study. The two plant species are common crops which have showed high potential in the phytoremediation (Huang et al. 1997; Ebbs and Kochian 1998). In this study, barley was cultured in Fe deficient-nutrition solutions. The root exudates of barley were collected and added to the soils with plants of peas to study their effects on the uptake of metals by peas. In addition, peas and barley were mixing-cultured to evaluate the effects of barley growth on the accumulation of metals by peas.

2 Materials and Methods

2.1 Collection of Root Exudates

The seeds of barley (*Hordeum vulgare* L. cv. Jian 4) were germinated in the dark on filter papers moistened with deionized water (DIW). After germination (4 d), twenty seedlings were transferred to each 2-L polyethylene vessels filled with a modified 0.2-strength Rorison nutrient solution with the following composition (in µmol L^{-1}): 400 $Ca(NO_3)_2$, 200 $Mg(SO_4)_2$, 50 K_2HPO_4, 300 KCl, 9.2 H_3BO_3, 1.8 $MnSO_4{\cdot}4H_2O$, 0.21 $Na_2MoO_4{\cdot}2H_2O$, 0.31 $CuSO_4{\cdot}5H_2O$, 10 $ZnSO_4{\cdot}7H_2O$, and 10.8 Fe-EDTA at pH 6.0 (Hewitt 1966). Two treatments of the control group (full-nutrient composition) and the Fe deficient treatment (without the addition of Fe-EDTA) were imposed 10 d after the seedlings were transferred to the nutrient solutions. Each treatment was replicated four times. Before the treatments were imposed, the roots of the seedlings were rinsed thoroughly with DIW. The nutrient solution was aerated continuously and renewed every two days, and the experiment was conducted in a glasshouse under natural light with the air temperature ranging from 25 to 34 ºC. Before the root exudates were collected (21 d after the plants were grown), the roots were cultured overnight in DIW. Root exudates were collected by bathing the roots in 200 ml autoclaved 0.1 mM $CaCl_2$ solution for 3 h after the onset of the light period, during which period the solution was aerated continuously (Zhao et al. 2001). After the exudates were collected, the roots of the barley were separated from the shoots, washed with DIW, and dried in an oven for dry weight measurement.

The root exudate solutions were filtered immediately after collection through a sterile 0.45 µm paper filter (Whatman [Maidstone, UK] 42) into an autoclaved glass tube. The filtered solutions were immediately concentrated to 10 ml using a rotary evaporator at 40 ºC. The root exudate solutions were immediately added to soils to study their effects on the solubility of metals.

2.2 Extraction of Metals from Soil with the Addition of Barley Root Exudates

Soil samples were collected from a disused agricultural field in the Yuen Long area of

Hong Kong. The samples were passed through a 2 mm sieve and air-dried for one week. The soils were artificially contaminated with Cu (400 mg kg^{-1} of soil) as $CuCO_3$ (copper carbonate); Pb (500 mg kg^{-1} of soil) as $Pb_3(OH)_2(CO_3)_2$ (lead hydroxide carbonate) and PbS (lead sulfide – galena, a common lead mineral in mining areas) at a Pb concentration ratio of 1:1; Zn (500 mg kg^{-1} of soil) as $ZnCO_3$ (zinc carbonate) and ZnS (zinc sulfide) at a Zn concentration ratio of 1:1; and Cd (15 mg kg^{-1} of soil) with $Cd(NO_3)_2{\cdot}4H_2O$ (cadmium nitrate). The soil pH was adjusted to 8.4 with lime, and measured by 0.01 mol $CaCl_2$ at a 1:5 ratio (w/v) using a pH meter. Basal fertilizers applied to the soil were 80 mg P kg^{-1} of dry soil, and 100 mg K kg^{-1} of dry soil as KH_2PO_4 (Shen et al. 2002). After the addition of heavy metals, the soils were equilibrated for two months, undergoing seven cycles of saturation with de-ionized water and air-drying processes.

The initial pHs of the root exudates are shown in Table 2. Root exudates were adjusted to pH 5.0 with 1 N HNO_3 and 1 N KOH. Two grams of soil (passed through a 2-mm sieve) were placed in a 20-mL polypropylene centrifuge tube and shaken with 10 ml root exudates for 30 min. After centrifugation (3500 g for 10 min), the supernatant was filtered through a 0.45 µm paper filter (Whatman [Maidstone, UK] 42), acidified with HNO_3 and analyzed for concentrations of metals by ICP-AES. The soil extracted with 0.1 mM pH 5.0 $CaCl_2$ in the same way served as the control for background concentrations of exchangeable metals in the soil.

2.3 Metal Accumulation in Peas with the Addition of Root Exudates from Barley

In this experiment, the soil used was the same as that described above. The seeds of peas

(*Pisum sativum* L. cv. Qinxuan No. 2) were directly sown in the soils, and the seedlings were thinned to four plants per pot. Barley root exudates from the full nutrient component treatment and the Fe deficiency treatment were collected in the same way as that described above. After the peas had been grown for 10 d, 1000 ml of the root exudate solutions from barley (pH was adjusted to 5.0) were concentrated to 50 ml and immediately added to the surface of the soil. Pea plants treated with 0.1 mM pH 5.0 $CaCl_2$ solutions in the same way were used as the control. Three replicates were conducted in each treatment. All of the experiments were conducted in a glasshouse under natural light. The air temperature ranged from 23 to 32 ºC. The plants were harvested 7 d after the application of root exudates. Shoots and roots were separated, washed with tap water, rinsed with DIW, and then dried at 70 ºC in an oven to a constant weight for dry weight measurements and further chemical analysis.

2.4 Mixed Cultures of Peas and Barley in Pot Experiments

Air-dried soils (500 g) were placed in plastic pots (12 cm i.d. x 12 cm height). The moisture of the soil was maintained at near field water capacity by adding DIW daily. The treatments consisted of a monoculture of peas and a mixed culture of peas and barley. The seeds of plants were directly sown in the soils, and the seedlings were thinned to four plants per pot. For the mixed-culture, two pea plants were grown on one side of the pot, and two plants of barley on the other side. Three replications were used in each treatment. All of the experiments were conducted in a glasshouse under natural light. The air temperature ranged from 23 to 32 ºC. The plants were harvested 14 d after planting.

Shoots and roots were separated, washed with tap water, rinsed with DIW, and dried at 70 ºC in a drying oven to a constant weight for dry weight measurements and further analysis.

2.5 Plant Analysis

Subsamples of ground shoot samples (200 mg) were digested in a mixture of concentrated HNO_3 and $HClO_4$ (4:1, by volume), and major and trace elements in the solutions were determined with ICP-AES (Chen et al., 2004a). A certified standard reference material (SRM 1515, apple leaves) of the National Institute of Standards and Technology, U.S.A., was used in the digestion and analysis as part of the QA/QC protocol. Reagent blank and analytical duplicates were also used where appropriate to ensure accuracy and precision in the analysis. The recovery rates were around 90 ± 6% for all of the metals in the plant reference material.

The concentration of dissolved organic C in root exudates was determined by a TOC analyzer (model 5000A), and the pH in exudate solutions was measured using a pH meter.

2.6 Statistical Analysis

The data reported in this paper were the mean values based on the results of the three replicated experiments. Statistical analyses of the experimental data, such as correlation and significant differences, were performed using SPSS® 11.0 statistical software.

3 Results

3.1. Effects of Root Exudates on the Solubilization of Metals in Soils

The DOC and pH of the root exudates of barley were measured (see Table 2). The amounts of DOC can be used to evaluate the root exudates (Zhao et al. 2003). The content of DOC in the root exudates from the Fe deficiency treatment was 2.9-fold that of the control group (full-nutrition cultured plants). The pH of root exudates in the Fe deficiency treatment was 0.7 units lower than that of the control plants (see Table 2).

The amounts of Cu, Pb, Zn, Cd and Fe extracted by barley root exudates are shown in Table 3. The addition of root exudates led to higher concentrations of soluble metals in the soil than the addition of 0.1 mM $CaCl_2$ (blank). Compared with the control (full-nutrient solution treatment), the addition of root exudates from the Fe deficiency treatment dramatically enhanced the mobilization of metals in the soil. On average, the amounts of Cu, Pb, Zn, Cd and Fe extracted by the root exudates from the Fe deficient plants were 4.7, 3.2, 9.7, 4.9 and 11.5 times those in the control.

3.2. Effects of Root Exudates on the Accumulation of Metals in the Shoots of Peas

Compared with the control (blank group) (to which 0.1 mM $CaCl_2$ solutions were added), the addition of barley root exudates significantly enhanced the concentrations of metals in the shoots of peas (Table 4). In addition, the improved effect was more significant for the root exudates from the Fe deficiency treatment than from the full-cultured plants. In the plants treated with barley root exudates from the Fe deficiency treatment, the concentrations of Cu, Pb, Zn, Cd and Fe were 3.8, 2.3, 1.6, 1.7 and 2.6 times the values in the group to which the root exudates from the full-cultured barley plants were applied.

The total amounts of metals phytoextracted from the soils are presented in Table 5. This is expressed on the basis of the amounts of root exudates added to the soil. In the calculation, the metal phytoextraction for the control (blank) group (adding 0.1 $CaCl_2$ mM solutions) was used as the basis for the plants treated with root exudates. The control group represents the pea plants treated with the root exudates from the full-cultured barley plants. The phytoextraction of Cu, Pb, Zn, Cd and Fe by the shoots of peas was significantly enhanced by adding the root exudates of the Fe deficiency treatment, being 3.8-, 2.7-, 8.5-, 2.6- and 8.2-fold that of the control group.

3.3 Effects of Barley Growth on the Accumulation of Metals in Pea Shoots

Pea plants appeared to be normal and healthy wherever they were grown in sole and mixed-culture systems (see Table 6). The mixed cropping system had no significant effect on the production of dry matter (Table 6). However, when peas were in a mixed culture with barley, the concentrations of Cu, Pb, Zn, Cd and Fe in the shoots of peas reached about 1.5, 1.8, 1.4, 1.4 and 1.3 times the concentrations of those grown in sole (see Fig. 1).

4 Discussion

The phytoremediation of soils contaminated with heavy metals is becoming more attractive because of its “green” characteristics and low cost. The results of the present study showed that growing peas and barley in a mixed culture led to the accumulation of a great amount of metals in the shoots of peas than growing plants in the absence of barley. The result indicated that changing the growing system of plants could improve the phytoextraction efficiency of metals from soil. In addition, this enhancement was achieved without the application of any additional chemicals. Thus, the technique led to a reduction in application costs. The leaching of metals to the surrounding environment, which is associated with the application of artificial chemicals, was also greatly eliminated.

Iron-deficiency is a common phenomenon in calcareous soils because of the extremely low solubility of soil Fe (Mengel 1994). Plants have been grouped into Strategy I plants and Strategy II plants, according to the mechanisms of iron (Fe) acquisition in higher plants. Strategy I plants include dicotyledons and non-graminaceous monocotyledons, which respond to Fe deficiencies by both extruding protons and reducing substances from the roots, and by enhancing ferric reduction activity at the root plasma membrane. Strategy II plants (graminaceous species) usually respond to Fe deficiencies by releasing phytosiderophores and by activating (or developing) a highly specific uptake system for ferrated phytosiderophores (Ma and Nomoto, 1996). Several plant and environmental factors, such as plant species/cultivars, temperature and light intensity can influence the rate at which phytosiderophores are released from plant roots (Cakmak et al. 1998). It was found that the release of phytosiderophores correlated positively with the content of $CaCO_3$ in soils (Awad et al., 1999). Usually, root exudates can influence the solubility of nutrients. Root exudates can have a direct impact on the uptake of metals by acidification, chelation, precipitation and oxidation-reduction reactions, and an indirect impact through their effects on microbial activity, and on the physical and chemical properties of rhizosphere and root growth patterns (Uren and Reisensuer 1988). Ma et al. (2003) concluded that the secretion of DMA (2'-deoxymugineic acid) from the roots of perennial grasses may be partially responsible for the conversion of insoluble soil Fe to forms available to fruit trees. The “re-greening effect” observed in fruit trees grown on calcareous soils after the introduction of grasses in the tree rows indicated that these grasses were able to improve the uptake of Fe by tree roots (Tagliavini et al. 2000; Ma et al. 2003). In the present study, when peas were mix-cultured with barley, the concentration of Fe in the shoots of peas increased by 26% in comparison with that grown in the absence of barley (Fig. 1). The enhanced uptake of Fe by peas, observed in the study on the mixed culture system, was likely caused by the root exudates secreted by barley.

In a chemically enhanced phytoremediation process, the first step is to produce a high biomass of candidate plants at contaminated sites, and the second step is to induce the accumulation of bioavailable metals in the shoots of these plants (Blaylock et al. 1997). Usually, the enhanced solubility of metals in the soil is accomplished by the addition of synthetic chelates. However, the potential leaching of metals associated with the application of chelates has limited the application of this technology in the field (Nowack 2002). It is now found that plants can naturally produce substances of phytosiderophores, which are capable of chelating not only Fe, but also Cu, Zn and Mn, thus mobilizing these metals from soils (Treeby et al. 1989). It is hypothesized that the release of phytosiderophores and other organics from graminaceous plants mobilizes heavy metals by forming corresponding complexes, thus increasing their availability to plants. In the present study, the addition of root exudates from barley significantly increased the concentration of soluble metals in the soil and resulted in a 2.8-, 1.7-, 7.5-, 1.6- and 7.2-fold increase in the phytoextraction of Cu, Pb, Zn, Cd and Fe, respectively, in the shoots of peas (Tables 4 and 5). Similarly, Chen et al. (2004) observed that a deficiency of Fe induced the uptake and accumulation of Cu in *Commelina communis*. Mench and Martin (1991) reported that the uptake of Cd from soils by the three plant species of *Nicotiana tabacum*, *Nicotiana rustica* and *Zea mays* followed the same order as the extent to which Cd was extracted by their root exudates. In the shoots of wheat plants precultured under a low Fe nutritional status, the concentrations of Zn, Ni, and Cd were between 25% and 200% higher than in the Fe precultured plants. (Römheld and Awad 2000). However, the results from Shenker et al (2001) suggested that the release of phytosiderophores does increase the uptake of Fe, Zn and Mn in barley and wheat, but does not increase the uptake of Cd in plants.

Of course, the use of the solution to mimic the interaction of the roots only reflects the rough direction and the relative extent of metal accumulation in plants, rather than the exact degree. This is because the root exudates were concentrated in the present study, which could explain the difference in the concentrations of metals in the shoots of peas shown in Fig. 1 and Table 4. When the barley root exudates (from the Fe-deficient nutrition solutions) were added into the pots grown with peas, the concentrations of metals in the shoots of peas reached 8.1, 6.6, 2.2, 2.4 and 3.5 times those of the control (without the addition of root exudates) for Cu, Pb, Zn, Cd and Fe, respectively (see Table 4). However, when peas were mixed-cultured with barley, the concentrations of metals in the shoots of peas only increased by 1.4, 1.5, 1.8, 1.4 and 1.3 times those of the plants grown in sole (see Fig. 1).

Besides the potential effects from root exudates, a pH change and microbial activity may also be involved in the process of solubilizing metals. The pH values of the root exudates from the barley were 4.5 for the Fe deficiency treatment and 5.2 for the full-nutrition culture, lower than that of the soil (8.2) (Table 2). In order to eliminate the possible effects of the pH of root exudates on the mobilization of metals in the soil, the pH of the root exudates was adjusted to 5.0 before they were added to the soils. Tao et al. (2004) observed that the effects of a change in pH on copper fractionation in calcareous soils were relatively insignificant compared with those of root exudate complexation because of the strong buffering capacity of calcareous soils, and that the acidification of the soil solution was not the reason for the increase in zinc availability (Knight et al. 1997). The effect of root exudates and microbial products could not be easily distinguished. Many components of root exudates can serve as carbon sources for microorganisms and facilitate microbial activity. This, in turn, leads to an increase in the release of organic acids, which can mobilize metals through the formation of metal-organic chelates (Tao et al. 2004). A reduction in the amount of enzymes released from microorganisms may also play a role in the solubilization of metals (Lombi et al. 1999).

The components of root exudates were comparatively complicated. In the present study, it was not clear which component of the root exudates was directly responsible for enhancing the metals in the shoots of peas in the mixed culture system. However, the present study showed a possible approach to the naturally enhanced phytoremediation of metal-contaminated soils.

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Table 1 The physicochemical properties of the soil used in the study

Physicochemical Properties	**Soils used in the study**
pH ($CaCl_2$)	8.4
Electrical conductivity at 25°C ($\mu S\ cm^{-1}$)	262
Sand (%) > 0.05 mm	79.5
Silt (%) 0.05-0.001 mm	13
Clay (%) < 0.001 mm	7.5
N_{Total} (%)	0.15
Organic matter (%)	2.7
Cation exchange capacity ($cmol\ kg^{-1}$)	4.2
Field water capacity (%)	39.7
Background total metal content ($mg\ kg^{-1}$)	
Cu	80
Pb	80
Zn	200
Cd	1.6

Table 2 The concentration of dissolved organic C (mg g^{-1} root DW) and the pH of the root exudates collected from barley treated in full (control) and Fe-deficient nutrition solutions

	DOC	**pH**
Control solution	6.7 ± 0.3a	5.2 ± 0.4b
Fe-deficient nutrition solution	19.5 ± 1.1b	4.5 ± 0.2a

Notes: The control represents the full-nutrient solution treatment. Values are means ± S.D. (n = 3); the different small letters stand for statistical significance at the 0.05 level with the LSD test.

Table 3 The amounts of metals in the soil extracted with the addition of root exudates (mg kg^{-1} root DW of barley)

	Blank	**Control**	**Fe-deficient nutrition solution**
Cu	1.36 ± 0.5a	23.6 ± 3.5b	110 ± 15c
Pb	0.73 ± 0.3a	8.3 ± 1.2b	26.5 ± 2.1c
Zn	1.67 ± 0.8a	23.3 ± 1.8b	225 ± 30c
Cd	0.06 ± 0.02a	0.09 ± 0.01a	0.44 ± 0.1b
Fe	22 ± 3a	103 ± 11a	1180 ± 85b

Notes: Blank and control groups represent 0.1 mM $CaCl_2$ and full-nutrient solution treatments, respectively. Values are means ± S.D. (n = 3); the different small letters stand for statistical significance at the 0.05 level with the LSD test.

Table 4 The concentration of metals (mg kg^{-1} DW) in the shoots of peas

	Blank	**Control**	**Addition of Fe-deficient nutrition**
Cu	14.5 ± 2.5a	31 ± 2.7a	118 ± 14b
Pb	0.32 ± 0.1a	0.9 ± 0.1b	2.1 ± 0.1c
Zn	70 ± 8.9a	97 ± 8.1a	157 ± 13b
Cd	0.2 ± 0.1a	0.29 ± 0.03a	0.48 ± 0.1b
Fe	102 ± 13a	138 ± 15a	359 ± 39b

Notes: Blank and control groups represent 0.1 mM $CaCl_2$ and full-nutrient solution treatments, respectively. Values are means ± S.D. (n = 3); the different small letters stand for statistical significance at the 0.05 level with the LSD test.

Table 5 The total phytoextraction of metals (mg kg^{-1} root DW of barley) in the shoots of peas

	Control treatment	Fe-deficient treatment
Cu	25.2 ± 3.1a	94.6 ± 11b
Pb	0.9 ± 0.1a	2.4 ± 0.3b
Zn	37.6 ± 2.9a	319 ± 23b
Cd	0.13 ± 0.02a	0.34 ± 0.1b
Fe	49.4 ± 5.5a	405 ± 24b

Notes: Control treatment represents the full-nutrient solution. Values are means ± S.D. (n = 3); the different small letters stand for statistical significance at the 0.05 level with the LSD test.

Table 6 The dry matter yields (g plant^{-1}) in the shoots of peas

Cropping system	**Dry weight**
Monoculture	0.48 ± 0.04a
Mixed-culture	0.51 ± 0.05a

Notes: Monoculture and mixed-culture represent peas grown in the absence and presence of barley, respectively. Values are means ± S.D. (n = 3); the different small letters stand for statistical significance at the 0.05 level with the LSD test.

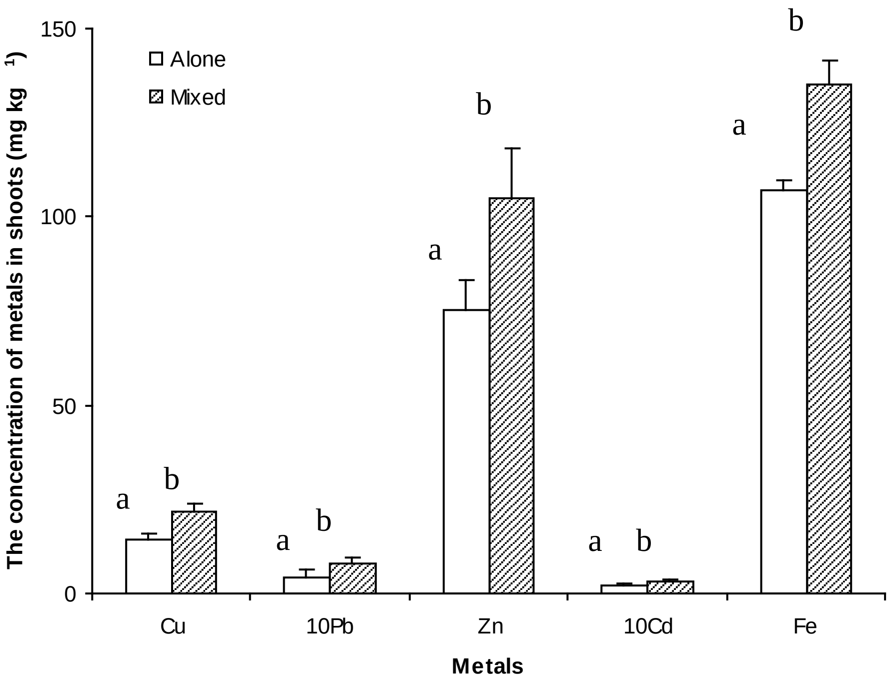

Fig. 1 The concentrations of Cd, Cu, Pb, Zn and Fe in the shoots of peas grown alone and mix cultured with barley. The data for Cd and Pb shown here are 10 times the original values. Values are means ± S.D. (n = 3); the different small letters stand for statistical significance at the 0.05 level with the LSD test.