Bioavailability assessment of trace metal contaminants in urban soils and partitioning of zinc, cadmium, lead, nickel, and copper in the roots, shoots, foliage, and seeds of *Chenopodium quinoa*

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by

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Abstract

This paper presents two studies on urban soils in Vancouver – the first is a case study for assessment of trace metals in urban soils, with particular emphasis on brownfield reclamation and food production. Three single-extraction procedures are evaluated for their usefulness for identifying the bioavailable fractions of trace metals in soil. Plant-available Zn, Cd, Pb, Ni, and Cu were estimated using elemental analysis, pH, organic matter, and soil texture. Specific geochemical factors such as oxidizing conditions and the presence of Fe-, Al-, and Mn-oxides suggest trace metal mobility in the soils. The use of 0.1M HCl for estimating the risk of trace metal bioaccumulation in crop urban plants is recommended. The second study assesses selected phytoremediation technology for removing trace metals from soil through successive cultivation of trace metal-accumulating crop plants. A pot study was conducted using *Chenopodium quinoa* (quinoa) to extract Zn, Cd, Pb, Ni, and Cu from variously spiked brownfield soil. *Chenopodium quinoa* is a grain that may be a high-value specialty crop in British Columbia. Previous research suggested that *Chenopodium quinoa* may be a useful plant for phytoextraction of trace metals. The reasons for using quinoa in the present experiment are twofold – (1) to evaluate potential human health risks involved with growing a metal-accumulating crop in potentially contaminated urban soils, and (2) to assess the above-ground partitioning and accumulation of trace metals for evaluating the usefulness of *Chenopodium quinoa* for phytoextraction in Vancouver, British Columbia. It was found that quinoa is a hyperaccumulator and there is a potential concern that grains may contain harmful levels of trace metals for human consumption.

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List of abbreviations

BC	-	British Columbia
BCF	-	Bioconcentration factor
C	-	Concentration
CEC	-	Cation exchange capacity
DAT	-	Days after transplant
EC	-	Electroconductivity
GHG	-	Greenhouse gas
ICP-OES	-	Inductively Coupled Plasma Optical Emission Spectrometer
nd	-	Undetectable
ns	-	Not significant
PAH	-	Polycyclic aromatic hydrocarbon
PCB	-	Polychlorinated biphenyl
TF	-	Translocation factor
UBC	-	University of British Columbia
USDA	-	United States Department of Agriculture
YTC	-	Yard trimmings compost

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1 General introduction

1.1 Problem statement

Urban agriculture is recognized as a positive activity that provides ecosystem services, connections to nature, and the offering of fresh local food to urban communities. Yet there are concerns about the environmental hazards to which urban crops are exposed. As urban agriculture gains popularity in Vancouver, underutilized land parcels are increasingly being repurposed as farms or gardens. These parcels, known as 'brownfields,' are often vacant when contamination caused by prior uses necessitates expensive and time-consuming assessment and remediation. Brownfield remediation is a growing concern, and with 4,000 to 6,000 brownfields in British Columbia alone (BC Brownfield Renewal, 2013) new methods of low-cost, in-situ assessment and remediation are needed in order transform these lots into useable space.

A primary impediment to site reclamation is economic -- contaminated sites remain undeveloped when the cost of remediation exceeds the estimated value of the land. Working within the provincial protocol, property managers must conduct a costly risk assessment to identify and evaluate the presence of harmful substances and the risk of human exposure to those substances. This assessment is used to develop site-specific hazard indices to estimate potential health risks associated with land use. The most common accepted solution for site management currently involves relocation of the contaminated material (BC Ministry of Environment, 1996).

Excavation and relocation are labour- and cost-intensive. Further, such practices do not remediate the valuable existing soil but rather transport the problem, disrupting ecosystems in the process.

Ideal techniques for contaminant removal should not damage soil structure, compromise existing fertility, or replace native soils with imported fill. Alternative methods for *in situ* remediation include adding metal-binding chelates and organic matter, increasing soil pH to make metals less available (which may also diminish availability of necessary micronutrients), or sowing plants that accumulate metals in their tissue, either to remove (phytoextraction) or to bind (phytostabilization) the contaminants (Gupta et al., 1996). For water-soluble contaminants, soil can be leached or washed.

Provincial standards for trace metal concentrations in soil are given in terms of total as opposed to bioavailable metals; the same is true for commercial laboratory analysis (Iverson et al., 2012). This method does not provide information on the binding strength or solubility of heavy metals, and thus may be an overestimation of the risks posed by soil contaminants to crop plants and human health (Houba et al., 1996).

The following research focuses on the determination of a useful and accurate measurement of the plant-available fractions of trace metals Zn, Cd, Pb, Ni, and Cu for a number of urban soils in Vancouver, BC. A subsequent study builds on this background information with an investigation of *Chenopodium quinoa* (quinoa) as a potential plant for phytoextraction in Vancouver, BC.

1.2 Scope and objectives

Soil contaminants can be broadly grouped as either organic or inorganic. Organic compounds such as PAHs (polycyclic aromatic hydrocarbons) and PCBs (polychlorinated biphenyls) may break down over a period of weeks or months with focused application of remediation technologies. Trace metal contaminants, on the other hand, cannot be broken down by biological processes and therefore cause a particular environmental pollution problem, as they accumulate in soil over time. Plants may be exposed to trace metal

contaminants either via uptake and accumulation from the soil solution or via atmospheric deposition of contaminants on the leaf surfaces. This paper focuses on the trace metal contaminants in soil, and specifically on trace metal availability and plant uptake in the acidic soils common to Vancouver, British Columbia.

The general objectives of this work are to:

(1) Determine a useful single extraction method for assessing the bioavailability of trace metals in Vancouver soils,
(2) Compare differences in trace metal plant-availability among urban soils formed on different parent materials, with consideration to pH, organic matter content, and soil texture,
(3) Evaluate *Chenopodium quinoa* as a potential plant for phytoextraction of trace metals and as an urban specialty crop safe for human consumption through investigation of plant uptake and aboveground partitioning of trace metals from soil, and
(4) Review and make recommendations for provincial contamination standards as they relate to site remediation and farming of urban brownfields in Vancouver.

1.3 Research plan and organization

1.3.1 Preliminary case study

An initial assessment of 8 soils from current and future food growing sites around Vancouver, BC was conducted to investigate potential correlations between bioavailability of trace metals in soils of different parent materials. The case study takes into account past and future land use, as well as anthropogenic additions to the soil at each site. Three different soil extraction procedures were considered as measurements of trace metal bioavailability in soil, taking into account the geochemical effects of pH, organic matter, and soil parent material. A review of relevant literature on extraction techniques and soil

factors specific to Vancouver, BC, were used to make recommendations for the adoption of a 0.1M HCl extraction procedure as a low-cost, reliable assessment of trace metal availability.

The preliminary case study is presented in Chapter 2.

1.3.2 Phytoextraction pot study

The Hastings site from the preliminary case study was chosen for further study based on its moderate trace metal contamination and its unique status as a potential site for expansion of an urban farm. Topsoil was removed from the site for use in a pot study where *Chenopodium quinoa* was grown both in the original soil and in the original soil spiked with two different amounts of a multi-metal solution. Tissue sampling was used at two stages of growth to determine both the aboveground accumulation of trace metals, which is useful for phytoextraction studies, and the trace metal accumulation in the edible portions of the plant, which is useful for evaluating potential human health risks should this plant be adopted for wider cultivation in urban areas.

The phytoextraction study is described in Chapter 3.

2 Factors affecting trace metal bioavailability in urban soils of Vancouver, British Columbia: A case study

2.1 Introduction

Plant uptake of trace metal contaminants is currently of concern as issues of industrial contamination cause delays in municipal policies dealing with land use changes, zoning, urban agriculture, and public health.

2.1.1. Brownfields

Vancouver, British Columbia is similar to other North American cities where shifting land-use from industrial and manufacturing to residential and commercial has left numerous brownfields throughout the city. Brownfields are defined as "abandoned, vacant, or underutilized commercial or industrial properties where past actions have resulted in actual or perceived

Figure 2.1: Brownfield site on E. Hastings Street, Vancouver BC

contamination and where there is an active potential for redevelopment" (BC Brownfield Renewal, 2013). As a result of the high costs and subsequent delays associated with traditional remediation practices, managers and owners of brownfields are encouraged to seek citizen engagement in finding interim uses for these parcels (BC Brownfield Renewal, 2013). As the City of Vancouver allocates more space for gardens in accordance with the 2010 Greenest City Action Plan, an issue of increasing concern is whether brownfields are safe sites growing produce in urban farms and community gardens (City of Vancouver, 2012b).

2.1.2 Trace metal contamination

Trace metal contaminants of concern to urban farmers include cadmium, copper, manganese, nickel, zinc, arsenic, and lead, which variously come from paints, fertilizers, batteries, combustion, pesticides, electroplating, preservatives, and other human industrial applications (Kabata-Pendias, 2004). Trace metals occur naturally in soils, but usually in much lower concentrations than those resulting from anthropogenic contributions (Craul, 1995). Further, pedogenically originated trace metals are often bound tightly to silicates within the soil matrix, rendering them relatively immobile and therefore less likely to move through ecosystems than anthropogenic additions (Kabata-Pendias, 2004). Plant uptake and groundwater leaching tend to be low, therefore metals persist in

Figure 2.2: Urban soil showing natural and anthropogenic contributions

soils for long periods of time, causing a unique environmental problem (Dudka et al., 1996). Potential health risks to humans include cancer, liver and kidney damage, reproductive aberrations, growth effects, neurological and immune disorders (McIntyre, 2003; Peralta-Videa et al., 2009).

Pathways of exposure to these contaminants depend on the both the organisms and the ecosystems concerned, and the time of exposure (McLaughlin et. al., 2000). Trace metal contaminants may pollute aquatic systems through leaching of soluble compounds or surface erosion, while volatilization of particles or suspended surface dust may contribute to air pollution. Plants may accumulate trace metal contaminants in the same way that they accumulate essential elements, via uptake from the soil solution. Bioconcentration of metals in tissues of the plants results can result in higher concentrations of contaminants in plants than in the soil (Kirkham, 2006).

There is particular concern, therefore, for humans consuming vegetables grown in urban soils, especially in urban garden plots where a large proportion of one's intake may come from the same area of land (DeKimpe & Morel, 2000). There are multiple pathways by which metals can contaminate plant tissue, namely through uptake from the soil to edible plant parts, atmospheric deposition of combustion emissions (i.e. roadways, vents) or adhesion of soil particles to roots or leaf surfaces in the form of dust. While accumulation in plant tissue is relatively unlikely for Pb, Cd is a particular concern as it is absorbed from the soil solution and can cause toxicity in relatively low quantities (Dudka et al., 1996).

2.1.3 Bioavailability assessment

Current government risk assessment standards in British Columbia provide a conservative estimate of short-term risks associated with plant uptake from soil. This is because the

standard values refer to the total[1] concentrations of trace metals in the soil, which represent a much larger fraction than those relevant for interpreting toxic effects (Houba et al., 2000). In other words, non-reactive forms of elements in the soil are considered just as hazardous as reactive ones (Sauvé et al., 1996). These 'total' values are useful for evaluating long-term remediation needs as they represent the maximum possible environmental contamination, however they are considered excessively conservative for short-term assessments of agricultural risk (Rao et al., 2007).

'Bioavailable' elements are easily mobilizable in the soil solution, and therefore available for plant uptake (Houba et al., 2000). The bioavailable fraction of metals in soil is impossible to determine as a fixed concentration, as availability is subject to a multitude of constantly changing soil factors. These include cation exchange capacity (CEC), pH, redox potential, organic matter, moisture, and texture as well as the species of plant and metal concerned (Kabata-Pendias, 2004).

2.1.4 Evaluation of single-extraction procedures

It has been widely recommended by researchers that soil contamination guidelines used by governments be based on the soil metal pool that actually may become bioavailable, rather than the total pool (Sauvé et al., 1996). It is thus important to have an understanding both of the pools and chemical forms of elements in the soil, as well as the conditions that will affect mobilization, availability, and subsequent toxicity to plants is variable by element and type (MacNicol & Beckett, 1985). The generally accepted idea that metal phytoavailability is related to free ion activity of the metal in the soil solution has been challenged in the last decade by research showing that metal-organic complexes, changes in specific metal speciation and soil types, and inorganic functional groups can increase the plant-availability of trace metals in soil (Huang et al., 1997; McLaughlin et al., 1997). McLaughlin et al. (2000) point out that just as agricultural soil nutrient analyses may use

[1] The term 'pseudototal' would be more accurate – these values do not reflect those trace metals bound in

one of a number of extractants based on soil type, cropping plans, and environmental conditions, to evaluate nutrients 'available' for crop plants, so should risk assessment for environmental hazard use extraction procedures that are relevant for food chain and ecosystem effects.

Selective extraction methods can be used to target soil phases or compounds bound to particular exchange sites and provide site-specific information for assessing effects based on metal species mobility, designated land use, and soil type (Rao et al., 2007). The frequently used five-step sequential extraction method identifies five 'pools' of elements in the soil matrix in the order of their binding strength and subsequent availability. These are: (1) exchangeable, (2) bound to carbonates, (3) bound to Fe- and Mn-oxides, (4) oxidizable, and (5) residual (Peijnenburg et al., 2007; Tessier et al., 1979). The single extraction procedures discussed in this paper, 0.01M $CaCl_2$, 0.1M HCl, and *aqua regia*, address trace metals bound in phases (1), (3), and (4), respectively.

Table 2.1: Selective extraction methods and target soil fractions

Extraction method	Target Fraction	References
Aqua regia	Mn or Fe oxide bound	(Peijnenburg et al., 2007, Tessier et al., 1979)
0.1M HCl	Oxidizable	(Kashem et al., 2007)
0.01M $CaCl_2$	Water-soluble	(Houba et al., 1996, 2000)

Aqua regia is a concentrated strong acid that provides an estimate of the 'total,' or non-residual fraction of elements in soil. This is the extraction procedure used for contaminated site risk standards in Canada and most other countries. Strong acid extractions access elements tightly bound within the soil matrix. The resulting high concentration values are not useful for evaluating the immediate environmental availability of trace metal contaminants. This extraction was therefore used for this study

only to provide a benchmark for comparing values to those used in the BC Contaminated Sites Regulation (2012).

A number of authors have suggested that neutral salt extraction using 0.01 $CaCl_2$ or 0.1M $NaNO_4$ provides the most accurate assessment of ion activity and mobilizable trace metals in the soil solution (Gupta et al., 1996; Houba et al., 2000). These procedures are most often used in Sweden and the Netherlands, where neutral pH and redox potential render these oxides more stable; consequently, metals bound to them are unlikely to become available (Kabata- Pendias, 2004).

In Vancouver, British Columbia, where soil acidity and oxidizing conditions contribute to ion exchange and metal activity, Fe-, Al-, and Mn-oxides are more prevalent and thus 0.1M HCl is considered more suitable for this assessment (Smith et al., 2007). A dilute strong acid, 0.1M HCl can be used to estimate that portion of minerals bound in Mn- and Al-oxides that may become available through natural weathering and decomposition processes. It is used here to refer to the 'plant-available' fraction of minerals in soil. This is a relatively low-cost extraction procedure often used in site specific assessments when remediation costs are limiting, and is useful for the extraction of metals sorbed to Mn- or Fe-oxides (McLaughlin et. al., 2000; Rao et al., 2007).

2.1.5 Soil factors affecting trace metal availability

This paper focuses on organic matter content, pH, and soil parent material as three of the main factors affecting plant-availability of trace metals in soil (McBride et al., 2009). Trace metals are more mobile in acidic soils where H^+ competition results in metal desorption from negatively charged binding sites along clay edges (Lasat, 2000). Binding and sorption with carboxyl groups present in organic matter limits the mobility of trace metals (Dube et al., 2001). Parent material may also affect the clay fraction of soil, which influences the

plant-availability of Ni, Cd, Cu, Zn and Pb. The negatively charged clay layers in the inorganic colloidal fraction soil fraction create a 'sink' for trace metals, which are immobilized through adsorption and exchange with hydrous Mn and Fe oxide polymer chains (Rao et al., 2007, Kabata-Pendias 2004, McLaughlin et. al., 2000). Vancouver has podzolic soils rich in Al, Fe, and Mn, and leaching during the rainy winter months increases acidity (Iverson et al., 2012). The consequent oxidizing conditions and high CEC therefore provide adsorption sites for the binding and immobilization of trace metals (McLaughlin et. al., 2000).

Iverson et al.'s (2012) urban soil map of Vancouver shows that the soils in Vancouver are formed on glacial till, glacial marine till, and marine parent materials rich in Mn, Fe, and Al. Alluminosilicate clays formed by weathering of chlorite parent material provide opportunities for trace metal cations to adsorb to oxygen or hydroxide anions through isomorphic substitution of Al, Mg, or Si ions (Dube et al., 2001). Manganese is prevalent in marine environments and therefore is abundant in soils formed on highly weathered marine deposits. Because it does not occur as the free metal, Mn forms hydrous oxides within the clay minerals, where it moves easily among oxidation states. In soils with pH above 6.0, Mn will bond with organic matter, becoming less mobile, but in soils with low pH and low redox potential, Mn is highly reactive. In the oxidizing environments common for Vancouver soils, Mn exchangeability has a major effect on ion activity of other metals (Nádaská et al., 2010).

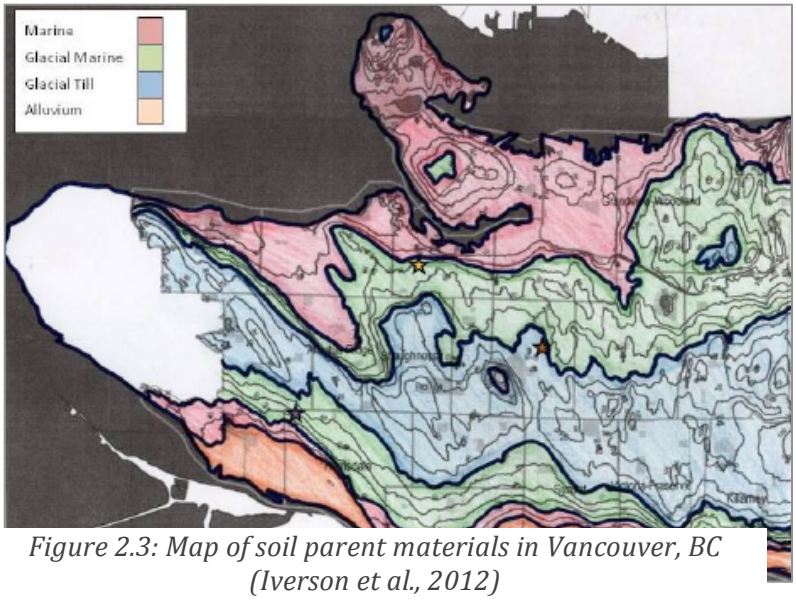

Figure 2.3: Map of soil parent materials in Vancouver, BC (Iverson et al., 2012)

2.2 Objectives

This paper presents an assessment case study of 8 different urban agriculture sites in Vancouver, British Columbia. Estimates of total and bioavailable Zn, Cd, Pb, Ni, and Cu for each site were determined using three single extraction procedures. Results were compared among sites and with BC provincial guidelines for contaminated sites. The differences in extraction procedures are compared as well as the geochemical effects of pH, organic matter, and soil parent material on bioavailability of trace metal contaminants in soil. The primary objectives of this study were:

(1) To compare the total, Fe- and Mn- oxide bound, and free ion metal concentrations of 8 different urban soils in Vancouver, British Columbia,

(2) To recommend the addition of bioavailability estimates for contaminated sites risk assessments,

(3) To evaluate geochemical effects of soil parent material on bioavailability of trace metals in urban soils, and

(4) To discuss the influence of pH and organic matter on trace metal availability in soil.

2.3 Materials and methods

Eight sites representing a range of parent materials, management characteristics, and geographic locations were sampled within the city of Vancouver based on their status either as current or planned food-producing areas [Figure 2.4]. The sites included three community gardens (Pine, 60th & E. Blvd, and 16 Oaks), two urban farms (41st & Blenheim and UBC Farm), and three sites where garden development is planned but has not yet begun (Hastings, Vernon, and UBC). This case study demonstrates several methods of site assessment for interpreting trace metal bioavailability for urban agriculture in Vancouver, British Columbia.

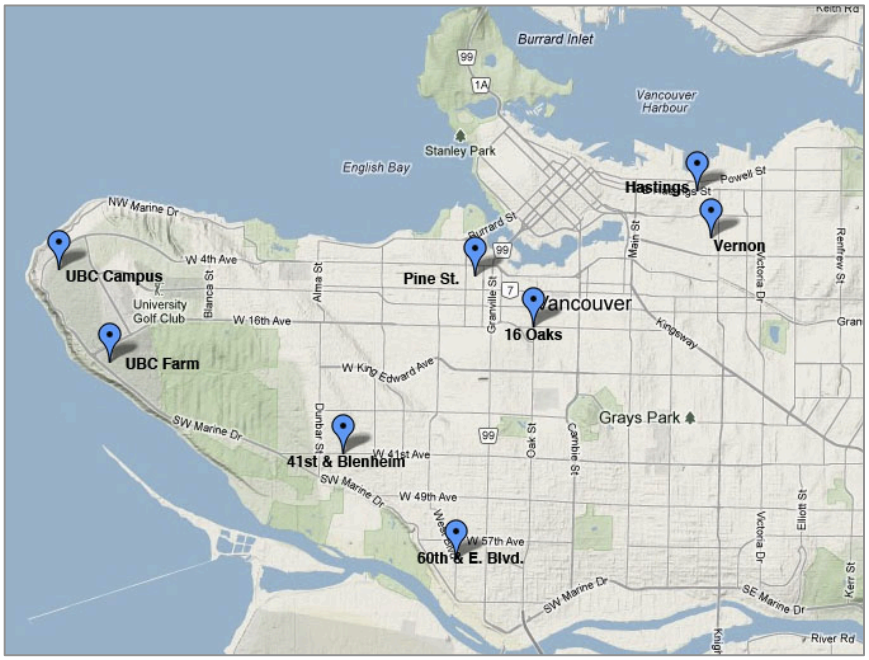

Figure 2.4 Map showing sampled sites

2.3.1 Sampling strategy

The subjective random sampling used in this study is recommended for urban and industrially contaminated sites where past or current land use can be used to as a guide for choosing representative sampling locations (Rao et al., 2007). At each site, 2x2m quadrats with no visible soil additions (i.e. compost or infill) were subjectively chosen in an effort to obtain samples that accurately represented the original or 'native' soil. Six soil cores to a depth of 15cm were taken from random points within each sampling quadrat, and mixed in a clean plastic bucket. Composite samples were air dried and passed through a 2mm sieve for analysis.

2.3.2 Assessment parameters

2.3.2.1 Organic matter

Percent organic matter was estimated using the loss-on-ignition method (Ball, 1964). Five grams of each sample was dried at 105° C, weighed, then heated overnight at 350° C. The ratio of change in mass to original mass represents the percent organic matter removed through ignition of carbonaceous material.

2.3.2.2 Electroconductivity

Electrical conductivity was measured at 1.5 µMHO scale using a solution of 0.01M KCl as standard and a 1:2 soil to water ratio (Miller & Curtin, 1993.)

2.3.2.3 pH

A pH meter was used to measure soil acidity in deionized H_2O and in 0.01M $CaCl_2$ at a ratio of 10g soil per 20 mL solution (Hendershot et al., 1993).

2.3.2.4 Texture

Soil texture was determined by hand texturing (USDA, 2001).

2.3.2.5 *Aqua regia* extraction

Soil samples previously heated at 350° C and weighing 0.5g were treated with 15mL of 1M HNO_3: 3M HCl (*aqua regia*) solution, heated to dryness, and made to 100 mL volume with 0.2M HNO_3. The solution was then passed through Whatman #42 filter paper. Samples were analyzed for metals with a Varian Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES).

2.3.2.6 0.1M HCl extraction

A 0.1M HCl solution was added to 5g of sample, shaken for 4 hours on a reciprocal shaker at 60 cycles per minute and covered for approximately 10 hours before passing the supernatant solution through Whatman #42 filter paper and adding 0.1M HNO_3 to a volume of 50mL (Chen & Ma, 2001). Solutions were analyzed by ICP-OES.

2.3.2.7 0.01M $CaCl_2$ extraction

Dry soil weighing 10g per sample was added to 100 mL 0.01$CaCl_2$ in 250 mL polythene bottles and shaken for 2 hours on a reciprocal shaker. After shaking 60mL of the suspension was subjected to centrifugation for 10 minutes at 1800 relative centrifugal force (RCF). 10mL per sample of the supernatant was transferred to a test tube and acidified with 0.1mL 1M HCl (Houba et al., 2000). Solutions were analyzed by ICP-OES.

2.3.3 Statistical analysis

Linear regression analysis, ANOVA, and Student's t-tests were performed for statistical analysis using JMP 10.0 statistical software to test the following hypotheses:

> **H_0:** There is no difference in concentrations of 0.1M HCl-extractable Zn, Cd, Pb, Ni, or Cu among soils formed on different parent materials.
>
> **H_0:** There is no difference between *aqua regia,* 0.1 M HCl, and 0.02 $CaCl_2$ extractions for estimating bioavailability of trace metals in Vancouver, British Columbia.
>
> **H_0:** There is no correlation between *aqua regia* and 0.1M HCl concentrations of trace metals at the sampled sites.

2.4 Results and discussion

2.4.1 Total concentrations

Aqua regia-derived trace metal concentrations at each site were compared with the BC Ministry of Environment guidelines for risk assessment of contaminated sites (Contaminated Sites Regulation, 2011). Of the sites studied, 16 Oaks and Hastings were in excess of these values for Cu, Pb, and Zn. Pine, 60th & E. Blvd., and 41st & Blenheim were in excess of these values for Pb and Zn only. Non-significant differences in total concentrations of Zn, Pb, Ni, and Cu among sites and among parent material groups suggest similar patterns of anthropogenic contribution.

Table 2.2: Mean total concentrations (mg kg^{-1}) of Zn, Cd, Pb, Ni, Cu & Mn (± SE) at selected sites compared with BC standards for agricultural use (nd=not detected)

	Hastings	Pine	16 Oaks	UBC Farm	UBC Campus	Vernon	60th & E. Blvd.	41st & Blenheim	BC Standards
Zn	419 ± 105	237 ± 63.7	887 ± 599	76.5 ± 6.85	88.4 ± 4.32	117 ± 44.3	184 ± 16.8	119 ± 12.3	150
Cd	nd	nd	nd	nd	nd	nd	nd	nd	1.5
Pb	208 ± 44.7	124 ± 34.5	185 ± 28.6	56.6 ± 7.52	75.3 ± 6.08	69.2 ± 33.6	105 ± 7.92	159 ± 12.3	100
Ni	35.2 ± 8.21	37.7 ± 22.8	26.3 ± 5.91	12.3 ± 3.09	35.5 ± 12.5	27.8 ± 3.05	11.6 ± 2.06	30.6 ± 10.9	100
Cu	482 ± 315	78.9 ± 19.7	127 ± 46.1	34.5 ± 5.44	40.9 ± 1.74	38.6 ± 13.3	68.5 ± 2.52	66.8 ± 11.5	90
Mn	279 ± 45.9	484 ± 143	490 ± 84.8	219 ± 63.3	249 ± 6.06	377 ± 55.0	885 ± 46.1	459 ± 25.9	N/A

Mean total concentrations of Mn and Fe were significantly different ($p < 0.05$) among sites as well as among parent material groups, supporting the hypothesis that parent material in the urban environment influences the concentrations in the surface layers (Rao et al., 2007, Kabata-Pendias, 2004) [Appendix A-1 & A-2]. Total concentrations of Pb were significantly higher at those sites

with marine or glacial marine parent material. However, as Pb is rarely observed in high concentrations in the natural environment this is more likely a consequence of greater anthropogenic activity in those areas than of parent material effects *(Table 2.2).*

2.4.2 'Available' concentrations

'Available' trace metal concentrations (determined by 0.1M HCl extraction) were correlated with total concentrations for Fe, Mn, and Cu, but were not correlated with total concentrations for other tested elements [Appendix A-3]. These results support Rao et al.'s (2007) statement that *aqua regia* is a poor predictor of the mobilizable forms, and therefore the environmental impact, of trace metal contaminants in soils. There were stronger correlations between total and available concentrations of essential plant minerals K, Mg, and Ca, which are bound to a more easily extractable fraction of the soil matrix. Concentrations of elements extracted with the 0.01M $CaCl_2$ had values for essential elements K, P, Mg, and Ca that were roughly commensurate with available nutrient levels in the region (Marx et al., 1999) [Appendix C-2]. Mn, however, is not present as the free metal in the soil solution, and therefore the 0.01M $CaCl_2$ extraction is insufficient for providing an estimate of those metals sorbed to Mn-oxides that may become available through decomposition (Nádaská et al., 2010). The 0.1M HCl extraction was therefore considered more useful for evaluating potential trace metal risks in Vancouver soils.

Based on the dynamic properties of Fe and Mn-oxide bonds, it is suggested that a 0.1M HCl extraction be as a rapid assessment for evaluating potentially mobilizable trace metal contaminants in Vancouver, BC. Mn, Fe, and Al form hydrous oxides that bind heavy metals through adsorption, ion exchange, surface complex formation, and co-precipitation (McLaughlin et al., 2000; Rao et al., 2007). Of the soils tested, those high in total Mn and Al had lower levels of available heavy metals, particularly when parent material consisted of marine origin *(Figure 2.5)*. Total levels of Fe showed significant negative correlations with

the percent of available Cu, Ni, and Zn, while the levels of Fe extractable by 0.1M HCl were positively correlated with the percent available Pb, Ni, and Cu [Appendix A-3]. These correlations may be stronger in soils with marine till parent material because Mn is more prevalent in the marine environment.

2.4.3 Influence of parent material

Using Iverson et al.'s (2012) map [Figure 2.3], samples were grouped by parent material to evaluate geochemical effects of clay minerals (including Mn- and Fe-oxides) on trace metal availability. As urban soils rarely exhibit the type of layered profile common for natural soils, it is more likely that mixing of parent material and anthropogenic soil additions may occur, resulting in a surface layer often as deep as 50cm (DeKimpe & Morel, 2000, Rao et al., 2007). The assumption that parent material was incorporated into the top 15cm of the soils sampled is supported by significant differences in available trace metal concentrations among sites reflecting different parent materials. Student's t-test was applied to mean concentrations of 0.1M HCl extractable metals, revealing significantly higher concentrations of HCl-extractable Pb, Ni, Fe, and Cu in the sites with marine parent material than in those with glacial till parent material [Appendices A-4 -A-7]. This is likely due to the fact that soils with marine or glacial marine parent materials had finer texture and higher clay content than the soils formed on glacial till parent material (Valentine, 1986). Tested soils formed on glacial till parent material had significantly higher concentrations of HCl-extractable Al, suggesting that trace metals in those sites may be bound to Al-oxides, rendering them less available for plant uptake.

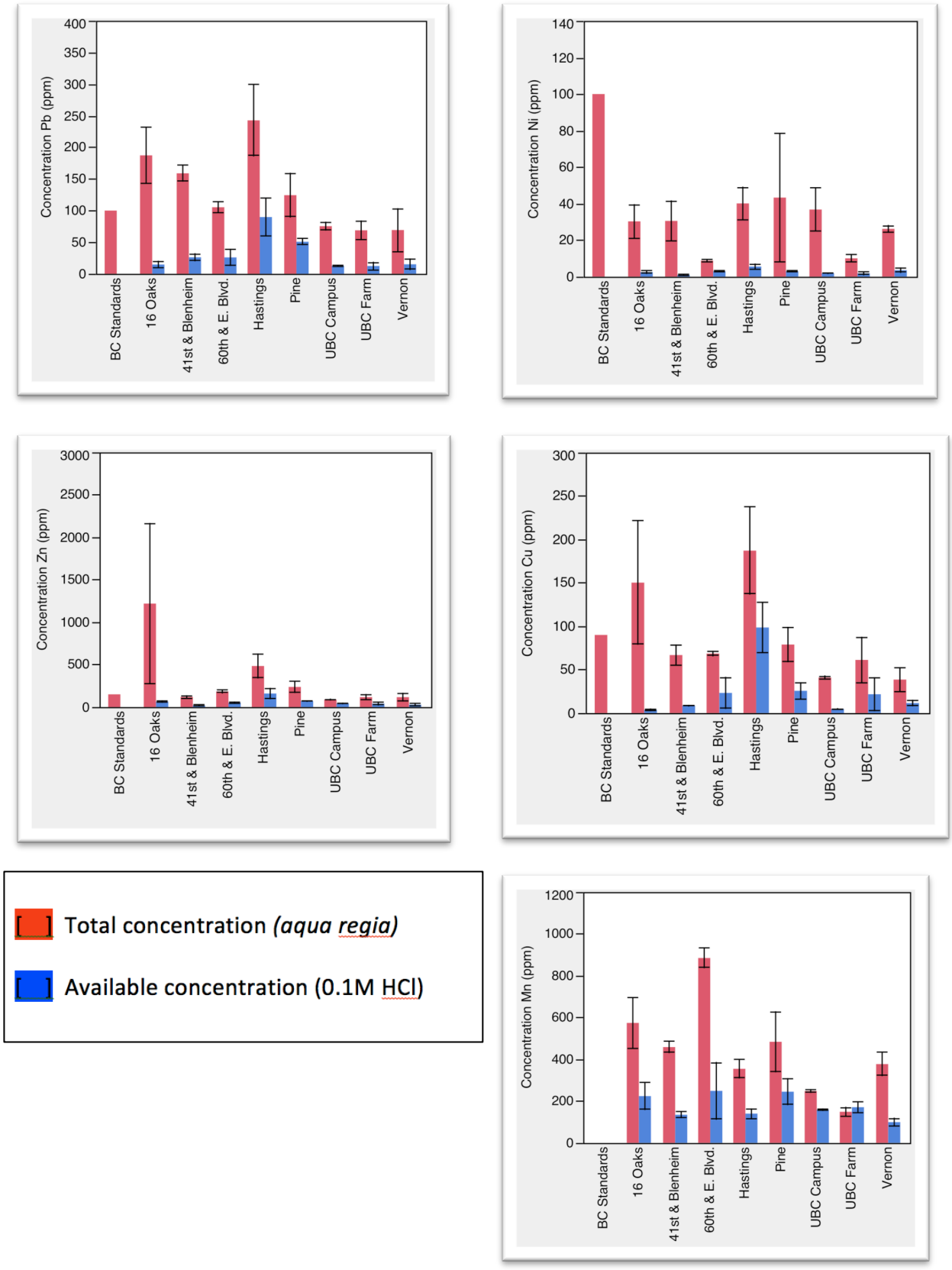

Figure 2.5: BC Standards and total/available concentrations (mg kg^{-1}) of Ni, Cu, Mn, Pb, and Zn by site

In the soils with marine or glacial marine parent materials, the level of HCl-extractable Fe was strongly correlated with the levels of extractable Ni, Pb, and Cu. Fe-oxides have stronger bonds than Mn-oxides in the soil matrix, and thus are not extensively extractable by the 0.1M HCl technique. Thus, the significantly higher HCl-extractable Fe concentrations in marine and glacial marine soil indicates two things : (1) increased mobility as a result of acidic, oxidizing conditions, and natural weathering and (2) high anthropogenic additions of Fe which are more easily mobile than natural deposits (Peijnenburg et al., 2007; Tessier et al., 1979).

Table 2.3: Percentage of mean available trace metals among sites and parent material groups

Parent Material	Site	Zn	Pb	Ni	Cu	Mn	Fe	Al
Marine	**Vernon** (n=3)	24.4	20.5	13.3	31.5	26.2	4.59	9.38
Glacial-marine	**Hastings** (n=7)	22.0	32.3	15.5	38.1	44.9	2.73	14.0
	16 Oaks (n=7)	20.7	7.26	7.80	4.71	36.9	1.07	7.78
	60th & E. Blvd. (n=2)	28.0	25.7	33.6	34.9	27.5	2.11	14.8
Glacial till	**Pine** (n=3)	33.9	50.2	18.3	40.6	59.3	3.11	21.0
	UBC Campus (n=4)	48.9	17.0	7.08	11.5	64.2	1.83	35.4
	UBC Farm (n=5)	30.6	15.5	17.1	18.2	91.3	1.66	24.1
	41st & Blenheim (n=3)	15.8	16.2	4.03	13.4	29.3	0.11	13.2

An approximate percentage value for trace metal availability was derived by calculating the ratio of 0.1M HCl-extractable concentrations to the total concentrations for each element (*Table 2.3*). Sites with marine parent material had significantly higher percentages of available Fe and Cu, while soils formed on glacial till had significantly higher percentages of available Mn [Appendices A-4 - A-7]. Concentrations of total and 0.01M HCl-extractable trace metals at the studied sites were compared with Luttmerding's (1981) background study on trace metals in the Lower Fraser Valley. Naturally occurring Fe, Cu, and Mn was much shown to be much lower than at the urban sites in soils of corresponding parent materials [Appendix A-10]. This

suggests greater anthropogenic contributions of those metals at the tested sites, which would increase the mobility of trace metals bound to those Fe and Mn oxides.

2.4.4 Organic matter

Organic matter was significantly lower in the marine sites than at the glacial till and glacial marine sites – this is likely because the only sites representing marine parent material those where food production was not yet active, and therefore had less biomass available for natural decomposition in the soil [Appendix A-8]. Although samples were taken from areas that appeared to have had minimal additions to the soil, it is possible that higher levels of organic matter reflect the incorporation of compost or mulching materials (*Table 2.4*).

Table 2.4: Summary of soil pH, texture, percent organic matter, and parent material by site

	pH	Texture	OM %	Parent material
16 Oaks	6.2	Sandy clay loam	11.5	Glacial marine
Hastings	4.9	Fine sandy loam	3.2	Marine
Pine	5.3	Sandy loam	9.1	Glacial marine
Vernon	5.9	Loamy sand	4.1	Marine
Blenheim & 41st	5.8	Loamy sand	6.0	Glacial till
60th & E. Blvd.	5.4	Sandy loam	16.9	Glacial marine
UBC Farm	6.8	Sandy loam	10.1	Glacial till
UBC Campus	6.1	Sandy loam	5.4	Glacial till

Sites with glacial-marine parent material showed significantly higher concentrations of Mn than either marine or glacial till sites for both *aqua regia* and HCl extractions. In this case, site history and land management may be a factor – the sites with glacial marine parent material (16 Oaks, 60th & E. Blvd., Pine) are the only established community gardens represented in this case study. The City of Vancouver offers yard trimmings compost (YTC) to community gardens at no cost (City of Vancouver, 2012a). As a result, it has been used as a soil amendment in many community gardens and its presence may be linked to some of the higher levels of Mn in those gardens. In a review of the agricultural use of municipal solid waste compost, Hargreaves et al. (2008) determined through sequential extraction

that total Mn concentrations in soil increased with addition of municipal solid waste compost, the largest portion of which was bound in the Fe- or Mn-oxide fraction. Compost is routinely tested to confirm that elemental concentrations fall within acceptable limits, yet metals may accumulate in soils over time, particularly as the decomposition of organic matter renders them more available in the soil solution (Forgie et al., 2004). Hargreaves et al. (2008) also noted that both total and neutral-salt extractable concentrations of Cu, Pb, and Zn increased with the application of municipal solid waste compost. A preliminary analysis of Vancouver YTC compost was used as a reference. Yard trimming compost may have contributed to anthropogenic additions of Mn at the tested sites. Mn binds with organic matter, accounting for the higher percentage of 0.1M HCl extractable Ni, Pb, and Zn at community garden sites (16 Oaks, Pine, 60th and E. Blvd.) where YTC compost may have been added *(Table 2.5).*

Table 2.5: Mean available concentrations (± SE) of Zn, Pb, Ni, Cu, & Mn at tested sites compared with reference values for available concentrations in yard trimmings compost (mg kg^{-1})

	16 Oaks	41st & Blenhiem	60th & E. Blvd.	Hastings	Pine	UBC Campus	UBC Farm	Vernon	YTC Compost
Zn	65.3 ± 7.78	19.1 ±3.80	51.4 ± 5.35	121 ± 43.8	69.8 ± 1.32	42.8 ± 2.80	40.1 ± 15.1	27.7 ± 10.1	181
Pb	13.2 ± 5.64	26.2 ± 4.46	26.0 ± 12.6	87.6 ± 30.0	50.7 ± 4.47	12.6 ± 0.50	12.4 ± 5.80	15.1 ± 8.27	58
Ni	2.19 ± 0.69	1.00 ± 0.14	2.96 ± 0.12	10.1 ± 3.52	3.01 ± 0.20	1.88 ± 0.07	1.1 ± 0.74	3.6 ± 1.16	4.6
Cu	3.49 ± 0.99	8.50 ± 0.31	23.2 ± 17.8	115 ± 31.9	25.6 ± 9.07	4.71 ± 0.23	21.7 ± 18.9	11.6 ± 3.05	54
Mn	235 ± 74.8	135 ± 13.2	249 ± 136	150 ± 21.6	246 ± 61.9	159 ± 3.23	171 ± 25.1	99.8 ± 18.7	184

2.4.5 Influence of pH

Total concentrations of Cu and Zn were negatively correlated with pH [Appendix A-9], however there were no significant relationships noted between pH and trace metal availability, although this trend has been widely documented in the literature (McBride et al., 2009). This may be due to the fact that pH was similar at most of the sites studied. The values for pH across all sites ranged from 4.9-6.8, with a mean of 5.4; the lack of noticeable effect of pH, therefore, on the availability of trace metals suggests that this slightly acidic value is not sufficient for affecting metal availability to plants.

2.5 Conclusions

In summary, the conclusions of this case study are:

(1) Contaminant risk assessment of urban brownfields for development and interim uses in Vancouver, BC should take into account the bioavailability of trace metals. The 0.1M HCl extraction technique, in addition to the standard strong acid extractions, may provide a first approximation of bioavailable trace metals. This echoes recommendations by other authors that government guidelines should be based on the soil metal pool that actually poses a threat to organisms (Sauvé et al., 1996; Kabata-Pendias, 2004).

(2) Soils formed from marine or glacial-marine till are richer in Mn-oxides, which contributes to the binding capacity and subsequent mobility of trace metals.

(3) In acidic, oxidizing soil conditions like those common to Vancouver, BC, extraction with 0.1M HCl provides an estimate of those trace metals that may become available through ion exchange and breakdown of Mn hydrous oxides. This extraction may therefore be a more accurate indicator of trace metal availability than *aqua regia* or 0.01M $CaCl_2$.

3 Phytoextraction and partitioning of Zn, Cd, Pb, Ni, and Cu in *Chenopodium quinoa*

3.1 Introduction

Trace metal contamnation of soils poses a long-term land management problem requiring human intervention. Elemental pollutants persist in soils causing health risks to humans and ecosystems (Luo & Rimmer, 1995; MacNicol & Beckett, 1985). In rural areas, bioaccumulation on agricultural land can reduce crop yields and quality (Wuana & Okieiman, 2011). In urban areas, soil contamination results in costly redevelopment delays as land use patterns change from industrial to residential and agricultural.

Industrial activity, mining waste disposal and biosolids application are some of the anthropogenic activities causing multi-metal contamination scenarios with high concentrations of Zn, Cd, Pb, Ni and Cu in the soil (Luo & Rimmer, 1995). Although potential mobility and ecosystem toxicity depends both on environmental conditions as well as the chemical forms of contaminants, Cd, Zn, and Ni are generally bioavailable in soil (Kabata-Pendias, 2004).

3.1.1 Phytoextraction

Chemical immobilization, soil washing, and phytoremediation are cost-effective technologies for rehabilitating contaminated sites (Wuana & Okieiman, 2011; Lasat 1999). Phytoremediation is an appealing land management option as it can be practiced in-situ and therefore causes minimal ecosystem disruption. The term 'phytoremediation' can refer to phytostabilization, in which contaminants are immobilized via sequestration in

plant roots, or phytoextraction, in which contaminants are concentrated in aboveground plant parts, harvested, and removed for safe disposal (Padmavathiamma & Li, 2009). Phytomining is an application of phytoextraction in which the ashed plant residues are smelted to recover valuable metals (McGrath & Zhao, 2003). This paper deals with phytoextraction, which is a promising technology despite several limitations.

Phytoextraction requires several sequential crops of hyper-accumulating plants to reduce soil metal concentrations to levels appropriate for most land uses (Kumar, et al., 1995). The technique is most effective for moderate to lightly contaminated soils, as plants are unlikely to thrive in extremely toxic soil. Plants must be easily harvestable, produce significant biomass, and have extensive root systems (Kumar et al., 1995). Uptake efficiency can be improved by amending soil with synthetic chelates, however this also increases bioavailability of essential plant nutrients that may subsequently out-compete metals for plant uptake.

Phytoextraction is a time-consuming procedure that is limited by the uptake efficiency of the plants used. Even in hyperaccumulating plant species, metal removal is highly variable depending on the soil condition, extent of contamination, and metal species present (Cobb et al., 2000). Many metal-tolerant plants documented in the phytoextraction literature, most notably *Thlaspi caerulescens*, are slow-growing and produce insufficient biomass to be immediately useful. McGrath and Zhao (2003) point out that even if plants used for phytoextraction were to produce the same average biomass as an agricultural crop, it would still take at least 10 crops to effectively halve metal concentrations. Affected plant material cannot be composted or returned to the soil, posing a problem for farmers or community operations without access to proper mechanisms for disposal (Huang & Cunningham, 1996).

Recent interest in the use of field crops for removal of metals from contaminated soils has led to wide adoption of the idea that high biomass-yielding species may be more promising for phytoextraction than those plants considered to be hyperaccumulators (Chaney et al., 1997; Vamerali et al., 2010). Fast growing, easily cultivated agronomic crops like sunflower (*Helianthus annus*), Indian mustard (*Brassica juncea*), and maize (*Zea mays*) may therefore be better for phytoextraction of trace metals than traditional hyperaccumulators, as their high biomass compensates for lower metal accumulation (Zuang et al., 2007). Such observations support McGrath & Zhao's (2003) suggestion that metal accumulation and biomass production be the main criteria for a plant's phytoextraction suitability, rather than numerical concentrations and translocation factors.

Perhaps the most pressing problem related to phytoextraction is the lack of practical adoption of the technology by municipalities and remediation efforts. Baker (2002) points out that despite abundant scientific research on the potential of phytoremediation, since the early 1980s, large-scale and even pilot applications of the technology are few. Remediation of contaminated sites in BC tends toward harsh, ex-situ techniques such as soil-washing[2], vitrification[3], and soil excavation[4] and replacement. Such techniques are expensive and damaging to soil, yet mild in-situ techniques like phytoremediation are infrequently used (Gupta et al. 1996).

3.1.2 Hyperaccumulator plants

The ability to concentrate trace metals in aboveground tissue is both an indicator of a plant's usefulness for phytoextraction and its potential health risk for human consumption of crop plants (Kumar et al., 1995). Plants respond to trace metals in the soil solution by three known physiological pathways: exclusion, passive accumulation, and hyperaccumulation. Excluder species tolerate high metal concentrations in soil by

[2] Squamish (British Columbia Ministry of Environment, 2009a)
[3] Burnaby (British Columbia Ministry of Environment, 2009b)
[4] Trail (British Columbia Ministry of Environment, 2009c)

restricting metal uptake to the roots. Passive accumulators store metals in the roots, sequestering non-essential elements in cell walls and vacuoles as a defense mechanism to prevent transport to leaves and shoots (Kirkham, 2006). Most plants adapted to soils with high concentrations of metals exclude these elements from the aerial plant parts, but hyper-accumulating species actually translocate high quantities - in concentrations 10 to 100 times those tolerated by normal accumulators - from the soil solution to leaf and shoot tissue (Krämer et al., 2000; Baker & Brooks, 1989; Kirkham, 2006). To date, less than 0.2% of all angiosperms are thought to be natural hyperaccumulators of trace metals, and Environment Canada maintains a database of 776 vascular plants worldwide that are thought to have some capacity for extraction and remediation of 19 different trace metal contaminants (McGrath & Zhao, 2003; Environment Canada 2003).

The concept of hyperaccumulating plants was first presented in the early 1980s, but still there is no set of unified criteria for determining which plants fall into this category (Baker, 1981). Increasingly research suggests that the plant mechanisms for tolerating high concentrations of toxic elements are related to vacuolar sequestration and internal detoxification in leaves, however a multitude of factors affect a plant's usefulness for phytoextraction (McGrath & Zhao, 2003). The translocation factor (TF), or root to shoot/leaf quotient is commonly used as a metric - TF greater than 1 indicates preferential partitioning of metals in aboveground plant parts (Baker, 1981; Pourrut, et al., 2011). Many hyperaccumulator plants with efficient root to shoot transport efficiency, however, are limited by small size or slow growth, making them less effective for metal removal than a plant with a lower translocation factor that produces more aboveground biomass. Identifying accurate concentrations of metals in root tissue can further compound the calculation of efficiency as it is difficult to distinguish by root concentration values those ions absorbed by cells from those adsorbed at the negatively-charged sites along root cell walls (Lasat, 1999).

Padmavathiamma and Li (2009) evaluated the suitability of 5 plant species for heavy metal phytoextraction using variously spiked soil from roadsides near Vancouver, BC. Based on similarities in scope and regional soil characteristics, their study and results were used as a guide for comparison. This experiment builds on a field study by Bhargava et al. (2008) comparing the bioconcentration and speciation of Cu, Zn, Cd, Ni, and Pb in the roots, shoots, seeds, and foliage of *Chenopodium quinoa* (quinoa) plants in three multi-metal contamination scenarios. This research assesses the potential of quinoa (*Chenopodium quinoa)* for remediating metal-contaminated sites in British Columbia and investigates health risks associated with human consumption of quinoa grown on contaminated soils.

Bhargava et al. (2008) found 18 genotypes of quinoa to hyperaccumulate heavy metals in a soil moderately contaminated with Fe, Zn, Cu, Ni, Cr, and Cd, suggesting the plant's usefulness for land remediation through phytoextraction. Samples of the edible foliage were harvested and analyzed for trace metals at 30 days after germination. The study did not analyze the plants at maturity, nor address partitioning of metals in different plant parts. Both areas are of particular interest as the nutritious quinoa seeds are commonly eaten. Trace metal accumulation and partitioning at different growth stages and various contamination levels has yet to be investigated in Vancouver's acidic mineral soils.

Quinoa may be particularly suited for remediation of trace metal-contaminated soils in British Columbia as it thrives in temperatures between 18° C and 25° C, which are typical of the southern coastal growing season. In its native Bolivia, quinoa is generally grown in the shoulder season after potato harvest, where it relies on residual fertilizers and occasional rainwater for survival. Morphological traits like a deep taproot and fibrous root system allow quinoa plants to access soil water and nutrients unavailable to other plants (Jacobsen et al., 2003). This may improve uptake efficiency for trace metals, which are often absorbed by plant roots via substitution when essential nutrients are not readily available (Das et al., 1997). Further, quinoa shares some of the halophytic properties common in the

Chenopodaceae family and this tolerance for saline conditions may allow it to thrive in soils where chelating agents have been added to chemically enhance phytoextraction efficiency (Jacobsen et al., 2003).

3.2 Objectives

The research in this study evaluates the usefulness of *Chenopodium quinoa* as a potential plant for phytoextraction of Zn, Cd, Pb, Ni, and Cu in urban soils of Vancouver, BC. Additional consideration is given to partitioning of contaminants in the aboveground plant portions for the purpose of evaluating potential human health risks involved with eating plants grown on contaminated sites. The specific objectives of this study are as follows:

(1) Determine bioconcentration factor (BCF) of roots, foliage, shoot and seeds and translocation factor (TF) for shoots, foliage, and seeds in a multi-metal contamination scenario at two stages of growth for one genotype of *C. quinoa* grown in soil collected from a single brownfield site in Vancouver BC,

(2) Compare total and plant-available trace metals in soil with guidelines provided by the British Columbia Contaminated Sites Regulation,

(3) Determine total metal uptake and total plant concentration at two sampling stages 30 DAT (days after transplant) and 110 DAT (Padmavathiamma & Li, 2009),

(4) Assess the usefulness of *Chenopodium quinoa* as an urban agricultural crop and/or a hyperaccumulator of trace metals in Vancouver, British Columbia,

(5) Evaluate trace metal concentrations in seeds as compared with international upper intake limits for human health, and

(6) Evaluate potential for phytoextraction of trace metals in urban soils of Vancouver, BC.

3.3 Materials and methods

Approximately 60kg from the 0-30cm portion of the soil was collected from the brownfield site on Glen and East Hastings (discussed in the previous chapter). The soil was formed on marine parent material, with mineral clays characteristic of Vancouver's iron-rich podzols. Partly because of leaching during the wet winter months, these soils are acidic, low in organic matter, and nutrient-poor (Iverson et al., 2012).

Soil was taken to the laboratory, mixed, dried and sieved through a 2mm sieve, and divided into treatment groups A, B, and C. Group A is the original un-amended soil collected on-site, while groups B and C were spiked with multi-metal solutions based on guidelines corresponding to the BC Contaminated Sites Regulation (Padmavanthiamma & Li, 2009; Contaminated Sites Regulation, 2011). Metals were added to air-dried soil by wetting soil with a solution of trace metals and carrier salts [Appendix D-1] dissolved in 1000mL distilled water (Padmavanthiamma & Li, 2009). Because soil A was already moderately contaminated with trace metals, it is considered a comparison rather than a control *(Table 3.1).*

Table 3.1: Mean trace metal concentrations (± SE) for treatment groups A, B, and C, compared with British Columbia guidelines for agricultural land use (mg kg^{-1})

Treatment Group	Zn	Cd	Pb	Ni	Cu
BC Standards moderate*	150	1.5	100	100	90
level C**	1500	20	1000	500	500
A	252 ± 157	223 ± 4.58	307 ± 6.52	243 ± 5.32	373 ± 6.26
B	309 ± 140	233 ± 4.78	375 ± 8.94	260 ± 5.39	389 ± 8.62
C	552 ± 144	242 ± 3.33	858 ± 48.0	293 ± 6.98	472 ± 14.9

* levels above which soil must be relocated to non-agricultural land (BC Contaminated Sites Regulation, Schedule 7, 2011)

** For exclusive commercial or industrial land use, level C is the remediation standard. For soils containing contaminants exceeding this level, all uses of the land will be restricted until remediation measures have reduced concentrations to levels less than those given.

3.3.1 Experiment design

Each 16cm diameter plastic pot was weighed, labeled, and filled with approximately 2kg soil from group A, B, or C. Thirty pots with ten replicates of each treatment were arranged in a completely randomized design for the greenhouse study. *Chenopodium quinoa* Willd. PI 510532 was seeded in sterile starting mix and transplanted into the treatment pots after emergence but before development of true leaves (Raven et al., 1996).

Two quinoa seedlings were planted per pot to facilitate comparisons between growth stages for each sample. Thirty days after transplant (DAT) one plant per pot was removed and prepared for analysis, along with roots and surrounding soil. Remaining plants and soil were harvested at 110 DAT, following seed set. Destructive sampling at these two

stages of growth allowed for the use of paired t-tests to evaluate changes in uptake and concentration within each sample over the plants' lifecycles. Two pots per treatment group were not planted to provide a reference for measuring natural changes in soil concentrations over time.

3.3.2 Assessment parameters

3.3.2.1 Biometric characters

Dry weight of seeds, shoots, leaves and foliage, number of leaves, stem length, and longest leaf length were recorded at each sampling [Appendix D-8], to assess the effects of metal concentration on biomass and seed yield (MacNicol & Beckett, 1985; Padmavathiamma & Li, 2009).

3.3.2.2 Organic matter

Elemental carbon was measured using loss on ignition; the resulting value was used as an estimate of organic matter percentage in the soil (Ball, 1964). Samples were dried overnight at 105°C, weighed, then heated for 16 hours in a muffle furnace at 375°C and weighed a second time. The percentage of weight lost between first and second measurement is presented as the loss-on-ignition value.

3.3.2.3 pH

Soil pH was measured both in water and a dilute salt solution (0.01M $CaCl_2$). In a beaker, 20mL distilled water was added to 10g air-dried soil and the suspension was stirred periodically over 30 minutes, then left to settle for one hour. Measurements were taken with a combined electrode pH meter and values recorded. To each beaker was then added 10mL 0.02M $CaCl_2$, and the procedure was repeated (Hendershot et al., 1993).

3.3.2.4 Electroconductivity

Electrical conductivity was measured at 1.5 mMHO scale using a 2:1 ratio with a solution of 0.01M KCl as standard (Miller and Curtin, 1993.)

3.3.2.5 Bioconcentration factor

In order to assess *C. quinoa*'s uptake and accumulation of soil metals in plant tissue, the bioconcentration factor (BCF) was calculated for roots (C_{roots}/C_{soil} = ratio of root concentration to soil concentration), shoots (C_{shoots}/C_{soil} = ratio of shoot concentration to soil concentration), seeds (C_{seeds}/C_{soil} = ratio of seed concentration to soil concentration), and foliage ($C_{foliage}/C_{soil}$ = ratio of leaf concentration to soil concentration) at 30 DAT and 110 DAT (Kumar et al., 1995; Padmavanthiamma & Li, 2009).

3.3.2.6 Translocation factor

The translocation factor (TF) refers to amount of metals transferred from roots to aboveground plant parts, and is an indicator of the phytoextraction potential for a given plant (Kumar et al. 1995; Padmavanthiamma & Li, 2009). TF was calculated for shoots and (C_{shoots}/C_{roots}) foliage ($C_{foliage}/C_{roots}$) at both stages of plant growth, and seeds (C_{seeds}/C_{roots}) at 110 DAT.

3.3.3 Elemental extraction

Elements were extracted with dilute strong acid (0.1M HCl) and concentrated strong acid (*aqua regia*) to estimate concentrations of plant-available and non-residual trace elements, respectively, in soil (Peijnenburg et al., 2007; Tessier et al., 1979). Samples were analyzed using a Varian Inductively Coupled Plasma-Optical Emission Spectrometer.

3.3.3.1 Plant tissue digestion

Plant material from both sampling events was separated into roots, shoots, foliage and seeds (where applicable). Roots were defined as plant parts with no visible chlorophyll, shoots included the main stem and branches, and foliage included leaves and petioles (Cobb et al., 2000). Biometric observations of stem length, number of leaves, and longest leaf length were recorded for each sample. All samples were washed thoroughly with tap water, then rinsed with distilled water before drying for 48 hours at 70°C (Kumar et al., 1995; Padmavathiamma & Li, 2009). A dry-ashing technique was chosen based on improved accuracy over wet ashing methods (Lambert, 1976). Samples were weighed after drying, then ashed in a muffle furnace at 500° C for 6 hours (Kumar et al., 1995; Hue et al., 2000; Richards, 1993). Following ashing, samples were ground with a mortar and pestle and passed through a 2-mm sieve. Ashed plant material was digested in aqua regia to determine total quantities of elements in tissue.

3.3.3.2 Soil digestion

For the strong acid digestion, samples weighing approximately 0 .5g were dissolved in 17 mL *aqua regia* in 250mL Erlenmeyer flasks and heated until evaporation. The remaining residue was dissolved in 20 mL 0.1M HNO_3 and passed through Whatman #42 filter paper. Samples were brought to 100mL volume with 0.02M HNO_3 (Chen & Ma, 2001).

For the dilute strong acid digestion, 50 mL of 0.1M HCl was added to 5.0g air-dried soil in a 100 mL plastic centrifuge tube,. Tubes were shaken for 1 hour and the resulting suspension filtered through Whatman #42 filter paper and brought to 100mL volume with 0.5M HNO_3 (Black, 1965). As mentioned in the previous chapter, concentration values derived from the 0.1M HCl extraction method are more accurate indicators of the plant-available portion of trace metals in soil; values discussed in this paper are therefore given in terms of 'available' concentrations unless otherwise indicated.

3.3.4 Statistical analysis

Paired t-tests were conducted to assess the significance of changes in concentration between 30 DAT and 110 DAT for each sample, and Kruskal-Wallis comparison of means test were used to evaluate significant ($p < 0.05$) differences between means across treatment groups. Linear regression analysis was used to evaluate the significance of correlations between soil concentrations and biometric characters. Principal components analysis was used as a multivariate test to compare factors accounting for major differences in the data. All statistical analyses were performed using JMP 10.0 statistical software to test the following hypotheses:

H_0: Seeds of quinoa grown on metal-contaminated sites are safe for human consumption.

H_0: There are no differences in trace metal concentrations among roots, shoots, seeds, and foliage of quinoa grown in contaminated soils.

H_0: There is no difference between the BCFs and TFs of trace metals in plant tissues at 30 and 110 days after transplant.

3.4 Results and discussion

The results of this experiment concern changes in contaminant concentrations in soil, transport and accumulation trends metals in shoots and foliage at both 30 DAT and 110 DAT, and the concentration and corollary health risks of heavy metals in quinoa seeds. Also of interest are the mechanisms of uptake and how they are affected by interactions

among essential and non-essential elements at various growth stages. These factors are considered in evaluating the phytoextraction potential of quinoa for Vancouver's soils.

3.4.1 Changes in soil concentrations

Concentrations of plant-available Cd, Ni, Cu, Pb, and Mg were significantly decreased from the original concentrations by 110 DAT in all treatment groups (Table 3.2). As plants in treatment group C did not survive beyond 30 days, only the changes in groups A and B are discussed. Significant differences between groups A and B were only observed for Pb [Appendix 2-B].

Table 3.2: Mean decrease in plant-available concentrations of Pb, Ni, Cu, Cd, and Zn in potted soil at 110 DAT (significance at $p < 0.05$)

Treatment	Pb	Ni	Cu	Cd	Zn
A	24.4 ± 4.3	12.9 ± 3.0	31.5 ± 5.2	11.3 ± 2.8	16.7 ± 2.6
B	48.1 ± 8.9	24.9 ± 5.1	35.6 ± 7.2	13.9 ± 3.6	ns

It is commonly accepted that the uptake and concentrations of metals in aboveground plant parts does not occur in a linear response to the metal concentrations in soil (Dudka et al., 1996). This is in part due to the species and solubility of metals in soil solution – Zn and Cd, for instance, occur primarily as free ions, making them easily exchangeable and plant-available (Lasat, 2000). Plant mechanisms for uptake and transport of non-essential trace metals are the same as for essential elements- once contaminants cross the root cell wall, translocation to aboveground plant parts is controlled by root pressure and leaf transpiration (McLaughlin et al., 1998; Lasat, 2000).

Table 3.3: Significant (p<0.05) correlations between soil concentrations and biometric characters

	Zn	Cd	Ni	Cu
Leaf Length (30 DAT)	**Decrease** r=-0.47 n=17			
Number of Leaves (30 DAT)		**Increase** r=0.76 n=17		**Increase** r= 0.57 n=17
Leaf Biomass (110 DAT)	**Decrease** r= -0.45 n=23		**Decrease** r=-0.42 n=23	**Decrease** r=-0.43 n=23
Stem Biomass (110 DAT)	**Decrease** r=-0.46 n=19			**Decrease** r=-0.53 n=19
Seed Yield (110 DAT)	**Decrease** r=-0.47 n=23	**Decrease** r=-0.50 n=23	**Decrease** r=-0.51 n=23	**Decrease** r=-0.53 n=23

Of the biometric characters recorded for each sample, all but Pb showed at least one relationship with trace metal concentrations in soil (*Table 3.3*). Pb in soil occurs as a precipitate and not as the free ion, it is less available to plants, and tends to be poorly correlated with plant uptake (Lasat, 2000).

3.4.2 Metal concentrations in leaves and shoots

The specific relationships between tissue concentrations and growth stage are likely the result of synergisms between essential plant nutrients involved in the early stages of plant growth (i.e. P, K) and certain trace metals.

Speciation of metals in plant parts is variable and may change over the duration of plant growth (MacNicol & Beckett, 1985). Concentrations of Zn, Cd, Pb, and Ni in foliage were significantly higher at 30 DAT than at 110 DAT. The change in leaf concentrations from 30

to 110 days was significantly greater in group B for Ni and Zn. There were no significant differences in BCF for foliage between the two sampling times, suggesting that plant uptake is a function of plant size [Appendix B-1].

At 110 DAT, Ni, Pb, and Cd had the highest aboveground concentrations in seeds, while Zn and Cu were highest in foliage *(Figure 3.1).* At 30 DAT, aboveground concentrations were highest in shoots for Cd, Pb, Ni, and Cu, and Zn concentrations were highest in foliage *(Figure 3.2)*. Of the elements studied, aboveground concentrations surpassed root concentrations for Pb, Cd, and Ni only at 30 DAT.

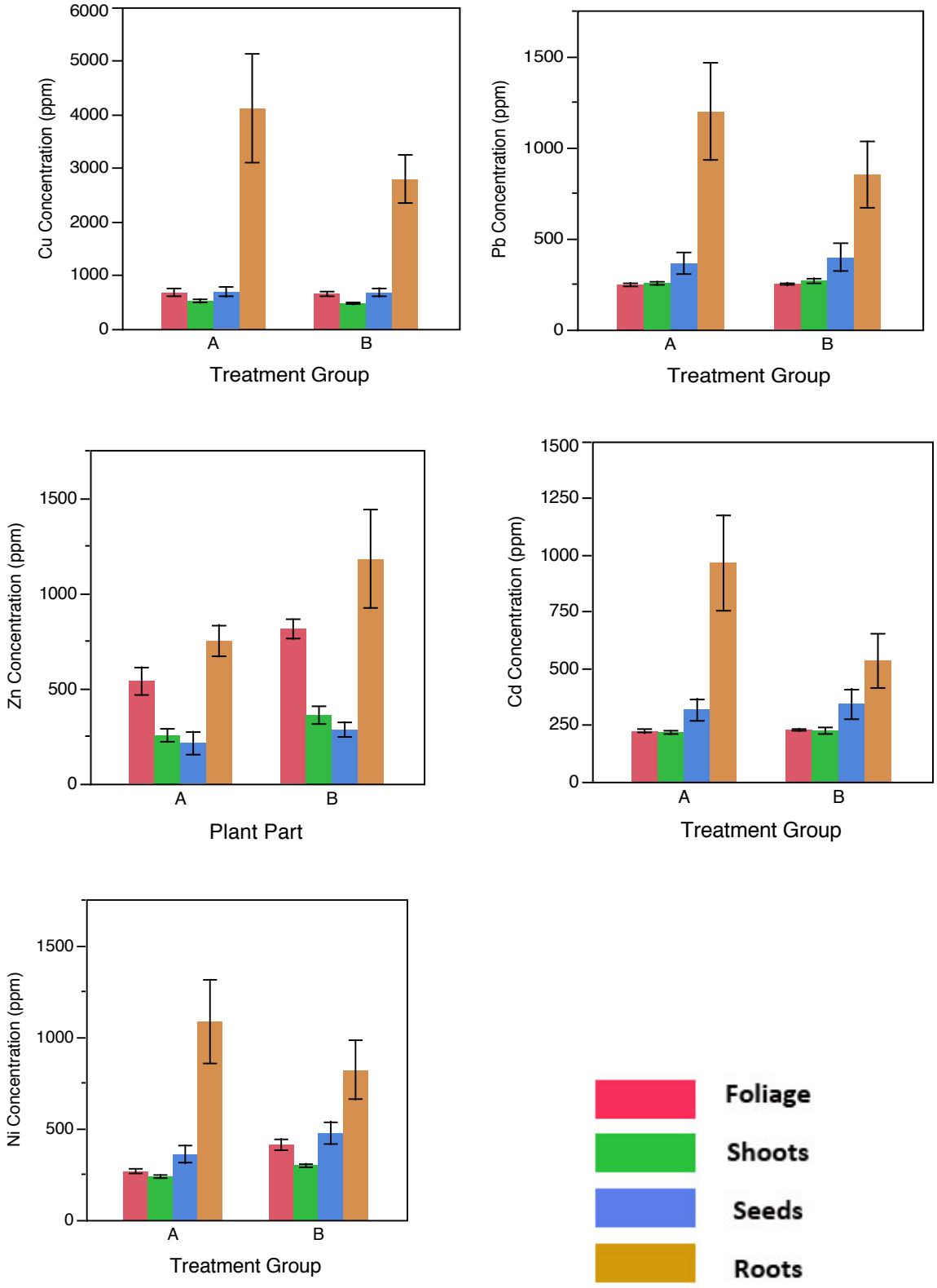

Figure 3.1: Concentrations (mg kg-1) of Zn, Cd, Pb, Ni, and Cu in foliage, shoots, seeds and roots at 110 DAT

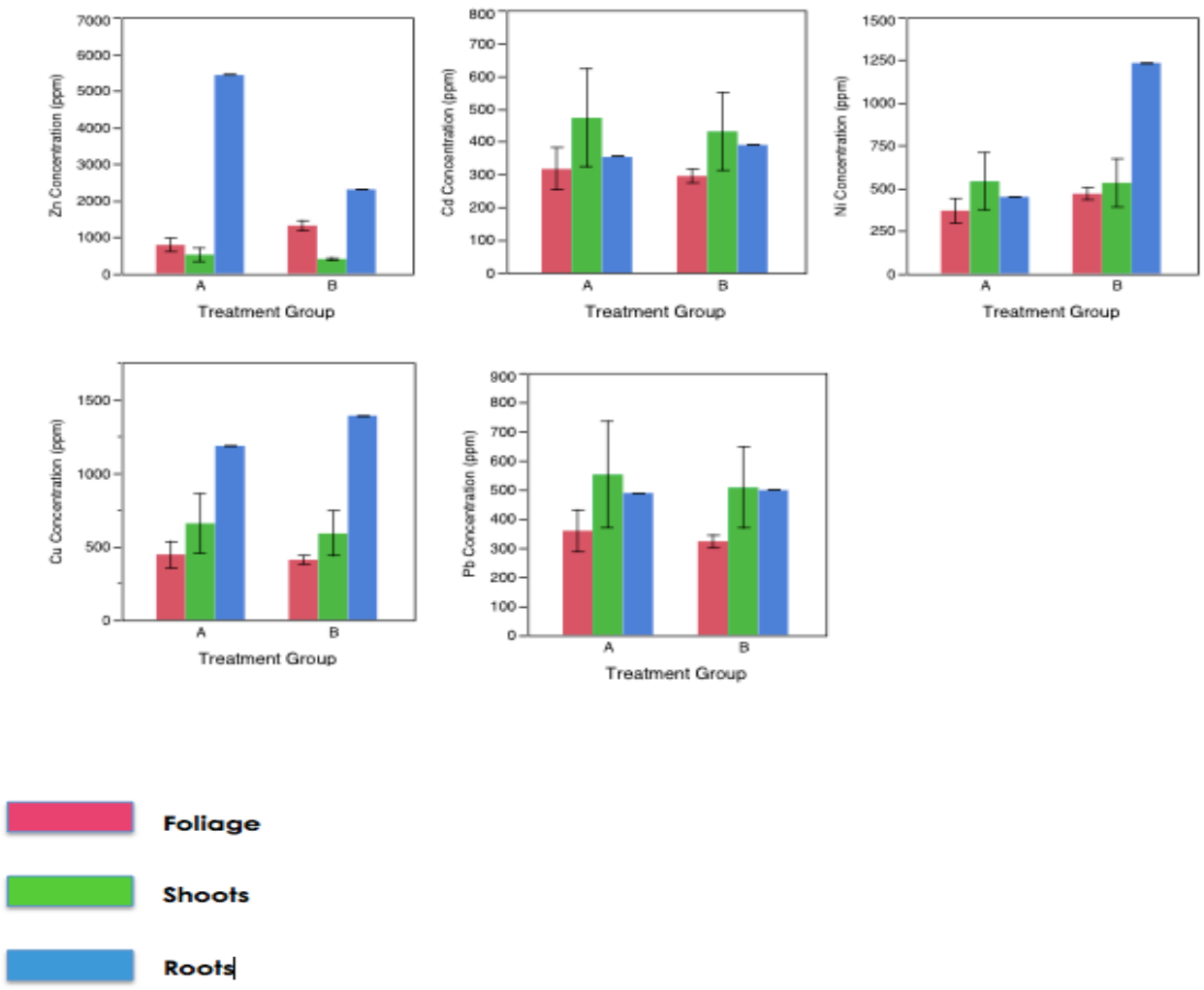

Figure 3.2: Partitioning of Zn, Cd, Ni, Cu and Pb in foliage, shoots, and roots at 30 DAT

3.4.3 Seed concentrations

The concentrations and amounts of trace metals in quinoa seeds are important human health considerations, as the seeds are most commonly eaten and are a staple of many diets. The BCF seeds was negatively correlated with both the dry weight of shoots and the dry weight of seeds for Cd, Pb, Ni, and Cu, indicating that the more biomass produced, the lower the concentration of contaminants [Appendix B-2].

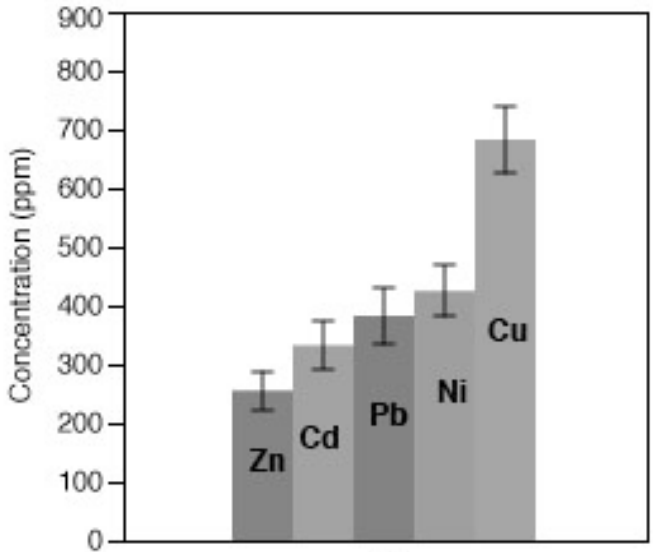

Figure 3.3: Seed concentrations of Zn, Cd, Pb, Ni, and Cu in mature quinoa plants

The results of this experiment showed the concentrations of elements in quinoa seeds follow the trend: Cu>Ni>Pb>Cd>Zn *[Figure 3.3].* There were no significant differences in seed concentration across treatment groups.

As most government guidelines for human consumption of heavy metals is given in terms of total amounts, determining the actual amounts of metals in plant tissue the best means of assessing health risks related to ingestion of contaminated plant materials. The weight (mg) in mg of Zn, Cd, Pb, Ni, and Cu in seeds was determined based on mean trace metal concentrations per 1 gram (mean dry weight of seeds per plant was 1.81g ± 0.35). Metal quantity in a full serving of quinoa, however, cannot be estimated based on these measurements, as the results of this study indicate that seed concentrations decrease with increased plant production. The mean total Zn, Cd, Pb, Ni, and Cu per plant was compared with the European Scientific Commission's Health Standards (Scientific Committee on Food, 2003; European Food

Table 3.4: Upper intake limits for human consumption compared with mean amounts of Zn, Cd, Pb, Ni, & Cu in quinoa seeds (± SE)

	Zn	Cd	Pb	Ni	Cu
Upper intake limit (mg/day for a person weighing 60 kg)	25 mg/day	0.15 mg/day	0.03 mg/day	0.72 mg/day	5-10 mg/day
Mean concentration (mg kg^{-1})	294 ± 41.0	333 ± 37.4	383 ± 43.8	322 ± 39.7	660 ± 53.9
Amount (mg/g) in quinoa seeds (N=14)	0.29	0.33	0.38	0.32	0.66

Safety Authority, 2010). It was concluded based on this comparison that seeds of quinoa grown on contaminated sites with similar levels of trace metal contamination would be too high in Cd and Pb to be safe for human consumption.

3.4.4 Element interactions

Interactions between essential and non-essential elements in the soil solution can influence the phytoavailability of some heavy metals (Kabata-Pendias, 2004).

Elemental analysis revealed relationships between K, P, and Ca and several trace metals including Cd, Pb, Ni, and Zn. Additional interactions between Cd and Zn and Zn and Cu affect plant uptake.

3.4.4.1 Cadmium and potassium

Because of Cd's mobility in the soil solution it is easily taken up by plants and transferred to aboveground plant parts (Ciećko et al., 2004). At 30 DAT, Cd had the highest translocation efficiencies from roots to leaves and shoots than any other heavy metals, although translocation and concentration was still less than that of essential plant nutrients K, P, and Mg. The concentrations of K in quinoa were strongly correlated with Cd, but the nature of these relationships was variable. Despite strong positive relationships between the bioconcentrations of Cd and K in the leaves, seeds, and particularly the shoots, there were strong negative correlations between Cd and K in the soil. The results of this experiment support those of Ciećko et al. (2004) who found that soil contamination with Cd caused either an increase or a decrease in K content depending mainly on plant species and specific plant organ.

The high concentration of K in immature quinoa plants shows the effect of growth stage on translocation of essential and non-essential elements. Young plants actively accumulate high concentrations of K as they grow, especially in the shoots. At 30 DAT, the soil concentration of K was negatively correlated with the concentration and translocation efficiency of Cd in the shoots, suggesting an antagonistic relationship between the two elements. The soil concentrations of Cd exhibited corresponding negative relationships with the uptake and bioconcentration of K in the shoots, seeds, and foliage as the plants reached maturity [Appendices B-3 – B-6].

In experiments with oat, yellow lupine, and radish, Ciećko et al. (2004) reported that high concentrations of Cd in the soil decreased the content of potassium in oat grain and shoots of the other plants. In addition their research showed that the content of K was positively correlated with plant yield. Building on that observation, this study found that neither soil nor organ concentrations of Cd affected quinoa yield (estimated here by the dry weight of seeds). However, K concentrations of shoots at 30 and 110 DAT were positively correlated with total plant yield [Appendix B-7].

3.4.4.2 Cadmium and zinc

Plants have no known physiological needs for Cd, thus it has been hypothesized that interactions during uptake result from plants' inability to distinguish Cd ions from Zn ions (Wuana & Okieiman, 2011; Lasat, 1999). Both are mobile elements in the soil solution, as they occur primarily in exchangeable form. In addition to their chemical similarities, Cd and Zn are widely observed in the same pollution scenarios, increasing the likelihood of interaction (McKenna et al., 1993).

Studies on the effects of Cd-Zn relationships on plant uptake have shown the behavior of the two elements to be synergistic or antagonistic depending on plant species (Luo & Rimmer 1995; McKenna et al., 1993). Quinoa showed a significant positive association between Cd and Zn in terms of plant yield (dry weight of aboveground plant parts) and total accumulation of both metals at 30 and 110 DAT. $TF_{foliage}$ and TF_{shoot} for each element were significantly higher at 110 DAT given higher concentrations of the other [Appendices B-8 & B-9].

3.4.4.3 Zinc and copper

The concentrations of Cu and Zn in aboveground tissues were approximately the same for plants at both 30 and 110 DAT, suggesting similar mechanisms for uptake of the two elements. Luo and Rimmer (1994) found that various amounts of added Cu increased Zn uptake by barley plants (1994). The Cu-Zn synergism apparent in quinoa echoes their hypothesis that Zn and Cu would effectively substitute for each other based on their chemical similarity.

The Zn-Cu synergism in quinoa is demonstrated by increased $TF_{foliage}$ values at both stages of growth as well as increased total plant uptake of both elements at 30 DAT. It is interesting to note that this relationship is significant only in the non-spiked soil (treatment A), while antagonistic concentrations of Zn and Cu in the treated soil (treatment B) were significant in the root concentrations, $TF_{foliage}$, and TF_{shoot} for both elements [Appendices B-8 & B-9]. Based on McLaughlin (1998) the evaluation of the effects of sulfate on Cd uptake by plants from soil, it can be posited that the apparent differences between treatment groups was unrelated to increasing concentrations of SO_4 and is therefore a function of differences in Zn and Cu concentrations in the soils of each treatment group [Appendix B-9].

Soil Zn was negatively associated with total plant yield (aboveground dry weight) in immature plants. This relationship is likely a reflection of the important role Cu plays in water transport and plant growth; substitution of Zn for Cu at this early growth stage may be responsible for diminished growth (Wuana & Okieiman, 2011) [Appendix B-10].

3.4.4.4 Lead

Pb is difficult for phytoextraction, as it occurs primarily as a soil precipitate rather than as the free metal; it is not readily absorbed or accumulated by plants (Lasat, 2000; Wuana & Okieiman, 2011). For most plant species, Pb is either adsorbed to the root membrane or absorbed by the roots where it is sequestered or excreted by plant detoxification systems. Thus, only a minor fraction is translocated to aboveground plant parts (Pourrut et al., 2011). When Pb does successfully enter the plant system, it can

inhibit photosynthesis, as demonstrated by the negative correlation between Pb concentration in roots and total plant yield in quinoa [Appendix B-11].

Soil Ca can have an effect on uptake of lead from the soil solution. Linear regression analysis showed negative correlations at 110 DAT between BCF_{roots} for Ca and TF_{seed} for Pb. These observations are supported by Garland and Wilkins (1980) who reported that calcium in root solutions was an effective repressor of Pb uptake. (The reduction in Pb accumulation in their experiments was significantly greater than would be expected from merely raising soil pH).

3.4.5 Phytoextraction potential for *C. quinoa*

Concentrations of trace metals in aboveground plant tissues, total elements accumulated in harvestable biomass, and the $TF_{foliage,}$ TF_{shoot}, and TF_{seed} were evaluated to assess the phytoextraction potential of quinoa plants. According to Lasat (1999), hyperaccumulator species will concentrate:

>100ppm Cd,

>1,000ppm Cu and Pb, and

>10,000ppm Ni and Zn.

By this metric, the genotype of quinoa studied is a hyperaccumulator of Cd in shoots, leaves and seeds at both stages of growth, and Cu and Pb during early growth.

Table 3.5: Translocation factors at 30 and 110 DAT

		30 DAT		110 DAT		
		Foliage	Shoots	Foliage	Shoots	Seeds
Zn	A	0.10	0.10	0.61	0.37	0.22
	B	0.56	0.18	1.22	0.45	0.47
Cd	A	0.81	1.34	0.24	0.30	0.36
	B	0.76	1.11	0.58	0.56	0.71
Pb	A	0.67	1.13	0.23	0.29	0.33
	B	0.64	1.02	0.43	0.46	0.57
Ni	A	0.74	1.20	0.26	0.28	0.35
	B	0.40	0.43	0.74	0.50	0.71
Cu	A	0.34	0.56	0.19	0.17	0.18
	B	0.30	0.42	0.26	0.19	0.25

Translocation (TF) values indicate that quinoa may be a more efficient accumulator of Zn, Cd, Pb, Ni, and Cu than suggested by the previous assessment (Table 3.5). Within the scope of this experiment, quinoa was more effective at concentrating contaminants in tissues during the early stages of growth, with TF_{shoot} > 1 for Cd, Pb, and Ni in shoots. At maturity, quinoa seems to hyperaccumulate Zn in tissues, which is common for

hyperaccumulators of Zn due to vacuolar sequestration in the leaves (McGrath & Zhao, 2003).

The results of this study clearly show that although BCFs and TCFs of metals were substantially lower after 110 days, the amount of total metals removed was considerably higher. This indicates that growing one crop of quinoa plants to maturity over 110 days would remove greater quantities of metals from soil than growing 3 or 4 crops of quinoa over successive 30-day periods. Such observations further suggest total metals accumulated in above-ground plant tissue may provide a more accurate assessment of a plant's phytoextraction potential than the translocation factor.

Bioconcentration values for Zn, Cd, Cu, and Ni in aboveground plant parts were compared between this study and the Bhargava et al. (2008) study at 30 days after germination. Both studies used the same genotype of quinoa, however, the study by Bhargava et al. did not distinguish between foliage and shoots, but combined all aboveground plant tissue. The plants in this study had higher bioconcentrations of Ni and Zn, but lower bioconcentrations of Cu and Cd than the earlier study. Different levels of contamination in soil, as well as weather and environmental effects may have caused these differences.

Table 3.6: Mean total accumulation of Zn, Cd, Pb, Ni, & Cu content (mg) per plant part

	30 DAT			110 DAT			
	Total	**Shoots**	**Foliage**	**Total**	**Shoots**	**Foliage**	**Seeds**
Zn (mg)	0.84	0.25	0.59	11.3	3.75	7.05	0.53
Cd (mg)	0.50	0.31	0.19	5.31	2.49	2.22	0.60
Pb (mg)	0.58	0.37	0.21	6.08	2.94	2.45	0.69
Ni (mg)	0.62	0.36	0.26	6.99	3.03	3.38	0.58
Cu (mg)	0.68	0.43	0.25	13.2	5.51	6.52	1.19

3.5 Conclusions

In summary, the findings of this research are:

(1) The seeds of *Chenopodium quinoa* grown in metal-contaminated soil are not appropriate for human consumption based on current limits for human consumption of trace metals in food.

(2) Uptake and concentration of Zn, Cd, Pb, Ni, and Cu in roots and tissues varied according to plant growth stage, plant organ, and concentrations of other essential and non-essential elements.

(3) Quinoa is a hyperaccumulator of Cd, Pb, and Ni in the early stages of growth, however it removes more metals from soil when grown to maturity (110-120 days). Due to its easy cultivation in poor soils and high biomass production, it is recommended for phytoextraction of Cd, Pb, and Ni in lightly- to moderately-contaminated sites with multi-metal contamination scenarios

4 Conclusions and recommendations

This research project was inspired by the questions and concerns of urban gardeners; the conclusions and recommendations that follow are therefore intended for practical, as well as academic applications. Despite widespread public interest in phytoremediation technologies, there has been low adoption by municipal governments, as large-scale civil engineering technologies are often required for quick remediation of commercially viable properties. While the impediment to brownfields re-development is often financial, the impediment to farmer/citizen- led remediation efforts is generally a lack of access to technical guidance and scientific resources. Some additional avenues of research are recommended, as are municipal and university partnerships that can transform phytoremediation research into safe, scientifically viable, and widespread practice.

> ***"... there appears to be a widening gap between science and practicality. Successful applications of metal-accumulating plants for remediation or metal recovery purposes, even at the pilot scale, are still alarmingly few and far between."***
>
> *- AJM Baker, 2002*

4.1 Recommendations for further research

Additional studies on the bioaccumulation of trace metal contaminants in the edible portions of common crop plants is an area of increasing interest for urban farmers and gardeners, as well as for those who purchase their produce at farmers' markets and locally-

sourced restaurants. In addition, a comprehensive and thorough review of existing literature on that subject would be particularly useful for practitioners, and may be used inform progressive municipal food policies.

Continued research on the availability, volume, and origin of atmospherically deposited trace metals on soil and crop tissue is particularly timely and should be conducted both for leaf surface contamination and soil contamination over time (Lum et al., 1987; McKendry et al., 2001).

A comparison of the trace metal contents in food crops produced in Vancouver with that of food crops produced in surrounding rural areas would also be a useful and valuable area of study. Such a comparison might build on De Pieri et al.'s (1997) research on micronutrient concentrations in crop tissues and soils in the Lower Fraser Valley.

4.2 Recommendations for partnerships

Partnerships between government, practitioners, and universities are highly recommended to develop practical pilot programs in urban brownfields. Government organizations can provide assistance with initiating these partnerships, as well as with identifying appropriate pieces of land and arranging for proper safe disposal of contaminated plant material. Citizen groups may provide the long-term commitment needed for such projects, and may benefit from increased access to vacant land. University partnerships are essential for the necessary soil testing and interpretation needed for evaluating the success of such projects.

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Appendix A: Statistical information for chapter 2

Appendix A- 1: Connecting letters report showing significant differences among sites for mean total concentrations of Mn and Fe (derived using Tukey-Kramer Honestly Significant Difference test)

Mn:

Level				Mean
60th & E. Blvd.	A			884.9
16 Oaks	A	B		574.4
Pine	A	B	C	484.1
41st & Blenheim	A	B	C	458.7
Vernon	A	B	C	377.4
Hastings		B	C	360.3
UBC Campus		B	C	248.6
UBC Farm			C	149.4

Fe:

Level			Mean
Hastings	A		36389
16 Oaks	A	B	26775
Vernon	A	B	22899
Pine	A	B	21601
41st & Blenheim	A	B	21071
60th & E. Blvd.	A	B	20801
UBC Farm		B	15050
UBC Campus		B	11729

Appendix A-2: Significant differences among parent material groups for mean total concentrations of Mn and Fe (derived using Tukey-Kramer Honestly Significant Difference test)

Mn:

Level			Mean
Glacial Marine	A		603.6
Marine		B	365.4
Glacial		B	259.8

Fe:

Level			Mean
Marine	A		32342
Glacial Marine	A	B	24486
Glacial		B	15448

Appendix A- 3: Linear correlations between total and available concentration of elements

Linear Fit	F value	Prob > F	N
AR Mg = 2245.975 + 4.1622668*HCl MG	31.5	.001	35
AR Ca = -3809.463 + 3.2151076*HCl CA	149	.0001	36
AR Cu = 64.284754 + 3.1458518*HCl Cu	4.82	.04	35
AR K = 494.27489 + 2.4618595*HCl K	93.8	.0001	36
AR Fe = 16230.37 + 10.649688*HCl Fe	4.63	.04	36
AR Mn = 118.06008 + 1.4776648*HCl Mn	17.7	.0002	35

Appendix A- 4: Significant differences in mean available Mn among parent material groups determined by oneway comparison of means (determined with Student's t-test)

Level	- level	Difference	Std. Error	p-value
Glacial-Marine	Marine	89.1	36.2	0.019*

Appendix A-5: Significant differences in mean available Cu by parent material (determined with Student's t-test)

Level	- level	Difference	Std. Error	p-value
Marine	Glacial	77.8	22.3	0.0013*
Marine	Glacial-Marine	76.3	21.9	0.0013*

Appendix A-6: Significant differences in mean available Ni by parent material (Student's t-test)

Level	- level	Difference	Std. Error	p-value
Marine	Glacial	6.81	2.18	0.004*
Marine	Glacial-Marine	5.55	2.13	0.014*

Appendix A- 7: Significant differences in mean available Fe by parent material (Student's t-test)

Level	- level	Difference	Std. Error	p-value
Marine	Glacial	757	124	0.0001*
Marine	Glacial-Marine	503	121	0.0002*
Glacial Marine	Glacial	254	124	0.048*

Appendix A- 8: Significant differences in percent organic matter by parent material (determined using Tukey-Kramer Honestly Significant Differences test)

Level	- level	Difference	Std. Error	p-value
Glacial-Marine	Marine	8.03	2.13	0.006*
Glacial	Marine	7.29	2.53	0.032*

Appendix A-9: Negative linear relationships between pH and total concentrations of Zn & Cu

Linear Correlation	F Value	Prob.>F	N
AR Zn = 1257 – 194 * pH $CaCl_2$	5.10	0.04*	17
AR Cu = 666 - 108*pH $CaCl_2$	11.1	0.005*	17

Appendix A-10: Reference values for trace metal concentrations (ppm) in Lower Fraser Valley soils of different parent materials compared with mean values of Cu, Zn, and Mn at tested urban sites

	Parent Material	Cu	Zn	Mn
Vancouver	Glacial till (n=12)	55.8	108	260
	Marine (n=11)	458	384	361
	Glacial Marine (n=12)	119	801	604
Lower Fraser Valley (Luttmerding, 1981)	Glacial till	10-12	11-26	0.10
	Marine	10-55	96-130	214-920
	Glacial Marine	20-53	65-85	99-790

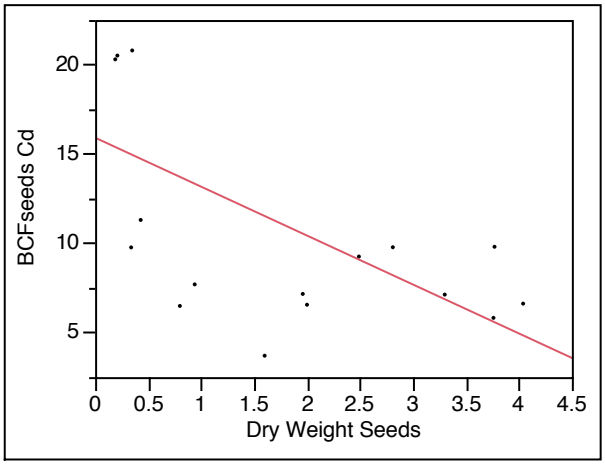
Appendix B: Statistical information for chapter 3

Appendix B- 1: Significant differences in foliage concentrations at 110 DAT and 30 DAT

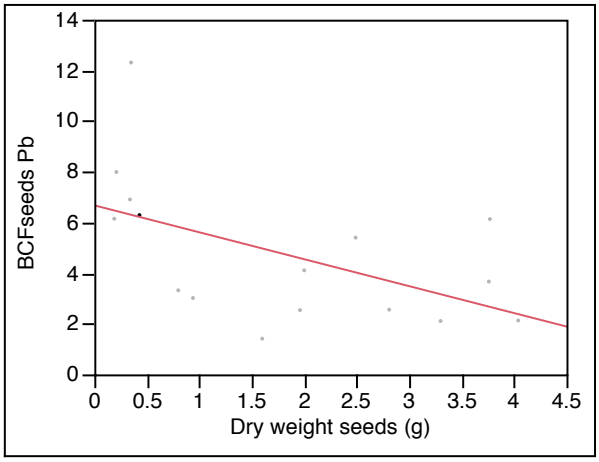
Difference	t-value	N
$C_{foliage}$ Ni	1.86	14
$C_{foliage}$ Cd	3.10	14
$C_{foliage}$ Zn	2.45	14
$C_{foliage}$ Pb	3.21	14
$C_{foliage}$ Cu	7.18	14

- values derived using paired t-test, all values significant at t<.05

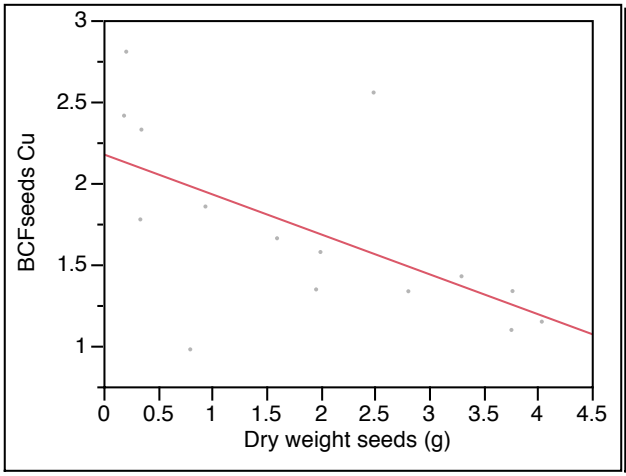
Appendix B- 2: Significant negative correlations between seed biomass and bioconcentration of Cd, Pb, Ni, & Cu



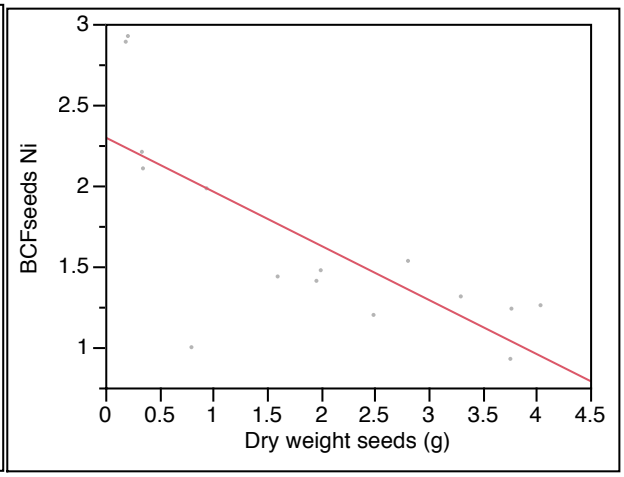
N=15, F=6.57, Prob.>F = 0.024



N=15, F=4.66, Prob.>F= 0.050

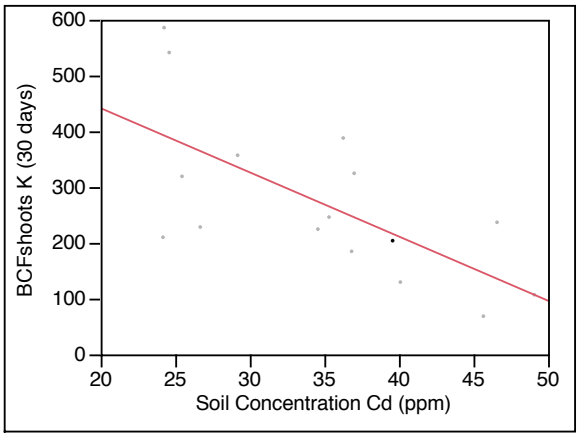


N= 15, F=7.42, Prob.>F=0.017



N=15, F= 16.5, prob>F = 0.0014

Appendix B- 3: Negative correlation between soil concentration Cd & bioconcentration of K in shoots at 30 DAT

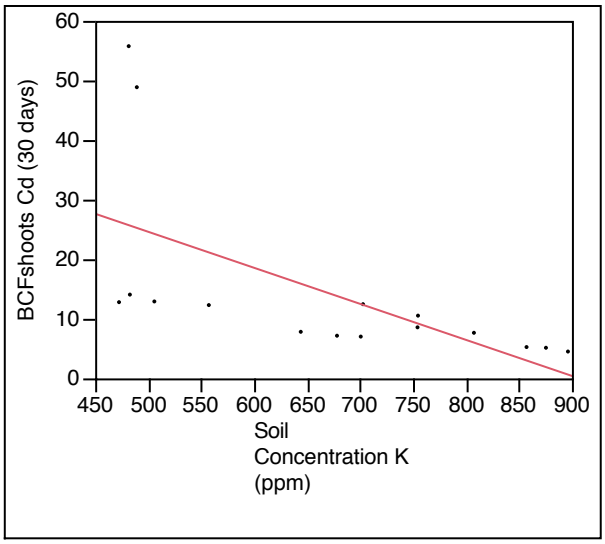


F= 10.5

N=15

Prob. >F = 0.0065*

Appendix B- 4: Negative correlation between soil concentration K and bioconcentration of Cd in shoots at 30 DAT

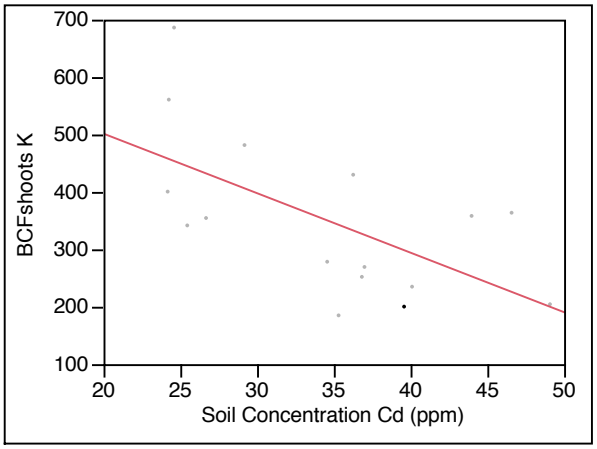


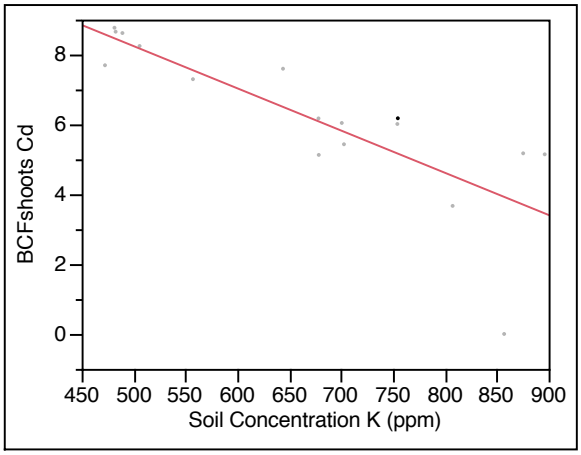
F= 8.21

N=16

Prob.>F = 0.013

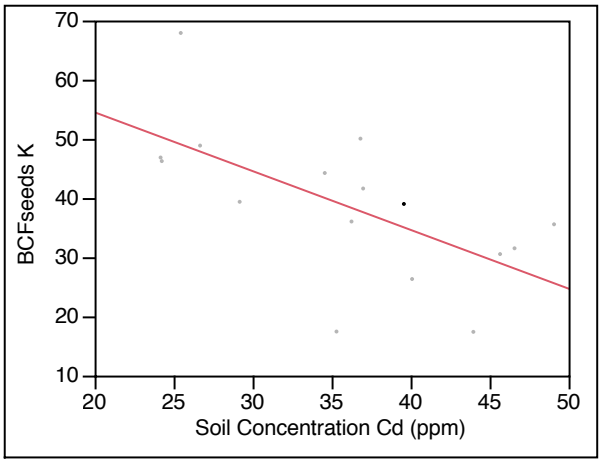
Appendix B- 5: Negative correlations between soil concentrations & BCF of Cd & K in shoots at 110 DAT

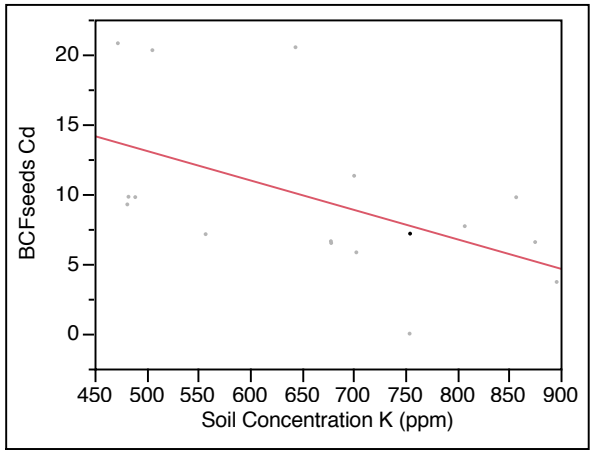




F = 8.8 N= 15 Prob. >F = 0.011 F = 28 N= 16 Prob. >F = 0.0001

Appendix B- 6: Negative correlations between soil concentrations & BCF of Cd & K in seeds at 110 DAT



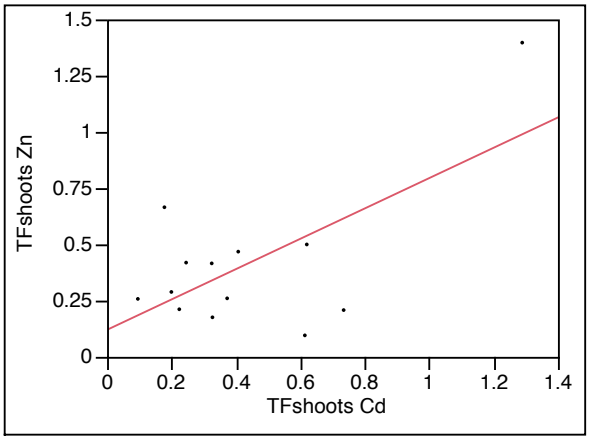


F = 14 N= 15 Prob. >F = 0.003 F = 5.6 N= 16 Prob. >F = 0.033

Appendix B- 7: Pairwise correlations between plant uptake of Cd & K

Variable	By Variable	R-value	N	Significant Prob.
BCF_{shoot} K (30 days)	Total plant Cd (110 days)	0.53	15	.0403
BCF_{root} Cd (30 days)	BCF_{shoot} K (110 days)	0.63	15	.0128
BCF_{root} Cd (30 days)	BCF_{shoot} K (30 days)	0.63	15	.0124
$TF_{foliage}$ Cd (30 days)	$C_{Foliage}$ K (30 days)	0.76	15	.0011
$TF_{foliage}$ Cd (30 days)	$BCF_{\mathbf{foliage}}$ K (30 days)	0.77	15	.0009
C_{root} Cd (30 days)	$C_{Foliage}$ K (110 days)	-0.56	14	.0395
$BCF_{\mathbf{shoot}}$ Cd (110 days)	BCF_{seed} K (110 days)	0.67	15	.0066
$BCF_{\mathbf{shoot}}$ Cd (110 days)	BCF_{shoot} K (110 days)	0.55	15	.0351
$BCF_{\mathbf{shoot}}$ Cd (110 days)	BCF_{shoot} K (30 days)	0.63	15	.0116
BCF_{shoot} Cd (30 days)	BCF_{seed} K (110 days)	0.60	15	.0171
BCF_{shoot} Cd (30 days)	BCF_{shoot} K (30 days)	0.60	15	.0173
$BCF_{foliage}$ Cd (110 days)	$C_{Foliage}$ K (30 days)	-0.76	14	.0011
$BCF_{foliage}$ Cd (110 days)	TF_{shoot} K (110 days)	-0.56	15	.0370
$BCF_{foliage}$ K (110 days)	BCF_{seed} K (110 days)	0.66	15	.0076
$BCF_{foliage}$ Cd (30 days)	$C_{Foliage}$ K (30 days)	0.68	15	.0056
$\mathbf{C_{Foliage}}$ (30 days)	$C_{foliage}$ K (30 days)	.071	15	.0030
$\mathbf{C_{Foliage}}$ Cd (30 days)	BCF_{shoot} K (110 days)	0.67	14	.0087
$\mathbf{C_{Foliage}}$ Cd (30 days)	BCF_{shoot} K (30 days)	0.69	14	.0086
$\mathbf{C_{Foliage}}$ Cd (30 days)	$BCF_{\mathbf{foliage}}$ 1F K (30 days)	0.74	15	.0016
$\mathbf{C_{soil}}$ Cd (HCl)	C_{seed} K (110 days)	0.63	13	.0207
$\mathbf{C_{soil}}$ Cd (HCl)	BCF_{seed} K (110 days)	-0.72	15	.0026
$\mathbf{C_{soil}}$ Cd (HCl)	BCF_{shoot} K (110 days)	-0.64	15	.0109
$\mathbf{C_{soil}}$ Cd (HCl)	BCF_{shoot} K (30 days)	-0.67	15	.0065

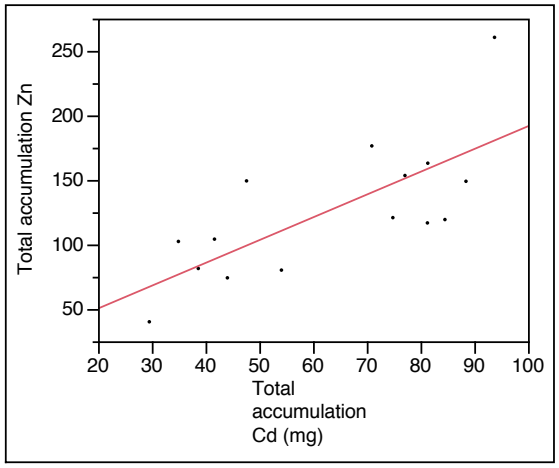
Appendix B- 8: Cd and Zn interaction in translocation efficiency of shoots, foliage, and total uptake at 110 DAT



F = 7.8

N= 13

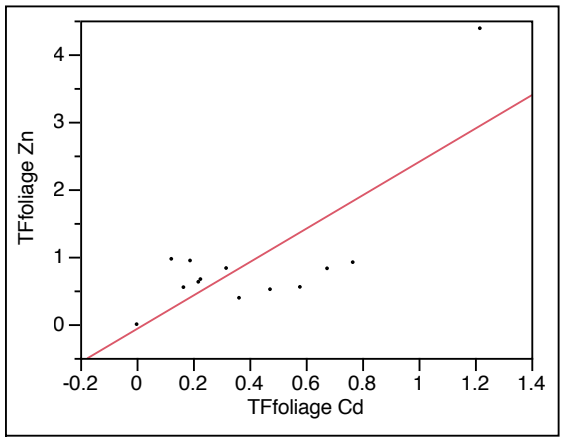
Prob. >F = 0.017



F = 15.7

N= 13

Prob. >F = 0.002

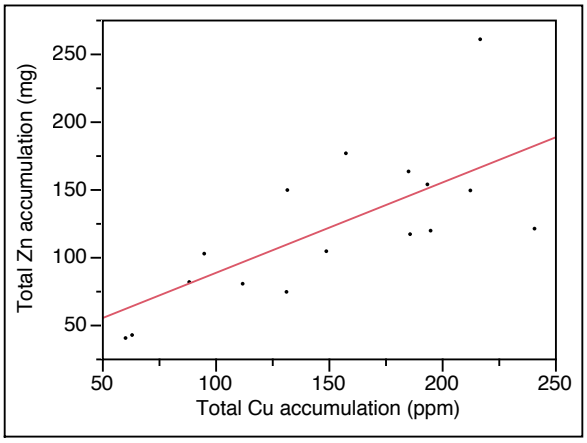


F = 15

N= 13

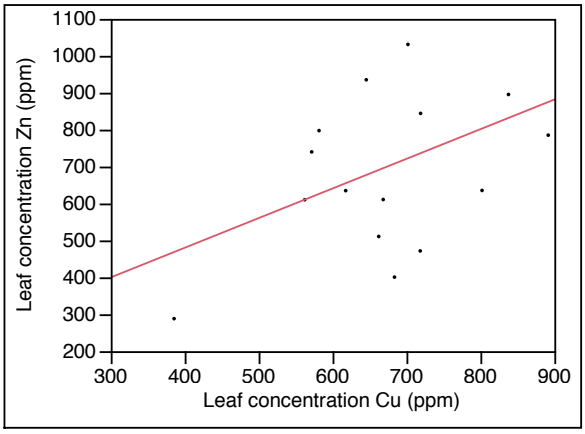
Prob. >F =0 .002

Appendix B- 9: Synergistic relationships between Cu & Zn



F= 11 N=15

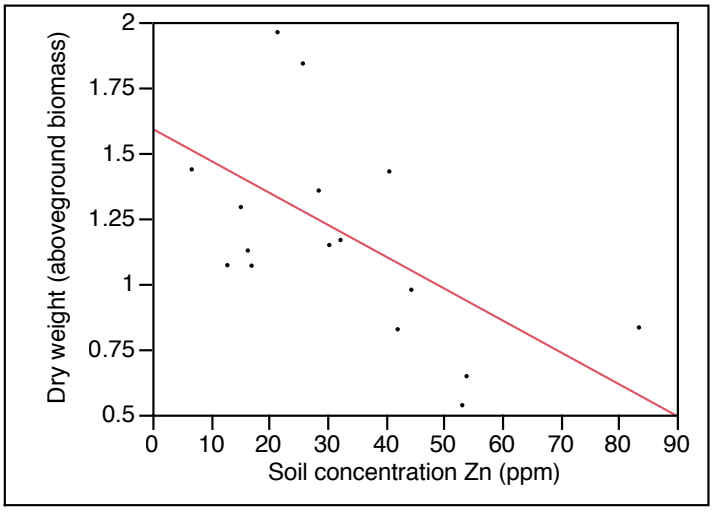
Prob.> F =0.006



F= 16 N=7

Prob.> F=0.012

Appendix B- 10: Negative correlation between soil Zn and overall plant yield (g) at 30 DAT

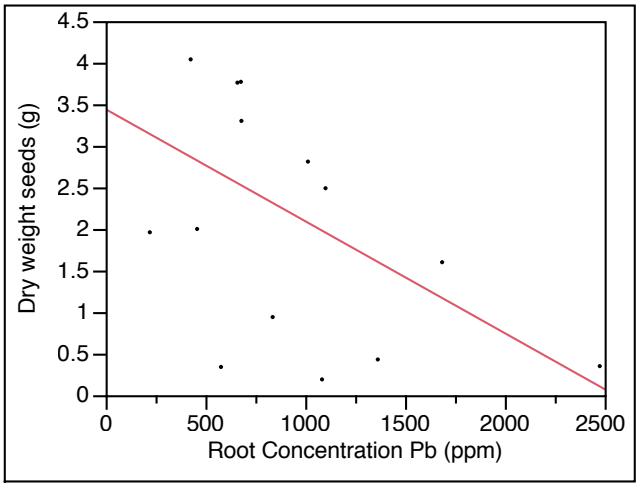


F=6.4

N=16

Prob.>F= 0.025

Appendix B- 11: Negative correlation between Pb root concentration & seed yield at 110 DAT



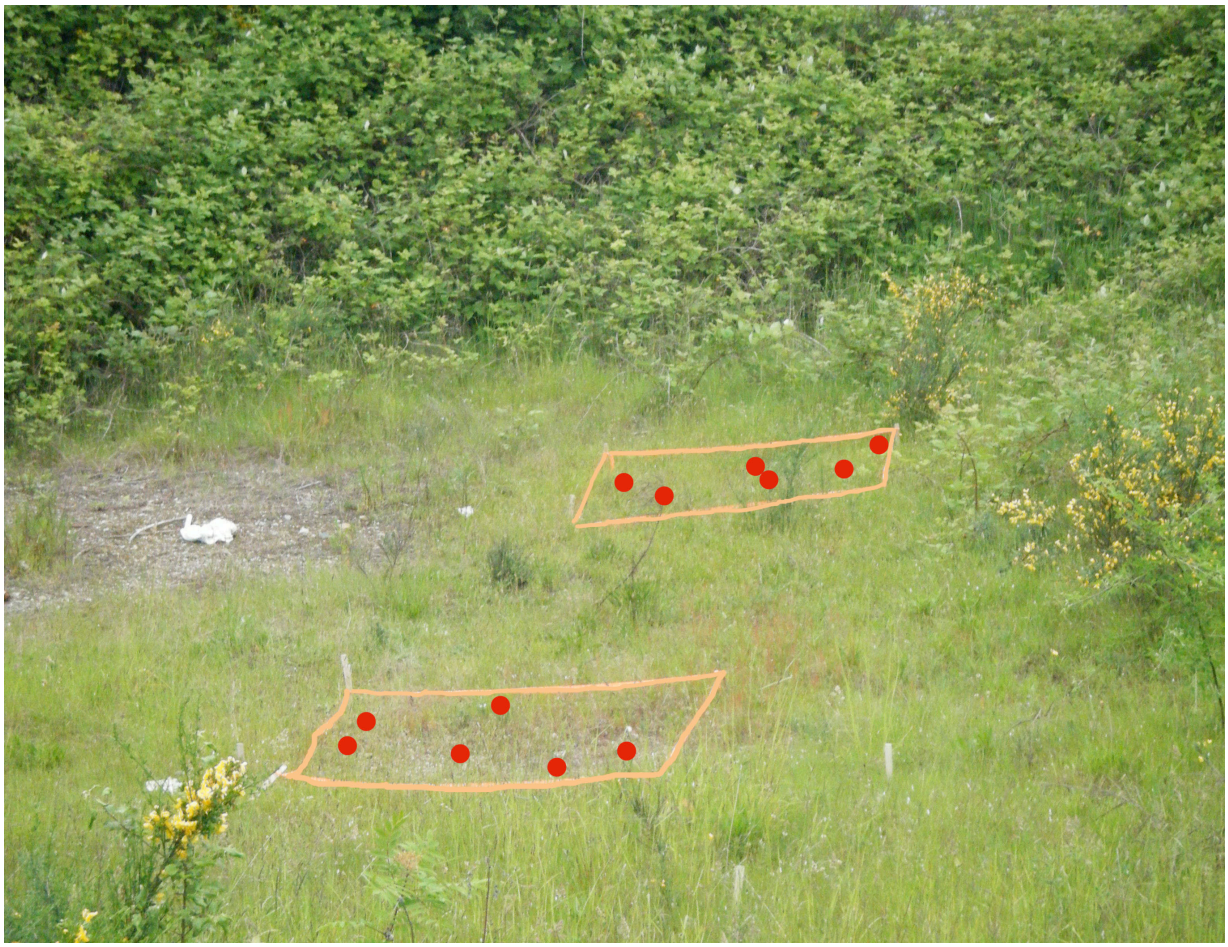
F=6.0

N=13

Prob.>F= 0.033

Appendix C: Case study details for chapter 2

Appendix C- 1: Example of subjective random sampling (red areas indicate random points for composite sampling)



Appendix C-2: Mean concentrations (± SE) from aqua regia, 0.1M HCl, and 0.01M $CaCl_2$ extractions for Hastings and 16 Oaks, with reference values for regional nutrient analysis (n=5)

	P	Mg	Ca	K	Cu	Zn	Mn	Fe
Low*	<20	<60	<1,000	<150	>0.6	>1.0	>1.5	
Med*	20-40	60-180	1,000-2,000	150-250				
High*	40-100	>180	>2,000	250-800				
Aqua regia **16 Oaks**	1,546 ± 508	4,886 ± 612	17,545 ± 5,594	1,511 ± 498	127 ± 46.1	887 ± 599	490 ± 84.8	25,476 ± 2,473
0.1M HCl **16 Oaks**	203 ± 54.0	925 ± 201	9,291 ± 2,304		9.74 ± 6.57	141 ± 77.7	127 ± 16.9	512 ± 20
0.01M $CaCl_2$ **16 Oaks**	3.23 ±0.56	198 ± 35.0			0.70 ±0.48	3.23 ± 0.56	3.02 ± 0.36	5.13
Aqua regia **Hastings**	268 ± 55.2	3,792 ± 138	3,818 ± 230	3,792 ± 107	446 ± 107	391 ± 99.9	282 ± 42.1	28,780 ± 5052
0.1M HCl **Hastings**	60.7 ± 14.9	304 ± 33.5	1,482 ± 181		152 ± 34.7	126 ± 38.6	151 ± 18.9	806 ± 99.0
0.01M $CaCl_2$ **Hastings**	1.59 ± 0.19	77.1 ± 14.6			2.09 ± 0.33	27.7 ± 9.34	5.21 ± 0.67	1.71

* reference values based on Marx, et al. 1999

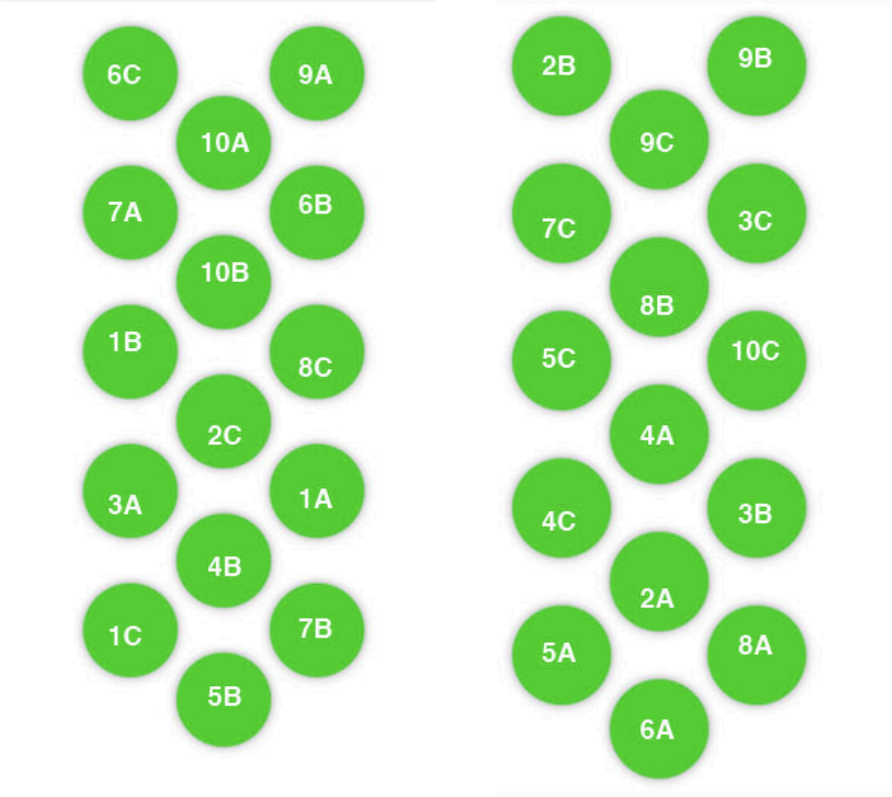
Appendix D: Experimental details for chapter 3

Appendix D-1: Quantities of trace metals and carrier salts (g kg^{-1}) added to soil for treatments A, B, & C

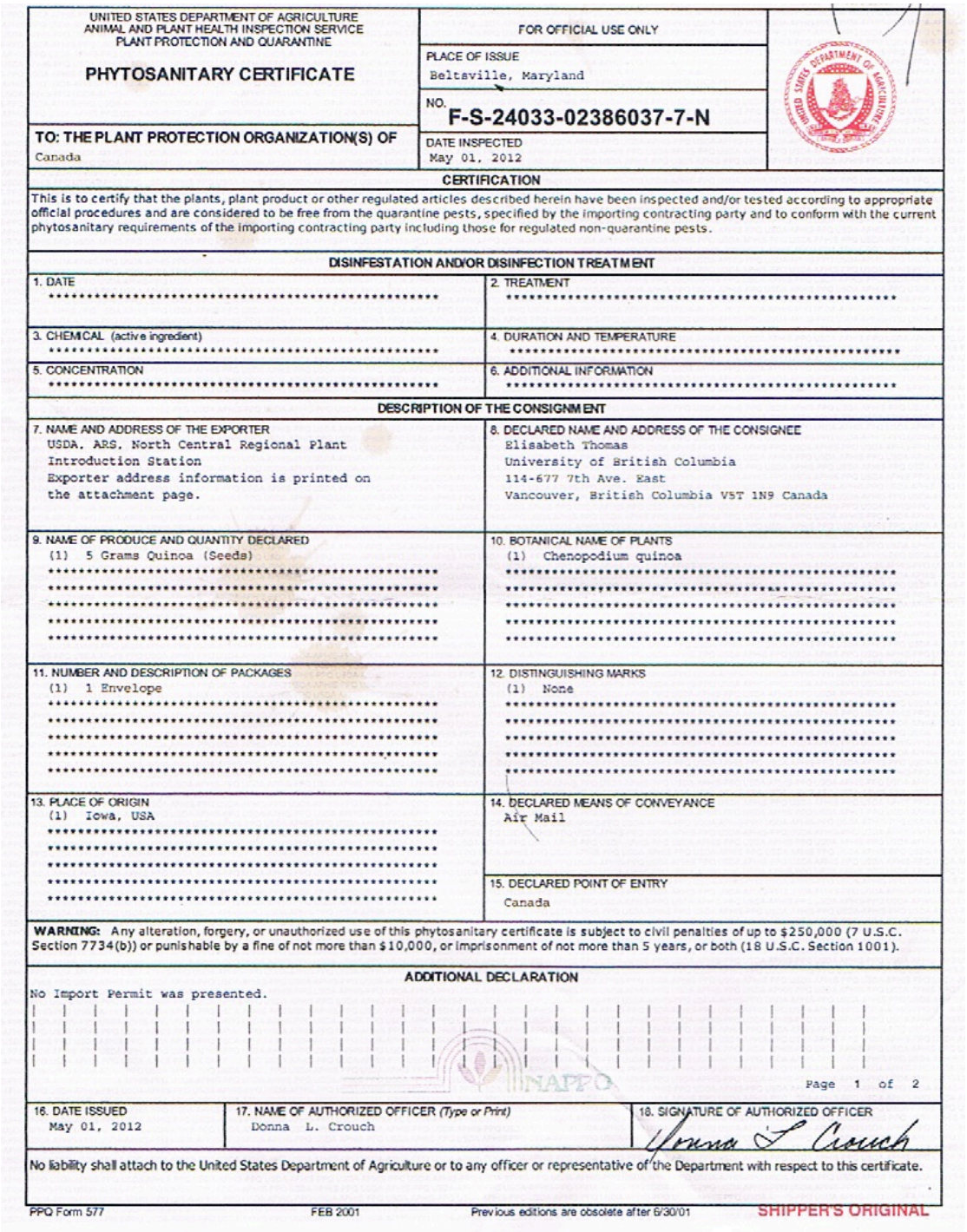
Treatment Group	$CuSO_4$	$Pb(C_2H_3O_2)_2$	$ZnSO_4$	$CdNO_3$	$NiNO_3$
A	n/a	n/a	n/a	n/a	n/a
B	0.10	0.15	1.51	0.002	0.11
C	0.57	1.13	1.61	0.02	0.31

Appendix D-2: Chenopodium quinoa seeds were started in sterile potting mix and transplanted after germination

Appendix D-3: Replicates 1-10 from treatment groups A, B, & C were arranged in a completely random block design in the UBC Horticulture greenhouse



Appendix D-4: Germplasm for Chenopodium quinoa Willd. PI 510532 was assessed from USDA Germplasm Resources Information Network



UNITED STATES DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE
PLANT PROTECTION AND QUARANTINE

PHYTOSANITARY CERTIFICATE

FOR OFFICIAL USE ONLY

PLACE OF ISSUE: Beltsville, Maryland

NO. F-S-24033-02386037-7-N

TO: THE PLANT PROTECTION ORGANIZATION(S) OF: Canada

DATE INSPECTED: May 01, 2012

CERTIFICATION

This is to certify that the plants, plant product or other regulated articles described herein have been inspected and/or tested according to appropriate official procedures and are considered to be free from the quarantine pests, specified by the importing contracting party and to conform with the current phytosanitary requirements of the importing contracting party including those for regulated non-quarantine pests.

DISINFESTATION AND/OR DISINFECTION TREATMENT

1. DATE	2. TREATMENT
**********	**********
3. CHEMICAL (active ingredient)	4. DURATION AND TEMPERATURE
**********	**********
5. CONCENTRATION	6. ADDITIONAL INFORMATION
**********	**********

DESCRIPTION OF THE CONSIGNMENT

7. NAME AND ADDRESS OF THE EXPORTER	8. DECLARED NAME AND ADDRESS OF THE CONSIGNEE
USDA, ARS, North Central Regional Plant Introduction Station Exporter address information is printed on the attachment page.	Elisabeth Thomas University of British Columbia 114-677 7th Ave. East Vancouver, British Columbia V5T 1N9 Canada
9. NAME OF PRODUCE AND QUANTITY DECLARED	10. BOTANICAL NAME OF PLANTS
(1) 5 Grams Quinoa (Seeds) **********	(1) Chenopodium quinoa **********
11. NUMBER AND DESCRIPTION OF PACKAGES	12. DISTINGUISHING MARKS
(1) 1 Envelope **********	(1) None **********
13. PLACE OF ORIGIN	14. DECLARED MEANS OF CONVEYANCE
(1) Iowa, USA **********	Air Mail
	15. DECLARED POINT OF ENTRY
	Canada

WARNING: Any alteration, forgery, or unauthorized use of this phytosanitary certificate is subject to civil penalties of up to $250,000 (7 U.S.C. Section 7734(b)) or punishable by a fine of not more than $10,000, or imprisonment of not more than 5 years, or both (18 U.S.C. Section 1001).

ADDITIONAL DECLARATION

No Import Permit was presented.

NAPPO

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16. DATE ISSUED	17. NAME OF AUTHORIZED OFFICER *(Type or Print)*	18. SIGNATURE OF AUTHORIZED OFFICER
May 01, 2012	Donna L. Crouch	Donna L Crouch

No liability shall attach to the United States Department of Agriculture or to any officer or representative of the Department with respect to this certificate.

PPQ Form 577 FEB 2001 Previous editions are obsolete after 6/30/01 SHIPPER'S ORIGINAL

Appendix D-5: Chenopodium quinoa at 30 DAT before destructive sampling

Appendix D-6: Mean bioconcentration factors (± SE) for foliage, shoots, roots, and seeds at 30 and 110 DAT (n=8)

		30 DAT			110 DAT			
		Foliage	**Shoot**	**Root**	**Foliage**	**Shoot**	**Seed**	**Root**
Zn	**A**	44.5 ± 22.4	24.8 ± 8.96	342 ± 69.4	30.0 ± 5.23	16.4 ± 5.67	12.9 ± 6.33	38.1 ± 10.6
	B	31.8 ± 6.31	10.4 ± 2.13	53.8 ± 5.93	19.2 ± 3.04	8.18 ± 1.13	6.12 ± 1.63	25.1 ± 8.25
Cd	**A**	8.86 ± 2.68	21.0 ± 5.93	10.8 ± 1.10	5.71 ± 1.01	5.88 ± 0.99	8.87 ± 1.87	26.2 ± 8.15
	B	8.69 ±0.89	14.0 ± 5.08	11.5 ± 0.95	6.66 ± 0.44	6.61 ± 0.59	10.4 ± 2.27	13.4 ± 3.30
Pb	**A**	5.33 ±1.67	12.2 ± 3.58	7.88 ± 0.74	3.35 ± 0.58	3.63 ± 0.61	5.35 ± 1.08	17.2 ± 5.30
	B	2.72 ± 0.18	4.41 ± 1.33	4.22 ± 0.20	2.09 ± 0.07	2.27 ± 0.16	3.49 ± 0.82	6.15 ± 1.49
Ni	**A**	9.19 ± 2.63	20.9 ± 6.00	12.2 ± 1.19	6.34 ± 1.13	5.81 ± 0.97	2.68 ± 1.40	26.4 ± 7.78
	B	9.12 ± 0.92	11.1 ± 3.56	24.0 ± 1.75	7.87 ± 0.58	5.83 ± 0.48	1.84 ± 0.24	13.3 ± 3.10
Cu	**A**	3.73 ± 1.05	8.10 ± 1.94	10.9 ± 0.73	5.30 ± 1.01	4.15 ± 0.72	2.20 ± 0.63	32.8 ± 11.7
	B	3.37 ± 0.22	4.96 ± 1.41	11.4 ± 0.41	5.33 ± 0.17	3.89 ± 0.24	1.75 ± 0.21	17.3 ± 4.77

Appendix D-7: Comparison of mean bioconcentration factors at 30 DAT for Chenopodium quinoa Willd. PI 510532 in two different studies (± SE, n=8)

	Shoots & Foliage **(Bhargava et**	**Foliage**	**Foliage**	**Shoots**	**Shoots**

	al., 2008)	Treament A	Treatment B	Treatment A	Treatment B
Zn	2.24	54.9 ± 22.4	31.8 ± 6.31	28.5 ± 8.99	10.4 ± 2,13
Cu	18.2	3.78 ± 1.05	3.37 ± 0.22	6.50 ± 1.94	4.96 ± 1.41
Ni	4.87	9.15 ± 2.63	9.12 ± 092	16.9 ± 6.00	11.1 ± 3.56
Cd	111.8	8.78 ± 2.68	8.69 ± 0.89	16.5 ± 5.93	14.0 ± 5.08

Appendix D-8: Mean values for biometric characters recorded at 30 and 110 DAT (± SE)

	30 DAT					110 DAT					
	Longest leaf (cm)	**Stem length** (cm)	**Number of leaves**	**Dry weight foliage** (g)	**Dry weight stem** (g)	**Longest leaf** (cm)	**Stem length** (cm)	**Number of leaves**	**Dry weight foliage** (g)	**Dry weight stem** (g)	**Dry weight seeds** (g)
Group A	5.76 ± 0.34	23.8 ± 2.20	33.2 ± 3.94	0.66 ± 0.11	1.03 ± 0.55	7.66 ± 0.26	127 ± 7.67	280 ± 53.3	14.3 ± 5.37	10.7 ± 2.75	1.39 ± 0.47
Group B	5.20 ± 0.33	24.8 ± 1.06	29.5 ± 2.66	0.53 ± 0.08	0.44 ± 0.09	8.51 ± 0.44	129 ± 9.19	294 ± 56.9	11.8 ± 1.46	15.1 ± 2.33	1.88 ± 0.50