Differential effects of plant root systems on nickel, copper and silver bioavailability in contaminated soil

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Highlights

 The labile pools of TE, notably Ag, Ni, and Cu, were differently affected by the presence of the plants.

 Availability of Ni and Cu is related to the level of dissolved organic carbon (DOC) in the soil.

 Different root systems can have a distinct influence on soil DOC, and thus can modify the labile pools of Ni and Cu.

Abstract

A pot experiment was conducted to investigate the effect of diverse plant species (four herbaceous and four woody species) on the labile pool of six metals (Ag, Cu, Pd, Zn, Ni and Se) present in their rhizosphere. After three months of cultivation, concentrations of trace elements (TE) in above and below-ground biomass of each species were determined. The labile and presumably bioavailable fraction of these TE in the rhizosphere as well as key soil parameters (e.g. pH, electrical conductivity (EC), percent of organic matter and dissolved organic carbon (DOC)) were also measured and compared as a function of plant species. The concentration of TE in plant tissues differed among species. In general and as expected, concentrations were higher in root tissues of tested plants than in above-ground tissues. While the labile pool of several TE in the rhizosphere, notably Ag, Ni, and Cu, was significantly and differently affected by the presence of the plants, pH, EC and percentage of organic matter remained unchanged. In contrast, DOC was higher in the rhizosphere of all tested plants than in soil of the control pots without plants. In addition, there was a positive relationship between Ni and Cu availability concentrations, and DOC levels. This suggests that root systems of different species can have a distinct influence on soil DOC and consequently modify the labile pools of Ni and Cu in the rhizosphere. These findings have important implications for plant selection in phytoremediation projects.

Abbreviations

TE	Trace elements
EC	Electrical conductivity
DOC	Dissolved organic carbon

INTRODUCTION

Labile pools of contaminants are usually considered to be biologically active (bioavailable) and soluble (mobile) in the matrix (Mench et al., 2009). They represent a fraction of the total contaminant pool; the remainder corresponds to the non-labile forms. The balance between these forms varies according to soil conditions such as pH, redox potential and the presence of organic ligands (NFESC, 2000; Schwartz et al., 2003). Therefore, bioavailability of TE can influence the efficiency of metal phytoextraction in metal-contaminated soil. The bioavailability of metals in soils depends on many factors, including their speciation (Salomons, 1995; NFESC, 2000), pH, organic matter content, cation exchange capacity and soil texture (Reichman, 2002; Jung, 2008; Violante et al., 2010; Fijałkowski et al., 2012). Some plant species, identified as hyperaccumulator plants, can concentrate and tolerate high levels of metals in their tissues due to their specific capacity for metal uptake and accumulation in detoxified subcellular fractions such as granules (Tu et al., 2004; Peer et al., 2006). In addition, TE bioavailability can be enhanced by using chelating agents such as ethylene diamine tetraacetic acid (EDTA) (Blaylock et al., 1997; Vassil et al., 1998; Kos and Leštan, 2004; Hernández et al., 2007; Surat et al., 2008; Suthar et al., 2014; Baek et al., 2005), humic acids (HA) (Evangelou et al., 2004; Topcuoğlu, 2012) and diethylene triamine pentaacetic acid (DTPA) (Zhao et al., 2007; Li et al., 2011), or by lowering soil pH using chemicals (e.g. elemental sulfur, aluminum sulfate, iron sulfate) (Everhart, 1994; Vossen, 2006; Longstroth, 2008). However, disadvantages of these methods are that synthetic chelators, such as EDTA, are barely degradable by microorganisms and their use on an operational basis can pose an environmental risk, especially to ground water (Chen et al., 2004). Also, lowering soil pH is a slow process and may require repeated treatments. Furthermore, the repeated application of aluminum or iron can result in a build-up of these metals in the soil up to toxic levels (Vossen, 2006). For these reasons, it is important to better understand how plant root systems could positively influence both the soil rhizosphere and the labile pool of metals.

The use of plants for remediating metal-contaminated soil has been subjected to extensive studies. Numerous investigations have focused on the use of hyperaccumulators and fast growing plants or secondary accumulator plant species to absorb and concentrate TE in their tissues (Koontz and Berle, 1980;

3

Hammer et al., 2003; Yanqun et al., 2003; Solhi et al., 2005; Chen et al., 2009; Soudek et al., 2010). These studies have proposed effective solutions for removal of TE from soil. Some studies have also addressed the role of roots in the phytoremediation of inorganic contaminants such as metals (McGrath et al., 2001;Fitz and Wenzel, 2002; Wenzel et al., 2004). From an operational point of view, some concerns remain regarding the use of exotic plants for phytoremediation. This is one of the reasons this study utilized different shrubs and herbaceous species indigenous to Eastern Canada. We also believe that perennial plant species are easier to manage, notably when repeated harvesting is involved, as in the case of phytoextraction of trace elements. Plants selected for this study have been shown to be easy to cultivate on harsh sites such as brownfields, and resprout well after coppicing or after mowing

The present study was undertaken to acquire knowledge on the effect of roots of different plant species on metal bioavailability in the soil. Consequently, this study aims to verify whether diverse plant species, characterized by different root systems, and physiological and nutritional demands, can positively influence both the rhizosphere and the labile pool of metals in soil. We propose two objectives: (1) to compare the levels of TE accumulated in different tissues of selected woody and herbaceous plant species; and (2) to compare the efficiency of these species in terms of their capacity to affect metal bioavailability in a TE polluted soil.

MATERIALS AND METHODS

Experimental design

A pot experiment examined eight plant species, which included the four woody species *Salix nigra*, *Populus sp*, *Cornus stolonifera*, Alnus *crispa*, and the four herbaceous species *Phalaris arundinacea, Trifolium repens, Panicum virgatum*, *Festuca arundinacea*. These species have all been commonly used in the context of phytoextraction studies in Canada (Pitre et al., 2010; Guidi Nissim et al., 2014; Guidi Nissim et al., 2015).

The four woody species selected were young seedlings produced in "Styroblock ®" containers and transplanted into pots containing the polluted soil (one seedling per pot). The herb species were seeded directly in the pots (10 grams of seed per pot). During plant growth, only tap water (125 ml per day) was

supplied by an automatic drip irrigation system, without the addition of fertilizers or any other amendments.

The soil used in this experiment was excavated from a brownfield located in an industrial zone in the eastern part of the City of Montreal (45°38'07"N 73°32'26"W). This soil was heterogeneous, and composed of filled material including gravel, sand and less than 3% organic material. Large stones and debris were removed from the soil, which was then thoroughly mixed and homogenized and used to fill the pots. A complete portrait of the chemical and agronomic characteristics is shown in Table 1. The trace element content was not considered very high, and silver was the only metal that could be considered an environmental risk according to the provincial guidelines used in Quebec, Canada (Beaulieu, 2016). The concentrations of zinc, copper and nickel were close to but not above levels considered acceptable (Table 1). This trial was carried out under a polyethylene transparent roof (to avoid interference from natural precipitation) mounted on a tunnel structure on the site of the Montreal Botanical Garden, Montreal, Canada (45°55'72'' N, 73°55'68'' W). The cultivation period lasted for three months during summer, from June 6th to September 3rd, 2014. Five-liter plastic pots were installed in a completely randomized block design with five replicates. Each block included the eight species planted in two pots (plus one control pot without any plants) for a total of 85 pots.

Chemical analysis

Sampling

Plants were harvested by cutting the above-ground tissues at the soil surface and removing the roots from the pots. Rhizosphere soil, the soil adhering to plant roots (Jaillard et al., 2002), was collected after gently shaking the roots. Soil samples were placed in plastic bags and air dried for several days until analysis. Roots were thoroughly washed in tap water, then quickly rinsed in deionized water, to remove adhering soil particles. Above-ground tissues and cleaned roots were oven-dried at 70 ^{0}C until they reached a constant weight.

Soil analyses

Chemical properties of the soil used for planting were determined based on analysis of five random samples taken prior to filling the pots. Soil pH was measured in deionised water at a 1:1 soil:water ratio (Hendershot et al. 2008). Electric conductivity (EC) was determined following the CEAEQ method (CEAQ 2015). Total organic matter was obtained by loss on ignition following the Rabenhorst (1988) method. Cation-exchange capacity (CEC) was estimated with the $BaCl_2$ displacement method used by Hendershot et al. (2008). A hot acid-extractable method was used to estimate soil TE concentrations. A 200 mg mass of 0.5 mm sieved dry soil was soaked overnight in 2 mL of concentrated nitric acid (trace metal grade, HNO_3) in 16×125 mm Pyrex ignition tubes. Samples were digested on a block digester at a mean temperature of 120°C for 5 h. The volume was adjusted to 50 mL with deionized water and suspensions were filtered through a Nylon membrane (0.45-µm). The suspension reacted on a reciprocal shaker for two hours, was centrifuged (1400 g) for 10 min, filtered with a Nylon membrane (0.45-µm) and stored in 0.2% trace metal grade HNO3. The TE concentrations of HNO_3 extracts were measured by ICP-mass spectrometry using appropriate dilution to reduce matrix effects (Perkin Elmer NexION 300).

Metal bioavailability

Water extraction was used to estimate water-soluble TE in the soil. Soluble metal concentrations are typically well correlated with plant uptake (Ivezić et al., 2013). Metals present in the water extract are considered water-soluble and potentially available for uptake, or bioavailable (Séguin et al., 2004).

Water extraction involved adding 40 ml of deionized H_2O and a 4 g soil sample (sieved through a 0.5 mm mesh) into a 50 ml acid-washed Falcon® tube. The tubes were then shaken end-over-end for 2 hours at room temperature. Subsequently, the samples were centrifuged at 1400 g for 15 minutes. The mixture was then filtered through a nylon membrane (pore size 0.45µm) using a vacuum system. 0.02 ml HNO_3 50% (TMG grade, Thermo-Fisher) was then added to 5 ml samples of the filtered solution. As for HNO_3 extracts described above, an ICP-mass spectrometer (Perkin ElmerNexION 300) was used for analysis of water-soluble TE (referred to as Ag-H_2O, Se-H_2O, Cu-H_2O, Ni-H_2O, Pb-H_2O, and Zn-H_2O).

Plant tissue digestion

Dry plant tissue samples (0.200 g) were digested in borosilicate tubes containing 2 ml nitric acid 70% (TMG grade from Thermo-Fisher) at 120°C for five hours. The entire contents of the borosilicate tubes were transferred into 50 ml Falcon® tubes and the volumes were adjusted to 50 ml with ultrapure water (MilliQ), to attain a final concentration of 4% nitric acid. The tubes were stirred and allowed to stand overnight. Samples were then filtered through a nylon membrane (pore size 0.45μm). Digestates were diluted to 2% HNO_3 before ICP-MS analyses.

Quality assurance and quality control for metal analyses

Laboratory equipment was acid-washed (20% HNO_3) and rinsed six times with deionized water. Quality controls were performed with certified natural water from the National Institute of Standards and Technology (NIST 1640a). The results yielded mean recoveries of 99 – 102% for all metals.

For the soil water extractions and other soil physicochemical measurements, internal soil control was used to test quality. The results yielded an accuracy of 82–133%. The detection limit of the method was calculated for each environmental matrix by multiplying by three the standard deviation obtained from multiple analyses of solutions having low solute concentrations (0.0008 - 0.2414 μg/l, depending on the TE).

DOC analysis

DOC concentrations were measured with an IO Aurora 1030 carbon analyzer. Briefly, DIC (dissolved inorganic carbon) was purged from samples after adding phosphoric acid (5% ACS grade, Fisher Scientific). DOC was oxidized by adding sodium persulfate, 10% (98%+ grade, Acros) and quantified against a potassium biphtalate (BDH) calibration curve (0-25 mg/L).

Statistical analysis

All variables were analyzed with fixed-model ANOVAs. This model is presented as Y= blocks.species.blocks*species, in which Y represents variables measured (pH, EC, percent organic matter, DOC, TE in the soil and TE in the plants), and blocks and species are nominal variables. For all tests, the

classical level of 0.05 was used as *p*-value threshold. Analyses were performed using JMP statistical software version 10.0 (SAS Institute, Cary, NC) and Excel (Microsoft Inc.) software packages.

RESULTS

Biomass

Total biomass varied between 4.6 g pot^{-1} to 16.8 g pot^{-1} for *A. crispa* and *P. virgatum*, respectively. Root biomass ranged from 1.4 g pot^{-1} in *A. crispa* to 7.3 g pot^{-1} in *F. arundinacea* and from 3.3 g pot^{-1} in *A. crispa* to 11.7 g pot^{-1} in *T. repens* for above-ground biomass (Figure 1). The largest root biomass was found for *F. arundinacea*, and *P. virgatum*. Above-ground tissue biomass was found to be highest in *T. repens*, and was three times higher than in *A. crispa.*

Among the eight plant species, *A. crispa* had the smallest biomass, which necessarily affects its capacity to accumulate metals. For example, Ni mass per pot in roots *A. crispa* (i.e., TE concentration × biomass) was 0.2 mg pot^{-1}, while roots of *T. repens* was able to take up 0.7 mg pot^{-1}.

Ag, Pb, Cu, Zn, Ni, Se in roots and above-ground tissues.

All tested species showed a low Ag concentration in their above-ground tissues (stem, leaves and twigs) (Figure 2A). The highest concentrations were observed in roots of *F. arundinacea* and *P. arundinacea* (P < 0.05). .

Cu concentrations in roots varied between 34.5 mg kg^{-1} (in *A. crispa*) and 292.7 mg kg^{-1} (in *P. arundinacea*). *P arundinacea* also presented the highest Cu concentration (P < 0.05) in its above-ground tissues with a value of 55.8 mg kg-1 (Figure 2B). (Figure 2B).

Zn translocation to above-ground tissues of *S. nigra* was the greatest (159.9 mg kg^{-1}), followed by *P. arundinacea* (104.4 mg kg^{-1}) and *Populus sp*. (96.2 mg kg^{-1}), while the lowest value was found in *C. stolonifera* (32.8 mg kg^{-1}); the corresponding Zn concentrations in roots varied between 62.7 mg kg^{-1} (in *A. crispa*) and 212.4 ± 14.0 mg kg^{-1} (in *S. nigra*) (Figure 2C).

Figure 2D shows that Ni concentrations in above-ground tissues were generally low and inferior to 3 mg kg^{-1}). Concentration in the roots was about twice that found in aerial parts, with the highest value found in *T. repens* (4.7 mg kg^{-1}).

Pb concentrations in aerial parts were also low for all species. The highest concentration was observed in *P. arundinacea* (2.72 mg kg^{-1}). In root tissues, *C. stolonifera* had the highest level of Pb (6.77 mg kg^{-1}). The lowest Pb concentrations were in root tissues of *P. virgatum* and *A. crispa* (2.05 and 2.46 respectively)(Figure 2E).

Se accumulation was found to be highest in the above-ground tissues of the two grass species, *F. arundinacea* and *P. arundinacea* but the concentrations were weak and did not exceed 0.25 mg kg^{-1}(Figure 2F).

In general, these results indicate that TE accumulation by plants differed among species and tissues. In addition, concentrations of TE accumulated by plants were mostly accumulated in roots. However the ratios between Ni and Zn concentrations in aerial to belowground tissues were higher in comparison to other TE. TE concentrations (mg kg^{-1}) in root tissues of the herb species tended to be greater than in those of the woody species because herb species grew faster, and their root system was more extensive than that of the woody species. Therefore, total TE removal (i.e., TE concentration × biomass) in herb species was greater than woody species. For example, Se, Zn, Cu, and Ni taken up by *T. repens* were 4.1 mg, 2.7 mg, 1.4 mg, and 1.4 mg respectively. On the other hand, quantities of Se, Zn, Cu, and Ni found in biomass of *A. crispa* were only 0.3 mg, 0.2 mg, 0.1 mg, and 0.1 mg respectively (data not shown).

In general, water-extractable fraction (considered to be potentially available for uptake) of TE was higher in rhizospheric soil than in unplanted control soil (Figure 3). For instance, Ag concentration in the water-extractable fraction in the rhizosphere was the highest in rhizosphere of *S. nigra* (Figure 3). Available concentrations of Cu were found to be greater in the rhizosphere of three herb species, namely *T. repens,F. arundinacea* and *P. virgatum.* It was not possible to observe a similar pattern for Ni and Cu (Figure 3).

EC, pH, and % organic matter, and DOC

Measurements were made to evaluate whether the cultivation of plants can induce some changes in EC, pH, and % organic matter in the rhizospheres of all species. No significant changes were observed for any of these particular parameters (data not shown).

In contrast, DOC levels in the rhizosphere of *T. repens* (6.7 mg l^{-1}) and *C. stolonifera* (6.3 mg l^{-1}) were significantly different from that measured in the control pots (4.7 mg l^{-1}) (Table 2).

Figure 4 presents changes in TE bioavailability according to the level of DOC measured in the soils. Only the most relevant results are shown in this figure. DOC in the rhizosphere of *S. nigra* was at an intermediate level compared to the rhizosphere of the other species, but the available concentration of Ag was highest. However, the highest DOC level as well as the highest concentrations of available Ni and Cu were found in the rhizosphere of *T. repens*.

After three months of plant growth, there was a positive and statistically significant correlation between the availability of Ni (adjusted R^2 = 0.67; p = 0.004) and Cu (adjusted R^2 = 0.59; p = 0.009), and DOC concentrations in the rhizosphere (Figure 4, Table 3). In the case of Ag, no significant relationship was found, even when excluding the extreme value of *S. nigra*. This suggests that different root systems had different abilities to directly affect DOC levels in the rhizosphere, and to indirectly influence the availability of Ni and Cu in the rhizosphere.

DISCUSSION

Concentrations of TE in tissues

The capacity of the plants in this experiment to absorb TE in their tissues was investigated. Ag rarely occurs in nature in a soluble form (Koontz and Berle, 1980), so available Ag for plant uptake is restricted. In this study, Ag concentration in soil was only 2.4 mg kg^{-1}, but concentrations of Ag accumulated in root tissues of *F. arundinacea* were up to 1.7 mg kg^{-1}. Ag was mainly stored and immobilized in the root, which suggests that *F. arundinacea* has potential for use in phytostabilization of Ag-contaminated soils (USEPA, 1999).

Among the TE tested in this experiment, Zn and Ni were translocated to aerial tissues in greatest amounts. *S. nigra* was more efficient than other species at translocating Zn from root to shoot tissues. Therefore, the efficient removal of Zn from the soil-plant system could be achieved through the harvest of aerial plant parts. Based on our findings, *S. nigra* would be a good candidate for use in phytoextraction in Zn-contaminated soils.

Se is a metalloid that can be lost to the atmosphere because it can be biomethylated to form volatile molecules (Guidi Nissim et al., 2015). Some plants, such as *Astragalus bisculatus* (Neuhierl and Bock, 1996), *Atriplex nummularia* and *Brassica juncea* (Smith, 1994), have been found to accumulate high

amounts of Se (up to several milligrams/gram dry tissue) by accumulating nonvolatile methyl selenate derivatives. In the current study, the highest accumulation of Se was in the roots of *S. nigra*, which reached 0.32 mg kg^{-1}, and in above-ground tissues of *F. arundinacea* and *P. arundinacea* with concentrations around 0,25 mg kg^{-1} although its concentration in soil was very low, with less than 1.0 mg kg^{-1}.

Cu is an element known to be essential to plant growth, however it can become toxic when it accumulates in shoots or leaves at levels exceeding 20 mg kg^{-1} (Krämer et al., 1997). Cu values in this study were above this toxic level in some tested species such as *P. arundinacea* (55.8 mg kg^{-1}), *T. repens* (41.8 mg kg^{-1}), and *P. virgatum* (29.4 mg kg^{-1}). These plant species may have good potential for use in remediation of Cu-contaminated soils, since they were able to tolerate high Cu levels.

In soil, Pb usually exists as insoluble precipitates (phosphates, carbonates and hydroxyoxides), which are largely unavailable for uptake by roots; the potential for Pb phytoextraction is therefore limited (Lasat, 2002). Pb accumulation was relatively higher in roots than in above-ground tissues for all tested plant species. This trend was similar to those found for other species. For instance, Pb accumulated in sunflower (hybrid Albena) that grew in soil that contained 554 mg kg^{-1} Pb as follows: 38.9 mg kg^{-1} in roots, 22.6 mg kg^{-1} in shoots, 4.3 mg kg^{-1} in leaves, 1.7 mg kg^{-1} in capsules, and 11.6 mg kg^{-1} in seeds (Lasat et al., 1998).

Availability of TE in the rhizozphere

Overall, the presence of plant species did not affect most measured parameters (pH, EC, percent organic matter), they did, however, affect DOC levels.

Regarding pH, various researchers have found that metal solubility increases greatly under acidic conditions (Harter, 1983; Chuan et al., 1996; Takáč et al., 2009). With increasing pH, the availability of most metals present in soil decreases due to their increased adsorption and precipitation in alkaline media (Takáč et al., 2009). Smith (1994) found a negative relationship between concentrations of Ni and Cu in ryegrass and soil pH (ranging from 4.2- to 7.0)). In this study, soil pH was more alkaline, ranging from 7.94 to 8.00. This indicates that the solubility of all the elements was likely limited by pH. The highest available concentrations of Ag, Cu and Ni were 1 μg kg^{-1} in *S. nigra*, 200.4 μg kg^{-1} and 32.3 μg kg^{-1} in *T. repens*, respectively. It is likely that the very narrow range of pH values precluded the detection of a relationship between pH and metal availability.

In contrast, we found strong and significant linear relationships between DOC and metal availability in the rhizosphere. Metals can form complexes with DOC (Welsh, 1996; Zhao et al., 2007; Merrington and Peters, 2009), and these complexes may increase the labile metal pool and thus also metal bioavailability (Rembe and Trefry, 2003 Antoniadis and Alloway, 2001; Carmona et al., 2008; Li et al., 2011). A previous study found that plants significantly increase the size of a number of dissolved nutrient pools, which include DOC and total phenolics in solution (Khalida et al., 2006). It is interesting to note that some studies have also shown that hyperaccumulator species exude more DOC in the rhizosphere than non-hyperaccumulators (Tu et al., 2004; Li et al., 2011). These results provide support to the hypothesis that certain plants may enhance metal solubility in the rhizosphere via root exudation, consequently increasing plant metal uptake (Li et al., 2011). This increased metal availability may either result directly from the formation of complexes between metals and organic ligand exudates, or it can be an indirect process by which root exudates in the form of organic acids destabilize metal bounds in the soil organic matter, making these metals available for uptake (Clarholm et al, 2015).

In this study, DOC concentrations were also significantly and differently affected by the presence of plants, with the lowest DOC levels found in the unplanted treatment. There was no clear difference between wood vs herbaceous species in their ability to modify DOC levels in rhizosphere. Positive relationships were found between the availability concentration of Ni and Cu, and DOC concentrations in the rhizosphere. This suggests that different root systems had different abilities to directly affect DOC pools in the soil and hence indirectly influence the bioavailable pool of TE and their uptake by roots. Further, this effect of plants on metal uptake is likely metal dependent, since we did not see a clear positive relationship between DOC and available Ag. This is to be anticipated, since different metal complexes will differ in solubility and will interact with metal binding sites on the root surface differently. There are few data in the published literature showing such relationships between DOC and available metals in the rhizosphere across many plant taxa, and further research should aim to identify what are the key metal complexes formed, and if the effect of root exudates is mainly via direct complexation or by metal destabilization of the soil organic matter. This would enhance our ability to predict metal uptake by plants. Our findings suggest that knowledge of the phytoremediation potential of the root systems of

individual species can significantly improve the outcome of phytoremediation projects, but ensuring that plant selection accommodates the needs specific to a project, whether phytostabilization or phytoextraction.

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TABLES

Table 1. Characteristics and means of HNO_3-extractable trace element concentrations of the soil used.

Characteristic	Mean
pH	7.9 ± 0.0
EC (μS cm^{-1})	131.0 ± 7.0
CaCO$_3$ (%)	41.4 ± 1.4
CEC (meq 100g^{-1})	34.6 ±15.7
Organic-C (%)	2.81± 0.89
Ag (mg kg^{-1})	2.4 ± 0.2
Cu (mg kg^{-1})	34.3 ± 0.8
Pb (mg kg^{-1})	60.7 ± 19.3
Zn (mg kg^{-1})	129.9 ± 2.1
Ni (mg kg^{-1})	29.9 ± 4.2
Se (mg kg^{-1})	0.9 ± 0.1

Table 2. Dissolved organic carbon in the rhizosphere of all plants after three months of in-pot

cultivation

(mg l^{-1})

Species	Least Sq Mean (mg l^{-1})
Trifolium rupens	6.7[a] ± 0.3
Cornus stolonifera	6.3[ab] ± 0.3
Festuca arundinacea	6.1[abc] ± 0.3
Alnus crispa	5.5[abc] ± 0.3
Panicum virgatum	5.5[abc] ± 0.3
Phalaris	5.3[abc] ± 0.3
arundinacea	5.3[abc] ± 0.3
Salix nigra	5.1[bc] ± 0.3
Populus sp	4.7[c] ± 0.3
Unplanted control	

Different letters within the column indicate statistically significant differences between species, $P < 0.05$.

Table 3. Regression test relationship between the availability of Ni and Cu, and DOC concentration in the rhizosphere after three months of cultivation in pots.

		Coefficients	Standard Error	df	Significance P
Ni	Regression			1	0.047*
	Intercept	0.003	0.007		
	X	0.002	0.001		
Cu	Regression			1	<0.001*
	Intercept	-0.127	0.027		
	X	0.042	0.005		

*: significance at level 5%

Y (Ni availability concentration (mg kg^{-1})) = 0.002X (DOC concentration (mg l^{-1})) + 0.003

Y (Cu availability concentration (mg kg^{-1})) = 0.042X (DOC concentration (mg l^{-1})) – 0.127

FIGURE CAPTIONS

Figure 1. Comparison of biomass produced after three months of cultivation in pots. Columns with different letters are significantly different at $P < 0.05$.

Figure 2. The concentration of TE (Ag, Ni, Cu, Pb, Zn, and Se) in roots and aboveground tissues of eight plant species after three months of cultivation in pots. Columns with different letters are significantly different at $P < 0.05$.

Figure 3. The water-soluble concentration of TE (Ag, Ni, Cu) in the rhizosphere of eight plant species after three months of cultivation in pots. Columns with different letters are significantly different at $P < 0.05$.

Figure 4. Changes in TE (Ag, Ni, Cu) bioavailability according to the level of DOC concentrations in the rhizosphere of eight plant species after three months of cultivation in pots.

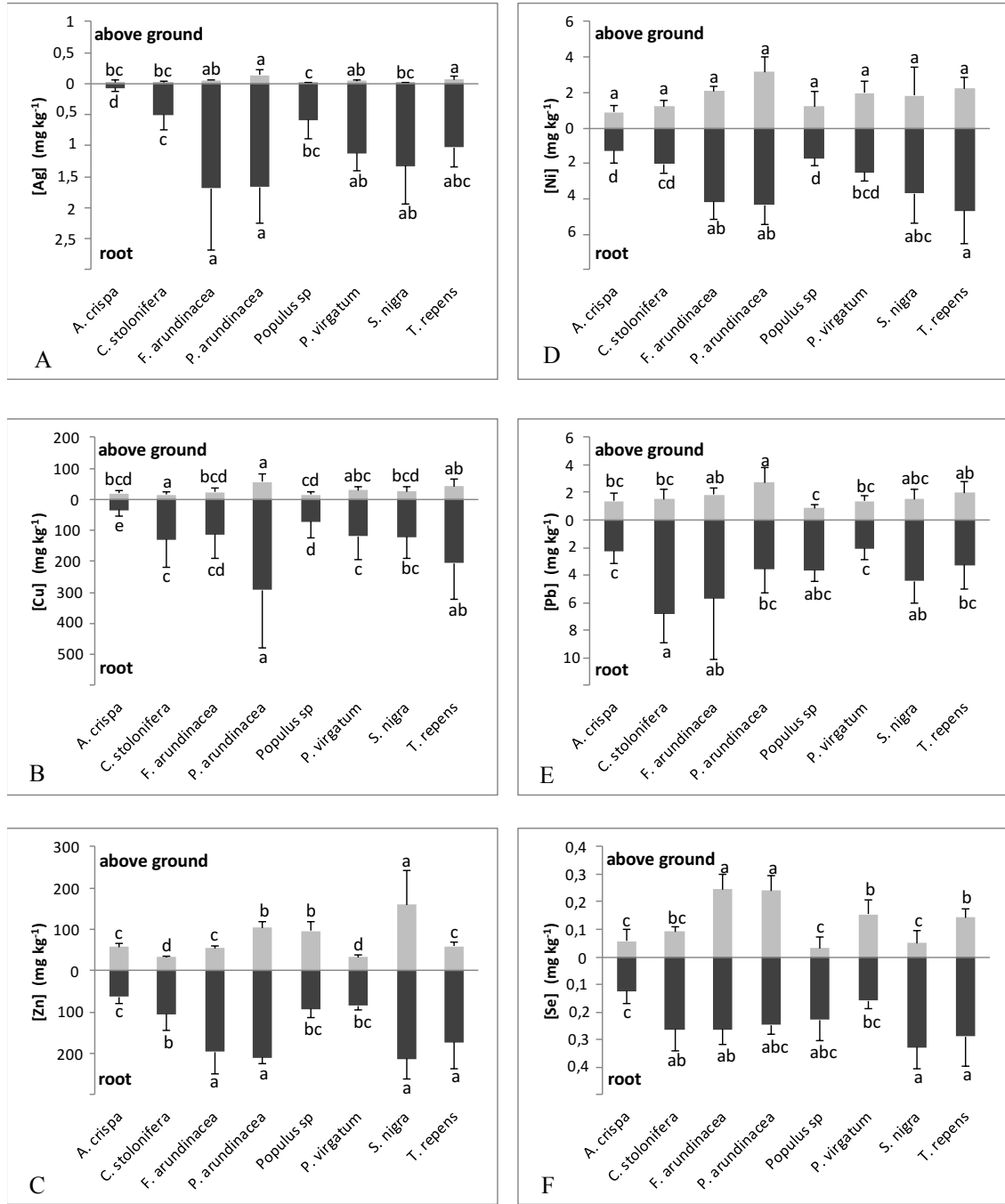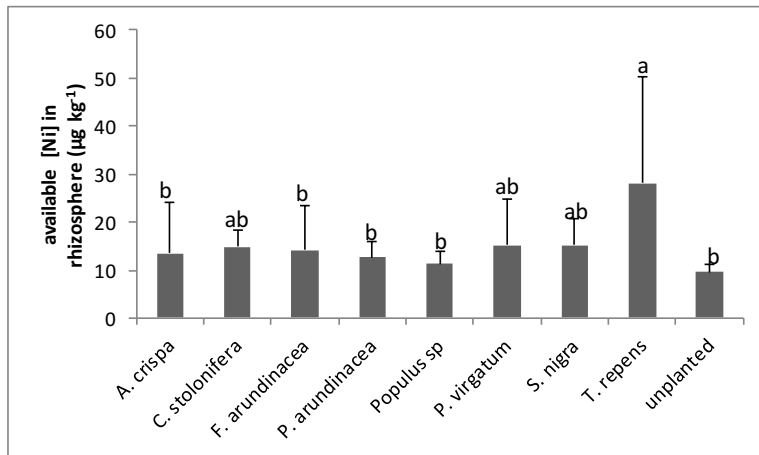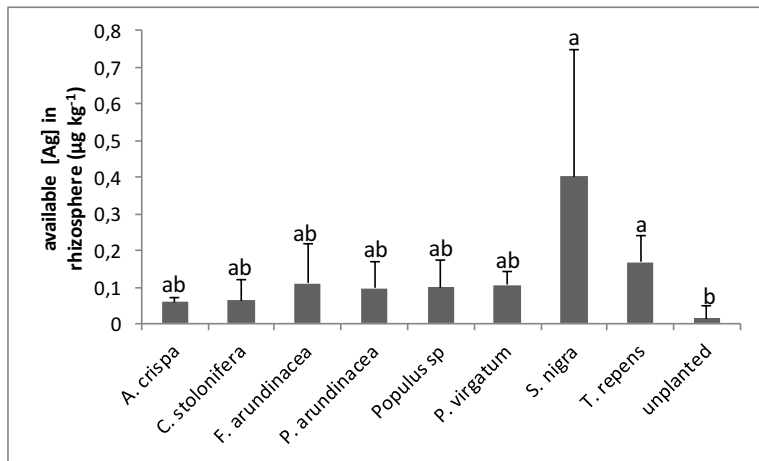FIGURE 1. Nguyen et al.
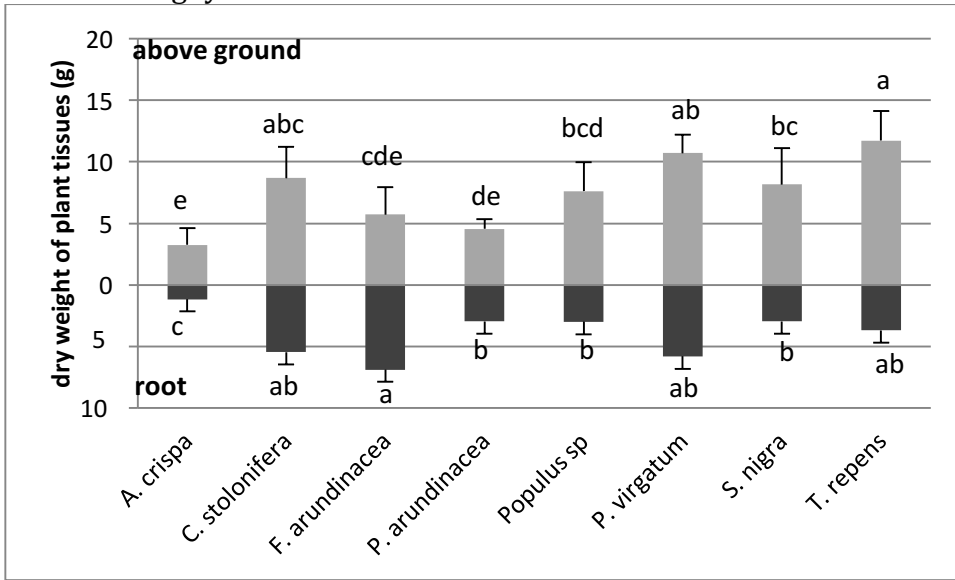
FIGURE 2. Nguyen et al.

FIGURE 3. Nguyen et al