Cu fractionation and plant accumulation in a soil contaminated with Cu and amended with lime, compost, and organic matter

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ABSTRACT
The extent of the hazard to the environment posed by Cu in the soil depends on its concentrations and chemical speciation. Studies were conducted to determine a feasible and practical phytoremediation strategy for Cu in soils with low to medium level contamination. This involved two studies, first to identify promising plant species capable of Cu stabilisation suited to soils and climatic conditions of British Columbia, and second to assess the effect of soil amendments in modifying the soil properties and thereby influencing the plants to immobilise Cu. The plants that were found to be promising in the first experiment study – *Lolium perenne*, *Festuca rubra* and *Poa pratensis* – were tested in the presence of soil amendments (lime, phosphate and compost, both individually and in combination) to assess the effect of chemo-phytostabilisation on Cu. The efficiency of treatments to stabilize Cu was assessed on the basis of Cu speciation in soil, partitioning of Cu in plants, and Cu uptake by the plants. Significant partitioning of Cu in immobile forms was noticed by the growth of *Festuca rubra.* Application of lime significantly reduced the exchangeable fraction of Cu, whereas phosphate application had an accelerating effect. The effects of amendment addition on plant uptake of Cu and on the Cu accumulation characteristics of plants are also discussed.

RÉSUMÉ
L'ampleur du risque environnemental posé par Cu dans le sol dépend de ses concentrations et de sa spéciation chimique. Des études ont été entreprises pour déterminer une méthode de phytoremediation du cuivre faisable et exploitable dans les sols faiblement à moyennement contaminés. Ceci a impliqué deux études. Tout d'abord il a fallu identifier des espèces adaptées aux sols et aux conditions climatiques de la Colombie-Britannique et capables de stabiliser le Cu, et, dans un second temps, évaluer l'effet de la fertilisation des sols en modifiant les propriétés de sol et en influençant de ce fait la réponse des plantes sur l'immobilisation du Cu. Les plantes qui se sont avérées prometteuses lors de la première étude - *Lolium perenne*, *Festuca rubra* et *Poa pratensis* - ont été examinées en présence de fertilisants (chaux, phosphate et compost, individuellement et couplés) pour évaluer l'effet de la phytostabilisation chimique du Cu. L'efficacité des traitements pour stabiliser le Cu a été évaluée a partir de la spéciation du Cu dans le sol, et de la répartition et de l'absorption du Cu par les plantes. La division significative du Cu sous ses formes immobiles a été notée lors de la croissance du *Festuca rubra*. L'application de la chaux a réduit de manière significative la fraction échangeable du Cu, tandis que l'application de phosphate a eu l'effet inverse. Les effets de l'addition de fertilisant sur la capacité d'absorption du Cu par les plantes et sur leurs caractéristiques d'accumulation y sont également discutés.

1 INTRODUCTION

Copper is a transition metal with three oxidation states: zero (Cu °, solid metal); plus one [Cu (I), cuprous ion]; and plus two [Cu (II), cupric ion] and it is classified as a heavy metal, with a density greater than 5g cm^{-3} (Forstner and Wittmann, 1979). Although Cu is a required element for plants as well as animals, at elevated levels, Cu becomes toxic and therefore, Cu levels in natural environments and its biological availability are important (Li et al., 2006). The sources of Cu in the soil are diverse, including sewage sludge, municipal compost, pesticides, fertilizers and emissions from municipal wastes incinerators, car exhausts (engine and break pad wear and lubricants), residues from metalliferous mines and smelting industries (Yang et al., 2002). Excess copper can bring about toxic effects in plants by catalyzing the production of highly toxic hydroxyl radicals from intracellularly generated hydrogen peroxide and affecting membrane properties by oxidation of membrane lipids (Brouwer and Brouwer, 1998; De-Vos et al., 1993 and Prasad et al., 2001). To clean up soils contaminated with Cu by traditional physiochemical methods can be very costly and also destructive to the soil. Phytoremediation, an emerging low-cost and ecologically benign technology for decontamination of soils, is defined as the process of utilizing plants to absorb, accumulate and stabilise contaminants in soil through physical, chemical and biological processes (Wenzel et al., 1999). The strategy for containment by reducing the mobility and phyto-availability of contaminants by changing its chemical state using plants is called phytostabilisation (Smith and Bradshaw, 1979). Addition of organic and inorganic soil amendments can complement the plant effect in reducing metal mobility by enhancement of its precipitation in the rhizosphere and this is termed chemo-phytostabilisation (Simon, 2005). High concentrations of Cu in soil may not cause pollution to the environment, if the mobile or bio-available fraction in the soil is reduced by altering the soil properties (Simon, 2005). For situations such as highway soil pollution, where metal removal is neither feasible nor practical due to financial and other physical constraints, this method can be adopted.

This study focused on Cu phytostabilisation in soil using plants and soil amendments. Promising plant species capable of phytoremediation of Cu were identified for the soils and climatic conditions of British Columbia. The effect of soil amendments in modifying the soil properties and influencing the plant species to immobilize Cu was then assessed. The study was conducted in soils collected from the back yard of Surrey fire hall, 1 km north of the intersection, HW 1 with 176th street in Surrey, B.C (a busy site with respect to traffic counts, >80,000 vehicles/day). The plants which were identified to be suitable for phytostabilisation from the previous experiment were tested with soil amendments (lime, phosphate and compost individually and in combination) to assess the effect of chemo-phytostabilisation on Cu. Cu speciation in the soil, accumulation characteristics and translocation properties of Cu in plants with and without amendment addition were studied. The study provided sufficient information to recommend Cu immobilisation in soils with low to medium multi-metal contamination.

2 MATERIALS AND METHODS

2.1 Identification of Plant Species

In this part of the study, the effect of plant growth on soil Cu fractionation, plant accumulation and uptake of Cu at two growth stages (90 and 120 days after sowing) were investigated. Six plant species (*Amaranthus hybridus, Helianthus annuus, Brassica napus, Lolium perenne, Poa pratensis and Festuca rubra*) were investigated for their efficiencies in phytoremediation of Cu from the soil. The collected soil that was contaminated with Cu, Pb, Mn and Zn at A level according to British Columbia standards for contaminated sites (Ministry of Environment, B.C, 1995) was spiked with multi-metals, Cu, Pb, Mn and Zn at A level (30, 50, 80 and 200 mg/kg) to simulate the polluted soils along highways (Padmavathiamma and Li, 2008). The experiment was conducted under outdoor field conditions in the Totem field, University of British Columbia.

2.2 Amendment Addition and Chemo-Phytostabilisation

To the spiked soil, amendments such as lime, compost and phosphate were added individually and in combination. Three plant species (*Poa pratensis*, *Lolium perenne* and *Festuca rubra*) based on the previous work were selected for the chemo-phytostabilisation study. The Experimental Program is given in Table 1.

Basic characteristics of the soil such as pH, E.C, total carbon and texture were estimated using standard procedures. Cu speciation in the soil was assessed by the selective sequential extraction following the procedure of Tessier et al (1979), as modified by Preciado and Li (2006). The distribution of Cu fractions before and after plant growth reveals the influence of plant growth on the mobility of Cu in the soil. The Cu concentration in plant was determined by dry ashing the plant samples (Lintern et al., 1997) and dissolving the ash in 10 mL of 1 M HCl and diluting it to 50 mL with de-ionized water. Both soil and plant extracts were analysed for Cu using a Varian Spectre AA 220 Multi-element Fast Sequential Atomic Absorption Spectrometer.

The statistical significance of differences among means was determined by one-way analysis of variance (ANOVA). ANOVA was performed to compare the effect of plant growth and amendment addition on soil Cu speciation, total soil Cu concentration as well as Cu uptake by plants. In order to assess the efficiency of plants for phytoextraction and phytostabilisation, the Enrichment Co-efficient (EC) of root (C_{roots}/C_{soil}, the ratio of root concentration to soil concentration) and shoot (C_{shoots}/C_{soil}, ratio of shoot concentration to the soil concentration) and Translocation Factor (TF = C_{shoots}/C_{roots}, ratio of shoot concentration to the root concentration) were calculated (Kumar et al., 1995).

Table 1 Experimental Program

Metals studied	Conditions/ Treatments	Plant species	Stage of sampling
Cu	Original soil with multi-metal concentrations (Cu – 52 ppm, Pb – 93 ppm, Mn – 215 ppm, Zn – 70 ppm), denoted as B0.	*Poa pratensis*	90 days after sowing
Pb		*Lolium perenne*	
Mn	Original soil + A level concn. (30ppm Cu, 50 ppm Pb, 80 ppm Zn and 200 ppm Mn), denoted as BA.	*Festuca rubra*	
Zn	BA plus lime (10 tons/ha).		
	BA plus phosphate (134 kg P_2O_5/ha).		
	BA plus compost (10 tons/ha).		
	BA plus lime plus phosphate plus compost (combined application).		

Design – Completely Randomized Design. 18 treatments (6 conditions and 3 plant species) and 3 replications.

3 RESULTS AND DISCUSSION

The texture of the studied soil is clay loam and the soil classification is Luvisolic humoferric podzol according to the Canadian System of Soil Classification (1998). Basic characteristics are: pH – 5.4, EC – 0.61, % carbon – 1.2, available phosphorus – 10.4 mg/kg. The total metal concentrations in the original soil and spiked soil were: Cu – 50 and 85 mg/kg, Pb – 90 and 143 mg/kg, Mn – 220 and 440 mg/kg and Zn – 75 and 162 mg/ kg.

3.1 Identification of Plant Species

Among the six studied plant species, only three plant species (*Lolium, Poa* and *Festuca*) survived in metal spiked soils, whereas all plant species except *Amaranthus* survived in the original soil. The concentrations and partitioning of Cu in plants (Figure 1), reveal the superiority of *Festuca* in phytoremediation of Cu. *Festuca* has been found to accumulate the highest amount of Cu when compared to other plant species (Figure 1). In *Festuca*, the shoot concentration for Cu was almost less than half of the root concentration, revealing the efficiency of *Festuca* for phytostabilisation of Cu. The Cu concentrations in all the plant species studied were higher at 120 days after sowing than at 90 days after sowing (an increase of 5 – 20 % Cu).

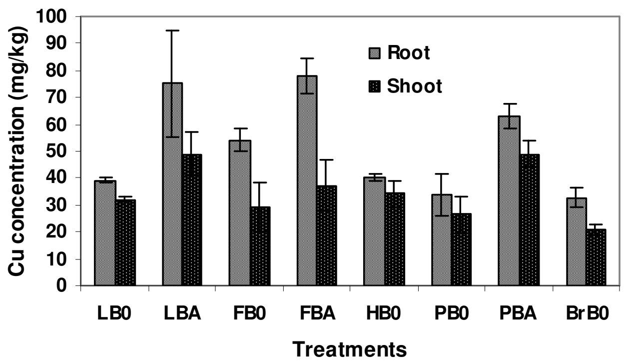

Figure 1. Cu concentration in plants at 120 days after sowing. LB0- *Lolium* B0 soil, LBA- *Lolium* BA soil, FB0- *Festuca* B0 soil, FBA- *Festuca* BA soil**,** PB0- *Poa* B0 soil, PBA- *Poa* BA soil, HB0- *Helianthus* B0 soil, BrB0- *Brassica* B0 soil. Error bars represent ±S.D. of means of three replicates. F significant at $P<0.05$.

The Cu speciation in the soil before and after plant growth (Table 2) showed that Cu was retained mainly in the organic fraction at 90 days after sowing, which later converted to the oxide fraction at 120 days after sowing. Compared to B0 soil (4 % exchangeable Cu), there was a significant reduction in exchangeable Cu at 90 days after sowing by the growth of *Festuca* (<1 %). *Festuca* partitioned more $Cu_{organic}$ to Cu_{oxide}, the relative partitioning of Cu in different fractions being $Cu_{residual} > Cu_{oxides} > Cu_{organic} > Cu_{exchangeable}$ at 120 days after sowing. The re-distribution of exchangeable Cu to oxide Cu in soil by the growth of *Festuca* makes it suitable as a phytostabilising plant for Cu. However the organic fraction of Cu was relatively high in soils with *Lolium* and *Poa*. This may be because of the well-developed fibrous root system of these plants, as observed from the high values of root biomass, which would increase the soil organic matter status, which in turn would help to retain more Cu in organic form. Thus from the results obtained, three plant species selected for the second study were *Lolium perenne, Poa pratensis* and *Festuca rubra*.

Table 2 Relative % partitioning of Cu in soils

Stage of sampling	Samples	Exch. (%)	Oxide (%)	Organic (%)	Residual (%)
Before plant growth	B0	4	15	31	**50**
	BA	3	25	**39**	33
90 days after sowing	LB0	2.6	21	**45**	31
	LBA	1.3	29	35	34
	FB0	<1	21	27	**51**
	FBA	<1	23	34	**42**
	HB0	2.0	18	30	**50**
	PB0	1.8	19	**48**	31
	PBA	2.0	34	32	33
	BrB0	4.1	19	33	**44**
120 days after sowing	LB0	4	29	15	**53**
	LBA	5	**39**	28	28
	FB0	<1	**42**	20	38
	FBA	<1	**41**	27	31
	HB0	4	32	16	**48**
	PB0	6	24	29	**41**
	PBA	7	**38**	23	32
	BrB0	5	21	26	**48**

B0 soil (original soil), BA soil (spiked soil), LB0 (Lolium B0 soil), LBA (Lolium BA soil), FB0 (Festuca B0 soil), FBA (Festuca BA soil), PB0 (Poa B0 soil), PBA (Poa BA soil), HB0 (Helianthus B0 soil) and BrB0 (Brassica B0 soil).

3.2 Amendment Addition and Chemo-Phytostabilisation

Addition of lime and compost had a depressing effect on Cu concentration in plants, whereas phosphate application had an enhancing effect, especially the root Cu concentration (Table 3). Compost application reduced the plant concentration of Cu providing evidence for Cu binding with insoluble organic fractions in the soil (Hsu and Lo, 2000). Combined application of amendments gave the lowest plant Cu concentrations in all the three plant species. The concentration of Cu in shoots and roots decreased by more than 40 % by the combined application of amendments. Since the plant biomass was higher in treatments, which received amendments in combination, the decreased uptake may be due to lower Cu absorption by plants. Low absorption and uptake of Cu by plants that received the combined application of amendments may be due to the decrease in the exchangeable fraction of Cu in the soil (Figure 2). The decrease in Cu mobility may be explained by the soil biochemical transformations brought about by the modified rhizosphere environment, created by plant growth and amendment addition (Kumpiene et al., 2007).

Table 3. Cu concentration in plants

	Poa		*Lolium*		*Festuca*	
	Root	Shoot	Root	Shoot	Root	Shoot
B0	42±3.2	31±3.5	69±1.5	33±2.6	71±1.0	48±4.5
BA	98±4.2	47±3.6	91±5.5	49±3.4	113±2.6	54±9.5
BAL	60±1.5	31±4.2	76±7.8	42±6.0	64±9.6	36±4.9
BAP	101±3.6	65±7.8	98±6.1	54±12.1	108±2.6	48±10.3
BAO	86±4.7	63±4.7	79±4.0	56±4.0	95±10.1	53±5.1
BALPO	72±3.1	45±4.9	68±3.8	42±4.6	87±3.6	43±6.5

Mean ± standard deviation, n = 3. F significant at $P<0.05$. B0 – Initial soil, BA – Spiked soil, BAL - Spiked soil plus lime, BAP - Spiked soil plus phosphate, BAO - Spiked soil plus compost, BALPO - Spiked soil plus lime, phosphate and compost.

Table 4. Enrichment Co-efficient (EC) and Translocation Factor (TF) in different treatment

Treatments	*Poa*			*Lolium*			*Festuca*		
	EC		TF	EC		TF	EC		TF
	EC_{root}	EC_{shoot}		EC_{root}	EC_{shoot}		EC_{root}	EC_{shoot}	
B0	0.85c	0.63b	0.74a	1.50a	0.71a	0.48c	1.39a	0.94a	0.68a
BA	1.22b	0.58b	0.48c	1.19b	0.64b	0.54c	1.41a	0.67b	0.48c
BAL	0.70cd	0.36c	0.52c	0.87c	0.48c	0.55c	0.73c	0.41c	0.56b
BAP	1.36a	0.87a	0.64b	1.25b	0.69a	0.54c	1.47a	0.65b	0.47c
BAO	1.10b	0.80a	0.73a	0.95c	0.67a	0.71a	1.21b	0.67b	0.56b
BALPO	0.91c	0.63b	0.69b	0.78cd	0.48c	0.62b	0.77c	0.45c	0.45c
F	*	*	*	*	*	*	*	*	*

Mean values, n = 3. F significant at $P<0.05$. Statistical significantly different values ($p<0.05$) according to the Least Significance Test. in each column are followed by different letters. B0 – Initial soil, BA – Spiked soil, BAL - Spiked soil plus lime, BAP - Spiked soil plus phosphate, BAO - Spiked soil plus compost, BALPO - Spiked soil plus lime, phosphate and compost.

Application of phosphates increased the EC (Enrichment Co-efficient) values of Cu (Table 4), whereas lime had a decreasing effect. Low EC and TF (Translocation Factor) values indicate a low absorption and low translocation of metals in the plants. The EC and TF values were significantly lowered (Table 4) by the combined application of amendments in all the three plant species, *Lolium, Poa* and *Festuca.*

However, *Festuca* with the combined application of amendments gave the lowest EC values (EC_{root} and EC_{shoot}, being 30 and 35 % lower than that of plants without amendments) as well as TF value. The differences between plant species in metal accumulation and translocation may be due to the differences in release of root exudates, e.g. organic acids, CO_2 and H^+ that change the pH and release the elements from the substrate (Kelly et al., 1998). The ionic competition and high soil pH may also be the reasons for the lower translocation of metals to the above ground parts of plants in amendment applied treatments (Kumpiene et al., 2007).

The effect of soil amendments and plant growth on Cu speciation in soil is discussed below. The partitioning of Cu in four soil fractions, exchangeable, carbonates and oxide bound, organic bound and residual before and after plant growth and amendment addition are given in Figure 2. Growth of *Poa* and *Lolium* had an enhancing effect on the exchangeable fraction, whereas *Festuca* had a lowering effect supporting the results obtained from the previous study (Padmavathiamma and Li, 2007). Since $Cu_{exchangeable}$, which is the bio-available fraction, was lowest in soils grown with *Festuca* along with the combined application of soil amendments (0.5 mg/kg), this treatment combination can be suggested as the best for phytostabilisation of Cu in soil.

Lime application reduced the exchangeable fractions of Cu ($P<0.05$) and this reduction was highest in *Festuca* for Cu, since almost all the exchangeable fraction was converted to the oxide fraction. Similar observations on the effect of lime in reducing the mobile metal fractions were reported by (Hooda and Alloway, 1996). Lime amendments increase soil pH (McBride et al., 1997) and favour the formation of oxides, metal-carbonate precipitates and complexes that decrease metal solubility (Mench et al., 2000). In the present study, when lime was applied, the pH increased from 5.5 to 7.1, whereas for phosphate-applied soils, a slight decrease in pH was noticed. Application of phosphates increased the exchangeable fraction of Cu in the soil (Figure 2). The organic bound Cu fraction, which is relatively immobile, was reported to account for 50 % of the total soil Cu by the growth of *Poa* with the combined application of amendments. This is consistent with the observations of several other authors (Clement et al., 2003 and Balasoiu et al., 2001) indicating that Cu forms very stable complexes with organic matter. Thus organic amendments can contribute to Cu immobilisation through the formation of stable complexes with hydroxyl or carboxyl groups on the solid surfaces of the organic polymers (Chirenje and Ma, 1999). The effect of combined amendment addition on changing the Cu partitioning in soils (Figure 2), may be due to the precipitation as hydroxides, carbonates, phosphates and several other anions as well as forming complexes with organic ligands (Mench et al., 2000).

4 CONCLUSIONS

- Exchangeable Cu fraction in the soil and plant tissue Cu concentrations decreased by increasing the soil pH with lime application.
- Phosphate application increased the exchangeable Cu content of soil and enhanced Cu uptake by plants, which is congenial for phytoextraction of Cu.
- Compost application reduced the plant concentration of Cu providing evidence for Cu binding with insoluble organic fractions in the soil.
- Maximum Cu immobilisation (partitioning of complete exchangeable to oxide Cu) was achieved in the soil by the combined application of amendments in conjunction with growth of *Festuca.*
- The organic bound Cu fraction (less mobile and phyto-available) was reported to account for 50 % of the total soil Cu by the growth of *Poa* with the combined application of amendments.
- The concentration of Cu in shoots and roots decreased by more than 40 % in *Festuca* plants when grown along with the combined application of amendments.
- Festuca plants when grown along with the combined application of amendments gave lowest EC and TF values for Cu.

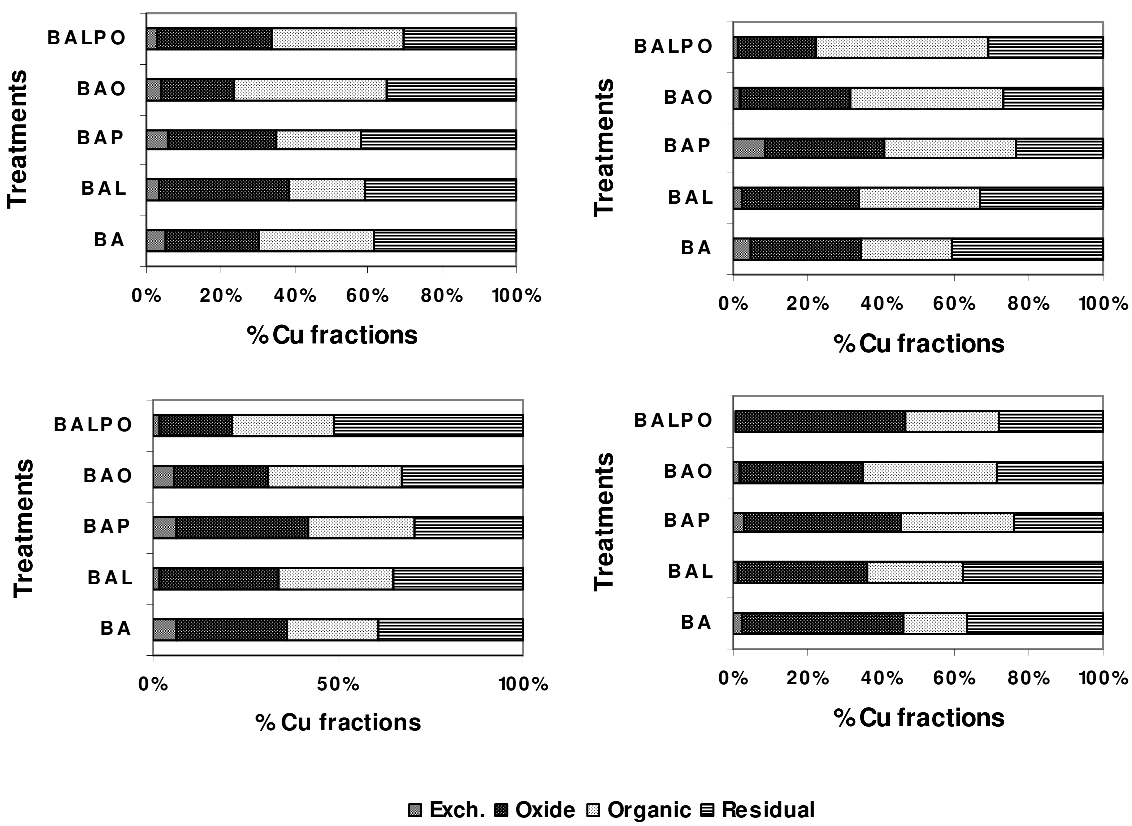

Figure 2. Relative partitioning of Cu in different fractions of soil under different conditions/treatments. (a) soil with amendments alone, (b) soil with amendments and *Poa,* (c) soil with amendments and *Lolium*, (d) soil with amendments and *Festuca*. -BA – Spiked soil, BAL - Spiked soil plus lime, BAP - Spiked soil plus phosphate, BAO - Spiked soil plus compost, BALPO - Spiked soil plus lime, phosphate and compost.

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