

Université de Montréal

Effects of diverse plant species on the bioavailability of contaminants in soil

par

Nguyen Thi Xuan Trang

Institut de recherche en biologie végétale

Département de sciences biologiques

Mémoire présenté à la Faculté des études supérieures
en vue de l'obtention du grade de Maîtrise en sciences biologiques

Décembre 2015

© Nguyen Thi Xuan Trang, 2015

Université de Montréal
Faculté des études supérieures et postdoctorales

Ce mémoire intitulé :

Effects of diverse plant species on the bioavailability of contaminants in soil

Présenté par :

Nguyen Thi Xuan Trang

Évalué par un jury composé des personnes suivantes :

Michel Labrecque, directeur de recherche

Marc Amyot, co-directeur de recherche

Frédéric Pitre, président-rapporteur

François Courchesne, membre du jury

Résumé

La phytoremédiation constitue une technologie alternative pour le traitement de sols contaminés en métaux. Toutefois, la biodisponibilité des métaux dans le sol peut limiter l'efficacité de cette approche. Nous émettons l'hypothèse que diverses espèces de plante, caractérisées par systèmes racinaires différents, peuvent affecter différemment la biodisponibilité des éléments traces (ET) dans le sol.

Une étude utilisant un dispositif expérimental en bloc aléatoire complet avec cinq réplicats a été conduite entre le 6 juin et le 3 septembre 2014, sur le site du Jardin botanique de Montréal. Dans ce contexte, l'impact de la présence de huit espèces de plantes, herbacées ou ligneuses, sur le pool labile de six métaux (Ag, Cu, Pd, Zn, Ni et Se) dans la rhizosphère de celles-ci a été étudié. Après trois mois de culture, la biomasse aérienne et souterraine de chaque espèce a été mesurée et la concentration en ET dans les tissus des plantes a été analysée. La fraction labile de ces ET dans la rhizosphère (potentiellement celle qui serait biodisponible) de même que d'autres paramètres édaphiques (le pH, la conductivité, le pourcentage de matière organique et le carbone organique dissous (COD)) ont aussi été mesurés et comparés en fonction de la présence d'une ou l'autre des espèces utilisées.

Les résultats montrent que pour la plupart des plantes testées, les plus fortes concentrations en ET ont été trouvées dans les racines alors que les plus faibles niveaux s'observaient dans les parties aériennes, sauf pour le Ni dans le *Salix nigra*. Ceci suggère que le Ni peut être extrait du sol par des récoltes régulières des tiges et des feuilles de cette espèce de saule. Les pools labiles de l'Ag, Ni et du Cu dans la rhizosphère étaient significativement et différemment affectés par la présence des plantes. Toutefois, la présence des plantes testées n'a pas affecté certains paramètres clés de la rhizosphère (ex. le pH, conductivité, et le pourcentage de matière organique). À l'opposé, les niveaux de COD dans la rhizosphère de toutes les plantes testées se sont révélés supérieurs en comparaison des témoins (sols non plantés). De plus, une corrélation positive a pu être établie entre la concentration disponible du Ni et la concentration en COD. Une relation similaire a été déterminée pour le Cu. Ceci suggère que certains systèmes racinaires pourraient modifier les niveaux de COD et avoir un impact indirect sur les pools labiles des ET dans le sol.

Mots-clés : Biodisponibilité, Métaux, Rhizosphère, Accumulation, Carbone organique dissous

Abstract

Phytoremediation is considered an alternative technology for treating metal-contaminated soil, however, its efficiency is usually limited by bioavailability of metals in soil. We hypothesized that diverse plant species have different root system types that can affect bioavailability of trace elements (TE) in soil differently.

The experiment was conducted during summer, from June 6th to September 3rd, 2014, at the Montreal Botanical Garden. The effect of eight herbaceous and woody species on the labile pool of six metals (Ag, Cu, Pd, Zn, Ni and Se) in their rhizosphere was studied using a completely randomized block design with five replicates. After three months of cultivation, the above- and below-ground biomass of each species was measured and the concentrations of TE in the plant tissues 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 results showed that in most tested plants, the highest concentrations of TE were found in the roots and the lowest levels were in the above-ground tissues, except for Ni in *Salix nigra*. This suggests that Ni can be removed from soil through the regular harvest of the shoots and leaves of this species. The labile pool of Ag, Ni, and Cu in the rhizosphere was significantly and differently affected by the presence of the plants. However, the presence of the tested plant species did not affect some key factors of the rhizosphere (e.g. pH, EC, percent of organic matter). In contrast, DOC levels in the rhizosphere of all tested plants were higher than those measured for the control pots without plants. In addition, a positive relationship was found between Ni availability concentration and DOC concentration, as well as Cu availability level and DOC level. This suggests that some types of root systems appear to modify soil DOC and indirectly impact the labile pool of TE in soil.

Keywords : Bioavailability, Metals, Rhizosphere, Accumulation, Dissolved organic carbon.

Table des matières

Résumé.....	ii
Abstract.....	iv
Abstract.....	iv
Table des matières.....	v
Liste des tableaux.....	vii
Liste des figures.....	viii
Liste des abréviations.....	ix
Remerciements.....	xi
Chapter 1: Introduction.....	1
1.1. Metal bioavailability in soil.....	1
1.2. Factors influencing metal bioavailability.....	3
1.3. Enhancing metal bioavailability.....	6
1.3.1. Chelating products.....	6
1.3.2. Products affecting pH.....	7
1.3.3. Hyperaccumulator plants.....	7
1.4. Objectives and hypotheses.....	10
Chapter 2: Effects of diverse plant species on the bioavailability of contaminants in soil ¹	11
2.1. Abstract.....	11
2.2. Introduction.....	12
2.3. Material and methods.....	13
2.3.1. Experimental design.....	13
2.3.2. Chemical analysis.....	14
2.3.2.1. Sampling.....	14
2.3.2.2. Metal bioavailability.....	15
2.3.2.3. Plant tissue digestion.....	15

2.3.2.4. Quality assurance and quality control.....	15
2.3.3. Statistical analysis.....	16
2.4. Results.....	16
2.4.1. Biomass.....	16
2.4.2. Ag, Pb, Cu, Zn, Ni, Se in roots and above-ground tissues.....	17
2.4.3. Availability of Ag, Pb, Cu, Zn, Ni, Se in the rhizosphere	21
2.4.4. Levels of pH, EC, % organic matter, and DOC.....	22
2.5. Discussion.....	24
2.5.1. The concentration of TE in tissues.....	24
2.5.2. Availability of TE in the rhizosphere.....	26
2.6. Acknowledgments.....	27
Chapter 3: Conclusion.....	28
Bibliographie.....	31

Liste des tableaux

Table 1. Characteristics of the soil used in pot experiment	14
Table 2. Dissolved organic carbon in the rhizosphere of all plants after three months of in-pot cultivation	22
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	24

Liste des figures

Figure. 1. Comparison of biomass produced after three months of in-pot cultivation.	17
Figure. 2. The concentration of TE (Ag, Ni, Cu, Pb, Zn, and Se) in roots and above-ground tissues of eight plant species after three months of in-pot cultivation.	19
Figure. 3. The water-soluble concentration of TE (Ag, Ni, Cu) in the rhizosphere of eight plant species after three months of in-pot cultivation.	21
Figure. 4. The water-soluble concentration of TE (Ag, Ni, Cu) and DOC concentration in the rhizospheres of eight plant species after a three months of in-pot cultivation.	23

Liste des abréviations

DOC	Dissolved organic carbon (carbone organique dissous)
DTPA	Diethylenetriaminepentaacetic acid (acide diéthylène triamine penta-acétique)
EC	Electrical conductivity (conductivité électrique)
EDTA	Ethylenediaminetetraacetic acid (acide éthylène diamine tétraacétique)
g g^{-1}	Gramme par gramme
g pot^{-1}	Gramme par pot
HA	Humic acid (acide humique)
ICP-MS	Inductively coupled plasma mass spectrometry (spectromètre de masse-à plasma à couplage inductif)
LMW	Low molecular weight (faible poids moléculaire)
mg kg^{-1}	Milligramme par kilogramme
mg l^{-1}	Milligramme par litre
$\mu\text{S cm}^{-1}$	Micro-Siemens par centimètre

“If we knew what it was we were doing, it would not be called research, would it?”

Albert Einstein (1879-1955)

Remerciements

J'aimerais exprimer ma sincère gratitude à tous ceux qui m'ont aidé tout au long de mon mémoire de maîtrise.

Je tiens tout d'abord à remercier mon directeur de recherche et aussi conservateur du Jardin botanique de Montréal, Michel Labrecque, qui m'a accueilli à bras ouverts dans son équipe de recherche, et m'a donné l'opportunité de travailler sur ce projet qui me tient beaucoup à cœur. Je remercie aussi mon co-directeur de recherche, Marc Amyot. Michel et Marc m'ont appuyée tout le long de mon projet de maîtrise, avec leurs conseils et leurs encouragements qui m'ont poussée à donner le meilleur de moi-même.

Je tiens également à remercier François Courchesne et Frédéric Pitre pour leurs précieux conseils pour l'expérience.

Merci à Stéphane Daigle pour son aide inestimable dans la réalisation de la partie statistique du présent mémoire. Aussi je voudrais remercier Marie-Claude Turmel et Dominic Bélanger, qui travaillent dans le laboratoire des sciences au sol du département de géographie et le laboratoire du département de sciences biologiques de l'Université de Montréal, pour m'avoir assisté tout au long de mon stage de recherche.

Je ne voudrais pas oublier de remercier Hafssa Kadri et Ahmed Jerbi, pour leur support logistique sur le terrain.

Enfin, je tiens à remercier ma famille, mon mari et mes amis pour leur gentillesse et leur encouragement continu essentiels pour la réalisation de mon mémoire de maîtrise.

Chapter 1: Introduction

In the 1970s, Hartman proposed using plants to remove pollutants from waste water. In 1983, the idea of using metal-accumulating plants to clean up heavy metals and other compounds was first introduced by Chaney (Chaney, 1983). Today, it has been proposed as an alternative technology for remediating metal-contaminated soil (Raskin et al., 1997), particularly using phytoextraction, which is considered a green and low-cost technology to remove metals from contaminated sites (Kumar et al., 1995; USEPA, 2000; Gisbert et al., 2003). In the last decade, many studies have been conducted to investigate techniques for enhancing the efficiency of phytoremediation, such as using hyperaccumulators (Brown et al., 1995; Gumaelius et al., 2004; Peer et al., 2006); chelating agents such as ethylene diamine tetraacetic acid (EDTA), humic acids (HA) or diethylene triamine pentaacetic acid (DTPA) (Cooper et al., 1999; Evangelou et al., 2004; Baek et al., 2005; Hernández et al., 2007); fast growing plant species (Hammer et al., 2003; Soudek et al., 2010); or by lowering soil pH using chemicals (Everhart, 1994; Vossen, 2006; Longstroth, 2008). In general, the main purpose of these methods is to increase the bioavailability of TE in soil and metal accumulation in plants. Therefore, bioavailability of TE in the soil can influence the efficiency of metal phytoextraction in metal-contaminated soil. However, disadvantages of these methods are that synthetic chelators, such as EDTA, are barely degradable by microorganisms and their utilization on an operational basis can pose an environmental risk, especially to ground water (Chen et al., 2004). Also, the process of lowering soil pH is slow and may require repeated treatments. In such a case, the repeated applications 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 can positively influence the soil rhizosphere as well as the labile pool of metals.

1.1. Metal bioavailability in soil

There are many different definitions and uses of the term *metal bioavailability* in toxicology. A widely used definition is the ratio of total metal that is available for incorporation into living organisms. Bioavailability is defined as “the availability of a

chemical to an animal, plant, or microorganism; it may be assayed by measurement of uptake, toxicity or biodegradability” (Linz and Nakles, 1997). Another definition, used by the American Society for Testing and Materials (ASTM, 1998), is “a measure of the fraction of the chemical(s) of concern in environmental media that is accessible to an organism for absorption”. The United States Environmental Protection Agency (USEPA, 2004) defines bioavailability of metal as “the extent to which bioaccessible metals adsorb onto or absorb into and across biological membranes of organisms, expressed as a fraction of the total amount of metal the organism is proximately exposed to (at the sorption surface) during a given time and under defined conditions”.

In an aquatic environment, Morel (1983) described the interaction between trace metals and aquatic organisms through the free ion activity model (FIAM). This model illustrates the binding of the free metal ion and other metal species to the ligand; the author also described the competition between metal species and other cations for binding at the surface, thereby influencing the concentration of metal at the biological receptor. The gill surface interaction model (GSIM), developed shortly after (Pagenkopf, 1983), expanded on this concept to include the effects of alkalinity, hardness, and pH on the binding of metal toxicity at gill sites. In 2001, the biological ligand model (BLM), a tool that goes beyond description to predict the effects of acute metal toxicity on living organisms in the aquatic environment was developed (Santore et al., 2001).

In the soil system, metals can exist in both solid and solution phases. The use of FIAM is possible in soil system, however it is more complex because metals are bound in soil by precipitation, ion exchange and adsorption (Peijnenburg et al., 1997). Precipitation processes occur when the solubility product for a reaction between a metal ion and a ligand in the system is exceeded (Peijnenburg et al., 1997). This process participates in the growth of new bulk solid phase (Apak, 2002). As a result, metals can precipitate as oxides, hydroxides, carbonates, sulfides, or phosphates onto soil. Ion exchange is also one of process that involves the loss of metal ions from aqueous phase to a contiguous solid phase (Apak, 2002). In this process, one positively charged cation is exchanged for another at a constant-charged surface (Peijnenburg et al., 1997). The third mechanism is adsorption. This process is discussed as the accumulation of matter at the solid-water interface. It is the most important process affecting on metals

bioavailability because it may retain ions in the soil and limits their availability in the soil solution by strong binding of metal ions with soil organic matter and variable charge minerals (Apak, 2002).

In the soil solution, metals are present as free ions or form complexes because metals can combine with dissolved organic and inorganic ligands (e.g. anions such as OH^- , CO_3^{2-} , SO_4^{2-} , NO_3^- , or Cl^-). Ions in the solution phase are more available for plant uptake and transport; however, if environmental conditions change, ions in the solid phase may become available as well (NFESC, 2000). To assess the environmental risk of metal contamination in soil, determining the bioavailability of metals in the soil solution is more relevant than measuring their total concentration. Depending on the type of soil, the speciation and bioavailability of a metal differ. For example, the bioavailability and toxicity of nickel in soil rich in organic matter has been shown to be less than in sandy and clay soil at a similar pH (Weng et al., 2004). Similarly, the capacity of absorption of the clays in the Guadiamar Valley was found insufficient to immobilize heavy metals, especially Zn, because of the high levels of pollution in the soil in this area (García-Sánchez et al., 1999).

1.2. Factors influencing metal bioavailability

The process of binding heavy metals is primarily based on the partition of metals between the solid and solution phases. The bioavailability of metals in soil depends on:

(a) Their forms: for instance, metal compounds associated with iron and manganese hydroxides will have average bioavailability (Salomons, 1995); elemental and sulfide forms of a metal are less soluble in biological fluids and hence less bioavailable than the oxide, hydroxide, carbonate and sulfate forms of the same metal (NFESC, 2000).

(b) Soil properties, which include: pH, organic matter content, cation exchange capacity, soil texture and interaction among the target elements (Reichman, 2002; Jung, 2008; Violante et al., 2010; Fijałkowski et al., 2012). The bioavailability of metal is also affected by the redox potential in soil (Chuan et al., 1996). Some soil properties particularly important for our investigation are discussed below:

➤ pH

Soil pH is defined as the negative logarithm of the hydrogen ion concentration. Hydrogen ions are strongly attracted to the surface negative charges, and they have the power to replace most other cations (Alloway, 1995). Soil pH is the most important factor controlling metal partitioning (Harter, 1983; Peijnenburg et al., 1997; Bradham et al., 2006; Jung, 2008). In soil, metal solubility is controlled by interactions (e.g. adsorption/desorption, precipitation/dissolution and complexation reactions) in liquid and solid phases (Silveira et al., 2003). Many studies have found that metal solubility increases markedly under acidic conditions (Harter, 1983; Chuan et al., 1996; Takáč et al., 2009). For positively charged ions such as Cd^{2+} , soil acidification invariably results in increased TE solubility due to increased competition from H^+ ions at the negatively charged binding sites (Robinson et al., 2005). Conversely, sorption of metal cations increases by increasing pH (Violante et al., 2010). With increasing pH, the availability of most metals from soil decreases because there is an increase of their precipitation as insoluble hydroxides, carbonates and organic complexes under alkaline conditions (Takáč et al., 2009).

➤ Organic matter content

Organic matter existing on the soil surface is originally produced by living organisms (plant or animal) and returned to the soil through the decomposition process (FAO, 2005). Organic matter is one of the important factors influencing sorption reactions since it is quite effective in retaining metals (Barry et al., 1995; Silveira et al., 2003). Depending on the nature of the organic matter, metal-organic complexes can occur both in solution and on solid surfaces (Silveira et al., 2003). For example, exchangeable Cu concentration can decrease because biosolids act as organic immobilizing agents (Kiikkila et al., 2002). On the other hand, the solubility of heavy metals such as Cu, Ni and Pb shows a strong positive relationship to the solubility of organic matter (Ashworth and Alloway, 2008). Soil organic matter contains mainly high molecular weight humin and humic acid compounds, as well as lower molecular weight fulvic acid (Ross, 1994).

➤ Dissolved organic carbon (DOC)

Dissolved organic carbon (DOC) is operationally defined as organic matter that is able to pass through a filter, most often 0.45 μm . This is usually the major form of carbon transported within soil solution and in streams (Kolka et al., 2008). Metal ions and complexes are able to combine with DOC (Welsh, 1996; Zhao et al., 2007; Merrington and Peters, 2009), and these new complexes enhance metal solubility (Rembe and Trefry, 2003). Therefore, DOC in soils can increase the labile pool of metals (Antoniadis and Alloway, 2001; Carmona et al., 2008; Li et al., 2011).

The nature of DOC includes a large range of molecules, ranging from simple amino acids, sugars and lipids to complex humic substances with high molecular weights (Schomakers et al., 2014). Depending on the molecules of DOC, metal-DOC complexes can influence plant uptake. In the apoplastic pathway in plants, low molecular weight solutes (e.g. ions, organic acids, amino acids, sugars) will pass through the wall of the root cell by diffusion or mass flow (Marschner, 2012). Leaf transpiration is replaced by water absorbed by the roots, thus plants will uptake the water in soil, and then a hydraulic gradient is created. Through this hydraulic gradient, soluble ions in the rhizosphere can move from soil to root surface, enter the root, and follow mass flow through the space between cells (USEPA, 2000). Consequently, plants uptake low molecular weight metal-DOC complexes more easily than high molecular weight DOC complexes.

➤ Cation exchange capacity

In soil science, cation-exchange capacity (CEC) is the maximum quantity of cations, of any class, that a soil at a given pH value is capable of holding available for exchange with the soil solution. Absorption of metal cations in the soil solution depends on the density of negative charges on the surface of the solid colloids (Alloway, 1995). Soil with a significant negative charge has high cation exchange capacity and low cation mobility (Kelley et al., 2002).

1.3. Enhancing metal bioavailability

1.3.1. Chelating products

Some specific chemicals, synthetic chelators, have been used to enhance metal bioavailability in phytoextraction.

Ethylenediaminetetraacetic acid (EDTA) is probably the compound used most widely to enhance metal uptake by plants (Vassil et al., 1998; Kos and Leštan, 2004; Baek et al., 2005; Hernández et al., 2007; Surat et al., 2008; Suthar et al., 2014). For example, the accumulation of metals (Pb, Cd, Cu, Ni, and Zn) in the shoots of *Brassica juncea* can be enhanced through the application of synthetic chelates-EDTA to the soil (Blaylock et al., 1997). Recent work by Suthar et al. (2014) indicates that EDTA induces increases of Pb and Cd 13.1-fold and 3.1-fold, respectively, in soil over the controls, with the addition of 5.0 mM EDTA per kg of soil.

Another synthetic chelator, hydroxyethyl-ethylenediamine-triacetic acid (HEDTA), has been applied for phytoremediation of metal-contaminated soil. HEDTA is one of the three most effective chelates, which enhance plant uptake of Pb (Huang and Cunningham, 1996; Cooper et al., 1999).

Diethylenetriaminepentaacetic acid (DTPA) is also considered a good candidate for predicting the availability of Pb and Zn in contaminated soil of lead-zinc mining areas (Zhao et al., 2011).

Humic acid (HA) is a potential chelating agent. Many studies have proven that HA enhances metal phytoextraction and accumulation. For example, cadmium concentration in shoots increased from 30.9 to 39.9 mg/kg with HA added at a rate of 2g per kg of soil (Evangelou et al., 2004). In another study, treating soil with HA at a rate of 2% increased the heavy metal (Zn, Cu, Ni, Pb and Cd) concentration in shoot tissue (Topcuoğlu, 2012).

In general, chemically-assisted phytoremediation uses organic acids. While chelates have been shown to be highly effective in increasing metal bioavailability, they have numerous disadvantages such as barely degradable by microorganisms (Chen et al., 2004),

cause groundwater pollution by uncontrolled metal dissolution and leaching (Grcman et al., 2001).

1.3.2. Products affecting pH

Soil pH plays an important role because it affects availability of nutrients in general and availability of metals in particular. Many studies have found that metal solubility increases markedly under acidic conditions (discussed in part 1.2). For this reason, pH is often adjusted in order to increase metal availability. Soil pH can be reduced by adding sphagnum peat, elemental sulfur, aluminum sulfate, iron sulfate, acidifying nitrogen or organic mulches (Everhart, 1994). However, lowering soil pH is a slow process that can take several months to be modified using these amendments (Vossen, 2006; Mullen et al., 2007).

Sulfur is the element most widely used to lower soil pH because it is inexpensive (Everhart, 1994; Longstroth, 2008) and least likely to harm plants (TRHS, 2011). In soil, bacteria convert the sulphur to sulphuric acid, simultaneously lowering pH (Mullen et al., 2007).

Aluminum sulfate and iron sulfate are also used as soil acidifiers. However, they can interfere with the availability of soil phosphorus when large quantities are applied (TRHS, 2011). In addition, repeated applications of aluminum or iron can result in a build-up of these metals in the soil up to toxic levels (Vossen, 2006; TRHS, 2011).

Because of disadvantages noted above, changing soil pH is usually not used in phytoremediation.

1.3.3. Hyperaccumulator plants

Hyperaccumulator plants are defined as those able to accumulate high concentrations of heavy metals, up to 100 times greater than levels in non-accumulator plants growing in the same substrates (Brooks et al., 1998). More than 450 species have been identified as capable of metal hyper-accumulation (Maestri et al., 2010). For instance, *Thlaspi caerulescens* is best known as a Zn and Cd hyperaccumulator model species (Brown et al., 1995; Peer et al., 2006), and the fern species, *Pteris vittata*, is considered a good candidate for the phytoremediation of As-contaminated soil (Gumaelius et al., 2004).

Hyper-accumulating plant species can increase metal uptake by various methods. Many studies have proposed that metal hyperaccumulation mechanisms include a much greater capacity for metal uptake, faster xylem loading and increased internal detoxification (Krämer et al., 1997; Lasat et al., 1998; Caille et al., 2005; Peer et al., 2006; Rascioa and Navari-Izzo, 2011). Hyperaccumulation mechanisms are discussed below:

One such mechanism is acidification of the rhizosphere through the action of plasma membrane proton pumps and secretion of ligands capable of chelating the metal (Peer et al., 2006). Root secretions released in the rhizosphere include organic ligands (e.g. carbohydrates, organic acids, humic acids, polypeptides, proteins, amino acids, nucleic acids, etc.) and inorganic ligands (e.g. Cl^- , SO_4^{2-} , NH_4^+ , CO_3^{2-} , PO_4^{3-} , etc.). These substances function as an energy source for microorganisms, and can also chelate heavy metal ions, influencing pH and other chemical characteristics of the rhizosphere (Dong et al., 2007). For example, root exudates from sunflower significantly affect mobility, toxicity and phytoavailability of Cd because Cd can interact with the proteins, polysaccharides and phenolic compounds in root exudates (Yang and Pan, 2012).

A second mechanism involves translocating elements from roots to shoots via xylem more rapidly and efficiently than non-hyperaccumulator plants are able to. To avoid metal poisoning, non-hyperaccumulator plants usually retain metals in root cells, detoxifying them by chelation in the cytoplasm or storing them in vacuoles (Rascioa and Navari-Izzo, 2011). By contrast, hyperaccumulator plants enhance translocation of metal ions to the shoot; this requires radial symplastic passage and active loading into the xylem. In Ni hyperaccumulator species such as *Alyssum lesbiacum* and *Brassica juncea*, histidine plays an important role as a Ni chelator, and Ni-histidine complex is transported into the xylem (Krämer and Kerkeb, 2003). Concentrations of the free amino acid histidine in Ni hyperaccumulator species have been found to be substantially higher than in related non-accumulators, which enhances release of Ni into the xylem, and root-to-shoot transport of Ni.

In addition, hyperaccumulator plants can increase detoxification of metals. Accumulation of heavy metals in high concentrations can be toxic and would certainly kill the common non-accumulator plant. The fact that hyperaccumulator species can attain such levels suggests the existence of a detoxification mechanism within the plant itself (Meharg and

Hartley-Whitaker, 2002; Rascioa and Navari-Izzo, 2011). The detoxification mechanisms in aerial organs of hyperaccumulators involve heavy metal complexation with ligands for their removal from metabolically active cytoplasm into inactive compartments such as vacuoles and cell walls (Rascioa and Navari-Izzo, 2011). Organic acid ligands immobilize metal ions in the cytoplasm, thereby enabling organo-metallic chelation for storage in vacuoles. Highly effective storage of metal and detoxification usually occur in the leaves, mostly inside the vacuoles of leaf cells (Küpper et al., 2000). Metal concentrations are thus substantially higher in the leaves than in the roots, making them poisonous to herbivores (Rascioa and Navari-Izzo, 2011). Arsenic, for instance, as inorganic arsenite in vacuoles, is a highly effective example of this mechanism in fronds of hyperaccumulator fern species in the Pteridaceae family (Zhao et al., 2009). Metal ions may also be converted to less toxic forms; for example, a detoxification pathway for arsenate (AsO_4^{3-}) following uptake is efficient arsenate reduction to arsenite (AsO_2^-) in plant cells (Meharg and Hartley-Whitaker, 2002).

In general, hyperaccumulator plants can increase metal uptake from soil, enhance root-to-shoot transportation and store metals, mainly in vacuoles of leaves. Due to increased internal detoxification, they can retain a high level of metals without toxic effects. However, limits of hyperaccumulator plants in phytoremediation are that some species are highly metal specific, but they have a small biomass, slow growth habit and require careful management for multiplications which are not suitable for commercial applications (Sarma, 2011).

1.4. Objectives and hypotheses

Phytoremediation has been considered an alternative technology for improving metal-contaminated soil, however, its effectiveness is usually limited by bioavailability of metals in soil. We expected that the root systems of diverse plant species could affect bioavailability of TE in soil differently.

The aim of this study was to evaluate the efficiency of diverse plant species for optimizing metals removal (phytoextraction) from contaminated soil.

The specific objectives of this study were:

- To compare the level of TE that can accumulate in different parts of the tested plant species.
- To compare the efficiency of diverse plant species in terms of their capacity to affect metal bioavailability in a trace-metal polluted soil.

Hypotheses:

- Plant species should have different reactions to contamination and have different concentration levels in their tissues.
- The root systems of diverse tested plant species will each have distinct characteristics that can affect the bioavailability of TE in their rhizosphere differently. Furthermore, in comparison to control, bioavailability of elements will be significantly higher in the presence of plants.
- The cultivation of plants should induce some changes in key soil parameters (e.g. EC, pH, and % organic matter, as well as DOC) in the rhizosphere of all species.

Chapter 2: Effects of diverse plant species on the bioavailability of contaminants in soil¹

Thi Xuan Trang Nguyen^a, Marc Amyot^b, Michel Labrecque^a

a. Institut de recherche en biologie végétale – Plant Biology Research Institute, 4101 Sherbrooke East Montreal, QC H1X 2B2, Canada.

b. Department of Biological Sciences, Université de Montréal, Pavillon Marie-Victorin, 90, avenue Vincent-d'Indy, Montréal, Québec H3C 3J7, Canada.

2.1. Abstract

*Phytoremediation is usually limited by bioavailability of metals in soil; different plant species may affect bioavailability of trace elements (TE) in soil in varying ways. A pot experiment was conducted to investigate the effect of eight herbaceous and 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, above and below-ground biomass of each species was measured and concentrations of TE in the plant tissues 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, as well as dissolved organic carbon (DOC)) were also measured and compared as a function of plant species. We found that the concentration of TE in plant tissues differed among species. The concentrations of TE in root tissues of tested plants were greater than in above-ground tissues, except for Ni in *Salix nigra*. This indicates that the removal of Ni from the soil-plant system could be achieved through harvestable plant parts. The labile pool of Ag, Ni, and Cu in the rhizosphere was significantly and differently affected by the presence of the plants. The pH as well as EC and percentage of organic matter in the rhizosphere were not affected. In contrast, DOC was higher in the rhizosphere of all tested plants than in control pots without plants. In addition, there was a positive relationship between Ni availability concentration and DOC concentration, as well as Cu availability level and DOC level. This suggests that different root systems can influence soil DOC, and therefore modify the labile pools of Ni, Cu in the rhizosphere.*

Keywords: Bioavailability, Metals, Rhizosphere, Accumulation, Dissolved organic carbon

¹ This article was formatted for submission to the journal Environmental and Experimental Botany (EEB).

2.2. 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 rest being associated with the solid phase (NFESC, 2000). 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 metal forms (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 (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; Baek et al., 2005; Hernández et al., 2007; Surat et al., 2008; Suthar et al., 2014), humic acids (HA) (Evangelou et al., 2004; Topcuoğlu, 2012), 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 utilization 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 involved 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 study. 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; 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. In addition, some studies also have 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). To complement this aspect, this study was undertaken to acquire knowledge on the effect of roots of different plant species on metal bioavailability in the soil, and thus we proposes two objectives: (1) to compare the levels of TE that can accumulate in the tested plant species in their different tissues; (2) to compare the efficiency of diverse plant species in terms of their capacity to affect metal bioavailability in a TE polluted soil.

2.3. Material and methods

2.3.1. Experimental design

A pot experiment was conducted to examine eight plant species, including four woody species, *Salix nigra*, *Populus sp*, *Cornus stolonifera*, *Alnus crispa*, and four herbaceous species, *Phalaris arundinacea*, *Trifolium rupens*, *Panicum virgatum*, *Festuca arundinacea*. These species have been commonly used in the context of phytoextraction research in Canada (Pitre et al., 2010; Nissim et al., 2014; Nissim et al., 2015). Moreover, woody and herbaceous species have different root system types, which may have different effects on TE in the rhizosphere.

The four woody species were young seedlings produced in “Styroblock ®” containers and transplanted into the 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 (125ml per day) was supplied by an automatic drip irrigation system, without the addition of fertilizers or any other amendments.

Characteristics of the soil used in this experiment are described in Table 1. Large stones and debris were removed from the soil, which was then thoroughly mixed and homogenized and used to fill the pots. 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 (45.5572° N, 73.5568° 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.

Table 1. Characteristics of the soil used in pot experiment

Characteristic	Mean
pH	7.9 ± 0.0
EC ($\mu\text{S cm}^{-1}$)	131.0 ± 7.0
CaCO ₃ (%)	41.4 ± 1.4
CEC (meq 100g ⁻¹)	34.6 ± 15.7
Organic-C (%)	2.8 ± 0.9
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

2.3.2. Chemical analysis

2.3.2.1. Sampling

Plants were harvested by cutting the above-ground tissues at the soil surface and removing the roots from the pots. Soil adhering strongly to the roots was considered rhizosphere soil (Jaillard et al., 2002), and samples of it were collected by gently shaking the roots, then carefully collected in plastic bags and labeled for storage. 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 °C until they reached a constant weight.

2.3.2.2. 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 H₂O and a 4g soil sample (sieved through a 0.5 mm mesh) into a 50ml acid-washed Falcon® tube. The tubes were then shaken end-over-end for 2 hours at room temperature. Subsequently, the samples were centrifuged at 1400g for 15 minutes. The mixture was then filtered through a nylon membrane (pore size 0.45µm) using a vacuum system. 0.02ml HNO₃ 50% (TMG grade, Thermo-Fisher) was then added to 5ml samples of the filtered solution. An ICP-mass spectrometer (Perkin ElmerNexION 300) was used for analysis of water-soluble TE (referred to as Ag-H₂O, Se-H₂O, Cu-H₂O, Ni-H₂O, Pb-H₂O, and Zn-H₂O).

2.3.2.3. Plant tissue digestion

Dry plant tissue samples (0.200g) were digested in borosilicate tubes containing 2 mL nitric acid 70% (TMG grade from Thermo-Fisher) at 120°C for five hours. Then the entire contents of the borosilicate tubes was transferred into a 50 mL Falcon® tube and the volume was adjusted to 50 mL with ultrapure water (MilliQ), to attain a final concentration of 4% nitric acid. After that, the contents of the tube was stirred and allowed to stand overnight. Samples were then filtered through a nylon membrane (pore size 0.45µm). Digestives were diluted to 2% HNO₃ before ICP-MS analyses.

2.3.2.4. Quality assurance and quality control

Laboratory equipment was acid-washed (20% HNO₃) and rinsed six times with deionized water. Calibration checks of instruments were performed with certified natural water from Environment Canada and the National Institute of Standards and Technology (NIST). In addition, certified reference materials (SCP Sciences, Natural Water Research Institute, NIST) were also included in each batch of samples to test the quality of tissue digestion with hot acid. The results yielded an accuracy of 99 – 136%.

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).

2.3.3. Statistical analysis

All variables were analyzed with fixed-model ANOVAs. This model is presented as $Y = \text{blocks} \cdot \text{species} \cdot \text{blocks} * \text{species}$, 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.

2.4. Results

2.4.1. 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. rupens* for above-ground biomass (Fig. 1). The largest root biomass was found for *F. arundinacea*, and *P. virgatum*. Above-ground tissue biomass was found to be highest in *T. rupens*, and was three times higher than in *A. crispa*.

In general, root systems of herb species, which included *F. arundinacea*, *T. rupens*, and *P. virgatum*, grew faster than root systems of woody species. 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. rupens* was able to take up 0.7 mg pot⁻¹.

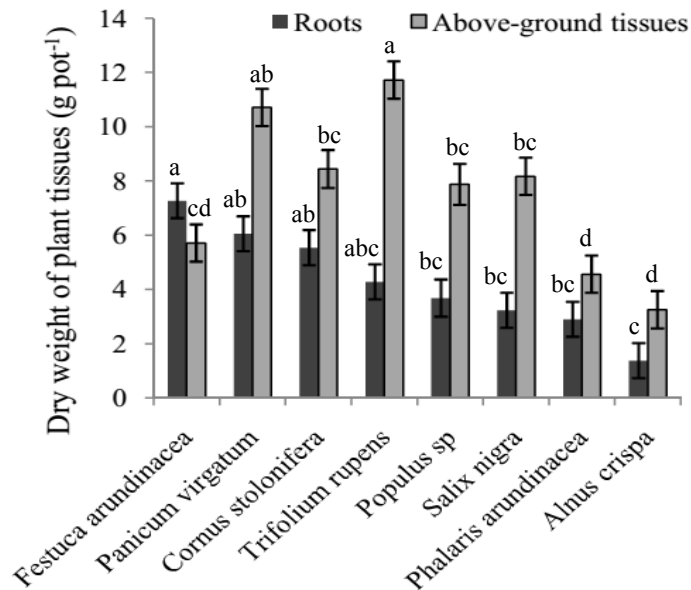


Figure. 1. Comparison of biomass produced after three months of in-pot cultivation. Error bars denote standard error for root biomass and aboveground biomass, respectively. Bars with different letters are significantly different at $P < 0.05$.

2.4.2. 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) (Fig. 2-A). The levels of Ag in roots ranged from $0.1 \pm 0.2 \text{ mg kg}^{-1}$ (in *A. crispa*) to $1.7 \pm 0.2 \text{ mg kg}^{-1}$ (in *F. arundinacea*), and from $0.0 \pm 0.0 \text{ mg kg}^{-1}$ (in *P. sp*) to $0.1 \pm 0.0 \text{ mg kg}^{-1}$ (in *P. arundinacea*) for Ag in above-ground tissues. *F. arundinacea* and *P. arundinacea* were the two species that accumulated the highest concentrations of Ag in their roots ($P < 0.05$).

Cu concentrations in roots varied between $34.5 \pm 24.5 \text{ mg kg}^{-1}$ (in *A. crispa*) and $292.7 \pm 24.5 \text{ mg kg}^{-1}$ (in *P. arundinacea*); the corresponding Cu concentrations in above-ground tissues ranged between $19.2 \pm 4.5 \text{ mg kg}^{-1}$ and $55.8 \pm 4.5 \text{ mg kg}^{-1}$ (Fig. 2-B).

Zn translocation to above-ground tissues of *S. nigra* was the greatest ($159.9 \pm 9.8 \text{ mg kg}^{-1}$), followed by *P. arundinacea* ($104.4 \pm 9.8 \text{ mg kg}^{-1}$), and the lowest value was found in *C.*

stolonifera ($32.8 \pm 9.8 \text{ mg kg}^{-1}$); the corresponding Zn concentrations in roots varied between $62.7 \pm 14.0 \text{ mg kg}^{-1}$ (in *A. crispa*) and $212.4 \pm 14.0 \text{ mg kg}^{-1}$ (in *P. arundinacea*) (Fig. 2-C).

Fig. 2-D shows that *S. nigra* was superior to other species in terms of its ability to concentrate Ni in above-ground tissues ($5.3 \pm 1.3 \text{ mg kg}^{-1}$); the aboveground-to-root concentration ratio was up to 1.5. In all other tested species, Ni concentration in above-ground tissues was much lower than in roots, as indicated by the aboveground-to-root concentration ratio, which varied between 0.5 (in *T. rupens*) and 0.8 (*P. virgatum*).

Pb concentrations in root tissues of *C. stolonifera* and *F. arundinacea* ranged from $6.8 \pm 0.7 \text{ mg kg}^{-1}$ to $5.7 \pm 0.7 \text{ mg kg}^{-1}$ respectively. The lowest Pb concentration was in root tissues of *P. virgatum*, $2.1 \pm 0.7 \text{ mg kg}^{-1}$ (Fig. 2-E).

Se accumulation was found to be highest in the above-ground tissues of the two grass species, *F. arundinacea* and *P. arundinacea*, two times higher than in *A. crispa* (Fig. 2-F).

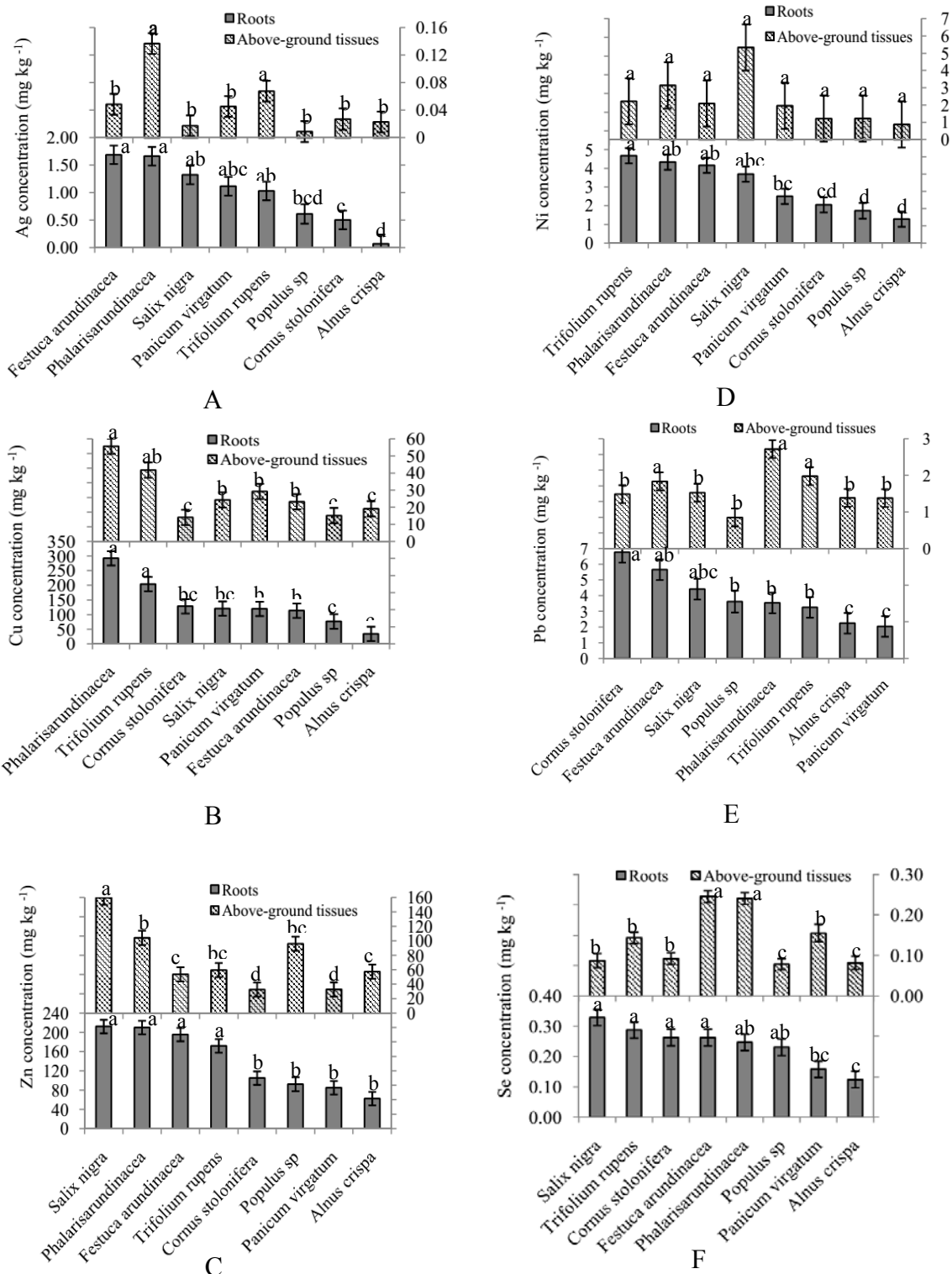


Figure 2. The concentration of TE (Ag, Ni, Cu, Pb, Zn, and Se) in roots and above-ground tissues of eight plant species after three months of in-pot cultivation. Error bars denote standard error for Ag, Cu, Zn, Ni, Pb, and Se concentration in individual tissues. Bars with different letters are significantly different at $P < 0.05$.

In general, these results indicate that TE accumulation by plants differed among species and tissues. In addition, concentration of TE accumulated by plants were mostly accumulated in roots, except for Ni in *S. nigra*. TE concentrations 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 \times biomass) in herb species was greater than woody species. For example, Se, Zn, Cu, and Ni mass per pot in *T. rupens* were 4.1 mg pot⁻¹, 2.7 mg pot⁻¹, 1.4 mg pot⁻¹, and 1.4 mg pot⁻¹ respectively. On the other hand, Se, Zn, Cu, and Ni mass per pot in *A. crispa* were only 0.3 mg pot⁻¹, 0.2 mg pot⁻¹, 0.1 mg pot⁻¹, and 0.1 mg pot⁻¹ respectively.

2.4.3. Availability of Ag, Pb, Cu, Zn, Ni, Se in the rhizosphere

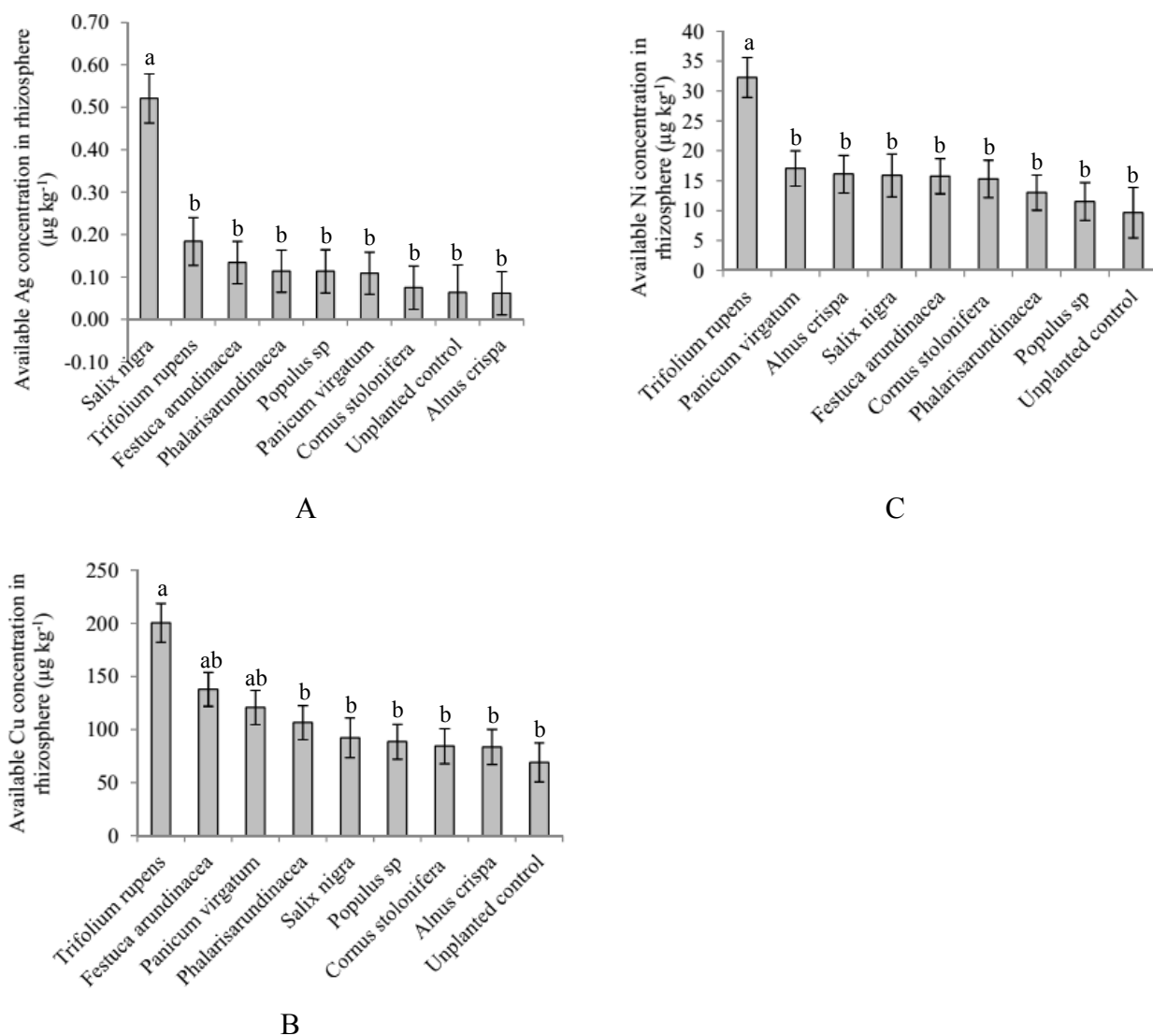


Figure 3. The water-soluble concentration of TE (Ag, Ni, Cu) in the rhizosphere of eight plant species after three months of in-pot cultivation. Error bars denote standard error for available Ag, Ni, and Cu concentration in the rhizosphere. Bars with different letters are significantly different at $P < 0.05$.

Ag concentration in the water-extractable fraction in the rhizosphere (considered to be potentially available for uptake) was the highest in *S. nigra*, 8.5 times higher than in *A. crispa* (Fig. 3). Available concentrations of Cu and Ni were found to be greater in the rhizosphere of

T. rupens than in the rhizosphere of the species; they were 2.9 and 3.3 times higher than in control pots without plants, respectively (Fig. 3).

2.4.4. Levels of pH, EC, % 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.

In contrast, DOC levels were different in the rhizosphere of each of the plants (Table 2). The highest DOC was found in the rhizosphere of *T. rupens* (6.7 ± 0.3 mg l⁻¹) and the lowest in the control (4.7 ± 0.3 mg l⁻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)
<i>Trifolium rupens</i>	6.7 ^(a) ± 0.3
<i>Cornus stolonifera</i>	6.3 ^(ab) ± 0.3
<i>Festuca arundinacea</i>	6.1 ^(abc) ± 0.3
<i>Alnus crispa</i>	5.5 ^(abc) ± 0.3
<i>Panicum virgatum</i>	5.5 ^(abc) ± 0.3
<i>Phalaris arundinacea</i>	5.3 ^(abc) ± 0.3
<i>Salix nigra</i>	5.3 ^(abc) ± 0.3
<i>Populus sp</i>	5.1 ^(bc) ± 0.3
Unplanted control	4.7 ^(c) ± 0.3

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

Fig. 4 shows that DOC in the rhizosphere of *S. nigra* was only at a medium level compared to the rhizosphere of the other species, but available Ag concentration was the greatest. However, we found in the rhizosphere of *T. rupens*, the greatest DOC level as well as the highest concentrations of available Ni and Cu.

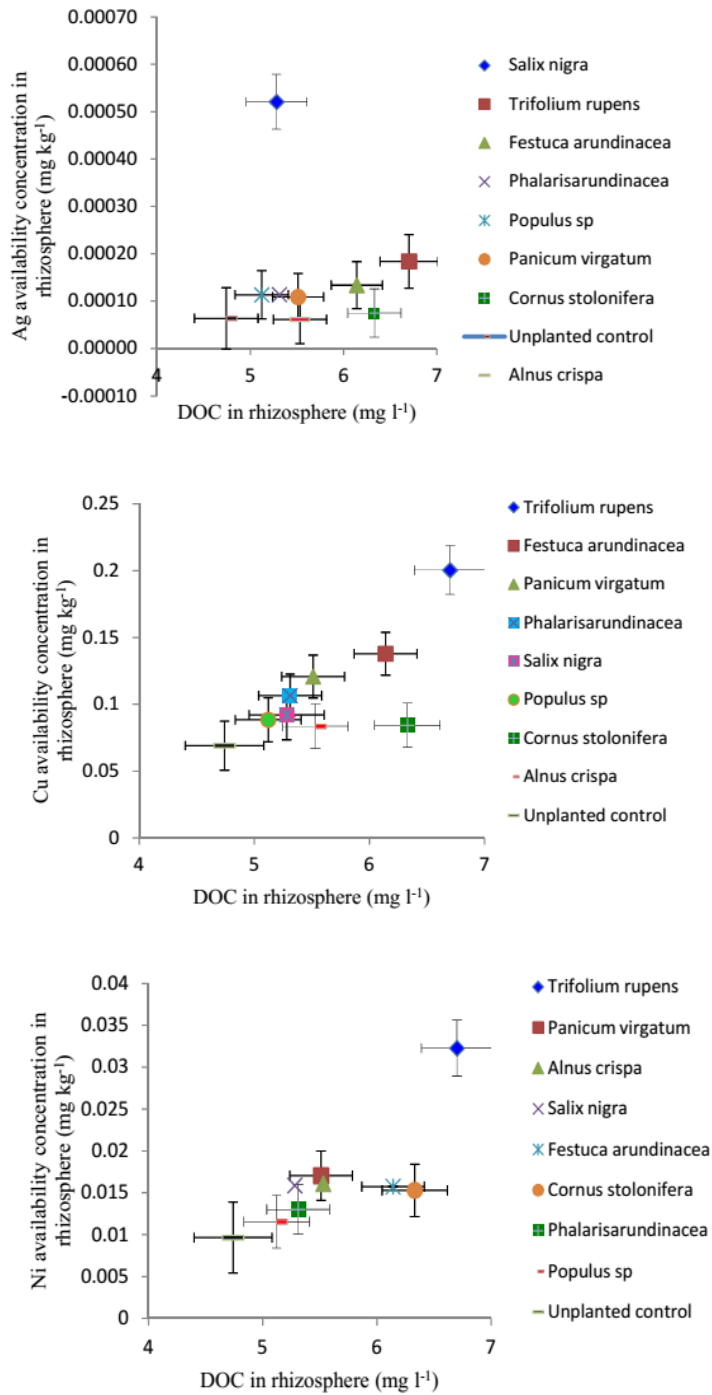


Figure. 4. The water-soluble concentration of TE (Ag, Ni, Cu) and DOC concentration in the rhizosphere of eight plant species after three months of in-pot cultivation. Vertical error bars denote standard error for available Ag, Ni, and Cu concentration in the rhizosphere. Horizontal error bars denote standard error for DOC in the rhizosphere of eight plant species.

After three months of plant growth, there was a positive relationship between the availability of Ni and Cu, and DOC concentrations in the rhizosphere (Fig. 4, Table 3). This suggests that different root systems had different abilities to directly affect the DOC level in the rhizosphere, and to indirectly influence the availability of Ni and Cu in the rhizosphere.

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 F
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%

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

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

2.5. Discussion

2.5.1. The concentration of TE in tissues

The capacity of the plants tested 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 $2.4 \pm 0.2 \text{ mg kg}^{-1}$, but concentration of Ag accumulated in root tissues of *F. arundinacea* was up to $1.7 \pm 0.2 \text{ mg kg}^{-1}$. 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).

In almost all tested plants, the highest concentration of Ni was in the roots and the lowest level in the above-ground tissues. The opposite trend was observed for Ni content in *S. nigra*, which indicates that *S. nigra* translocated Ni from root to shoot via the xylem more rapidly and efficiently than the other species. Therefore, the efficient removal of Ni from the soil-plant system could be achieved through the harvest of plant parts (shoots and leaves). Based on our findings, *S. nigra* is a potential candidate for Ni phytoextraction because of its hyperaccumulation mechanisms. Numerous studies have suggested that hyperaccumulators are able to retain a high level of metals internally without toxic effects due to mechanisms such as a much greater metal uptake capacity, faster xylem loading from root to shoot, an ability to store metals mainly in vacuoles of leaves, and increased internal detoxification (Krämer et al., 1997; Lasat et al., 1998; Caille et al., 2005; Peer et al., 2006; Rascio and Navari-Izzo, 2011)

Se is a metalloid that can be lost to the atmosphere because it can be biomethylated to form volatile molecules (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 plant roots of *S. nigra*, which reached $0.3 \pm 0.0 \text{ mg kg}^{-1}$, and $0.3 \pm 0.0 \text{ mg kg}^{-1}$ in above-ground tissue of *F. arundinacea*, although Se 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 a toxic level in some tested species such as *P. arundinacea* ($55.8 \pm 4.5 \text{ mg kg}^{-1}$), *T. rupens* ($41.8 \pm 4.5 \text{ mg kg}^{-1}$), and *P. virgatum* ($29.4 \pm 4.5 \text{ mg kg}^{-1}$). These plant species may have good potential for use in remediation of Cu-contaminated soils.

In soil, Pb usually exists as insoluble precipitates (phosphates, carbonates and hydroxy-oxides), 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).

2.5.2. Availability of TE in the rhizosphere

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). A negative relationship was also found between concentrations of Ni and Cu in ryegrass and soil pH (ranging from 4.2- to 7.0) (Smith, 1994). As well, the uptake of Ni²⁺ by *Lathyrus sativus* increased with increasing pH up to 5.0, then decreased as pH increased up to 8.0 (Chen et al., 2009). 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 $1 \pm 0.1 \mu\text{g kg}^{-1}$ in *S. nigra*, and $200.4 \pm 3.3 \mu\text{g kg}^{-1}$ and $32.3 \pm 18.1 \mu\text{g kg}^{-1}$ in *T. rupens*, respectively.

Metal forms are able to bind with DOC (Welsh, 1996; Zhao et al., 2007; Merrington and Peters, 2009), and these complexes may enhance metal solubility (Rembe and Trefry, 2003). Therefore, DOC in soils can increase the labile pool of metals (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). 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 hyperaccumulators may enhance metal solubility in the rhizosphere via root exudation, consequently increasing plant metal uptake (Li et al., 2011). In this study, DOC concentrations were also significantly and differently affected by the presence of plants.

The presence of the tested plant species did not affect some key factors (pH, EC, percent organic matter), however, DOC was affected. Moreover, positive relationships existed between the availability 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.

2.6. Acknowledgments

This study was supported by the NSERC CREATE Mine of Knowledge program through a scholarship to Nguyen Thi Xuan Trang. We would like to thank Golder and Associates for their partnership in funding this research program. The authors also wish to thank Marie-Claude Turmel and Dominic Bélanger for skillful technical assistance in the laboratory; Kadri Hafssa and Ahmed Jerbi for assistance in the field. We gratefully acknowledge advice from Professors François Courchesne and Frédéric Pitre for the experimental set-up as well as elemental analyses. M.A. received support from the Canada Research Chair and NSERC Discovery programs.

Chapter 3: Conclusion

We conducted a preliminary pot trial to evaluate the effect of eight herbaceous species and woody species on the TE of Ag, Cu, Pd, Zn, Ni and Se in their rhizosphere. The concentration of the TE in plant tissues, biomass and key soil parameters (e.g. pH, EC, percent of organic matter, and DOC) were also measured and compared as a function of plant species.

After three months of cultivation, all tested plant species grew very well and adapted quickly in slightly alkaline soil ($\text{pH} = 7.94 \pm 0.02$), except *A. crispa*. The highest total biomass was 16.8 g pot^{-1} in *P. virgatum*, whereas biomass of *A. crispa* was only 4.6 g pot^{-1} . Therefore, its capacity to accumulate TE (i.e., metal concentration \times biomass) was rather low. Root systems of herb species grew faster than those of woody species, and extended throughout the potted soil. Above-ground tissue biomass was much greater than root- biomass.

Various hypotheses were formulated early in the study. The first was that plant species have differing ability to accumulate TE in their tissues, which results in different TE concentrations in above- and below-ground parts. Our findings show that TE accumulation by plants differed among species and tissue bodies. Among eight plant species, *A. crispa* had low uptake for all elements. Two species, *F. arundinacea* and *P. arundinacea*, accumulated more Ag than the other plant species. *P. arundinacea* had the highest Cu concentration in both above- and below-ground tissues. Ni and Zn concentration in *S. nigra*, Pb concentration in *C. stolonifera*, and Se concentration in *F. arundinacea* were higher than in other species. Element uptake was relatively higher in roots than in above-ground tissues for most of the tested plant species. The translocation of TE from roots to above-ground tissues was low, except for Ni in *S. nigra*, for which the above-ground-to-root concentration ratio was up to 1.45. This suggests that efficient removal of Ni from the soil-plant system is possible through harvestable plant parts (shoots and leaves).

Our second hypothesis was that diverse tested plant species have different root systems that can affect the bioavailability of TE in their rhizosphere differently. Furthermore, in comparison to control, bioavailability of elements was expected to be significantly higher in the presence of plants. After cultivation in alkaline soil (pH ranging from 7.94 to 8.00), Ag, Cu, and Ni concentrations in the water-extractable fraction (considered to be potentially

available for uptake) in the rhizosphere were affected differently by the presence of the plants. The highest Ag availability concentration was observed in *S. nigra* and was 8.5 times higher than that in *A. crispa*. Cu and Ni availability concentrations were found to be greater in the rhizosphere of *T. rupens* than in that of other species, and were 2.9 times and 3.3 times greater than in control pots without plants, respectively. This suggests that the tested plant species affect the bioavailability of TE in their rhizosphere differently.

Finally, the cultivation of plants should induce some changes in key soil parameters (e.g. EC, pH, and % organic matter, as well as DOC) in the rhizosphere of all species. This hypothesis is only partially confirmed. Some parameters, including EC, pH, and % of organic matter in the rhizosphere of all species, were not affected by plants. In contrast, DOC in the rhizosphere of all plants differed between rhizospheres. Moreover, DOC levels in the rhizosphere of all tested plants were higher than those measured for the control pots without plants. This suggests that different root systems appear to have varying effects on soil DOC. Interestingly, the positive relationships existing between concentrations of available TE and DOC in the rhizosphere further suggest that available TE concentrations may increase along with those of DOC. These observations call for additional studies to improve our understanding of how plant root systems can affect the soil rhizosphere and the labile pool of metals. Further, these promising findings may help improve the success of phytoremediation.

The results of this pot experiment provide information about the effects of plant species on TE accumulation in above- and below-ground parts, availability of TE in the rhizosphere and key soil parameters (EC, pH, and % organic matter, as well as DOC). This study therefore increases our understanding of the interactions between plant species and TE in soil, as well as plant species and key soil parameters in the presence of low TE levels. We still know very little about TE accumulation in plants and TE availability in the rhizosphere as these species adapt to soils rich in heavy metals. Metal concentrations in plants depend on the concentrations of total metals in the soil in which the plants are grown (Intawongse, 2007). Furthermore, plant species have different reactions to TE concentration in soil, for instance, a silver nitrate (AgNO_3) concentration as high as $0.027 \mu\text{M}$ may result in a significant reduction of biomass productivity and a decrease in stomatal conductance of willows over the first week of exposure (Nissim et al., 2015). Future studies could expand testing of these plant species, to

investigate their performance in highly contaminated soil or at different levels of contamination.

This study also demonstrates that the presence of the tested plant species affects DOC in soil. However, the nature of DOC includes a large range of molecules, from simple amino acids, sugars and lipids to complex humic substances with a high molecular weight (Schomakers et al., 2014). Plants uptake LMW metal DOC-metal complexes more easily than complexes with higher molecular weights, through their apoplastic pathway (Marschner, 2012). Therefore, depending on the DOC molecules, metal-DOC complexes can affect plant uptake. The nature of DOC as it relates to root systems would need to be examined more closely in future studies in order to evaluate the full potential of these plants to influence the labile pool of TE and extract metals from contaminated soil.

Bibliographie

- Alloway, B.J. (1995). Heavy Metals in Soils, Springer Science & Business Media.
- Antoniadis, V., Alloway, B. J. (2001). The role of dissolved organic carbon in the mobility of Cd, Ni and Zn in sewage sludge - amended soils. *Environmental Pollution*. 117, 515–521.
- Apak, R. (2002). Adsorption of heavy metal ions on soil surfaces and similar substances: theoretical aspects. In: Hubbard A. (ed.), Encyclopedia of Surface and Colloid Science New York, Marcel Dekker: 484–509.
- Ashworth, D.J., Alloway, B.J. (2008). Influence of dissolved organic matter on the solubility of heavy metals in sewage-sludge-amended soils. *Communications in Soil Science and Plant Analysis*. 39, 538–550.
- ASTM (1998). Standard guide for conducting laboratory soil toxicity or bioaccumulation test with the lumbricid earthworm *Eisenia foetida*. *American Society for Testing and Materials*.
- Baek, K.H., Kim, H.H., Bae, B., Chang, Y.Y., Lee, I.S. (2005). EDTA-assisted phytoextraction of lead from lead-contaminated soils by *Echinochloa crusgalli* var. *frumentacea*. *Journal of Environmental Biology*. 26(1), 151-154.
- Barry, G.A., Chudek, P.J., Best, E.K., Moody, P.W. (1995). Estimating sludge application rates to land based on heavy metal and phosphorus sorption characteristics of soil. *Water Research*. 29(9), 2031–2034.
- Blaylock, M.J., Salt, D.E., Dushenkov, S., Zakharova, O., Gussman, C., Kapulnik, Y. (1997). Enhanced accumulation of Pb in Indian mustard by soil-applied chelating agents. *Environmental Sciences and Technology*. 31, 860-865.
- Bradham, K.D., Dayton, E.A., Basta, N.T., Schroder, J., Payton, M., Lanno, R.P. (2006). Effect of soil properties on lead bioavailability and toxicity to earthworms. *Environ Toxicol Chem*. 25(3), 769-775.
- Brooks, R.R., Chambers, M.F., Nicks, L.J., Robinson, B.H. (1998). Phytomining. *Trends Plant Sci*. 3, 359-362.

- Brown, S.L., Chancy, R.L., Angle, J.S., Baker, A.J.M. (1995). Zinc and Cadmium Uptake by Hyperaccumulator *Thlaspi caerulescens* Grown in Nutrient Solution. *Soil Sci. Soc. Am. J.* 59, 125-133.
- Caille, N., Zhao, F.J., McGrath, S.P. (2005). Comparison of root absorption, translocation and tolerance of arsenic in the hyperaccumulator *Pteris vittata* and the nonhyperaccumulator *Pteris tremula*. *New Phytologist.* 165(3), 755–761.
- Carmona, D.M., Cano, Á.F., Arocena, J. M. (2008). Dissolved organic carbon and metals release in amended soils. *Resumen Workshop Macla. 10*, 115-117.
- Chaney, R.L. (1983). Plant uptake of inorganic waste constituents. In *Land Treatment of Hazardous Wastes* (J. F. Parr, P. B. Marsh & J. M. Kla, Eds), 50-76.
- Chen, B.D., Shen, H., Li, Z., Feng, G., Christie, P. (2004). Effects of EDTA application and arbuscular mycorrhizal colonization on growth and zinc uptake by maize (*Zea mays* L.) in soil experimentally contaminated with zinc. *Plant and Soil.* 261, 219-229.
- Chen, C., Huang, D., Liu, J. (2009). Functions and Toxicity of Nickel in Plants: Recent Advances and Future Prospects. *Clean.* 37, 304 – 313.
- Chuan, M.C., Shu, G.Y., Liu, J.C. (1996). Solubility of heavy metals in a contaminated soil: effects of redox potential and pH. *Water Air Soil Pollut.* 91, 543-556.
- Cooper, E.M., Sims, J.T., Cunningham, S.D., Huang, J.W., Berti, W.R. (1999). Chelate-assisted phytoextraction of lead from contaminated soils. *Journal of Environmental Quality.* 28, 1709-1719.
- Dong, J., Mao, W.H., Zhang, G.P., Wu, F.B., Cai, Y. (2007). Root excretion and plant tolerance to cadmium toxicity – a review. *Plant soil environ.* 53(5), 193-200.
- Evangelou, M. W., H., Daghan, A., Schaeffer (2004). The influence of humic acids on the phytoextraction of cadmium from soil. *Environmental Pollution.* 132, 113–120.
- Everhart, E. (1994). How To Change Your Soil's pH. *Iowa State University of Science and Technology.* 467(7), 42-43.
- FAO (2005). The importance of soil organic matter. Rome, Food And Agriculture Organization of The United Nations: 3407–3418.
- Fijałkowski, K., Kacprzak, M., Grobelak, A., Placek, A. (2012). The influence of selected soil parameters on the mobility of heavy metals in soil. *Inżynieria i Ochrona środowiska.* 15(1), 81–92.

- Fitz, W.J., Wenzel, W.W (2002). Arsenic transformations in the soil–rhizosphere–plant system : fundamentals and potential application to phytoremediation. *Journal of Biotechnology*. 99, 259–278.
- García-Sánchez, A., Alastuey, A., Querol, X. (1999). Coupled mobilization of dissolved organic matter and metals (Cu and Zn) in soil columns. *Science of The Total Environment*. 242(1-3), 179–188.
- Gisbert, C., Ros, R., De Haro, A., Walker, D.J., Pilar Bernal, M., Serrano, R., Navarro-Aviñó, J. (2003). A plant genetically modified that accumulates Pb is especially promising for phytoremediation. *Biochem Biophys Res Commun*. 302(3), 440-445.
- Grcman, H., Velikonja-Bolta, S., Vodnik, D., Kos, B., Lestan, D. (2001). EDTA enhanced heavy metal phytoextraction: metal accumulation, leaching and toxicity. *Plant Soil*. 235, 105-114.
- Gumaelius, L., Lahner, B., Salt, D.E., Banks, J.A. (2004). Arsenic hyperaccumulation in gametophytes of *Pteris vittata*. A new model system for analysis of arsenic hyperaccumulation. *Plant Physiol*. 136(2), 3198-3208.
- Hammer, D., Kayser, A., Keller, C. (2003). Phytoextraction of Cd and Zn with *Salix viminalis* in field trials. *Soil Use and Management*. 19, 187–192.
- Harter, R.D. (1983). Effect of soil pH on adsorption of lead, copper, zinc, and nickel. *Soil Science Society of America Journal*. 47, 47-51.
- Hernández, A.J., Garbisu, C., Barrutia, O., Becerril, J.M. (2007). EDTA-induced heavy metal accumulation and phytotoxicity in cardoon plants. *Environ Exp Bot*. 60, 26-32.
- Huang, J.W., Cunningham, S.D. (1996). Lead phytoextraction: species variation in lead uptake and translocation. *New Phytologist*. 134(1), 75–84.
- Intawongse, M. (2007). Uptake of heavy metals by vegetable plants grown on contaminated soils, their bioavailability and speciation. *Doctoral thesis, Northumbria University, England*.
- Ivezić, V., Lončarić, Z., Engler, M., Kerovec, D., Singh, B.R. (2013). Comparison of different extraction methods representing available and total concentrations of Cd, Cu, Fe, Mn and Zn in soil. *PoljoPrivreda*. 19, 53-58.

- Jaillard, B., Plassard, C., Hinsinger, P. (2002). Measurements of H⁺ fluxes and concentrations in the rhizosphere. In: Rengel Z. (Ed.), *The handbook of soil acidity*, Marcel Dekker, in press.
- Jung, M.C. (2008). Heavy Metal Concentrations in Soils and Factors Affecting Metal Uptake by Plants in the Vicinity of a Korean Cu-W Mine. *Sensors*. 8, 2413-2423.
- Kelley, M., Brauning, S., Kelley, M.E. (2002). *Assessing Oral Bioavailability of Metals in Soil*, Battelle Press, Columbus Ohio
- Khalida, M., Solemana, N., Jonesa, D.L. (2006). Grassland plants affect dissolved organic carbon and nitrogen dynamics in soil. *Soil Biology & Biochemistry* 39, 378 – 381.
- Kiikkila, O, Pennanen, T., Perkiomaki, J., Derome, J., Fritze, H. (2002). Organic material as a copper immobilising agent: a microcosm study on remediation. *Basic and Applied Ecology*. 3, 245-253.
- Kolka, R., Peter, W., Mats, F. (2008). Measurement and Importance of Dissolved Organic Carbon. In: *Field Measurements for Forest Carbon Monitoring*, Springer Netherlands, 171-178.
- Koontz, H.V. , Berle, K.L. (1980). Silver Uptake, Distribution, and Effect on Calcium, Phosphorus, and Sulfur Uptake. *Plant Physiol*. 65, 336-339.
- Kos, B., Leštan, D. (2004). Chelator induced phytoextraction and in situ soil washing of Cu. *Environmental Pollution*. 132(2), 333–339.
- Krämer, L., Kerkeb, U. (2003). The role of free histidine in xylem loading of nickel in *Alyssum lesbiacum* and *Brassica juncea*. *Plant Physiol*. 131(2), 716-724.
- Krämer, U., Smith, R.D., Wenzel, W.W., Raskin, I., Salt, D.E. (1997). The role of metal transport and tolerance in nickel hyperaccumulation by *Thlaspi goesingense* Hálácsy. *Plant Physiol*. 115(4), 1641–1650.
- Kumar, P. B. A.N., Dushenkov, V., Motto, h., Raskin, I. (1995). Phytoextraction: The use of plants to remove heavy metals from soils. . *Environ. Sci. Technol*. 29(5), 1232–1238.
- Küpper, H., Lombi, E., Zhao, F.J., S.P., McGrath (2000). Cellular compartmentation of cadmium and zinc in relation to other elements in the hyperaccumulator *Arabidopsis halleri*. *Planta*. 212(1), 75-84.
- Lasat, M.M. (2002). Phytoextraction of Toxic Metals: A Review of Biological Mechanisms. *J. Environ. Qual*. 31, 109–120.

- Lasat, M.M., Baker, A.J.M., Kochian, L.V. (1998). Altered Zn compartmentation in the root symplasm and stimulated Zn absorption into the leaf as mechanisms involved in Zn hyperaccumulation in *Thlaspi caerulescens*. *Plant Physiol.* 118(3), 875–883.
- Li, T., Di, Z., Yang, X., Sparks, D.L. (2011). Effects of dissolved organic matter from the rhizosphere of the hyperaccumulator *Sedum alfredii* on sorption of zinc and cadmium by different soils. *Journal of Hazardous Materials.* 192, 1616–1622.
- Linz, D.G., Nakles, D.V. (1997). Environmentally Acceptable Endpoints in Soil. *American Academy of Environmental Engineers, Annapolis, MD*, 217-221.
- Longstroth, M. (2008). Lowering Soil pH with Sulfur. *Michigan State University Extension*.
- Maestri, E., Marmiroli, M., Visioli, G., Marmiroli, N. (2010). Metal tolerance and hyperaccumulation: Costs and trade-offs between traits and environment. *Environmental and Experimental Botany.* 68(1), 1-13.
- Marschner, P. (2012). Marschner's Mineral Nutrition of Higher Plants, Third Edition, Academic Press.
- McGrath, S.P., Zhao, F.J., Lombi, E. (2001). Plant and rhizosphere processes involved in phytoremediation of metal contaminated soils. *Plant Soil.* 232, 207-214.
- Meharg, A.A., Hartley-Whitaker, J. (2002). Arsenic uptake and metabolism in arsenic resistant and nonresistant plant species. *New Phytologist.* 154(1), 29–43.
- Mench, M., Schwitzguéb, J.B., Schroeder, P., Bert, V., Gawronski, S., Gupta, S. (2009). Assessment of successful experiments and limitations of phytotechnologies: contaminant uptake, detoxification and sequestration, and consequences for food safety *Environ Sci Pollut.* 16, 876–900.
- Merrington, G. , Peters, A. (2009). The importance of dissolved organic carbon in the assessment of environmental quality standard compliance for copper and zin. *Water Framework Directive-United Kingdom Technical Advisory Group* (ISBN: 978-1 - 906934-29-3).
- Mullen, R., Lentz, E., Watson, M. (2007). Soil Acidification: How to Lower Soil pH. *Ohio State University Extension Fact Sheet*.
- Neuhierl, B., Bock, A. (1996). On the mechanism of selenium tolerance in selenium-accumulating plants Purification and characterization of a specific selenocysteine

- methyltransferase from cultured cells of *Astragalus bisculatus*. *Eur. J. Biochem.* 239, 235-238
- NFESC, Naval Facilities Engineering Service Center (2000). Guide for incorporating bioavailability adjustments into Human Health and Ecological Risk Assessments at US Navy and Marine Corps Facilities (Part 2: Technical Background Document for Assessing Metals Bioavailability). Prepared by Battelle and Exponent, Washington DC.
- Nissim, G.W., Hasbroucq, S., Kadri, H., Pitre, F. E., Labrecque, M. (2015). Potential of selected Canadian plant species for phytoextraction of trace elements from selenium-rich soil contaminated by industrial activity. *International Journal of Phytoremediation (sous-presse)*
- Nissim, G.W., Pitre, F. E., Kadri, H., Desjardins, D., Labrecque, M. (2014). Early Response of willow to increasing silver concentration exposure. *International Journal of Phytoremediation.* 16(7-8), 660-670.
- Pagenkopf, G.K. (1983). Gill surface interaction model for trace-metal toxicity to fishes: role of complexation, pH and water hardness. *Environ. Sci. Technol.* 17(6), 342–347.
- Peer, W.A., Baxter, I.R., Richards, E.L., Freeman, J.L., Murphy, A.S. (2006). Phytoremediation and hyperaccumulator plants. *Molecular Biology of Metal Homeostasis and Detoxification. Topics in Current Genetics.* 14, 299-340.
- Peijnenburg, W.J., Posthuma, L., Eijsackers, H.J., Allen, H.E. (1997). A conceptual framework for implementation of bioavailability of metals for environmental management purposes. *Ecotoxicol Environ Saf.* 37(2), 163-172.
- Pitre, F., Teodorescu, T.I., Labrecque, M. (2010). Brownfield phytoremediation of heavy metals using Brassica and Salix supplemented with EDTA: Results of the first growing season. *Jour. of Environ. Science and Engi.* 4, 51-59.
- Rascioa, N., Navari-Izzo, F. (2011). Heavy metal hyperaccumulating plants: How and why do they do it? And what makes them so interesting? *Plant Sci.* 180(2), 169-181.
- Raskin, Y., Smith, R.D., Salt, D.E. (1997). Phytoremediation of metals: using plants to remove pollutants from the environment. *Current Opinion in Biotechnology.* 8(2), 145-266.

- Reichman, S.M. (2002). The Responses of Plants to Metal Toxicity. A Review Focusing on Copper, Manganese and Zinc. *Australian Minerals and Energy Environment Foundation*, 14-59.
- Rembe, R.D., Trefry, J.H. (2003). Increased concentrations of dissolved trace metals and organic carbon during snowmelt in rivers of the Alaskan Arctic. *Geochimica et Cosmochimica Acta*. 68, 477–489.
- Ross, S.M. (1994). Toxic Metals in Soil-Plant Systems, Wiley.
- Salomons, W. (1995). Environmental impact of metals derived from mining activities: processes, predictions, prevention. *Geochem Explor*. 52, 5-23.
- Santore, R.C., Di Toro, D.M., Paquin, P.R., Allen, H.E., Meyer, J.S. (2001). Biotic ligand model of the acute toxicity of metals. 2. Application to acute copper toxicity in freshwater fish and Daphnia. *Environ Toxicol Chem*. 20(10), 2397-2402.
- Sarma, H. (2011). Metal Hyperaccumulation in Plants: A Review Focusing on Phytoremediation Technology. *Journal of Environmental Science and Technology*. 4, 118-138.
- Schomakers, J., Mentler, A., Mayer, h. (2014). Determination of dissolved organic carbon in soils with UV spectroscopy, ultrasonic dispersion pre-treatment and separation with size exclusion chromatography. *Spanish Journal Of Soil Science*. 4(2), 127-142.
- Séguin, V., Gagnon, C., Courchesne, F. (2004). Changes in water extractable metals, pH and organic carbon concentrations at the soil-root interface of forested soils. *Plant Soil*. 260, 1-17.
- Silveira, M.L.A., Alleoni, Luís, R.F., Luiz, R.G.G. (2003). Biosolids and heavy metals in soils. *Sci. agric. (Piracicaba, Braz.)* 60.
- Smith, S.R. (1994). Effect of soil pH on availability to crops of metals in sewage sludge-treated soils. I. Nickel, copper and zinc uptake and toxicity to ryegrass. *Environ Pollut*. 85, 321-327.
- Solhi, M., Hajabbasi, M.A. , Shareatmadari, H. (2005). Heavy Metals Extraction Potential of Sunflower (*Helianthus annuus*) and Canola (*Brassica napus*). *Caspian J. Env. Sci*. 3(1), 35-42.

- Soudek, P., Petrová, Š. , Benešová, D. , Vaněk, T. (2010). Phytoextraction of toxic metals by sunflower and corn plants. *Journal of Food Agriculture & Environment*. 8 (3&4), 383-390.
- Surat, W., Kruatrachue, M., Pokethitiyook, P., Tanhan, P., Samranwanich, T. (2008). Potential of *Sonchus Arvensis* for the Phytoremediation of Lead-Contaminated Soil. *Int J Phytorem* 10, 325 - 342.
- Suthar, V., Memon, K.S., Mahmood-ul-Hassan, M. (2014). EDTA-enhanced phytoremediation of contaminated calcareous soils: heavy metal bioavailability, extractability, and uptake by maize and sesbania. *Environ Monit Assess* 186(6), 3957-3968.
- Takáč, P., Szabová, T., Kozáková, L., Benková, M. (2009). Heavy metals and their bioavailability from soils in the long term polluted Central Spiš region of SR. *Plant Soil Environment*. 55, 167–172.
- Topcuoğlu, B. (2012). The influence of humic acids on the metal bioavailability and phytoextraction efficiency in long-term sludge applied soil. *Conference on International Research on Food Security, Natural Resource Management and Rural Development, Georg-August Universität Göttingen and University of Kassel-Witzenhausen, Germany*.
- TRHS (2011). Acidifying soil. *The Royal Horticultural Society*.
- Tu, S., Ma, L., Luongo, T. (2004). Root exudates and arsenic accumulation in arsenic hyperaccumulating *Pteris vittata* and non-hyperaccumulating *Nephrolepis exaltata*. *Plant and Soil*. 258, 9-19.
- USEPA, United States Environmental Protection Agency (1999). Phytoremediation Resource Guide.
- USEPA, United States Environmental Protection Agency (2000). The Use of Plants for the Removal of Toxic Metals from Contaminated Soil.
- USEPA, United States Environmental Protection Agency (2004). Issue paper on the bioavailability and bioaccumulation of metals.
- Vassil, A.D., Kapulnik, Y., Raskin, I., Salt, D.E. (1998). The Role of EDTA in Lead Transport and Accumulation by Indian Mustard. *Plant Physiol*. 117(2), 447–453.

- Violante, A., Cozzolino, V., Perelomov, L., Caporale, A.G., Pigna, M. (2010). Mobility and bioavailability of heavy metals and metalloids in soil environments. *J. Soil Sci. Plant Nutr.* 10(3), 268-292.
- Vossen, P. (2006). Changing pH in Soil. *University of California Cooperative Extension*.
- Welsh, P.G. (1996). Influence of dissolved organic carbon on the speciation, bioavailability and toxicity of metals to aquatic biota in soft water lakes. *University of Waterloo*.
- Weng, L.P., Wolthoorn, A., Lexmond, T.M., Temminghoff, E.J.M., Riemsdijk, F.H.V (2004). Understanding the Effects of Soil Characteristics on Phytotoxicity and Bioavailability of Nickel Using Speciation Models. *Environ. Sci. Technol.* 38(1), 156–162
- Wenzel, W.W., Lombi, E., Adriano, D.C. (2004). Root and rhizosphere processes in metal hyperaccumulation and phytoremediation technology. In: *Heavy Metal Stress in Plants*, Springer Berlin Heidelberg: 313-344.
- Yang, J., Pan, X. (2012). Root exudates from sunflower (*Helianthus annuus* L.) show a strong adsorption ability toward Cd(II). *Journal of Plant Interactions.* 8(3), 263-270.
- Yanqun, Z., Yuan, L., Schwartz, C., Langlade, L., Fan, L. (2003). Accumulation of Pb, Cd, Cu and Zn in plants and hyperaccumulator choice in Lanping lead-zinc mine area, China. *Environ Int.* 30(4), 567-576.
- Zhao, F.J., Ma, J.F., Meharg, A.A., McGrath, S.P. (2009). Arsenic uptake and metabolism in plant. *New Phytol.* 181(4), 777-794.
- Zhao, H.Y., Lin, L.J., Yan, Q.L., Yang, Y.X., Zhu, X.M., Shao, J.R. (2011). Effects of EDTA and DTPA on Lead and Zinc Accumulation of Ryegrass. *Journal of Environmental Protection.* 2(7), 932.
- Zhao, L.Y.L., Schulin, R., Weng, L., Nowack, B. (2007). Coupled mobilization of dissolved organic matter and metals (Cu and Zn) in soil columns. *Geochimica et Cosmochimica Acta.* 71, 3407–3418.