EFFECT OF CADMIUM BIOAVAILABILITY ON PHYTOEXTRACTION FEASIBILITY AND ECOLOGICAL RISK IN A COMPOST-BASED SOIL

EFFET DE LA BIODISPONIBILITÉ DE CADMIUM SUR LA FAISABILITÉ D'UTILISER LA PHYTOEXTRACTION AINSI QUE LES RISQUES ÉCOLOGIQUES ASSOCIÉS DANS LES SOLS À BASE DE COMPOST

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ABSTRACT

Michele Alexandra Parisien. M.Sc., Environmental Sciences. Royal Military College of Canada. November, 2014. Effect of cadmium bioavailability on phytoextraction feasibility and ecological risk in a compost-based soil. Supervisors: Dr. Barbara A. Zeeb and Dr. Allison Rutter.

The effect of cadmium (Cd) bioavailability on phytoextraction feasibility and ecological risk was investigated in soil amended with municipal solid waste (MSW) and sewage sludge (SS) compost at the Peterborough Gun Club in Peterborough, Canada. Plant uptake was low due to sorption of Cd to stable soil fractions, and it was therefore determined that phytoextraction was not a feasible method of remediating these MSW/SS compost-based soils. Low plant bioavailability limited exposure of higher order receptors to Cd, and an ecological risk assessment (ERA) indicated no potential for risk to any of the seven ecological receptors evaluated at this site. For comparison, risk was also characterized at a nearby PCB-contaminated site using the same seven receptors. Previous research had shown that phytoextraction would be an effective method of remediating this PCB site due to high plant uptake of the contaminant, and an ERA revealed that five of the seven receptors evaluated were potentially at risk of experiencing adverse health effects from PCBs. As ERAs characterize risk under present environmental conditions, the long-term potential for biochar to sorb Cd and limit its bioavailability was evaluated at the Peterborough site. Trends indicated decreasing Cd bioavailability in biochar-amended soil relative to the un-amended control, and this coincided with a significant decrease in organic matter content in the control soil. The results of this thesis emphasize the influence of contaminant bioavailability on phytoextraction feasibility and ecological risk, and demonstrate the potential for biochar to limit long-term Cd bioavailability and risk in MSW/SS compost-amended soil.

Key Terms: Cadmium, municipal solid waste, sewage sludge, compost, bioavailability, phytoextraction, ecological risk, biochar.

RÉSUMÉ

Michele Alexandra Parisien. M.Sc., sciences de l'environnement. Effet de la biodisponibilité de cadmium sur la faisabilité d'utiliser la phytoextraction ainsi que les risques écologiques associés dans les sols à base de compost. Le collège militaire royal du Canada. Novembre, 2014. Directeurs : Dr. Barbara A. Zeeb et Dr. Allison Rutter.

L'effet de la biodisponibilité de cadmium (Cd) sur la faisabilité d'utiliser la phytoextraction ainsi que les risques écologiques associés ont été étudiés dans les sols ou des déchets municipaux solides (DMS) et des boues d'épuration (BE) compostées ont été ajoutés au Club de Tir Peterborough (CTP) à Peterborough, Canada. La phytoextraction de Cd par les plantes était faible grâce à la sorption de Cd sur les fractions de sol stables et la phytoextraction par les plantes n'est donc pas une méthode possible pour rétablir les sols contaminés avec le Cd par l'application de compost produit avec DMS/BE. Cependant, la faible phytoextraction de Cd par les plantes réduit l'exposition du Cd pour les récepteurs écologiques des niveaux trophiques plus élevés sur ce site, et une évaluation des risques écologiques a indiqué qu'il n'y a aucun potentiel de risque. Pour comparaison, les risques écologiques ont aussi été caractérisés sur un site contaminé par des biphényles polychlorés (BPC) en utilisant les mêmes sept récepteurs. Des recherches antérieures avaient montré que la phytoextraction serait une méthode efficace de réhabiliter ce site à cause de la biodisponibilité des BPC élevée, et une évaluation des risques écologiques a indiqué qu'il existe le potentiel de risque à cinq des sept récepteurs évaluées. Étant donné que les évaluations des risques écologiques caractérisent le risque dans les conditions environnementales actuelles, cette étude a permis d'évaluer le potentiel à long terme pour le biocharbon à adsorber le Cd au CTP, et la limitation de sa biodisponibilité a également été évaluée. Les tendances indiquent une diminution de biodisponibilité de Cd dans les sols avec le biocharbon par rapport aux sols de contrôle (sols sans biocharbon) ce qui a coïncidé avec une diminution significative de la teneur en matière organique dans le sol de contrôle et non dans le sol avec biocharbon. Les résultats de cette thèse soulignent l'influence de la biodisponibilité des contaminants sur l'efficacité des plantes à absorber le Cd ainsi que les risques écologiques et démontrent le potentiel pour le biocharbon de limiter la biodisponibilité de Cd à long terme et les risques dans les sols avec DMS/BE compostés.

Mots clés: cadmium, déchets municipaux solides, boues d'épuration, compost, biodisponibilité, phytoextraction, risque écologique, biocharbon.

CO-AUTHORSHIP STATEMENT

The student's contributions to the thesis manuscript are as follows:

- Active participant in the initial development of research ideas and projects.
- Primary researcher responsible for the successful implementation and completion of laboratory experiments conducted at the Royal Military College of Canada (RMCC; Kingston, ON), greenhouse experiments conducted at Queen's University (Kingston, ON), and field experiments conducted at the Peterborough Gun Club (PGC; Peterborough, ON).
- Completed all analytical work at both the Analytical Services Unit (Queen's University) and in the Phytotechnologies Laboratory (RMCC).
- Primary risk assessor at the Cd-contaminated PGC and the PCB-contaminated site in Lindsay, ON (Chapter 4).
    - Raw data for the risk assessment of the Lindsay site was taken from the M.Sc. thesis of Smith *et al.*, 2012, however all data interpretation and risk calculations for the site were completed by M. Parisien.
- Principal author on all three research papers (two of which have been submitted to peer-reviewed journals).

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Chapter 5: Parisien MA, Rutter A, Zeeb B. Effect of biochar on cadmium fractionation with organic matter degradation in a soil amended with municipal solid waste and sewage sludge compost.

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LIST OF ABBREVIATIONS

AUF	Area Use Factor
BA	Bioavailability
BAF	Bioaccumulation Factor
BW	Body Weight
CCME	Canadian Council of Ministers of the Environment
CEC	Cation Exchange Capacity
C_i	Contaminant concentration of a food item
C_s	Soil contaminant concentration
Cd	Cadmium
DDI	Double De-Ionized
DOM	Dissolved Organic Matter
DQRA	Detailed Quantitative Risk Assessment
EC	Environment Canada
EDI	Estimated Daily Intake
ERA	Ecological Risk Assessment
F_i	Fraction of the diet composed of food item
FIR	Food Ingestion Rate
HQ	Hazard Quotient
HTT	Highest Treatment Temperature
ICP-OES	Inductively Coupled Plasma Optical Emission Spectrometry
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
MOE	Ontario Ministry of the Environment
M.Sc.	Master of Science
MSW	Municipal Solid Waste
NOAEL	No Observed Adverse Effects Level
OM	Organic Matter
PCB	Polychlorinated Biphenyl
PGC	Peterborough Gun Club
PQRA	Preliminary Quantitative Ecological Risk Assessment
PSD	Particle Size Distribution
ROS	Reactive Oxygen Species
SIR	Soil Ingestion Rate
SS	Sewage Sludge
TF	Translocation Factor
TRV	Toxicity Reference Value
US EPA	United States Environmental Protection Agency

1. GENERAL INTRODUCTION

Cadmium (Cd) is a xenobiotic metal that causes adverse health effects in humans, plants, and animals. Cadmium can mimic essential nutrients such as iron, calcium, and magnesium (Adriano, 2001; Bridges and Zalups, 2005), and its high water solubility allows it to cross biological membranes and interfere with cell function (Tran and Popova, 2013; Stohs *et al.*, 2001). Cadmium may be accumulated by low trophic level organisms and transferred up the food chain (Hunter, 1982). Exposure to Cd can lead to ecological and human health risks including low biomass, chlorosis, and necrosis in plants (Cosio *et al.*, 2006; Cunha *et al.*, 2008), and bone, kidney, and liver disease in humans and other animals (Klaassen *et al.*, 2009; Singh, 2005).

Although Cd occurs naturally in the environment, anthropogenic loading of Cd to soils has increased considerably over the past century due to its use by industry and its many consumer applications (Kabata-Pendias and Mukherjee, 2007; Hutton, 1983). Improper disposal of Cd-containing products and runoff of Cd from roads and fields cause Cd to accumulate in municipal solid waste (MSW) and sewage sludge (SS) (Dean and Suess, 1985). Composted MSW/SS is widely applied to degraded soils to improve soil quality due to its high organic matter and nutrient contents, but amendment with MSW/SS compost can also result in soil Cd concentrations that exceed the guideline of 1.2 $\mu g \cdot g^{-1}$ Cd set by the Ontario Ministry of the Environment (MOE) (MOE, 2011). As Cd does not degrade, it persists for long periods of time in Cd-contaminated soils (Kabata-Pendias, 2000) such as those amended with MSW/SS compost.

Common methods of remediating Cd-contaminated soil include excavation and landfilling, incineration, soil washing, solidification/stabilization, and vitrification (Monferrán and Wunderlin, 2013; Khan *et al.*, 2004; Kabata-Pendias, 2000). Although effective, these methods can negatively affect soil quality, result in soil destruction, or limit future land use. There is therefore a demand for environmentally friendly remediation technologies that maintain soil integrity throughout the remedial process.

Phytoextraction is one such technology, as it uses vascular plants *in-situ* to extract soil contaminants into plant roots and translocate them to the shoots, which are harvested and disposed of (Salt *et al.*, 1998; Cunningham and Ow, 1996). Research has shown that several phytoextractors have the ability to extract significant amounts of Cd from contaminated soil and accumulate them in their shoots (Li *et al.*, 2012; Liu *et al.*, 2004; Schwartz *et al.*, 2003; Ebbs *et al.* 1997). Phytoextraction relies on contaminant mobility and bioavailability (Lasat, 2000), and therefore the successful implementation of phytoextraction requires an understanding of bioavailability and the factors that affect it. Contaminant speciation, soil properties, and other environmental conditions can impact the availability of a contaminant in soil and have a positive or negative impact on phytoextraction success (Kirkham, 2006; He and Singh, 1993; Haghiri, 1974). The results of studies examining plant uptake of Cd from MSW/SS compost-amended soils are conflicting, with some reporting low Cd bioavailability (Shuman *et al.*, 2002; Wen *et al.*, 2002; Sims and Kline, 1991) and others reporting high Cd bioavailability (Kaschl *et al.*, 2002; Simeoni *et al.*, 1984). To date, there are no studies investigating the use of phytoextraction to remediate soil contaminated with Cd from the application of MSW/SS compost.

Ecological risk assessment (ERA) is a useful tool that can be used to estimate the potential for risk associated with phytoextraction. Phytoextraction results in high contaminant concentrations in the aboveground plant tissue, and this might increase the exposure of plant-eating receptors to Cd (Angle and Linacre, 2005). An ERA can be used to estimate the potential risk to receptors from phytoextraction. If contaminant bioavailability is too low for phytoextraction to be feasible, the exposure of receptors to soil contaminants might be low enough to eliminate the potential health risks. In this case, remediation might cause more harm than good, and ERA can be used to determine whether remediation is necessary.

Another environmentally-friendly method of remediating Cd-contaminated soil is to immobilize it in the soil matrix with biochar. Biochar is the carbon-rich product of biomass pyrolysis, and it has a high sorption capacity for cations such as Cd^{2+}. Its addition to soil reduces the water-solubility of Cd in soils (Park *et al.*, 2011b) and significantly reduces Cd uptake by plants (Bian *et al.*, 2014; Suppadit *et al.*, 2012; Beesley and Marmiroli, 2011; Cui *et al.*, 2011). Because it is a stable form of organic matter that is estimated to persist in soils for several thousands of years (Spokas, 2010; Zimmerman, 2010), biochar might limit Cd bioavailability over the long-term. In addition, its sorption capacity for cations increases as it ages in soil due to the slow oxidation of its surface (Liang *et al.*, 2006), and therefore Cd bioavailability in soil should decrease with time as biochar ages. However, further research is required to ascertain the long-term effect of biochar on Cd bioavailability.

This M.Sc. thesis explores the relationship between contaminant bioavailability, phytoextraction, and ecological risk in an organic matter-rich soil contaminated with Cd from the application of MSW/SS compost at a site in Peterborough, Ontario. Chapter two provides a literature review of Cd toxicity and bioavailability in the environment, Cd phytoextraction and stabilization with biochar, and the process of conducting a preliminary quantitative ecological risk assessment (PQRA). In chapter three, the feasibility of using phytoextraction with native and naturalized plant species to remediate Cd-contaminated soil at the Peterborough site is evaluated. This chapter also includes the results of a Cd sequential extraction study completed for soil at the site with a discussion of its implications for Cd bioavailability to plants. In chapter four, the potential for risk to wildlife receptors from Cd phytoextraction at the PGC was calculated using a PQRA. For comparison, the ecological risk from phytoextraction was also calculated at a PCB-contaminated site located 45 km away in Lindsay, Ontario, where previous studies had demonstrated high PCB bioavailability and effective phytoextraction. Risk was calculated at these sites using the same risk calculations and receptors, and the same kind of site-specific data. In chapter five, organic matter breakdown and Cd fractionation during the ageing process of soil amended with MSW/SS compost is explored, and the long-term effects of biochar on these factors is examined. Chapter 6 includes a discussion of the major findings and conclusions of this thesis as well as directions for future research. Finally, raw data and quality assurance and quality control results are included in appendices A-E.

2. LITERATURE REVIEW

2.1 CADMIUM IN THE ENVIRONMENT

Cadmium (Cd) is a toxic metal with no known biological function. In its natural form, Cd is usually found associated with zinc (Zn) in parent rock (Hutton, 1983), and is redistributed throughout the environment via volcanic eruptions, forest fires, and dust storms (Naidu *et al.*, 1997). When Zn ores are mined, smelted, and refined, Cd is recovered as a by-product and is used in the production of nickel (Ni)-cadmium batteries, pigments, alloys, and stabilizers for PVC plastics (Adriano, 2001; Mulligan *et al.*, 2001). The production and disposal of these products has led to anthropogenic loading of Cd to the environment, particularly via emissions from industry, runoff from roads and fields, and dumping of Cd-containing liquids, which cause Cd to wash into water treatment systems and accumulate in sewage sludge (Hutton, 1983).

Accumulation and redistribution of Cd via sewage sludge is of particular concern in Canada, as Canada produces up to 2.5 million wet tons of sewage sludge annually (CCME, 2012). Disposal of this waste is costly (US EPA, 2003), and this financial burden has led to a push towards the land application of composted sewage sludge as an alternative to disposal (Pascual *et al.*, 2000). Sewage sludge compost is high in organic matter (OM) and improves soil properties such as water holding capacity, soil aeration, and nutrient content (Aggelides and Londra, 2000), and its application to soil significantly increases plant biomass (Indoria *et al.*, 2013). Sewage sludge can also contain high concentrations of toxic metals such as Cd (Breslin, 1999), and though regulations exist to control the allowable concentrations of toxic substances in sewage sludge composts, metal concentrations in soil can nonetheless increase with repeated application (Hinesly *et al.*, 1984) and cause adverse health effects in humans, plants, and animals.

2.2 CADMIUM TOXICITY

Plants are primarily exposed to Cd via uptake from the soil into the roots, while animal exposure occurs primarily through ingestion of Cd-contaminated soil, plants, and animals. Cadmium has a chemical structure resembling that of essential nutrients including Zn, Ca, and Fe, allowing it to compete with these metals for cell uptake in a process called 'ionic and molecular mimicry' (Adriano, 2001; Bridges and Zalups, 2005).

2.2.1 Cadmium Toxicity to Plants

Cadmium induces toxic effects in plants by disrupting plant cell homeostasis and inhibiting the antioxidant response, resulting in increased concentrations of reactive oxygen species (ROS) (Tran and Popova, 2013). Reactive oxygen species cause lipid peroxidation (Mohamed *et al.*, 2012; Romero-Puertas *et al.*, 2004; Chaoui *et al.*, 1997) that disrupts lipid membrane function (Fodor *et al.*, 1995).

Visible symptoms of Cd toxicity in plants include chlorosis, necrosis, defoliation, and reduced biomass (Cosio *et al.*, 2006; Cunha *et al.*, 2008). The ability of plants to withstand these toxic effects is species-specific, and though some hyperaccumulators such as *Thlaspi caerulescens* (alpine pennycress) can accumulate high Cd concentrations (>1000 $\mu g \cdot g^{-1}$; Yanai *et al.*, 2006) without exhibiting toxicity

symptoms, most species experience adverse health effects at much lower concentrations (<55 $\mu g \cdot g^{-1}$; Khan and Frankland, 1983).

2.2.2 Cadmium Toxicity to Animals

Cadmium interferes with the antioxidant response in animals (Stohs *et al.*, 2001), increasing the ROS abundance and the oxidation of lipids and DNA (Wang *et al.*, 2004; Bagchi *et al.*, 1996). When animal cells are exposed to chronic, low levels of Cd, the production of metallothionein (MT; a low molecular weight protein) is up-regulated to mediate Cd toxicity (Park *et al.*, 2001). Metallothionein binds Cd, and the inactive Cd-MT complex is transported to the kidney where it is filtered and excreted (Wolff *et al.*, 2006; Horner and Smith, 1975). When animal cells are exposed to acute, high levels of Cd, however, the amount of pre-synthesized MT is insufficient to prevent Cd from exerting toxic effects (Squibb *et al.*, 1976). Acute Cd toxicity primarily targets the liver and causes hepatotoxicity (Klaassen *et al.*, 2009).

Although the adverse effects of Cd on human health have been studied for over 150 years (Nordberg, 2009), widespread concern over Cd in the environment was only raised following a mass Cd poisoning in the Jinzu river basin of Japan in the 1940s, when people consuming rice contaminated with Cd from upstream industrial activity developed symptoms of severe Cd toxicity (Singh, 2005). This disease, now known as itai-itai ("ouch-ouch") disease, is caused by chronic Cd exposure resulting in bone, kidney, and liver diseases (Singh, 2005). As a result of this incident, much work has been done to study Cd loading to the environment, implement guidelines for safe Cd concentrations in soil, and develop cost-effective and environmentally friendly methods of remediating soil contaminated with Cd.

2.2 REMEDIATION

2.2.1 Phytoextraction

Phytoextraction involves the uptake of contaminants into plant roots, and the subsequent translocation of those contaminants into the above ground part of the plant (Salt *et al.*, 1998). Phytoextraction is achieved by planting successive crops of a high biomass, fast-growing plant species with expansive root systems that can tolerate high concentrations of a contaminant in soil and accumulate it in their shoots (Garbisu and Alkorta, 2001). Shoots are harvested and removed from the site, and usually undergo a volume and weight reduction step prior to disposal (Salt *et al.*, 1998). This step is accomplished by ashing or composting to reduce the mass and volume of the waste product, which is then either incinerated or directly disposed of in a landfill (Sas-Nowosielska *et al.*, 2004). Theoretically, phytoextraction is an ideal form of remediation, as it is an *in-situ*, low-cost, aesthetically pleasing, and environmentally friendly way to clean up contaminated sites.

Phytoextraction of metals such as Cd has been well-studied, and several plant species have demonstrated the ability to extract Cd into their shoots (Jun and Ling, 2012; Li *et al.*, 2012; Liu *et al.*, 2004; Baker *et al.*, 2000; Ebbs *et al.*, 1997). The two qualities used to measure a plant's phytoextraction effectiveness are its bioaccumulation factor (BAF) and translocation factor (TF) (Fayiga *et al.*, 2004). The BAF is the ratio of the Cd concentration of the plant to that of the soil, and indicates a plant's ability to extract contaminants from soil (Ji *et al.*, 2011). In most cases, a BAF >> 1 is required for effective phytoextraction (Krämer, 2005; McGrath and Zhao, 2003). The TF is the ratio of the Cd concentration of the shoot to that of the root, and demonstrates the ability of a plant to translocate Cd to the aboveground

portion of its tissue; the higher the TF the better, as the aboveground biomass is the portion that is removed from the site (Ji *et al.*, 2011).

Cadmium phytoremediation works best in moderately contaminated soils (~5 $\mu g \cdot g^{-1}$ Cd) (Milan *et al.* 2012; Vysloužilová *et al.*, 2003), as phytoextractors growing in highly contaminated soil often display signs of plant toxicity including chlorosis, necrosis, stunted growth, and/or wilting (Ghnaya *et al.*, 2007). A number of laboratory, greenhouse, and field-scale studies have indicated the potential of certain plant species to extract Cd. *Thlaspi caerulescens* is a particularly efficient Cd phytoextractor (Schwartz *et al.*, 2003), and its ability to accumulate high concentrations of Cd in its tissue (>100 $\mu g \cdot g^{-1}$) classifies it as a Cd hyperaccumulator (Baker *et al.*, 2000). There are few hyperaccumulators of Cd; the only ones identified to date include *T. caerulescens*, *Viola baoshanensis* (violet), and possibly *Amaranthus hypochondriacus* (Prince-of-Whales feather) (Li *et al.*, 2012; Liu *et al.*, 2004; Baker *et al.*, 2000). However, hyperaccumulation alone is insufficient for effective phytoextraction; a high biomass yield is also required (McGrath and Zhao, 2003), and in some cases its importance can exceed that of a plant's accumulation capacity. In a study by Ebbs *et al.* (1997), *Brassica juncea* (Indian mustard, a high biomass, moderate Cd-accumulator) was more effective than *T. caerulescens* (a low biomass, high Cd-accumulator) in removing Cd and Zn from sewage sludge-amended soil.

2.2.1.1 Soil Properties Affecting Cadmium Phytoavailability

Successful phytoextraction requires Cd to be phytoavailable (Lasat, 2000), meaning that in order to be extracted, Cd must be present (or have the potential to be present) in the soil solution, as it is from here that plants assimilate nutrients and minerals (Kashem and Singh, 2001; Naidu *et al.*, 1997; Xian, 1989). The speciation of Cd in soil is largely controlled by soil properties and environmental conditions.

Cadmium Speciation

Cadmium is predominantly present in the soil solution as the free divalent cation, Cd^{2+} (Alloway, 2013). Cadmium (II) can be retained in solution or sorbed to the solid phase, with major sorbents being organic matter particulates, silicate clay minerals, and Fe and Mn oxides (Naidu *et al.*, 1997; Zachara *et al.*, 1992). The relative importance of these sorbents varies with their abundance in soil. Cadmium (II) can be bound to these sorbents via inner-sphere (specific) adsorption (Sparks, 2003), which occurs when Cd^{2+} forms a covalent bond with the negatively charged O atom of a functional group on a soil colloid (Brady and Weil, 2013). This chemical bond binds Cd specifically, and is not easily broken. Cadmium (II) can also be bound by outer-sphere (nonspecific) adsorption (Guo *et al.*, 2006), which occurs when one or more water molecules are present between Cd^{2+} and the colloidal surface, causing Cd^{2+} to be sorbed to the negatively charged O-containing functional group via electrostatic attraction instead of covalent bonding (Brady and Weil, 2013). Cations held by outer-sphere adsorption are more weakly held than those bound by inner-sphere adsorption, and can be replaced by other, similarly charged cations in solution in a process called cation exchange (Naidu *et al.*, 1997).

Though the solid phase dominates Cd sorption in soil, Cd^{2+} can also form complexes with soluble inorganic or organic ligands in solution that mobilize Cd in soil (Alloway, 2005). Cadmium can form organo-metallic complexes with various dissolved organic matter (DOM) compounds in soil such as fulvic acids and low molecular weight organic acids released by microbes and plant roots (Kim *et al.*, 2010). In saline soils, Cd^{2+} can bind to Cl^- or SO_4^{2-} to form $CdCl_2$ and $CdSO_4$, respectively, both highly soluble Cd species (Alloway, 2005; Naidu *et al.*, 1997) that are bioavailable to plants (Kookana *et al.*,

1999). These soluble Cd-ligand complexes along with the free Cd^{2+} ions in solution and those released by cation exchange represent the fraction of Cd in soil that is most available for plant uptake.

Under strong reducing conditions, SO_4^{2-} is reduced to S^{2-}, which can precipitate with Cd^{2+} to form CdS (greenockite), an insoluble Cd mineral that is unavailable for plant uptake (Alloway, 2005). Cadmium can also precipitate with CO_3^{2-} to form $CdCO_3$ (otavite), another insoluble Cd mineral, though this reaction only occurs in the presence of abundant anions at high pH (>7.0) and high Cd concentration (>100 $\mu g \cdot g^{-1}$) (Alloway, 2005; Bataillard *et al.*, 2003).

Organic Matter Content

Organic matter (OM) is an important soil characteristic influencing soil Cd sorption (Shaheen, 2009; Kirkham, 2006; Hooda and Alloway, 1998). Soil OM (or soil humus) is characterized by its dark color, its ability to form stable complexes with metal cations, and its high buffering, cation exchange, and water holding capacities (Sparks, 2003). Soil OM is composed of non-humic substances, which are easily decomposable and have a short residency time in soil, and humic substances, which are high molecular weight compounds that persist in soil for long periods of time (Sparks, 2003). Humic substances can be further divided into fulvic acids, humic acids, and humin, and these are distinguished based on their solubility in acid or base.

Humic substances are present in soil either as colloids in the solid phase or as dissolved organic matter (DOM) in the solution phase, with considerable implications for the mobility and phytoavailability of Cd (Naidu *et al.*, 1997); DOM increases metal phytoavailability, whereas metals bound to solid phase OM are removed from the soil solution and are unavailable for plant uptake.

DOM in the soil solution is part of the labile fraction of soil OM, and consists primarily of low molecular weight organic acids such as fulvic and amino acids (Krishnamurti and Naidu, 2003). These compounds can increase Cd mobility in soil by forming soluble organo-metal complexes with Cd in solution (Kaschl *et al.*, 2002; Almås *et al.*, 2000). As these DOM complexes can be taken up by plants, sorption to DOM increases not only the mobility of Cd, but also its bioavailability (Antoniadis and Alloway, 2002).

However, dissolved humic and fulvic acids makes up only a small proportion of total soil OM; solid phase humic and fulvic acids are the predominant form of OM in soils (Young, 2013; Almås *et al.*, 2000), and they sorb and immobilize Cd (Shaheen, 2009; Ge and Hendershot, 2005). This solid state OM has a large concentration of negatively charged carboxyl and phenolic functional groups on its surface that can bind divalent cations (such as Cd) by both inner-sphere and outer-sphere complexation (Loganathan *et al.*, 2012; McLean and Bledsoe, 1996). The sorption capacity of OM is positively correlated with the surface area of the OM colloids due to the higher number of metal sorption sites on the colloid surface (Ge and Hendershot, 2005; Hooda and Alloway, 1998). Organic matter is variably charged, and its ability to sorb metal cations increases with pH (Morais *et al.*, 1976; Vinkler *et al.*, 1976). At high pH, OM-rich soils can significantly decrease plant bioavailability of Cd due to sorption by cation exchange (Haghiri, 1974).

Cation Exchange Capacity

A soil's cation exchange capacity is the total amount of exchangeable cations that it can adsorb at a given pH (Bache, 1976). Plant uptake of Cd is influenced by the cation exchange capacity (CEC) of the soil, with increasing CEC leading to decreased plant uptake (Miller *et al.*, 1976). Cation exchange is an

outer-sphere adsorption reaction that occurs when a cation in solution displaces a cation sorbed to the negatively-charged O atom of the functional groups on the surface of soil colloids (Brady and Weil, 2013). This newly sorbed cation can then in turn be displaced by another cation in solution in a continuous process. Cation exchange is pH-dependent; at low pH, the ratio of H^+:Cd^{2+} in the soil solution is high, and these ions will adsorb and desorb from the colloidal surface by cation exchange until an equilibrium is reached whereby the ratio of H^+:Cd^{2+} ions adsorbed to soil colloids is equal to that of its ratio in solution (Brady and Weil, 2013). Therefore, more Cd^{2+} will be present in the soil solution at low pH due to the high proton concentration. However, as pH increases, H^+ cations dissociate from the O atom of functional groups on the surface of clay and organic matter colloids, increasing the net negative charge of the soil and the available sorption sites for Cd binding (Morais *et al.*, 1976).

Cadmium competes with other metal cations for exchange sites in soil. These cations can come in the form of nutrients such as Na^+, Mg^{2+}, or Ca^{2+}, with Ca^{2+} being the strongest competitor for exchange sites on soil colloids (Naidu *et al.*, 1997). Soils rich in Ca can therefore have a considerable impact on Cd sorption and mobility. In multi-metal polluted soils, Zn^{2+}, Pb^{2+}, and Cu^{2+}, among other toxic metals, can compete with Cd^{2+} for sorption sites (Fontes *et al.*, 2000). In these soils, Cd is usually present at a much lower concentration than other metal cations, and its competition with these metals for sorption sites can decrease its sorption coefficient significantly (Kookana *et al.*, 1999).

Soil pH

There is general agreement in the literature that pH is the single most important factor affecting Cd sorption processes in variably charged soils (Naidu *et al.*, 1997), and as sorption strongly influences Cd solubility, pH ultimately affects Cd bioavailability to plants. As pH increases, H^+ cations are released from the O-containing functional groups of Fe, Al, and Mn oxyhydroxides (e.g. Al—O^-), organic matter (e.g. R—OO^-), and clay silicates (Morais *et al.*, 1976). This release of protons increases the negative surface charge density on the soil colloid, attracting and binding metal cations such as Cd^{2+} by outer-sphere adsorption (Karak *et al.*, 2005). Both outer-sphere and inner-sphere Cd adsorption increase rapidly with pH until they reach a plateau at which sorption has reached its maximum capacity (Tiller *et al.*, 1984).

Other Factors Affecting Cadmium Sorption to Soil

Metals have different characteristics that result in varying affinities for the surfaces of soil particles (Shaheen, 2009). Metals with a relatively small hydrated radius, high electronegativity, and a low hydrolysis constant (pK_H) have a higher affinity for sorption sites (Shaheen, 2009; Alloway, 2005; McBride, 1994). A small hydrated radius decreases the distance between the metal cation and the negatively-charged exchange sites at the surface of the soil or biochar particle, which increases the force of attraction between the two in accordance with Coulomb's Law (Shaheen, 2009). The higher the valency of a metal ion, the better its ability to replace an ion attached to the soil surface (Alloway, 2005). A metal with a relatively high electronegativity is better at attracting the surface negative charges on a soil colloid than a metal with a low electronegativity (McBride, 1994).

Cadmium sorption is also affected by competition with other cations for sorption sites. Cadmium is usually found alongside Zn and Pb in soils, and studies comparing the soil sorption of Cd, Zn, and Pb demonstrate the differences in affinities among these metals due to the characteristics mentioned above (Shaheen, 2009; *et al.*, 2008; Saha *et al.*, 2002). Saha *et al.* (2002) correctly predicted the Pb >> Zn > Cd sequence of sorption affinity to two different substrates based on the metals' respective pK_H. Shaheen

(2009) studied the sorption of Pb and Cd in soils from Egypt and Greece and found that Pb has a higher distribution coefficient (K_d) than Cd, meaning that Pb is retained more strongly than Cd by soils. This was probably due to the difference in characteristics between the two metals; Pb^{2+} has a smaller hydrated radius, a lower pK_H, and is more electronegative than Cd^{2+} (Shaheen, 2009).

2.2.2 Biochar

Biochar is the carbon-rich product of biomass pyrolysis in a low oxygen and relatively low temperature (<700°C) environment (Lehmann and Joseph, 2009). The process used to produce biochar is similar to that used to produce charcoal, though these two products differ based on their intended use; while charcoal is intended to provide a source of fuel, biochar is used as a soil amendment (Lehmann and Joseph, 2009). There are four traditional reasons to produce and amend soils with biochar: 1) soil improvement; 2) waste management; 3) energy production; and 4) mitigation of climate change (Lehmann and Joseph, 2009). Biochar also has a fifth emerging purpose: 5) the sorption of contaminants such as Cd.

Contaminant-sorbing biochars are divided into two main categories: those produced from plant material (wood chips, construction waste, crop and yard waste, etc.), and those produced from animal material (manure, bones, etc.). These biochars confer different advantages to soil based on their physical and chemical properties, with plant-based biochars primarily improving soil structure and porosity, and manure-based biochars primarily improving the soil nutrient content (Cantrell *et al.*, 2012; Uzoma *et al.*, 2011; Uchimiya *et al.*, 2010; Steiner *et al.*, 2007).

2.2.2.1 Physical Properties

As the biochar feedstock is pyrolyzed, it undergoes a reduction in volume and mass as its organic components are volatilized (Azargohar *et al.*, 2014; Kloss *et al.*, 2012; Laine *et al.*, 1991). The basic physical structure of the feedstock is retained throughout this process, resulting in an end product with a high density of micro-, meso-, and macro-pores that greatly increase its surface area (Laine *et al.*, 1991). Highest treatment temperature (HTT), the highest temperature achieved during pyrolysis, is an important factor influencing the physical properties of biochar. As the HTT increases, particle size distribution (PSD) decreases and OM volatilization increases, increasing the pore density and surface area of the biochar (Sun *et al.*, 2014; Dai *et al.*, 2013). A high surface area has positive implications for remediation, as it increases the space available for contaminant sorption.

2.2.2.2 Chemical Properties

Biochar can sorb Cd and other cations by cation exchange due to the high density of carboxyl groups on its surface (Uchimiya *et al.*, 2012; Appel *et al.*, 2008). Biochars generally have a high pH (>7.0), and can increase the pH of acidic soils (Cantrell *et al.*, 2012; Kloss *et al.*, 2012). This further contributes to the immobilization of Cd in soil due to the positive correlation between pH and CEC (Beesley and Marmiroli, 2011).

The HTT strongly influences the ability of biochar to sorb metal cations. Biochars produced at low HTTs (≤400°C) have a relatively high negative surface charge density, high CEC, high hydrogen to carbon ratio (H:C), low pH, and low stability (Sun *et al.*, 2014; Kloss *et al.*, 2012). Biochars produced at high HTTs (>400°C) have a relatively low negative surface charge density, low CEC, low H:C, and high stability due to its high degree of aromaticity (Azargohar *et al.*, 2014; Sun *et al.*, 2014; Kloss *et al.*, 2012). Therefore, biochars produced at low HTTs will be more effective at sorbing contaminants, but will also degrade faster than biochars produced at high HTTs.

Exposure to air results in the slow oxidation of the biochar surface as it ages in soil, leading to the formation of oxygen-containing functional groups on its surface (Cheng *et al.*, 2006). The incorporation of oxygen into the graphite-like structure of biochar results in a loss of positive surface charge and a gain in negative surface charge (Cheng *et al.*, 2008), increasing the CEC of the biochar and its sorption capacity for metal cations (Liang *et al.*, 2006).

2.2.2.3 Biochar in Organic Matter Rich Soils

The degree to which biochar will affect contaminant immobilization depends on the sorption capacity of the soil it is amending; metal immobilization by biochar is higher in soils with low sorption capacities than those with high sorption capacities (Uchimiya *et al.*, 2011). As OM-rich soils have a high CEC, biochar should have a more pronounced effect on Cd immobilization in low OM soils than in high OM soils (Shaheen *et al.*, 2009). However, OM breaks down over time, and soil Cd concentrations might increase as it is desorbed from soil throughout the degradation process. Biochar (particularly those produced at high HTTs) is a highly stable form of OM with an estimated half-life on the order of 10^2-10^7 years (Spokas, 2010; Zimmerman, 2010). As it also has a CEC that increases with its residency time in soil (Liang *et al.*, 2006), biochar has the potential to limit the long-term bioavailability of Cd in OM-rich soils and the associated human and environmental health risks.

2.2 ECOLOGICAL RISK ASSESSMENT

Ecological risk assessment (ERA) is a tool used to evaluate the potential for receptors to experience adverse effects from exposure to a chemical stressor present in the environment (CCME, 1996; MOE, 1996). In an ERA, the receptors are the plant and animal communities at a site, and the stressor is the chemical contamination present at that site. Ecological risk assessment is used to aid risk-based decision making for contaminated sites, particularly when there are significant ecological concerns, unacceptable data gaps, or special site characteristics that should be considered prior to remediation (CCME, 1996).

Risk assessments are either probabilistic or deterministic, and the differences between the two are primarily based on variability and uncertainty (Stark, 2000). A probabilistic risk assessment generates a range of risk values and indicates the probability that risk will occur given a particular set of circumstances, while a deterministic risk assessment is based on a single, "worst-case" estimate of exposure and does not account for any variability associated with risk (Stark, 2000). Deterministic risk assessments use highly conservative exposure and toxicity data, and results indicating no potential for risk can therefore be accepted with a high degree of confidence (CCME, 1996). The deterministic risk assessment is the most common type of risk assessment, and it follows a tiered approach to risk analysis.

2.2.1 The Tiers of ERA

There are three tiers of an ERA: 1) the screening assessment; 2) the preliminary quantitative risk assessment (PQRA); and 3) the detailed quantitative risk assessment (DQRA). Each tier includes the same four components: receptor characterization, exposure assessment, hazard assessment, and risk assessment. The ERA process begins with a screening assessment, and if, upon its completion, the risk is considered to be adequately characterized, the process ends and the results are reported to risk managers. If the risk is not considered to be adequately characterized, the process moves upwards through the tiers, with each tier reducing uncertainty, refining conceptual models and endpoints, and becoming increasingly quantitative, predictive, and site-specific.

2.2.1.1 Screening Assessment

The screening assessment uses existing information from the literature or from previous studies of the site, and usually requires no additional sampling (CCME, 1997). In this tier, the receptor characterization component establishes the receptors of concern by identifying potentially exposed, sensitive, or otherwise significant species at the site and the surrounding area and compiling their life histories (CCME, 1996). Exposure assessment involves characterizing the contaminant of concern, including its transport pathways and fate in the environment, the extent of contamination at the site, identification of possible exposure pathways, and estimation of uptake into higher trophic organisms. Hazard assessment relates the level of contamination at the site to its potential toxicity to receptors by extrapolating from toxicity data in the literature. Finally, risk characterization combines the previous components of the screening assessment to qualitatively characterize risk as being high, low, or negligible. Risk characterization also includes uncertainty analysis, wherein data gaps are identified and uncertainty is determined to be either acceptable or unacceptable (US EPA, 1998). When extrapolating from the literature, the most conservative values are always used to provide a conservative estimate of risk.

2.2.1.2 Preliminary Quantitative Ecological Risk Assessment

The PQRA builds on the information collected in the screening assessment, and fills data gaps by collecting site-specific data through field sampling programs. Receptor characterization for the PQRA includes the identification of the types of habitats available in the area, their proximity to the contaminated site, the type of surface cover, soil type, etc. (CCME, 1997). Life history data is compiled for each receptor of concern, including dietary components, food and soil ingestion rates, body weight, migration, and home range size (CCME, 1997). Exposure assessment for this stage of ERA involves additional field sampling of potentially contaminated media to further elucidate patterns of contamination and to determine its distribution at the site. Potential sources of exposure including food items (plants and worms, among others), soil, water, and air are sampled to provide estimates of exposure to receptors (CCME, 1996). A receptor's estimated daily intake (EDI) of a contaminant is calculated using its various exposure pathways and the contaminant concentration of the media it is exposed to (Sample and Suter, 1994):

$$Estimated\ Daily\ Intake = \frac{[\sum(C_i \times F_i \times FIR_i) + (C_s \times SIR)] \times AUF \times BA \times EF}{BW}$$

C_i = Contaminant concentration of food item (i) ($\mu g \cdot g^{-1}$ ww)
F_i = Fraction of the diet composed of food item (i) (unitless)
FIR_i = Food Ingestion Rate for food item (i) ($kg \cdot day^{-1}$ ww)
C_s = Soil contaminant concentration ($\mu g \cdot g^{-1}$ dw)
SIR = Soil ingestion rate ($kg \cdot day^{-1}$ dw)
AUF = Area use factor (unitless)
BA = Bioavailability (1)
EF = Exposure Frequency (weeks/year)
BW = Bodyweight (kg ww)

The hazard assessment quantitatively estimates the toxic effects of site contamination to receptors. Toxicity tests are completed using the species of concern or an appropriate surrogate, though toxicity data can also be extrapolated from the literature (CCME, 1996). The dose or concentration at which there are no observed adverse effects is called the toxicity reference value (TRV). Risk characterization for a PQRA includes a quantitative indication of risk using the quotient method, wherein a receptor's EDI is divided by its TRV to yield the hazard quotient (HQ):

$$Hazard\ Quotient = \frac{Estimated\ Daily\ Intake}{Toxicity\ Reference\ Value}$$

If the HQ is greater than one, then there is a potential for risk to this receptor group. If the HQ is less than one, then no risk is assumed for that receptor group. Preliminary quantitative risk characterization also includes an estimate of uncertainty associated with the HQ (US EPA, 1998; CCME, 1996).

2.2.1.3 Detailed Quantitative Ecological Risk Assessment

In most situations, ERAs can be completed to satisfaction with a PQRA. However, if the uncertainty associated with an individual ERA component is unacceptable, a DQRA can be completed for that component (CCME, 1996). This tier of ERA is highly detailed and complex, and is usually reserved for sites that are particularly large or contain sensitive or essential habitat. The risk assessment included in this thesis was completed to satisfaction using a PQRA, and therefore a DQRA was not applicable to this thesis.

3. FEASIBILITY OF USING PHYTOEXTRACTION TO REMEDIATE A COMPOST-BASED SOIL CONTAMINATED WITH CADMIUM

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3.1 ABSTRACT

The application of municipal solid waste (MSW) and sewage sludge (SS) composts to soils may affect soil organic matter (OM) and hence phytoextraction feasibility. Greenhouse and *in-situ* field experiments were used to determine the potential for phytoextraction to remediate soil contaminated with cadmium (Cd) from MSW/SS compost application at a site in Peterborough, Canada. For the greenhouse experiment, one native (*Chenopodium album*) and three naturalized (*Poa compressa, Brassica juncea, Helianthus annuus*) plant species were planted in soil containing no detectable Cd (<1.0 $\mu g \cdot g^{-1}$), and soil collected from the site containing low Cd concentrations (5.0±0.3 $\mu g \cdot g^{-1}$ Cd), and high Cd concentrations (16.5±1.2 $\mu g \cdot g^{-1}$ Cd). Plant uptake was low for all species, as indicated by root BAFs ≤0.5 for all but *P. compressa* in the low Cd treatment (BAF 1.0). Only *B. juncea* accumulated Cd in its shoots, though uptake was low (BAF ≤0.3) and there was no significant difference in shoot BAF between the low and high Cd treatments ($p>0.05$). For the field experiment, *B. juncea* was planted *in-situ* in areas of low and high Cd concentrations. *Brassica juncea* Cd uptake was low as indicated by root and shoot BAFs <0.2 for both treatments. Sequential extraction analysis indicated that Cd is retained primarily by soil fractions with relatively low bioavailability, and phytoextraction is therefore not a feasible method of remediation at this site. Though low Cd bioavailability has negative implications for Cd phytoextraction from soils amended with MSW/SS compost, it may limit receptor exposure to Cd sufficiently to eliminate the potential for risk at this site.

Key Terms: Cadmium, municipal solid waste, sewage sludge, compost, phytoextraction, organic matter, cation exchange capacity

3.2 INTRODUCTION

Soil is a valuable natural resource that is largely being depleted due to pollution and erosion (Pimentel, 2006). Efforts to economically restore degraded soils and preserve them for future use include amendment with municipal solid waste (MSW) and sewage sludge (SS) compost (Pascual *et al.*, 2000). The organic component of MSW is largely composed of residential food, leaf, and yard waste (Environment Canada, 2013), while SS is the solid or semi-solid organic waste produced during the wastewater treatment process. Municipal solid waste and SS are composted either together or separately to generate nutrient- and organic matter (OM)-rich compost that improves soil properties such as cation exchange capacity (CEC), pH, bulk density, and water retention (Bertoncini *et al.*, 2008; Cheng *et al.*, 2007). However, MSW/SS compost can contain high concentrations of metals such as cadmium (Cd) (Smith, 2009). Cadmium is a xenobiotic metal that enters the environment anthropogenically through the production and disposal of products such as nickel (Ni)-Cd batteries, pigments, alloys, and stabilizers for polyvinyl chloride (PVC) plastics (Adriano, 2001; Mulligan *et al.*, 2001). Cadmium is toxic to humans, plants, and animals, and despite regulations governing the allowable metal concentrations for the use of MSW/SS compost as soil amendments, their application can increase Cd concentrations in soil (Alloway and Jackson, 1991; Baker *et al.*, 1979).

Phytoextraction is an environmentally-friendly alternative to excavation and chemical remediation methods, as it uses vascular plants *in-situ* to extract soil contaminants into the roots and translocate them to the shoots, which are harvested and compacted for off-site disposal (Salt *et al.*, 1998). Using site-specific native or naturalized species further reduces the environmental impact of phytoextraction (Ghosh and Singh, 2005), as it minimizes disturbance to the site's ecology. Native plants are those that have originated in a region naturally without human involvement (Pysek *et al.*, 2004), whereas naturalized plants are non-native species that are able to reproduce and sustain populations through several life cycles (Richardson *et al.*, 2000) while remaining in balance with the ecological community to which they belong (Hallett, 2006). Because phytoextraction preserves the soil matrix and maintains soil properties, it is an attractive remediation option for high quality soils such as those amended with MSW/SS compost.

Only metals present in the soil solution are readily available for plant uptake. Therefore, OM influences phytoextraction success in two opposing ways: solid-state OM can sorb Cd and reduce its phytoavailability (Kukier *et al.*, 2010; Kaschl *et al.*, 2002), while dissolved organic matter (DOM) in the soil solution forms soluble complexes with Cd, increasing its mobility and phytoavailability (Wong *et al.*, 2007; Antoniadus and Alloway, 2002). Although solid state OM colloids are the predominant form of OM in soil humus, DOM is nevertheless present at lower levels in the soil solution due to the excretion of root exudates by plants (Li *et al.*, 2011) and the decomposition of colloidal OM (Martínez and McBride, 1999).

Fresh, uncomposted MSW/SS can significantly increase metal solubility and phytoavailability due to the composition and high concentrations of DOM (Salati *et al.* 2010; Ashworth and Alloway, 2008; Antoniadis and Alloway, 2002; Zhou *et al.*, 2000). In contrast, composted MSW/SS has a smaller fraction of DOM (Gao *et al.*, 2005; Castaldi *et al.*, 2005) and a greater proportion of large, humified, and insoluble organic acids (Jouraiphy *et al.*, 2005; Kaschl *et al.*, 2002; Chefetz *et al.*, 1996). This increase in stable OM in composted MSW/SS increases metal binding to the solid phase, and decreases metal solubility in soil and its availability to plants (Shuman *et al.*, 2002; Wen *et al.*, 2002; Sims and Kline, 1991). While most studies of composted MSW/SS follow this trend, some report high metal phytoavailability despite composting (Kaschl *et al.*, 2000; Simeoni *et al.*, 1984). The results of these studies indicate that other soil factors also play an important role in governing Cd complexation and its uptake by plants, and this affects the potential of phytoextraction to remediate MSW/SS compost amended soils.

In this study, greenhouse and field experiments are conducted using one native (*Chenopodium album*) and three naturalized (*Poa compressa, Brassica juncea, Helianthus annuus*) plant species (Cutright *et al.*, 2010; Abe *et al.*, 2008; Ebbs *et al.*, 1997; Park *et al.*, 2011b; Lai *et al.*, 2008) to extract Cd from a MSW/SS compost-amended soil in Peterborough, Canada. The objectives are to determine whether phytoextraction can be used to successfully remediate Cd contamination in MSW/SS compost-amended soils, and to elucidate the relationship between Cd fractionation and bioavailability in soils using sequential extraction.

3.3 METHODS AND MATERIALS

3.3.1 Site Description

In 2010, a Cd-contaminated compost was used to restore a 0.805 ha disturbed area at the Peterborough Gun Club (PGC) in Peterborough, Ontario, Canada (Figure 3-1). The disturbed area was originally a natural vegetated habitat situated at the edge of a drumlin, which was flattened and denuded of vegetation to create a clay floor for a firing range. In 2009, the decision was made to restore the area rather than complete the construction of the new range. Compost produced from MSW and SS was obtained from a Kingston, Ontario company and mixed with topsoil and mulch to cover the clay layer. Application of this compost at the site resulted in soil Cd concentrations up to 21.4 $\mu g \cdot g^{-1}$ Cd, exceeding the standard of 1.2 $\mu g \cdot g^{-1}$ Cd set by the Ontario Ministry of the Environment (MOE; MOE, 2011). The compost was applied in a ~30 cm thick layer across the site, and the level of Cd contamination is uniform throughout the depth of this soil layer. The underlying clay layer does not have a detectable concentration of Cd. Despite this contamination, the soil at the site supported an abundant and diverse community of native and naturalized plant and animal species at the time of sampling in 2012.

Figure 3-1. Map of southern Ontario with the relative locations of Peterborough and Kingston. The Peterborough Gun Club property is shown in the black box, and the contaminated site is outlined in red.

3.3.2 Soil and Plant Collection

Fifty nine discreet soil samples (~200 g wet weight) were collected from a 0-20 cm depth in a grid pattern (at 10 m intervals in a 100×70 m grid) to determine the concentrations and distribution of Cd in the soil (Appendix A - Figure A-1). Soil samples were stored in Whirlpak® bags at −15°C until analysis. Subsequently, bulk soil was collected from known areas of low and high Cd contamination for characterization and use in greenhouse studies. Bulk soil was air dried, sieved to <2 mm, and homogenized using the one-dimensional Japanese slab cake method (Pitard, 1993) and stored at room temperature until use.

3.3.3 Greenhouse Study Experimental Design

Plants were grown in soil in triplicate in three treatments: i) a control treatment using clean uncontaminated potting soil (<1.0 $\mu g \cdot g^{-1}$ Cd; n=12); ii) a low (5.0±0.3 $\mu g \cdot g^{-1}$ Cd; n=12) Cd treatment; and iii) a high (16.5±1.2 $\mu g \cdot g^{-1}$ Cd; n=12) Cd treatment using bulk soil collected from the PGC site. Plants were grown in the Queen's University (Kingston, Ontario) greenhouse at 28.2±5.7 °C, and soil was maintained at 30% moisture. Plants were harvested after 50 days.

3.3.4 In-situ Field Study Experimental Design

Plots measuring 1 × 2 m were established in areas of low (8.9±2.0 $\mu g \cdot g^{-1}$ Cd) and high (19.3±1.9 $\mu g \cdot g^{-1}$ Cd) Cd concentrations at the PGC for a field-scale phytoextraction feasibility study. Plots were established by removing the existing vegetation and rototilling the soil to a depth of 20 cm to homogenize the soil. Seeds of *B. juncea* were sown in all plots at a density of 12 plants/m^2. Plots were watered weekly for three months, at which time six plants were randomly harvested from each plot. Roots were separated from shoots, washed, and stored in Ziploc® bags at −15 °C until analysis.

3.3.5 Analytical Procedures

3.3.5.1 Soil Characterization

Soil characterization included particle size distribution (PSD), pH, cation exchange capacity (CEC), organic matter (OM) content, total Cd concentration, and Cd fractionation. All analyses were conducted at the Analytical Services Unit at Queen's University except for PSD, which was conducted at the Royal Military College of Canada (RMCC) by mechanical sieving. The pH was measured by mixing dry soil sample with double deionized (DDI) water at a 1:2 ratio, manually shaking for two minutes, allowing the solid residue to settle, and measuring the supernatant with a pH meter. The OM content was determined by loss on ignition, and the CEC was measured using the sodium acetate method according to Laird and Fleming (2008).

3.3.5.2 Cadmium in Soils

For total Cd analysis, soil samples were air-dried, homogenized, sieved to <2 mm, and ground with a mortar and pestle. Subsamples (0.5 g) were measured into glass DigiPrep tubes with 7 mL double deionized (DDI) water, 2 mL HNO_3, and 6 mL HCl prior to digestion at 95°C for 330 minutes. Samples were cooled and diluted to 25 mL with DDI water, then filtered into glass tubes using Whatman® No. 40 filter paper.

The sequential extraction scheme of Tessier *et al.* (1979) was used to determine the fractionation of Cd in soil, and was modified to include a water-soluble fraction as in Ma and Rao (1997). Briefly, a 1 g soil sample was sequentially extracted into five operationally-defined fractions: F1) water-soluble (double de-ionized (DDI) water, agitated for 2 hr); F2) exchangeable (1 M $MgCl_2$ (pH 7.0)); F3) bound to carbonates (1 M NaOAc (pH 5.0 with HOAc)); F4) bound to iron (Fe) and manganese (Mn) oxides (0.04 M NH_2OH-HCl in 25% (v/v) HOAc (pH 2.0)); F5) bound to OM (0.02 M HNO_3 plus 30% H_2O_2 (pH 2.0)); F6) residual (*aqua regia* (3:1 HCl/HNO_3)). Following each extraction, the samples were centrifuged, the supernatants were filtered through 0.45 µm filters, and analyzed by inductively coupled plasma optical emission spectrometry (ICP-OES).

3.3.5.3 Cadmium in Plants

To analyze plant Cd concentrations, plants were washed with water, patted dry, roots were separated from shoots, and dried in a vented oven at 70°C for 24 h. Plant samples were ground using a coffee grinder, and 0.5 g subsamples were measured into glass crucibles and dry-ashed in a muffle

furnace for 20 minutes at 150 °C, one hour at 250 °C, and three hours at 500 °C. Ashed samples were digested on a hot plate under watchglass cover for four hours with 1 mL HNO_3 and 3 mL HCl. Watchglass covers were removed, two drops H_2O_2 were added to each sample, and sample volumes were reduced to 2 mL. Samples were diluted to 12.5 mL with DDI water and filtered into glass ICP tubes using Whatman® No. 40 filter paper. Plant samples collected from the greenhouse experiment were analyzed by ICP-OES, and plant samples collected from the *in-situ* field study were analyzed by inductively coupled plasma mass spectrometry (ICP-MS).

3.3.6 Quality Assurance/Quality Control

One method blank, one certified reference material, and two analytical duplicates were included for every 14 samples analyzed. The mean relative standard deviation for all duplicate samples was 7.6±9.2% (n=28). All blanks were below the ICP-OES and ICP-MS detection limits for Cd of 1.0 and 0.05 $\mu g \cdot g^{-1}$ Cd, respectively. Certified reference materials SS-2 and NIST-1570A had mean percent recoveries of 100.0±13.3% (n=6), and 90.6±11.6% (n=9), respectively, as compared to the lab certified values.

3.3.7 Statistical Analysis

Statistical analyses were performed using S+ version 8.2 (Tibco Software Inc., USA). All Cd concentrations and plant weights are reported on a dry weight (g) basis and recorded with the standard deviation of the mean. Data were tested for normality using the Kolmogorov-Smirnov test. All non-normal data were transformed. One-way analysis of variance (ANOVA) and two-sample t-tests were used with significance level α=0.05.

3.4 RESULTS AND DISCUSSION

3.4.1 Soil Characterization

While pH, PSD, and CEC were similar between low and high Cd soil, Cd concentration and OM content were significantly higher in the high Cd soil relative to the low Cd soil (Table 3-1). Cadmium concentrations in the soil ranged from <1.0 to 21.4 $\mu g \cdot g^{-1}$ Cd with a mean of 6.1±4.8 $\mu g \cdot g^{-1}$ Cd. This Cd heterogeneity is likely due to the uneven distribution of compost throughout the site, with areas that received more compost having a higher Cd concentration than areas that received less compost. As the compost is the source of Cd, soils with higher Cd concentrations also have higher OM contents. The OM contents of the PGC soils are considerably higher than average global soil OM content (<5%; Sparks, 2003; Schnitzer, 1991), and this likely contributed to the high CEC of the PGC soils (Asadi *et al.*, 2009).

Table 3-1. Soil characterization results for the control soil (potting soil) and low and high Cd soils collected from the PGC for use in the greenhouse experiment. Significant differences among treatments are indicated by different letters. All parameters were measured in triplicate except for pH and CEC.

	Particle size distribution (%)		**pH**	**CEC**	**OM**	**Cd**
	fine (<0.5 mm)	coarse (0.5-4.7 mm)		$cmol \cdot kg^{-1}$	(%)	$\mu g \cdot g^{-1}$
Control Soil	50.0 ± 4.5^{a}	45.8 ± 1.3^{a}	5.4	150.4	87.7 ± 1.4^{c}	<1.0
Low Cd Soil	59.6 ± 1.4^{b}	39.4 ± 1.1^{b}	7.6	38.1	13.9 ± 1.3^{a}	5.1 ± 0.3^{a}
High Cd Soil	56.0 ± 4.3^{ab}	43.3 ± 4.3^{ab}	7.7	46.2	24.5 ± 1.9^{b}	16.6 ± 1.2^{b}

3.4.2 Cadmium Fractionation in Soil

Cadmium in the low and high Cd soils was separated into six operationally defined fractions, with Cd bioavailability decreasing with each successive fraction (Xian, 1989). The Cd concentrations in each fraction are reported as a percentage of the total concentrations (Figure 3-2). Cadmium fractionation was not completed in triplicate for each soil due to time and cost limitations, but soils were thoroughly homogenized prior to sequential extraction analysis.

The proportion of Cd in the OM- and residual-bound fractions of the high Cd soil was twice that of the low Cd soil, while the proportion of Cd in the exchangeable and carbonate-bound fractions of the high Cd soil were half that of the low Cd soil. This indicates higher Cd bioavailability in the low Cd soil compared to the high Cd soil. There was little difference in the proportion of Cd bound to Fe/Mn oxides between the low and high Cd soil, and the Cd concentration in the water-soluble fraction was below the detection limit of the ICP-OES.

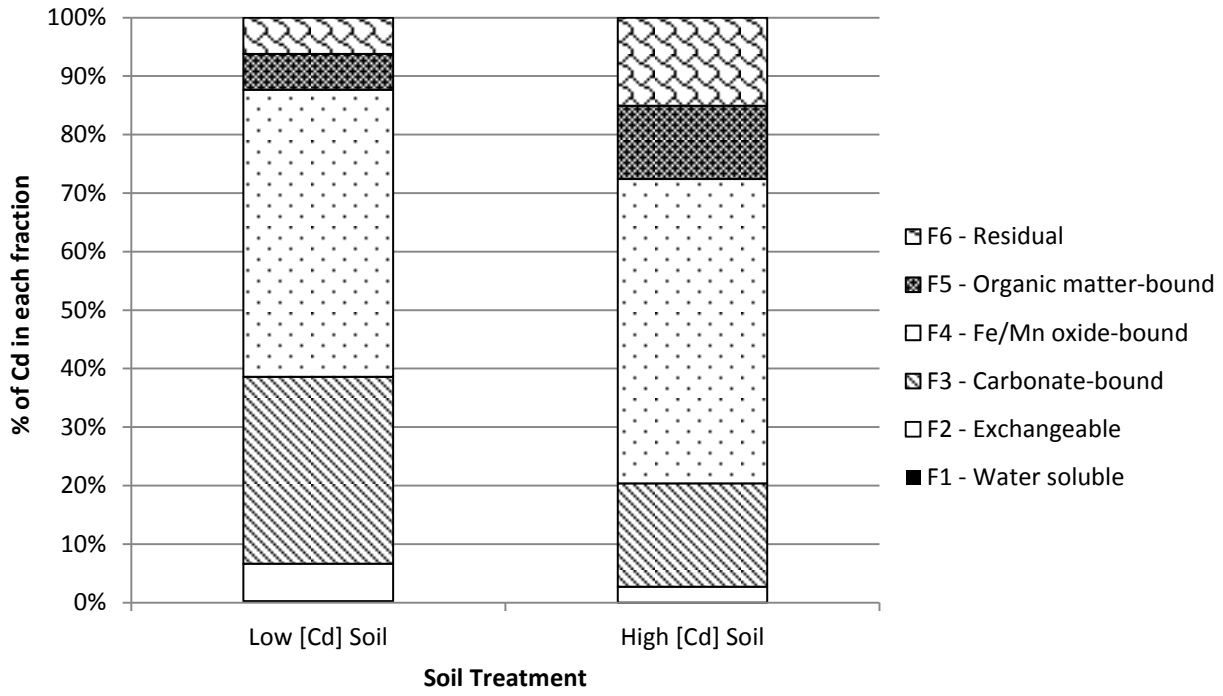

Figure 3-2. Fractionation of Cd in the low and high [Cd] soil into six operationally defined fractions. The Cd concentration in the F1 fraction was below the ICP-OES reporting limit of 0.025 mg/L.

3.4.3 Plant Biomass

Plants growing in all treatments in both the greenhouse and field studies did not display signs of reduced biomass, chlorosis, necrosis, leaf-rolls, or any other physical symptoms of Cd toxicity. Contrary to many published studies, three of the four plant species in the greenhouse experiment had a higher biomass in the high Cd soil relative to the low Cd soil (Mishra *et al.*, 2006; Ghosh and Singh, 2005; Shukla *et al.*, 2003). As the high Cd soil treatment also has a higher OM content, there were likely more nutrients and generally better growing conditions in the high compared to the low Cd soil.

In the greenhouse study, *P. compressa* shoot weight was significantly higher in the high Cd (12.0±0.1 g) compared to the low Cd (5.4±1.9 g) and control (3.5±0.3 g) treatments ($p<0.05$). *P. compressa* roots were too tangled to determine representative root weights for each treatment. *Brassica juncea* shoot weight was significantly higher in the control (7.9±2.7 g) compared to the low Cd treatment (1.4±0.3 g; $p<0.05$), but not the high Cd (3.8±1.3 g) treatment. There were no significant differences in *B. juncea* root weights among treatments ($p>0.05$). *Helianthus annuus* shoot weight was significantly higher in the control treatment (13.2±4.0 g) compared to the low Cd treatment (4.6±0.6 g; $p<0.05$), but not the high Cd treatment (7.6±2.5 g; $p<0.05$). *Helianthus annuus* root weights were significantly higher in the control (2.2±0.3 g) compared to the low (0.5±0.0 g) and high Cd (0.9±0.2 g) treatments ($p<0.05$). There were no significant differences in mean *C. album* shoot or root weights among treatments ($p>0.05$).

There were no significant differences in *B. juncea* shoot or root weights between treatments in the *in-situ* field study ($p>0.05$).

3.4.4 Plant Cadmium Uptake

3.4.4.1 Greenhouse Study

All plant species had low uptake of Cd into their roots (Figure 3-3), as indicated by a bioaccumulation factor (BAF; the ratio of the Cd concentration of the plant to that of the soil) of ≤0.5 for all species except for *P. compressa* in the low Cd soil (BAF 1.0±0.1). In the high Cd treatment, the root BAF for *P. compressa* was low (BAF 0.5±0.1), but significantly greater than those of *C. album*, *H. annuus*, and *B. juncea* ($p<0.05$).

The only plant to accumulate Cd in its shoots was *B. juncea*, and there was no significant difference in its mean shoot BAFs (both <0.5) between treatments ($p>0.05$).

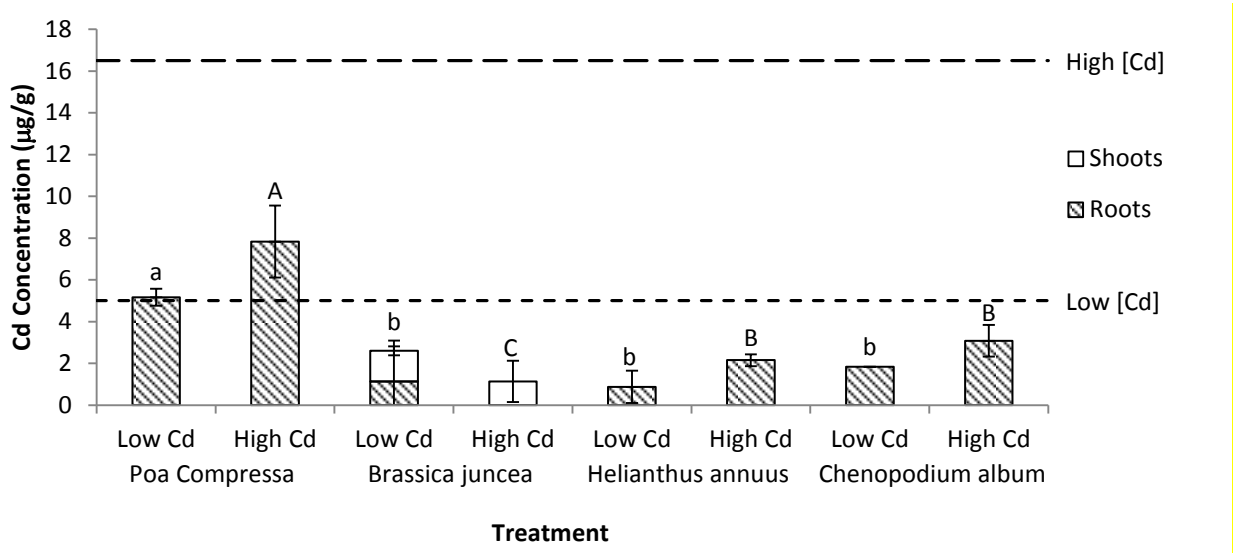

Figure 3-3. Mean Cd concentrations in plant root and shoot tissue for each treatment in contaminated soil. Soil concentrations for the Low Cd treatment and the High Cd treatment were 5.0±0.3 and 16.5±1.2 $\mu g \cdot g^{-1}$ Cd, as indicated by the dashed lines. The Cd concentrations in the control plants were all <1.0 $\mu g \cdot g^{-1}$ Cd and were not included in this figure. A one-way ANOVA was used to compare plant root Cd concentrations among species within the low treatment and the high treatment. Significant differences among species in the low Cd treatment are indicated by the lower-case letters, and significant differences among species in the high Cd treatment are indicated by the upper-case letters.

3.4.4.2 In-situ Field Study

Brassica juncea had low Cd uptake in both low and high Cd treatments (Figure 3-4), as indicated by mean root and shoot BAFs of <0.2 for both. There was no significant difference in *B. juncea* root BAFs between treatments ($p > 0.05$), but the mean shoot BAF was significantly higher in the low Cd (BAF 0.19±0.04) than in the high Cd treatment (BAF 0.10±0.03; $p < 0.05$).

There was no significant difference in *B. juncea* Cd uptake between treatments at the PGC for either roots or shoots ($p > 0.05$), though shoots accumulated significantly more Cd than roots in both the low and high Cd treatments ($p < 0.05$) (Figure 3-4).

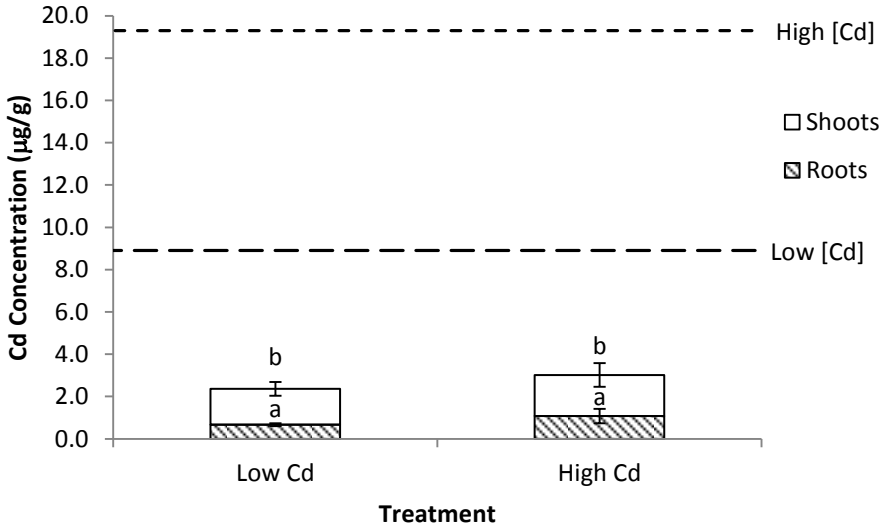

Figure 3-4. Mean Cd concentrations in root and shoot tissue for *B. juncea* growing in low and high Cd soil at the Peterborough Gun Club. Soil concentrations for the low and high Cd soils were 8.9±2.0 and 19.3±1.9 $\mu g \cdot g^{-1}$ Cd, respectively, as indicated by the dashed lines. A one-way ANOVA was used to compare plant Cd concentrations among treatments. Differing letters indicate significant differences.

Plant Cd uptake was low for all species in both the greenhouse and field experiments, with no plant having a BAF>1.0. These results are particularly surprising for *B. juncea*, a known Cd phytoextractor that usually achieves a BAF between 3-52 (Park *et al.*, 2011b; Lai *et al.*, 2008; Ishikawa *et al.*, 2006; Quartacci *et al.*, 2005; Ghosh and Singh, 2005; Su and Wong, 2004; Kumar *et al.*, 1995). Low Cd uptake by *B. juncea* indicates that there are factors limiting Cd bioavailability, and this is likely related to the fractionation of Cd in the PGC soil. The bulk of the Cd in the PGC soil is retained by the residual, OM, and Fe/Mn oxide fractions, and is not available for plant uptake (Figure 3-2).

The PGC soil has a high OM content relative to the global soils (Sparks, 2003) due to amendment with MSW/SS compost. Composted MSW/SS is composed primarily of stable, solid state OM that is resistant to microbial degradation (García-Gil *et al.*, 2000) and that can sorb Cd via inner-sphere complexation, wherein Cd is specifically sorbed to negatively-charged surface functional groups by covalent bonding (Brady and Weil, 2013). The formation of strong metal-organic complexes limits the release of metals into the soil solution and reduces their bioavailability (Smith, 2009).

Inorganic fractions of MSW/SS amendments (such as carbonates and Fe/Mn oxides) are also important in sorbing Cd and influencing its bioavailability (Achiba *et al.*, 2009; Zinati *et al.*, 2004; Li *et al.*, 2001; Illera *et al.*, 2000; Li *et al.*, 2000). In this experiment, 31.9% and 17.7% of Cd was sorbed to carbonates in the low and high Cd soil, respectively, and 49.1% and 52.0% of Cd was sorbed to Fe/Mn oxides in the low and high Cd soil, respectively. The presence of Fe/Mn oxides in MSW/SS amendments increases the ability of the amended soil to specifically adsorb Cd and limit its bioavailability (Chaney *et*

al., 2000). As the Fe/Mn oxide fraction retains the greatest proportion of Cd in both the low and high Cd soils, it likely plays an important role in limiting plant uptake of Cd in the PGC soil.

The results of this study agree with those reporting low mobility of Cd in soils amended with composted MSW/SS (Farrell *et al.*, 2010; Hanc *et al.*, 2009; Castaldi *et al.*, 2006). The high CEC and pH of the PGC soil is characteristic of composted MSW/SS (Pengcheng *et al.*, 2008; He *et al.*, 1995; Shiralipour *et al.*, 1992), and they confer on it a large sorptive capacity for metals such as Cd. The PGC soil's high pH buffering capacity is also characteristic of composted MSW/SS (García-Gil *et al.*, 2004), and it can moderate fluctuations in soil pH that might otherwise cause increased bioavailability of Cd from the exchangeable and carbonate-bound fractions (Wu *et al.*, 2004; Horckmans *et al.*, 2007). Soils that have these properties when amended with MSW/SS are those most likely to limit Cd solubility and plant uptake (McBride, 2003a). This study clearly indicates that phytoextraction is not a feasible method of remediating the PGC soil. However, low plant uptake of Cd limits Cd loading to the plant-based food chain, and may reduce animal exposure to Cd with positive implications for ecological risk. Further study is required to quantify the ecological risk associated with Cd from soils amended with MSW and SS compost.

4. ECOLOGICAL RISK ASSOCIATED WITH PHYTOEXTRACTION OF SOIL CONTAMINANTS

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4.1 ABSTRACT

Contaminant bioavailability is an important factor influencing the applicability of phytoextraction, and the potential for risk at contaminated sites. Low contaminant bioavailability leads to ineffective phytoextraction, while high bioavailability can lead to considerable contaminant uptake by plants, increasing the exposure of these and higher order receptors to contaminants. In this study, phytoextraction feasibility and ecological risk were investigated at two contaminated sites in Ontario. The first site (Peterborough) was contaminated with Cd, and the second site (Lindsay) with PCBs. At the Peterborough site, low Cd bioavailability limited phytoextraction effectiveness. A preliminary quantitative risk assessment (PQRA) based on ecological exposure to Cd indicated no potential for risk from oral exposure (hazard quotient <1), possibly reducing the need for remediation. Phytoextraction of PCBs using *Cucurbita pepo* was highly effective at the Lindsay site, and a PQRA based on ecological exposure indicated risk (hazard quotient > 1) from PCBs to five of seven receptor species examined. These results demonstrate the relationship between contaminant bioavailability, phytoextraction, and risk, and emphasize the importance of incorporating risk assessment into the remediation process.

KEY TERMS: Phytoavailability, receptor exposure, polychlorinated biphenyls, cadmium, organic matter, hazard quotient

4.2 INTRODUCTION

Contaminants accumulate in soils from a variety of sources including waste and emissions from industry, agricultural runoff, landfill leachate, and the application of soil amendments such as sludge (SS) and municipal solid waste (MSW) composts. They can be taken up by soil organisms and enter the food chain, where they may cause adverse health effects in humans, plants, and animals. Traditional methods of soil remediation, such as chemical washing and/or excavation followed by landfilling, are expensive and disruptive to the environment. Extensive work has therefore been completed in the past 20 years to study the potential of more environmentally friendly remediation alternatives, including phytoextraction. In theory, phytoextraction may provide an excellent remediation solution for contaminants that cannot be degraded (e.g., cadmium (Cd)) or are not effectively degraded (e.g., polychlorinated biphenyls (PCBs)) by soil microorganisms. However, phytoextraction success is greatly affected by contaminant bioavailability (Petruzzelli *et al.*, 2013), which is in turn affected by contaminant speciation and soil properties including pH, organic matter (OM) content, and cation exchange capacity (CEC), (Naidu, 2008; Domínguez *et al.*, 2008; Shuman, Dudka, and Das, 2002). If contaminants have low bioavailability, they will not be effectively accumulated and phytoextraction will not occur (Petruzzelli *et al.*, 2013). In this case, exposure to receptor organisms through the plant pathway is limited, and risk to these receptors might not exist. Conversely, if contaminants have high bioavailability and phytoextraction is effective, higher order ecological receptors might encounter risk from ingestion of contaminated plants. Therefore, the presence or absence of risk to ecological receptors at a site should be evaluated when considering phytoextraction as a remediation method. This is accomplished using ecological risk assessment (ERA) (Sample and Suter, 1994).

Ecological risk assessment is a useful tool to guide site remediation. Management of contaminated sites in North America is moving towards a risk-based approach (US EPA, 1997; CCME, 1996), with the goal of remediation being to reduce risk to an acceptable level (Johnson *et al.*, 1993). Even when contaminant concentrations at a site are several times higher than the prescribed federal or provincial guidelines, factors such as soil properties and contaminant speciation can limit contaminant bioavailability and reduce the potential for risk at a site. In this case, invasive remediation techniques may cause more harm than good (Efroymson *et al.*, 2004) as they generally involve disturbing the established ecological community (i.e. excavation and landfill) or mobilizing contaminants that are otherwise stable in the soil (i.e. soil washing).

In this study, the ecological risk associated with food and soil ingestion at two phytoextraction sites located in Southern Ontario, Canada, is calculated with a preliminary quantitative risk assessment (PQRA) (Environment Canada (EC), 2012; CCME, 1996; US EPA, 1998) using site-specific contaminant concentrations in plants and soil invertebrates derived in earlier studies. The sites were chosen for their proximity to each other (45 km) and their differences in contaminant type and soil quality. The first site is located in Peterborough and is characterized by an organic matter (OM)-rich soil contaminated with Cd from the application of a MSW/SS compost, and the second is a brownfield site located in Lindsay that is characterized by a degraded soil contaminated with PCBs.

4.3 MATERIALS AND METHODS

4.3.1 Site #1: Peterborough, ON (Cd)

In 2010, a Cd-contaminated MSW/SS compost was used to improve the soil in a 0.805 ha area at a firing range located near Peterborough, Ontario, Canada. Subsequently, it was determined that the soil

Cd concentrations ranged from <1.0-21.4 $\mu g \cdot g^{-1}$, exceeding the site condition standard of 1.2 $\mu g \cdot g^{-1}$ Cd set by the Ontario Ministry of the Environment (MOE) (MOE, 2011). The high OM content, high cation exchange capacity (CEC), and neutral pH of the soil indicated that the soil was in good condition (Table 4-1; Parisien *et al.*, Chapter 3 – this thesis), and the site supported a diverse community of native and naturalized plant and animal species.

Table 4-1. Properties of soil with low and high Cd concentrations from the Peterborough Site (Parisien *et al.*, Chapter 3 – this thesis). Asterisks indicate significant differences between soils.

	Particle size distribution (%)		**pH**	**CEC**	**OM**	**Cd**
	fine	coarse	(H_2O)	cmol/kg	(%)	$\mu g \cdot g^{-1}$
Low [Cd] Soil	59.6±1.4	39.4±1.1	7.6	38.1	13.9±1.3	5.1±0.3
High [Cd] Soil	56.0±4.3	43.3±4.3	7.7	46.2	24.5±1.9*	16.6±1.2*

A Cd phytoextraction feasibility study was completed in 2014 by Parisien *et al.* (Chapter 3 – this thesis). Briefly, one native (*Chenopodium album*) and three naturalized (*Brassica juncea*, *Helianthus annuus*, and *Poa compressa*) plant species were grown in triplicate in a control soil (potting soil; <1.0 $\mu g \cdot g^{-1}$ Cd; n=12), soil with low (5.0±0.3 $\mu g \cdot g^{-1}$ Cd; n=12) and high Cd concentrations (16.5±1.2 $\mu g \cdot g^{-1}$ Cd; n=12), both collected form the Peterborough site. The bioaccumulation factor (BAF; the ratio of the contaminant concentration in the plant to the contaminant concentration in the soil) was <1 for all species including *B. juncea*, a plant that usually has a BAF between 3-52 (Park *et al.*, 2011b; Lai *et al.*, 2008; Ishikawa *et al.*, 2006; Quartacci *et al.*, 2005; Ghosh and Singh, 2005; Su and Wong, 2004; Kumar *et al.*, 1995). Sequential extraction analysis indicated that Cd was bound primarily by soil fractions with relatively low bioavailability (Fe/Mn oxides, OM, and residual fractions). From this data it was determined that phytoextraction was not appropriate for this site (Parisien *et al.*, Chapter 3 – this thesis).

An earthworm bioavailability experiment was conducted to determine the exposure of higher order ecological receptors to Cd from earthworm ingestion. Earthworms (*Eisenia fetida*) purchased from the 'The Worm Factory' (Westport, ON) were added to 1.32 L pots in triplicate at a density of 20 worms/750 g soil. Soils used in this experiment were the same as those used in the phytoextraction feasibility study (Parisien *et al.*, Chapter 3 – this thesis), and included the same control, low Cd, and high Cd treatments described above. Soil moisture was maintained at 30% and worms were harvested after 50 days.

Analytical Procedures: Earthworms were washed with double de-ionized (DDI) water, patted dry, and depurated at 4°C for 72 h prior to being dried in a vented oven at 25°C for 24 h. Earthworm samples were chopped and homogenized, and 0.5 g subsamples were added to digestion tubes with 2 mL HNO_3 and 6 mL HCl and digested overnight on a hot plate at 200°C. Samples were diluted to 12.5 mL with DDI water, filtered into glass ICP tubes using Whatman® No. 40 filter paper, and analyzed by inductively coupled plasma optical emission spectroscopy (ICP-OES).

Quality Assurance and Quality Control (QA/QC): One blank, one certified reference material, and two analytical duplicate samples were included for every 14 samples analyzed. The certified reference material (Tort-2; NRCC, 1994) was within the accepted range, and had a percent recovery of 83.5 (n=1). Blank samples were below the ICP-OES detection limit (<1.0 $\mu g \cdot g^{-1}$ Cd). The mean relative standard deviation for duplicate samples was 5.5±1.5% (n=2).

Statistical Analysis: Statistical analyses were performed using S+ version 8.2 (Tibco Software Inc., USA). Earthworm Cd concentrations are reported on a dry weight (g) basis and recorded with the standard deviation of the mean. Data were found to be non-normal using the Kolmogrov-Smirnov test, and were therefore transformed. A one way analysis of variance (ANOVA) was used with significance level α=0.05.

4.3.2 Site #2: Lindsay, ON (PCBs)

The second study site was a former industrial site in Lindsay, ON that had soil contaminated with PCB Aroclor-1248 (Low *et al.*, 2010). The PCB concentrations at the site ranged from <2.1 to 22.5 $\mu g \cdot g^{-1}$ PCB (Low *et al.*, 2010). The soil was predominantly clay with 4.3% total organic carbon (Low *et al.*, 2011). Several field trials were completed at the site to study PCB phytoextraction with pumpkins and native colonizers (Ficko *et al.*, 2011; Greenwood *et al.*, 2011; Low *et al.*, 2011; Ficko *et al.*, 2010; Low *et al.*, 2010).

Ecological risk assessment inputs for the Lindsay site were generated by Smith (2012). Briefly, *Cucurbita pepo.* ssp *pepo* var. Howden was planted in triplicate in PCB-contaminated soil (4.8±1.6 $\mu g \cdot g^{-1}$; n=24) from the site with earthworms (*E. fetida*), fertilizer, and perlite. *C. pepo* total plant BAFs for PCBs were >15. The results of Smith (2012) corroborated the results of previous research demonstrating the effectiveness of using phytoextraction with *C. pepo* to remediate PCB-contaminated soil at this site (Ficko *et al.*, 2011; Greenwood *et al.*, 2011; Low *et al.*, 2011; Low *et al.*, 2010).

4.3.3 Ecological Risk Assessment

4.3.3.1 Receptor Organisms

Risk was calculated for the same seven receptor species at both sites as they are <50 km apart. The receptors included: two herbivorous mammals (eastern cottontail and meadow vole); two omnivorous mammals (raccoon and deer mouse); one invertivorous mammal (short-tailed shrew); one omnivorous bird (American Robin); and one invertivorous bird (American woodcock) (Table 4-2). Species were selected based on taxonomic guild, trophic level, life history traits, and relevance to the Peterborough/ Lindsay region.

Table 4-2. Ecological receptors included in the ecological risk assessments of the Peterborough and Lindsay sites.

	Common Name	Species Name
Herbivorous Mammal	Eastern Cottontail	*Sylvilagus floridanus*
	Meadow Vole	*Microtus pennsylvanicus*
Omnivorous Mammal	Raccoon	*Procyon lotor*
	Deer Mouse	*Peromyscus maniculatus*
Invertivorous Mammal	Short-Tailed Shrew	*Blarina brevicauda*
Omnivorous Bird	American Robin	*Turdus migratorius*
Invertivorous Bird	American Woodcock	*Scolopax minor*

Eastern Cottontail (*Sylvilagus floridanus*)

Eastern cottontails are widely distributed across North America, occupying habitat with dense, woody vegetation, and digging underground dens in abandoned fields (Chapman and Litvaitus, 2003). Their home range varies between 0.8 ha (Trent and Rongstad, 1974) and 5.98 ha (Bond *et al.*, 2001), with males having a higher home range than females (especially during the breeding season). Cottontails consume a large quantity of herbaceous plants during the growing season, and woody plants during the winter months (Chapman and Litvaitus, 2003).

Meadow Vole (*Microtus pennsylvanicus)*

Meadow voles are abundant in many types of field habitat across North America including grass-sedge marshes, old-field successional meadows, and grasslands (Madison, 1980; Getz, 1961). They occupy habitats as large as 0.085 ha (Getz, 1961) and as small as 0.0002 ha during the winter months (Douglass, 1976). Meadow voles' diets consist of shoots and leaves during the spring and summer, and roots and seeds during the fall and winter (Lindroth and Batzli, 1984). In this study, meadow voles represent small mammals exposed to contaminants primarily through plant ingestion.

Raccoon *(Procyon lotor)*

Raccoons are present throughout North America. They occupy a wide range of habitats, including forests and urban areas (Bartoszewicz *et al.*, 2008). Raccoons consume plants, mammals, insects, earthworms, and amphibians (Bartoszewicz *et al.*, 2008; Hamilton, 1951). Their home range varies depending on resource density, though most occupy a home range ≥ 39 ha (Lotze, 1979). Raccoons can be exposed to contaminants through consumption of contaminated plants and earthworms, as well as through incidental soil ingestion associated with these food items.

Deer Mouse (*Peromyscus maniculatus)*

Deer mice have highly variable home range sizes (0.012-0.935 ha) in field habitats across North America (Sheppe, 1966; Whitaker, 1966). They are omnivorous mammals that consume plants and invertebrates, including earthworms (Sheppe, 1966; Whitaker, 1966), and might therefore be exposed to contaminants through ingestion of plant and animal material.

Short-Tailed Shrew (*Blarina brevicauda)*

Short-tailed shrews are insectivorous mammals that live in burrows in the soil beneath vegetative cover and occupy a home range between 0.03-0.22 ha (Platt, 1976). Their fast metabolism requires them to consume a large quantity of food per day (Platt, 1974), primarily in the form of earthworms, plants, and small vertebrates (Hamilton, 1941). Because they live in the soil and their primary food item also lives in (and consumes) soil, short-tailed shrews may experience high exposure to soil contaminants.

American Robin *(Turdus migratorius)*

American robins are abundant throughout North America, and occupy relatively small home ranges (0.11-0.21 ha; Young, 1955; Howell, 1942) in suburban areas (Howell, 1942). They are frugivores during the fall and winter, and switch to an omnivorous diet composed mostly of invertebrates for the spring and summer (Wheelwright, 1986). As earthworms comprise a portion of their spring and summer diet (Howell, 1942), robins may be exposed to contaminants due to earthworm accumulation of contaminants. Earthworm consumption may also increase contaminant exposure due to incidental ingestion of soil on the surface of the earthworm body, and as foraging for them requires robins to probe the earth with their beaks.

American Woodcock *(Scolopax minor)*

American woodcocks are ground-nesting birds that occupy large home ranges (3.1-73.6 ha; Hudgins *et al.* 1985) in forest and field habitats with sufficient soil moisture to support abundant earthworm populations, as these invertebrates make up a large fraction of their diet (Dessecker and McAuley, 2001). Because woodcocks probe the soil for food items (Rabe, Prince, and Beaver, 1983) and primarily consume invertebrates that live in and eat soil, they are more exposed to contaminants than birds with other dietary and foraging characteristics.

4.3.3.2 Exposure Pathway Investigation

Air exposure was not considered to be a relevant exposure pathway at either site, as Cd is non-volatile and air measurements detected no PCBs. Water was also not considered to be a relevant exposure pathway, as there was no water source at either site. Furthermore, Cd had low water solubility at the Peterborough site due to sorption to low bioavailability soil fractions, and its downward movement through the soil profile is further limited by a clay layer beneath the contaminated top soil (Parisien *et al*.,

Chapter 3 – this thesis). As PCBs exhibit low water solubility due to their hydrophobicity, the potential for off-site migration and mobilization of contaminants to ground water are low at both sites. Contaminant exposure to ecological receptors from ingestion of food items was assessed using concentrations of Cd and PCBs in plants and earthworms.

Plant Bioavailability

The Cd and PCB concentrations used to represent exposure from plant ingestion were developed by taking the mean plant Cd and PCB concentrations from the treatments with the highest plant uptake in the phytoextraction feasibility studies, as they represent the most conservative contaminant concentrations. These concentrations were converted to wet weight (ww) using the conversion factor 0.15 (assumes a moisture content of 85%) (US EPA, 1993) to yield plant concentrations of 0.53 $\mu g \cdot g^{-1}$ for Cd and 9.75 $\mu g \cdot g^{-1}$ for PCBs.

Earthworm Bioavailability

Cd-Contaminated Soil: The earthworm Cd concentration used in this risk assessment was established by taking the average Cd concentrations of earthworms harvested from the low and high Cd treatments of the earthworm bioavailability experiment as there was no significant difference between them. This yielded an earthworm Cd concentration of 39.5 $\mu g \cdot g^{-1}$ (dry weight (dw)), which was converted to ww using the conversion factor for earthworms of 0.16 (assumes a moisture content of 84%) (US EPA, 1993) to yield 6.32 $\mu g \cdot g^{-1}$ Cd (ww).

PCB-Contaminated Soil: The earthworm PCB concentration used in this risk assessment is the mean concentration (110 $\mu g \cdot g^{-1}$ PCBs (dw)) from the PCB phytoextraction study (Smith, 2012). This concentration was converted to ww using the conversion factor for earthworms of 0.16 (assumes a moisture content of 84%) (US EPA, 1993) to yield 17.6 $\mu g \cdot g^{-1}$ PCBs (ww).

4.3.3.3 Risk Calculations

A preliminary quantitative risk assessment was conducted for both sites using the quotient method (EC, 2012; CCME, 1996; US EPA, 1998). Each receptor's estimated daily intake (EDI) of Cd and PCBs was calculated using the most conservative estimates from the literature in the equation below from Sample and Suter (1994).

$$Estimated\ Daily\ Intake = \frac{[\sum(C_i \times F_i \times FIR_i) + (C_s \times SIR)] \times AUF \times BA \times EF}{BW}$$

C_i = Contaminant concentration of food item (i) ($\mu g \cdot g^{-1}$ ww)
F_i = Fraction of the diet composed of food item (i) (unitless)
FIR_i = Food Ingestion Rate for food item (i) ($kg \cdot day^{-1}$ ww)
C_s = Soil contaminant concentration ($\mu g \cdot g^{-1}$ dw)

SIR = Soil ingestion rate (kg·day^{-1} dw)
AUF = Area use factor (unitless)
BA = Bioavailability (1)
EF = Exposure Frequency (weeks·year^{-1})
BW = Bodyweight (kg ww)

All exposure factors used in the EDI equation are shown in Table 4-3. The same plant and earthworm contaminant concentrations were used for all receptor species, while the remaining exposure factors were receptor-specific.

Table 4-3. Exposure factors used to determine the receptor-specific estimated daily intakes of Cd and PCBs at the Peterborough and Lindsay sites, respectively.

	Receptor						
Exposure Factors (EFs)	Eastern Cottontail Rabbit	Meadow Vole	Raccoon	Deer Mouse	Short-Tailed Shrew	American Robin	American Wood-cock
EFs specific to Cd							
C_i (μg·g^{-1})							
Plant	0.53	0.53	0.53	0.53	0.53	0.53	0.53
Worm	n/a	n/a	6.32	6.32	6.32	6.32	6.32
C_s (μg·g^{-1})	21.4	21.4	21.4	21.4	21.4	21.4	21.4
EFs specific to PCBs							
C_i (μg·g^{-1})							
Plant	9.75	9.75	9.75	9.75	9.75	9.75	9.75
Worm	n/a	n/a	n/a	17.6	17.6	17.6	17.6
C_s (μg·g^{-1})	4.8	4.8	4.8	4.8	4.8	4.8	4.8
Receptor-Specific EFs							
F_i (%)							
Plant	1.00[d]	1.00[e]	0.587[e]	0.640[k]	0.171[e]	0.298[e]	0.105[e]
Worm	n/a	n/a	7.20E-02[e]	1.70E-02[e]	0.418[e]	0.150[e]	0.678[e]
FIR (kg·day^{-1})	0.272[c]	5.26E-03[g]	0.961[c]	1.01E-02[j]	7.95E-03[e]	0.117[l]	0.130[e]
SIR (kg·day^{-1})	2.57E-03[d]	1.89E-05[h]	8.99E-03[h]	1.99E-05[h]	2.21E-05[h]	8.36E-04[h]	1.33E-03[h]
AUF[a]	1.00[e]	1.00[e]	2.10E-02[e]	1.00[e]	1.00[e]	1.00[e]	0.260[e]
BA[b]	1.00	1.00	1.00	1.00	1.00	1.00	1.00
BW (kg)	1.15[f]	1.60E-02[i]	3.67[e]	1.30E-02[e]	1.60E-02[e]	7.70E-02[e]	0.134[e]
EF	1.00	1.00	1.00	1.00	1.00	0.750[e, m]	0.673[e, n]

[a] If size (ha) of HR < site, AUF=1; if HR > site, AUF = site/HR
[b] 100% contaminant bioaccessibility is assumed for all receptors
[c] Kroner and Cozzie (1999)
[d] Extrapolated from Arthur and Gates (1988)
[e] US EPA (1993)
[f] Rongstad (1966)
[g] Extrapolated from Nagy (1987)

[h] Beyer *et al.* (1984)
[i] Merritt (1986); Barrett and Stueck (1976)
[j] Millar (1981)
[k] Jameson (1952)
[l] Calculated from Scorupa and Hothem (1985)
[m] Speirs (1953)
[n] Sepik *et al.* (1993)

The receptor's EDI was divided by its toxicity reference value (TRV) to yield the hazard quotient (HQ). The TRVs used in this PQRA are the contaminant concentrations in a medium at which a receptor demonstrates no adverse effects (NOAEL; No Observed Adverse Effects Level) and were derived from laboratory-based NOAELs by applying allometric scaling (as described in Sample *et al.*, 1996).

4.4 RESULTS AND DISCUSSION

4.4.1 Risk Characterization

4.4.1.1 Site # 1: Peterborough

Preliminary quantitative risk assessments assume that if the HQ < 1, there is no potential for risk to that receptor group, and if HQ > 1, there is potential for risk (CCME, 2006). Hazard quotient calculations indicate that there is no risk from Cd at the Peterborough site to any of the receptors involved in this PQRA (Table 4-4), despite soil Cd concentrations more than five times higher than the Canadian Council of Ministers of the Environment (CCME)'s soil quality guideline (SQG_E) for the protection of environmental health (SQG_E = 3.8 $mg \cdot kg^{-1}$) (CCME, 1999).

Risk to herbivores was limited by low Cd bioavailability to plants. This is supported by risk calculations that were made for a hypothetical case wherein the plant Cd concentration was equal to a BAF=1.00 (5.35 $\mu g \cdot g^{-1}$ Cd). In this case, the HQ surpasses 1.00 for the American robin, the eastern cottontail, and the deer mouse, indicating risk to these species due to increased Cd exposure from plant ingestion.

The plant bioavailability of Cd exhibited by this compost-based soil is low (Chen and Cutright, 2001; Gupta and Sinha, 2007). As OM can sorb Cd by cation exchange and reduce the water soluble fraction of Cd in the soil (the portion available for plant uptake; Shuman, Dudka, and Das, 2002), the high OM content of the soil is likely the factor limiting Cd bioavailability at this site. As this PQRA indicates no current potential for risk at the site, a leave-in-place strategy could be applied, as it has minimal financial and environmental costs. Although studies have demonstrated that the concentration of water soluble Cd in MSW/SS compost-amended soil can remain stable for several years (Brown *et al.*, 1998; Hyun *et al.*, 1998), OM breaks down over time and plant Cd bioavailability may increase in the future as it desorbs from degrading OM (McBride, 2003b). Therefore, a long-term monitoring program should be implemented at the site to monitor future ecological risk.

Table 4-4. Receptor-specific estimated daily intakes and mammalian and avian toxicity reference values for Cd and PCBs, as well as the hazard quotient for each receptor at the Peterborough and Lindsay sites. The EDIs were calculated using the exposure factors in Table 3, and the TRVs for Cd and PCBs were extrapolated from laboratory tests. The HQ was obtained by dividing the EDI by the TRV, and bolded numbers indicate potential ecological risk.

	Receptor						
	Eastern Cottontail Rabbit	Meadow Vole	Raccoon	Deer Mouse	Short-Tailed Shrew	American Robin	American Wood-cock
Peterborough, ON							
Cadmium							
EDI	0.174	7.10E-05	5.32E-03	0.381	1.39	1.44	0.621
TRV	0.716[a]	2.09[a]	0.536[a]	2.20[a]	2.09[a]	1.45[b]	1.45[b]
HQ	0.243	3.40E-05	9.93E-03	0.173	0.664	0.990	0.428
Lindsay, ON							
PCBs							
EDI	2.32	2.91E-04	3.87E-02	5.09	4.49	6.36	1.75
TRV	0.125[c]	0.364[c]	0.094[c]	0.384[c]	0.364[c]	0.180[d]	0.180[d]
HQ	**18.5**	8.00E-04	0.412	**13.2**	**12.3**	**35.3**	**9.73**

[a] Sutou *et al.* (1980), as cited in Sample *et al.* (1996)
[b] White and Finley (1978), as cited in Sample *et al.* (1996)
[c] Aulerich and Ringer (1977), as cited in Sample *et al.* (1996)
[d] Dahlgren *et al.* (1972), as cited in Sample *et al.* (1996)

4.4.1.2 Site # 2: Lindsay

In contrast, at the Lindsay site the American robin, American woodcock, short tailed shrew, eastern cottontail, and deer mouse are potentially at risk, while the meadow vole and raccoon are not (Table 4-4). The robin has the highest potential for risk, followed by the cottontail, deer mouse, short-tailed shrew, and American woodcock. The robin, woodcock and shrew have the highest earthworm intake, and this likely contributed to their high HQs as earthworms accumulated a high PCB burden. The deer mouse has a high food ingestion rate relative to the other small mammalian receptors, and therefore has high exposure to PCBs through food ingestion. The eastern cottontail consumes a large quantity of plant material and has less resistance to contaminant toxicity due to its low metabolic rate relative to smaller mammals (Sample *et al.*, 1996), and these factors might have contributed to its high HQ.

As there is potential for risk from PCBs to five of seven tested receptor groups at this site, precautionary measures must be taken to limit exposure of these receptors. This can be accomplished by netting or fencing, though it is difficult to limit the presence of small mammals such as shrews, which have small home ranges and might spend most of their time in a phytoextraction plot (Angle and Linacre, 2005). Identifying potential risks early in the remediation process can aid in determining the suitability of phytoextraction to remediate a site. While phytoextraction may pose temporary risk to ecological receptors, it may still be more acceptable than more traditional remediation methods such as excavation and landfilling, a process that is both expensive and environmentally destructive (Angle and Linacre,

2005). With proper implementation of safety precautions, risks can be managed in order to produce a safe environment for receptors while maximizing plant extraction of contaminants.

4.5 CONCLUSIONS

Bioavailability of a contaminant is essential for successful phytoextraction. Low plant availability of Cd in the Peterborough soil due to sorption to low bioavailability soil fractions prevented plants from extracting and accumulating a sufficient amount of Cd for effective phytoextraction. This low Cd availability also reduced plant uptake enough to eliminate the potential for ecological risk to receptors at the site, which in turn reduces the need for remedial action.

High bioavailability of PCBs in the Lindsay soil facilitated phytoextraction of this contaminant. However, high plant uptake of PCBs also increased receptor exposure to this contaminant, resulting in the potential for risk for five of seven receptor groups evaluated. Determining what receptor groups might be at risk due to phytoextraction is an important component of the planning process, as it allows site managers to implement appropriate risk management strategies to control risk during remediation.

The two sites considered in this PQRA are at opposite ends of the spectrum, and they emphasize the relevance of risk assessment to the remediation process as well as the relationship between contaminant availability, phytoextraction, and ecological risk. This study indicates that the exceedance of generic soil guidelines is not enough to determine whether a contaminated site poses ecological risk; contaminant bioavailability should be considered before bypassing site-specific risk assessment in favor of immediate remediation.

5. EFFECT OF BIOCHAR ON CADMIUM FRACTIONATION WITH ORGANIC MATTER DEGRADATION IN A SOIL AMENDED WITH MUNICIPAL SOLID WASTE AND SEWAGE SLUDGE COMPOST

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5.1 ABSTRACT

Biochar is a stable form of organic matter (OM) that can sorb and immobilize metals such as cadmium (Cd), potentially limiting its long-term mobility and bioavailability in soil. A laboratory experiment was conducted to investigate the effect of biochar on Cd mobility as OM degrades in a soil amended with municipal solid waste (MSW) and sewage sludge (SS) compost. Soil contaminated with Cd (21.7 $\mu g \cdot g^{-1}$) from MSW/SS compost application at a site in Peterborough, Ontario, was placed into glass jars in a control treatment (soil only; n=3) and a biochar treatment (soil amended with 4% biochar (dry weight); n=3) and maintained at 40°C for 185 days. The OM content significantly decreased (from 30.7±1.5% to 27.4±0.6%) in the control treatment between the start and end of the experiment, but did not change significantly in the biochar treatment. Although there were no significant changes in the proportion of Cd in each soil fraction for either treatment, the trends in Cd fractionation suggest that Cd is moving from soil fractions with high bioavailability to those with low bioavailability. The length of this experiment was likely too short for significant trends to develop, and it should therefore be repeated for a longer period of time to properly characterize the changes in Cd fractionation that occur as OM degrades.

Key words: Cadmium, MSW/SS compost, biochar, organic matter, bioavailability, sequential extraction, soil ageing

5.2 INTRODUCTION

Municipal solid waste (MSW) and sewage sludge (SS) composts are widely applied to degraded soils to improve soil quality and plant yield (Singh and Agrawal, 2008). Though concerns have been raised regarding their high concentrations of metals such as Cd, research has demonstrated low bioavailability of metals associated with these composts (Parisien *et al.*, Chapter 3 – this thesis; Hanc *et al.*, 2009; Wen *et al.*, 2002; Gigliotti *et al.*, 1996). Composting causes metals to shift from soil fractions with relatively high bioavailability (water soluble, exchangeable, and carbonate-bound) to those with relatively low bioavailability (Fe/Mn oxide-, OM-, and residual-bound) (He *et al.*, 2009; Castaldi *et al.*, 2006; Greenway and Song, 2002).

While Cd bioavailability is initially low in soils amended with MSW/SS compost, it increases over time as OM degrades (McBride, 2003b; Chang *et al.*, 1997) or if soils become water-logged and reducing conditions cause Fe/Mn oxides to dissolve and release Cd (Brown *et al.*, 1989). Little research has focused on the long-term availability of Cd in MSW/SS compost-amended soils, and the results are mixed for studies of non-composted MSW/SS; while some find little to no change in Cd bioavailability over time (McGrath *et al.*, 2000; McGrath and Cegarra, 1992; Brown *et al.*, 1998; Hyun *et al.*, 1998), others report high uptake into plants several years after the termination of MSW/SS application (McGrath *et al.*, 2000; Sloan *et al.*, 1997).

The lack of long-term data necessitates precautionary measures or long-term monitoring at sites contaminated with Cd from the application of MSW/SS composts in order to limit possible future risk to human and ecological receptors. Because the potential for increased risk from these soils stems primarily from the degradation of OM or the dissolution of Fe/Mn oxides, one possible method of limiting the long-term mobility of Cd is to amend soil with biochar. Biochar is the carbon-rich product of biomass pyrolysis, and it is an emerging technology for the remediation of contaminated soils (Denyes *et al.*, in press; Beesley *et al.*, 2011). Biochar has a high density of negatively charged functional groups on its surface that sorb cations such as Cd (Uras *et al.*, 2012; Wu *et al.*, 2012). Amending soil with biochar shifts Cd from the water-soluble fraction of soil to the carbonate-, OM-, and Fe/Mn oxide-bound fractions (Bian *et al.*, 2014; Park *et al.*, 2011b). While several studies have demonstrated the ability of biochar to immobilize Cd in soil and reduce its uptake into plants (Bian *et al.*, 2014; Suppadit *et al.*, 2012; Zhang *et al.*, 2012; Beesley and Marmiroli, 2011; Cui *et al.*, 2011), there is a lack of research examining the effect of biochar on long-term Cd mobility and bioavailability.

This study will evaluate the potential of biochar to sorb Cd and limit its long-term mobility and bioavailability in soil contaminated with Cd from the application of a MSW/SS compost at a site in Peterborough, Ontario. The Cd in this soil has low bioavailability as it is bound primarily by Fe/Mn oxides, OM, and residual soil components (Parisien *et al.*, Chapter 3 – this thesis). The results of an ecological risk assessment have indicated that there is currently no potential for risk to receptors at this site (Parisien *et al.*, Chapter 4 – this thesis). However, because Cd solubility may increase in the future due to dissolution of Fe/Mn oxides and OM degradation, this study investigates long-term Cd mobility and bioavailability.

5.3 METHODS AND MATERIALS

5.3.1 Site Description

In 2010, a MSW/SS compost originating from a Kingston, ON company was used to restore a disturbed 0.805 ha area at the Peterborough Gun Club (PGC) in Peterborough, Ontario. Native plants

were planted in the restored area to encourage the establishment of a native ecological community (BioLogic, 2009). The MSW/SS compost was later discovered to have Cd concentrations up to 21.4 $\mu g \cdot g^{-1}$, exceeding the Ontario Ministry of the Environment (MOE) site condition standard of 1.2 $\mu g \cdot g^{-1}$ Cd (MOE, 2011). Despite the high soil Cd concentrations, the site supported a diverse community of native and naturalized plant species.

5.3.2 Experimental Design

Soil was aged under accelerated conditions to promote microbial degradation of OM. Four hundred g (wet weight) of PGC soil with a Cd concentration of 21.7±1.5 $\mu g \cdot g^{-1}$ (n=3) were added to 1 L glass jars (n=3) in a control treatment (soil only) and a biochar treatment (soil amended with biochar at 4% dry weight). Biochar was obtained from Burt's Greenhouses in Odessa, Ontario, and was fully characterized in Denyes *et al.* (2014). Analyses included pH, cation exchange capacity, particle size distribution, specific surface area, organic carbon, moisture percentage, and proximate and ultimate analysis. Soils were maintained at ~60% soil moisture (Donnelly *et al.*, 1990) in an oven at 40ºC with continuous oxygen flow, and mixed twice per week for 185 days. After harvesting, soils were air-dried, homogenized, sieved to <2 mm, and ground for further analysis.

5.3.3 Analytical Methods

Soils were measured for total Cd by measuring 0.5 g subsamples into glass DigiPrep tubes with 7 mL double de-ionized (DDI) water, 2 mL HNO_3, and 6 mL HCl prior to digestion at 95°C for 330 minutes. Samples were cooled and diluted to 25 mL with DDI water, filtered into glass ICP tubes using Whatman® No. 40 filter paper, and analyzed by ICP-OES.

Cadmium was sequentially extracted from soils before and after 185 days of ageing according to the procedure of Tessier *et al.* (1997), with the addition of an extraction step for the water-soluble fraction as described in Ma and Rao (1997) (Table 5-1). This sequential extraction scheme partitions Cd into six soil fractions: 1) water-soluble; 2) exchangeable; 3) carbonate-bound; 4) Fe/Mn oxide-bound; 5) Organic matter-bound; and 6) residual. Cadmium bioavailability decreases with each successive extraction step, with the water-soluble fraction having the highest Cd bioavailability and the residual fraction having the lowest (Dudka and Chlopecka, 1990; Xian, 1989). Sequential extraction was carried out on 1 g soil samples in 50 mL polyethylene centrifuge tubes. Following each extraction, the samples were centrifuged at 3,000 rpm for 5 min, and the supernatants was filtered through 0.45 µm filters and analyzed by ICP-OES.

Table 5-1. Method used for sequential extraction of Cd from soils.

Fraction	Phase	Reagent	Time/Temperature
1	Water-soluble	15 mL double distilled (DDI) water	Shake 2 h at 22°C
2	Exchangeable	8 mL 1 M $MgCl_2$ (pH 7.0)	Shake 1 h at 22°C
3	Carbonate-bound	8 mL 1 M NaOAc (pH 5.0)	Shake 5 h at 22°C
4	Fe/Mn oxide-bound	20 mL 0.04 M NH_2OH-HCl in 25% (v/v) HOAc (pH 2.0)	6 h at 96°C
5	Organic matter-bound	3 mL 0.02 M HNO_3 + 5 mL 30% H_2O_2 (pH 2.0) / 3 mL 30% H_2O_2 (pH 2.0) / 5 mL 3.2 M NH_4OAc in 20% (v/v) HNO_3	2 hr at 85°C / 3 hr at 85°C / shake 30 min at 22°C
6	Residual bound	6 mL HCl + 2 mL HNO_3 (*aqua regia*)	5 hr at 95°C

5.3.4 Quality Assurance and Quality Control (QA/QC)

One blank, one control, and one analytical duplicate sample were included for every 14 samples analyzed. The certified reference material (SS-2) had a mean percent recovery of 101.0±19.9 (n=3). All blanks were below the ICP-OES reporting limit for soil (<1.0 $\mu g \cdot g^{-1}$) and water (<0.6 $\mu g \cdot g^{-1}$) (n=3). The mean relative standard deviation was 5.6±2.5% (n=5) for all duplicate samples. All calibration controls were within the accepted range. Cadmium recovered from each of the six fractions was summed for each sample and compared against the total Cd concentration (as determined by *aqua regia* digestion), and the mean percent recovery was 110.6±6.1% (n=4).

5.3.5 Statistical Analysis

Statistical analyses were performed using S+ version 8.2 (Tibco Software Inc., USA). Soil Cd concentrations are reported on a dry weight (g) basis and recorded with the standard deviation of the mean. Welch modified two-sample t-tests were used with significance level α=0.05.

5.4 RESULTS AND DISCUSSION

5.4.1 Cadmium Fractionation

There was no significant difference in the proportion of Cd in each soil fraction between one and 185 days for either the control or the biochar treatment ($p > 0.05$) (Figure 5-1). However, there is a considerable difference in the percent change in Cd concentration that occurred within soil fractions over the course of the experiment. The proportion of Cd in the residual fraction (F6) increased by 45.7% for the control treatment, compared to a 59.8% increase in the biochar treatment. During the same time frame, the proportion of Cd in the OM fraction (F5) decreased in the control treatment by 29.8%, but

increased in the biochar treatment by 10.3%. The percent change in Cd in the Fe/Mn oxide fraction (F4) was similar between the control and the biochar treatments, with decreases of 6.5% and 8.1%, respectively. Only small changes occurred in the carbonate and exchangeable fractions, and the Cd concentrations in the water soluble fraction were negligible (<0.1 $\mu g \cdot g^{-1}$) for both treatments. Trends indicate decreasing Cd bioavailability in the biochar treatment relative to the control treatment, and this conforms to the expected effects of biochar amendment and incubation time. Although these trends are not significant after 185 days, they might increase to statistical significance if this experiment is conducted over a longer time period.

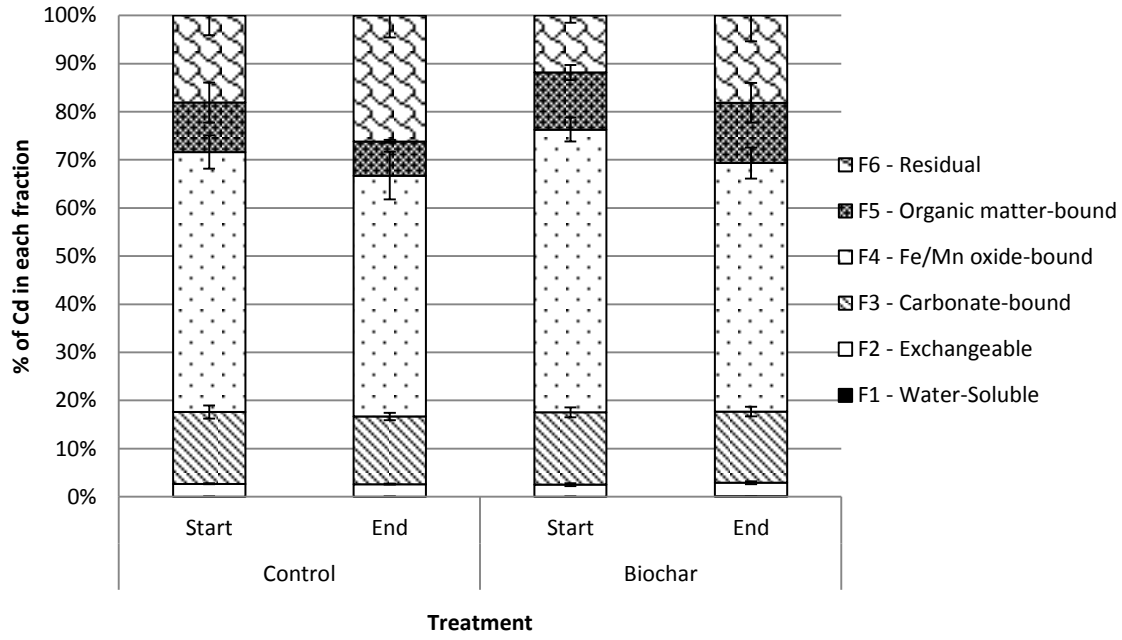

Figure 5-1. Fractionation of Cd in the Control and Biochar treatments into six operationally defined fractions between one and 185 days. The Cd concentration in the F1 fraction was below the ICP-OES detection limit of 0.6 $\mu g \cdot g^{-1}$ Cd. Two-sample t-tests indicated no significant differences in the percentage of Cd in each soil fraction within treatments between the start and end of the experiment.

5.4.2 Organic Matter Content

There was a significant decrease (from 30.7±1.5% to 27.4±0.6%; $p < 0.05$) in the soil OM content in the control treatment between one and 185 days. Despite this significant decrease in %OM, there was only a small (non-significant; $p > 0.05$) decrease in the proportion of Cd held in the OM-bound fraction. These results suggest that Cd in this fraction is sorbed primarily by the most stable portion of the soil OM, though the length of this experiment was likely too short for a significant loss of OM-bound Cd to occur.

There was no significant difference in the soil OM content in the biochar treatment between one and 185 days. Biochar is OM-rich and sorbs Cd, and therefore it is likely responsible for the small increase in the proportion of Cd bound to the OM fraction that occurred during this experiment (Park *et*

al., 2011b). Biochar provides a source of stable OM that is not susceptible to microbial degradation (Kuzyakov *et al.*, 2014), and its addition to soil likely compensated for the degradation of the more labile OM. As the native soil OM decomposes and releases Cd into the soil solution, biochar is expected to re-sorb Cd from solution and limit its long-term mobility and bioavailability. This effect will become more pronounced with time, as sorption of Cd by biochar increases with incubation time in soil due to the slow oxidation of its surface and the resulting increase in its negative surface charge density and sorption capacity (Major *et al.*, 2010; Zimmerman, 2010).

5.5 CONCLUSIONS

Although the changes in the proportion of Cd bound to each soil fraction between the beginning and end of the experiment were not significant for either treatment, the trend in Cd fractionation for both treatments indicates that Cd is moving from the more bioavailable fractions to the less bioavailable fractions with time. This trend is more pronounced in the biochar treatment.

Changes in the proportion of Cd bound to OM (though not significant) reflected the loss of OM that occurred in the control treatment and the input of biochar OM that occurred in the biochar treatment. This work highlights the potential for biochar to immobilize soil contaminants over the long term by stabilizing the OM. This experiment should be conducted over a longer period of time (1+ years) to allow significant trends to develop with further OM degradation and biochar incubation.

6. DISCUSSION AND CONCLUSIONS

Cadmium is a toxic metal with no known biological function, and exposure to Cd can cause adverse health effects to humans, plants, and animals. Although Cd is naturally present at low levels in the environment, anthropogenic activity, such as the use of municipal solid waste (MSW) and sewage sludge (SS) compost, can considerably increase Cd concentrations in soils. Cadmium-contaminated soil is commonly remediated using conventional technologies such as soil washing and excavation and landfilling. As these methods can be expensive and damaging to the soil matrix, efforts are being made to develop environmentally-friendly and cost-effective alternatives.

Phytoextraction uses vascular plants to extract Cd into the roots and translocate them to the shoots, which are harvested and disposed of (Salt *et al.*, 1998; Cunningham and Ow, 1996). Several plant species have successfully extracted significant concentrations of Cd into their aboveground tissue, and considerable research has been done over the past 20 years to identify new Cd phytoextractors and enhance plant uptake though the use of various soil amendments. Studies have demonstrated that plant uptake, and therefore phytoextraction success, depends on environmental factors such as soil properties and Cd speciation.

In this thesis, the feasibility of using phytoextraction to remediate Cd in a MSW/SS compost-based soil was investigated at a site in Peterborough, Ontario. Greenhouse and field studies showed low plant uptake into all species evaluated, with each having a mean BAF ≤ 1 (Chapter 3). Sequential extraction was used to determine the fractionation of Cd in the Peterborough soil, as the partitioning of Cd among soil fractions is representative of Cd bioavailability to plants. Cadmium in the Peterborough Gun Club (PGC) soil was retained primarily by the Fe/Mn oxide, organic matter, and residual fractions, with relatively little held in the water-soluble, exchangeable, and carbonate-bound fractions. As the latter three make up the plant available Cd pool, only 38.3% and 20.3% of Cd was potentially available for plant uptake in the low and high Cd treatments, respectively. Results of greenhouse, field, and sequential extraction experiments indicated low bioavailability to plants, and hence phytoextraction was deemed an infeasible method of remediating Cd-contaminated soil at the PGC.

Although low plant uptake eliminated phytoextraction as a remediation option, it also reduced exposure of plant-eating receptors to Cd, and might therefore limit the associated health risks. The potential for risk at the PGC was calculated for seven site-specific ecological receptors using a preliminary quantitative risk assessment (Chapter 4). This risk assessment was completed using site-specific Cd data for soil, plants, and earthworms in order to accurately characterize risk. Despite soil Cd concentrations up to 18 times higher than the MOE soil guideline of 1.2 $\mu g \cdot g^{-1}$ Cd, no potential for risk was found for any of the seven receptors evaluated. For comparison, risk was also calculated for the same seven receptors at a PCB-contaminated site in Lindsay, ON. This site differed from the Peterborough site based on its soil quality, the contaminant of concern, and contaminant bioavailability. Previous research has shown that phytoextraction would be an effective method of remediating this site due to high plant uptake of PCBs, and an ecological risk assessment revealed that five of the seven receptors were potentially at risk of experiencing adverse health effects from PCBs. The results of these risk assessments demonstrate the importance of considering contaminant bioavailability when estimating risk, as guideline exceedance alone does not accurately predict the potential for risk to ecological receptors.

While risk assessments characterize the potential for risk under existing environmental conditions, over time, changing environmental conditions can cause fluctuations in soil chemistry, leading to increased contaminant bioavailability in soil and increased potential for risk. This is an important consideration when characterizing ecological risk from Cd in soils amended with MSW/SS compost, as the degradation of organic matter and dissolution of Fe/Mn oxides may result in an increase in the proportion of Cd in high-bioavailability soil fractions in the future. Biochar is a carbon-rich amendment

that may limit long-term Cd bioavailability, as it has a high sorption capacity for Cd that increases over time as it is oxidized in soil. Therefore, a study was completed to investigate the changes in Cd fractionation in ageing MSW/SS compost-amended soil both with and without the addition of biochar (Chapter 6). While the soil organic matter content decreased significantly in the un-amended control after 185 days of ageing, there was no significant change in the biochar treatment. Sequential extraction of Cd from aged soils demonstrated trends of decreasing Cd bioavailability in the biochar treatment relative to the control, suggesting that biochar has the potential to limit Cd bioavailability in the PGC soil over the long-term, thus minimizing risk to ecological receptors.

This thesis is the first study investigating the relationship between the bioavailability, phytoextraction, and ecological risk associated with Cd in MSW/SS compost-amended soil. Although Cd bioavailability is low in these soils, future work should focus on Cd bioavailability in those amended with fresh, uncomposted MSW/SS, as its high content of dissolved organic matter improves Cd solubility and may therefore increase phytoextraction feasibility. Future work should also further investigate the effect of biochar on Cd bioavailability in compost-based soils over time, as this thesis demonstrates that it has the potential to be an effective long-term remediation technology for soils amended with MSW/SS compost.

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8. APPENDICES

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8.1 APPENDIX A

Additional site information
Soil Cd concentrations at the Peterborough Gun Club

Table A-1. Cadmium concentrations in soil samples collected from the Peterborough Gun Club.

Figure A-1. Map of sample locations at the Peterborough Gun Club corresponding to the sample numbers in Table A-1.

Table A-1. Cadmium concentrations in soil samples collected from the Peterborough Gun Club. Quality assurance and quality control data are included at the bottom of the table.

Sample Number	Soil [Cd] ($\mu g \cdot g^{-1}$)
6001	2.7
6002	3.1
6003	2.7
6004	1.4
6005	1.9
6006	2.6
6007	5.4
6008	6.9
6009	11.5
6010	7.7
6011	6.9
6012	2.3
6013	1.5
6014	5.8
6015	2.2
6016	2.7
6017	2.7
6018	8.6
6019	21.4
6020	11.3
6021	1.7
6022	5.2
6023	2.5
6024	2.7
6025	17.4
6026	3.8
6027	2.7
6028	5.4
6029	3.4
6030	3.4
6031	4.7
6032	4.1
6033	1.3
6034	1.5
6035	<1.0
6036	3.4
6037	5.6
6038	5.7
6039	5.9
6040	13.3
6041	16.7

6042	13.9
6043	8.7
6044	4.0
6045	6.0
6046	3.0
6047	6.0
6048	5.0
6049	10.3
6050	10.7
6051	6.6
Blank	<1.0
Blank	<1.0
Blank	<1.0
Blank	<1.0
SS-2	1.7
SS-2	1.7
SS-2 Target	1.8
6004	1.4
6004-D	1.4
6010	10.4
6010-D	9.9
6017	2.8
6017-D	2.7
6023	2.4
6023-D	2.6
6028	5.1
6028-D	5.8
6036	3.2
6036-D	3.6
6049	10.1
6049-D	10.5

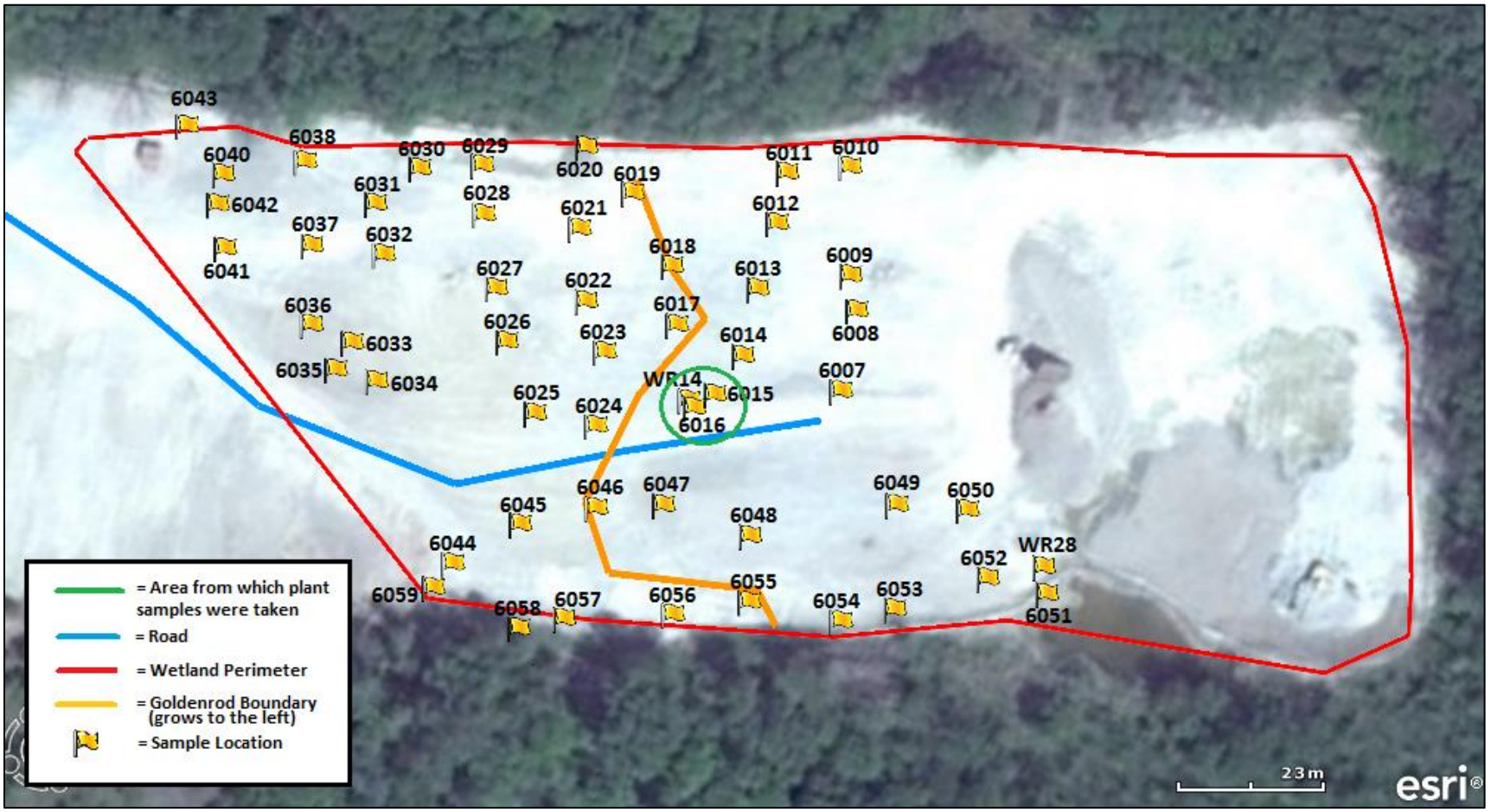

Figure A-1. Map of sample locations at the Peterborough Gun Club corresponding to the sample numbers in Table A-1.

8.2 APPENDIX B

Raw data for Chapter 3

Feasibility of using phytoextraction to remediate a compost-based soil contaminated with cadmium.

Table B-1. Particle size distribution data for all treatments.

Table B-2. Cation exchange capacity values for all treatments.

Table B-3. pH values for all treatments.

Table B-4. Soil organic matter content of all treatments determined by loss on ignition (LOI).

Table B-5. Cadmium concentrations of soil, roots, and shoots in each pot of the greenhouse experiment.

Table B-6. Soil Cd concentrations of the low and high Cd field plots at the PGC, and root and shoot Cd concentrations of *Brassica juncea* grown in the field experiment.

Table B-7. Sequential extraction results for low Cd and high Cd soils.

Table B-8. Final root and shoot dry weights (dw) for plants grown in the greenhouse pot experiment.

Table B-1. Particle size distribution data for the control, low Cd, and high Cd soil treatments. Duplicates are included at the bottom of the table.

		Sieve Numbers									
Sample Name	Sample Mass	4	10	18	35	60	100	200	Pan	% Coarse	% Fine
Low Cd 1	198.24	0.01	0.02	17.97	20.80	20.01	18.15	17.74	5.30	38.80	61.20
Low Cd 2	197.00	0.22	0.17	19.46	21.38	20.74	20.90	13.36	3.77	41.23	58.77
Low Cd 3	198.49	0.01	0.15	18.26	21.06	21.10	23.55	11.79	4.07	39.48	60.52
High Cd 1	178.72	0.07	0.22	20.99	27.28	19.67	11.98	10.21	9.58	48.56	51.44
High Cd 2	190.48	0.02	0.05	16.36	24.14	21.45	14.80	13.75	9.43	40.57	59.43
High Cd 3	197.71	0.02	0.08	16.77	24.88	21.02	14.47	12.28	10.49	41.75	58.25
Control soil1	51.53	9.74	14.75	13.35	13.55	17.37	18.16	11.51	1.57	51.39	48.61
Control soil 2	55.27	6.64	12.99	12.74	13.99	15.11	13.75	19.41	5.37	46.35	53.65
Control soil 3	57.84	4.84	14.11	13.40	14.25	15.84	14.06	16.91	6.60	46.59	53.41
Low Cd 3	198.49	0.01	0.15	18.26	21.06	21.10	23.55	11.79	4.07	39.48	60.52
Low Cd 3-D	190.30	0.01	0.16	18.32	21.09	21.88	22.80	12.03	3.71	39.58	60.42
Control soil 3	57.84	4.84	14.11	13.40	14.25	15.84	14.06	16.91	6.60	46.59	53.41
Control soil 3-D	60.02	4.46	14.75	12.55	15.35	15.87	13.49	17.54	6.00	47.10	52.90

Table B-2. Cation exchange capacity values for all treatments. Quality assurance and quality control data are included at the bottom of the table.

Sample Name	CEC (cmol/kg)
Control	150.4
Low Cd	38.1
High Cd	46.2
Blank	<3.0
Anders	11.2
Anders-D	9.6
Control (ICV)	**[Cd] (mg/L)**
Control	16.7
Control Target	16.0

Table B-3. pH values for all treatments. Quality assurance and quality control data are included at the bottom of the table.

Sample Name	pH
Control	5.4
Low Cd	7.6
High Cd	7.7
Control[a]	4.0
Low Cd	7.6
Low Cd-D	7.6

[a] Solution with known pH of 4.0

Table B-4. Soil organic matter content of all treatments determined by loss on ignition (LOI). Quality assurance data is included at the bottom of the table.

Sample Name	Treatment	Organic Matter (%LOI)
Exp1-05	Control	89.3
Exp1-06	Control	86.5

Exp1-07	Control	87.2
Exp1-21	Low Cd	15.2
Exp1-22	Low Cd	14.6
Exp1-23	Low Cd	15.5
Exp1-24	Low Cd	14.8
Exp1-25	Low Cd	12.5
Exp1-27	Low Cd	12.7
Exp1-28	Low Cd	11.7
Exp1-31	Low Cd	13.4
Exp1-35	High Cd	23.5
Exp1-36	High Cd	26.8
Exp1-37	High Cd	27.8
Exp1-38	High Cd	24.9
Exp1-40	High Cd	21.5
Exp1-41	High Cd	23.6
Exp1-42	High Cd	21.8
Exp1-43	High Cd	25.6
Exp1-46	High Cd	25.4
Exp1-23	Low Cd	14.8
Exp1-23-D	Low Cd	15.1
Exp1-35	High Cd	23.5
Exp1-35-D	High Cd	24.1
Exp1-37	High Cd	27.8
Exp1-37-D	High Cd	25.8

Table B-5. Cadmium concentrations of soil, roots, and shoots in each pot of the greenhouse experiment. Quality assurance and quality control data are included at the bottom of the table.

Sample Name [a]	Treatment	Soil [Cd] ($\mu g \cdot g^{-1}$)	Root [Cd] ($\mu g \cdot g^{-1}$)	Shoot [Cd] ($\mu g \cdot g^{-1}$)
Exp1-05/53	Poa compressa Control A	<1.0	<1.0	<1.0
Exp1-06/54	Poa compressa Control B	<1.0	<1.0	<1.0
Exp1-07/55	Poa compressa Control C	<1.0	<1.0	<1.0
Exp1-20/56	Poa compressa Low Cd A	4.9	5.6	<1.0
Exp1-21/57	Poa compressa Low Cd B	5.0	4.8	<1.0
Exp1-22/58	Poa compressa Low Cd C	4.9	5.1	<1.0
Exp1-35/59	Poa compressa High Cd A	16.0	7.1	<1.0
Exp1-36/60	Poa compressa High Cd B	16.5	6.6	<1.0
Exp1-37/61	Poa compressa High Cd C	17.6	9.8	<1.0
Exp1-11/65	Helianthus annuus Control A	<1.0	<1.0	<1.0
Exp1-12/66	Helianthus annuus Control B	<1.0	<1.0	<1.0

Exp1-13/67	Helianthus annuus Control C	<1.0	<1.0	<1.0
Exp1-26/68	Helianthus annuus Low Cd A	5.3	<1.0	<1.0
Exp1-27/69	Helianthus annuus Low Cd B	4.8	1.5	<1.0
Exp1-28/70	Helianthus annuus Low Cd C	4.6	1.1	<1.0
Exp1-41/71	Helianthus annuus High Cd A	15.9	2.0	<1.0
Exp1-42/72	Helianthus annuus High Cd B	14.8	2.5	<1.0
Exp1-43/73	Helianthus annuus High Cd C	17.8	2.0	<1.0
Exp1-08/74	Brassica juncea Control A	<1.0	<1.0	<1.0
Exp1-09/75	Brassica juncea Control B	<1.0	<1.0	<1.0
Exp1-10/76	Brassica juncea Control C	<1.0	<1.0	<1.0
Exp1-23/77	Brassica juncea Low Cd A	4.6	3.4	1.7
Exp1-24/78	Brassica juncea Low Cd B	4.6	<1.0	1.2
Exp1-25/79	Brassica juncea Low Cd C	4.9	<1.0	1.52
Exp1-38/80	Brassica juncea High Cd A	16.4	<1.0	1.62
Exp1-39/81	Brassica juncea High Cd B	18.1	<1.0	<1.0
Exp1-40/82	Brassica juncea High Cd C	15.3	<1.0	1.79
Exp1-14/86	Chenopodium album Control A	<1.0	<1.0	<1.0
Exp1-15/87	Chenopodium album Control B	<1.0	<1.0	<1.0
Exp1-16/88	Chenopodium album Control C	<1.0	<1.0	<1.0
Exp1-29[b]	Chenopodium album Low Cd A	5.0	-	-
Exp1-30[b]	Chenopodium album Low Cd B	5.6	-	-
Exp1-31/89	Chenopodium album Low Cd C	5.5	1.8	<1.0
Exp1-44/90	Chenopodium album High Cd A	15.5	3.3	<1.0
Exp1-45/91	Chenopodium album High Cd B	16.2	2.2	<1.0
Exp1-46/92	Chenopodium album High Cd C	18.3	3.7	<1.0

Sample Name	[Cd] ($\mu g \cdot g^{-1}$)	Matrix
Blank	<1.0	Soil
Blank	<1.0	Soil
Blank	<1.0	Soil
Blank	<1.0	Soil
Blank	<1.0	Plant
Blank	<1.0	Plant
Blank	<1.0	Plant
Blank	<1.0	Plant
Blank	<1.0	Plant
Blank	<1.0	Plant
SS-2	1.7	Soil
SS-2	1.9	Soil
SS-2	1.8	Soil
SS-2 Target	1.8	Soil
NIST-1570A	2.2	Plant

NIST-1570A	1.7	Plant
NIST-1570A	2.4	Plant
NIST-1570A	2.0	Plant
NIST-1570A	2.0	Plant
NIST-1570A	2.4	Plant
Target		
Exp1-4	13.1	Soil
Exp1-4-D	13.5	Soil
Exp1-13	<1.0	Soil
Exp1-13-D	<1.0	Soil
Exp1-18	<1.0	Soil
Exp1-18-D	<1.0	Soil
Exp1-27	5.3	Soil
Exp1-27-D	4.1	Soil
Exp1-32	5.5	Soil
Exp1-32-D	5.5	Soil
Exp1-41	16.3	Soil
Exp1-41-D	15.5	Soil
Exp1-46	19.6	Soil
Exp1-46-D	17.0	Soil
Exp1-54S	<1.0	Plant
Exp1-54S-D	<1.0	Plant
Exp1-59R	5.6	Plant
Exp1-59R-D	9.2	Plant
Exp1-62S	<1.0	Plant
Exp1-62S-D	<1.0	Plant
Exp1-67R	<1.0	Plant
Exp1-67R-D	<1.0	Plant
Exp1-70S	<1.0	Plant
Exp1-70S-D	<1.0	Plant
Exp1-75S	<1.0	Plant
Exp1-75S-D	<1.0	Plant

Exp1-78S	1.3	Plant
Exp1-78S-D	1.3	Plant
Exp1-83S	<1.0	Plant
Exp1-83S-D	<1.0	Plant
Exp1-85S	2.7	Plant
Exp1-85S-D	2.5	Plant
Exp1-87S	<1.0	Plant
Exp1-87S-D	<1.0	Plant
Exp1-92S	<1.0	Plant
Exp1-92S-D	<1.0	Plant

[a] Numbers before the dash represent soil samples, and those after the dash represent plant samples
[b] Plant did not germinate

Table B-6. Soil Cd concentrations of the low and high Cd field plots at the PGC, and root and shoot Cd concentrations of *Brassica juncea* grown in the field experiment. Quality assurance and quality control data are included at the bottom of the table.

Sample Name[a]	Treatment	Soil [Cd] ($\mu g \cdot g^{-1}$)	Root [Cd] ($\mu g \cdot g^{-1}$)	Shoot [Cd] ($\mu g \cdot g^{-1}$)
PGC13-041/065	Low Cd A	7.8	0.62	1.94
PGC13-042/066	Low Cd B	11.1	0.76	2.10
PGC13-043/067	Low Cd C	7.6	0.75	1.86
PGC13-044/068	Low Cd D	n/a	0.57	1.31
PGC13-045/069	Low Cd E	n/a	0.65	1.65
PGC13-046/070	Low Cd F	n/a	0.65	1.32
PGC13-053/077	High Cd A	17.2	1.47	2.04
PGC13-054/078	High Cd B	20.9	0.97	1.78
PGC13-055/079	High Cd C	19.9	0.63	2.10
PGC13-056/080	High Cd D	n/a	1.01	1.24
PGC13-057/081	High Cd E	n/a	1.47	2.89
PGC13-058/082	High Cd F	n/a	0.90	1.59

Sample Name	[Cd] ($\mu g \cdot g^{-1}$)	Matrix
Blank	<0.05	Plant
Blank	<0.05	Plant

Blank	<1.0	Soil
SS-2	1.9	Soil
SS-2 Target	1.8	
NIST-1570A	2.11	Plant
NIST-1570A	2.71	Plant
NIST-1570A	2.26	Plant
NIST-1570A	2.32	Plant
NIST-1570A Target	2.40	
PGC13-95	6.9	Soil
PGC13-95-D	6.8	Soil
PGC13-110	22.1	Soil
PGC13-110-D	24.9	Soil
PGC13-44	1.41	Plant
PGC13-44-D	1.21	Plant
PGC13-053	1.99	Plant
PGC13-053-D	2.08	Plant
PGC13-058	1.26	Plant
PGC13-058-D	1.91	Plant
PGC-068	0.55	Plant
PGC-068-D	0.59	Plant
PGC-077	1.62	Plant
PGC-077-D	1.31	Plant
PGC-082	0.89	Plant
PGC-082-D	0.90	Plant

[a] Numbers before the dash represent soil samples, and those after the dash represent plant samples

Table B-7. Sequential extraction results for low Cd and high Cd soils. Samples were converted from mg/L to $\mu g \cdot g^{-1}$ by multiplying the value in mg/L by the final sample volume (mL) and dividing the product by the final sample weight (g). Quality assurance and quality control data are included at the bottom of the table.

Sample Name	Treatment	Fraction	[Cd] mg/L	Final Sample Volume (mL)	Final Sample Weight (g)	[Cd] ($\mu g \cdot g^{-1}$)
PGC13-094	Low Cd Soil	F1	0.002	15	1.0147	0.0
		F2	0.066	8	1.0147	0.5
		F3	0.330	8	1.0147	2.6
		F4	0.271	15	1.0147	4.0
		F5	0.025	20	1.0147	0.5
		F6	0.021	25	1.0147	0.5
PGC13-104	High Cd Soil	F1	0.002	15	1.0702	0.0
		F2	0.074	8	1.0702	0.6
		F3	0.501	8	1.0702	3.7
		F4	0.788	15	1.0702	11.0
		F5	0.143	20	1.0702	2.7
		F6	0.137	25	1.0702	3.2

Sample Name	[Cd] ($\mu g \cdot g^{-1}$)
Blank	<0.6
SS-2	1.4
SS-2 Target	1.8
PGC13-095	8.0
PGC13-095-D	7.7

Control (ICV)	[Cd] (mg/L)
Control	3.9

Control Target	4.0
Control	0.41
Control Target	0.40
Control	3.8
Control Target	4.0
Control	0.42
Control Target	0.40
Control	4.2
Control Target	4.0
Control	0.39
Control Target	0.40

Table B-8. Final root and shoot dry weights (dw) for plants grown in the greenhouse pot experiment. Quality assurance and quality control data are included at the bottom of the table.

Treatment	Root dry weight (g)	Shoot dry weight (g)
Poa compressa Control A	-	3.80
Poa compressa Control B	-	3.31
Poa compressa Control C	-	3.41
Poa compressa Low Cd A	-	7.02
Poa compressa Low Cd B	-	5.75
Poa compressa Low Cd C	-	3.28
Poa compressa High Cd A	-	11.99
Poa compressa High Cd B	-	12.14
Poa compressa High Cd C	-	11.98
Helianthus annuus Control A	1.89	8.62
Helianthus annuus Control B	2.45	15.04
Helianthus annuus Control C	2.33	16.02
Helianthus annuus Low Cd A	0.51	5.04
Helianthus annuus Low Cd B	0.59	4.81
Helianthus annuus Low Cd C	0.51	3.94
Helianthus annuus High Cd A	1.05	10.25
Helianthus annuus High Cd B	0.93	5.41
Helianthus annuus High Cd C	0.61	7.17
Brassica juncea Control A	0.95	5.77
Brassica juncea Control B	1.23	10.9
Brassica juncea Control C	2.36	6.94
Brassica juncea Low Cd A	0.38	1.00
Brassica juncea Low Cd B	0.23	1.49
Brassica juncea Low Cd C	0.47	1.58
Brassica juncea High Cd A	0.90	3.71
Brassica juncea High Cd B	1.29	5.16
Brassica juncea High Cd C	0.49	2.55
Chenopodium album Control A	0.57	2.44
Chenopodium album Control B	0.49	2.94
Chenopodium album Control C	0.68	3.33
Chenopodium album Low Cd A	-	-
Chenopodium album Low Cd B	-	-
Chenopodium album Low Cd C	0.7	4.43
Chenopodium album High Cd A	0.6	3.79
Chenopodium album High Cd B	0.75	4.21
Chenopodium album High Cd C	0.18	1.18

8.3 APPENDIX C

Raw data for Chapter 4
Ecological risk associated with phytoextraction of soil contaminants.

Table C-1. Cadmium concentrations in earthworms.

Table C-1. Cadmium concentrations in earthworms. Quality control and quality assurance data are included at the bottom of the table.

Sample Name	Treatment	[Cd] ($\mu g \cdot g^{-1}$)
Exp2-01	Control A	8.2
Exp2-02	Control B	1.5
Exp2-03	Control C	6.0
Exp2-04	Low Cd A	33.2
Exp2-05	Low Cd B	31.7
Exp2-06	Low Cd C	24.8
Exp2-10	High Cd A	45.0
Exp2-11	High Cd B	51.2
Exp2-12	High Cd C	50.9
Blank	-	<1.0
Tort-2	-	22.3
Exp2-004	-	32.2
Exp2-004-D	-	34.3
Exp2-013	-	43.9
Exp2-013-D	-	48.2

8.4 APPENDIX D

Raw data for Chapter 5

Effect of biochar on cadmium fractionation with organic matter degradation in a soil amended with municipal solid waste/sewage sludge compost.

Table D-1. Cadmium concentrations in soil fractions of the control and biochar treatment soils before and after the soil ageing experiment.

Table D-2. Total (*aqua regia*) Cd concentrations in the control and biochar treatment soils.

Table D-3. Organic matter content of the control and biochar treatment soils at the start and end of the soil ageing experiment.

Table D-1. Cadmium concentrations in soil fractions of the control and biochar treatment soils before and after the soil ageing experiment. Samples were converted from mg/L to $\mu g \cdot g^{-1}$ by multiplying the value in mg/L by the final sample volume (mL) and dividing the product by the final sample weight (g). Quality control and quality assurance data are included at the bottom of the table.

Sample Name	Treatment	Fraction	[Cd] (mg/L)	Final Sample Volume (mL)	Final Sample Weight (g)	[Cd] ($\mu g \cdot g^{-1}$)
SAE-01	Start – Control A	F1	<0.025	15.0	1.0156	-
		F2	0.084	8.0	1.0156	0.7
		F3	0.513	8.0	1.0156	4.0
		F4	0.904	15	1.0156	13
		F5	0.079	20	1.0156	1.6
		F6	0.231	25	1.0156	5.7
SAE-02	Start – Control B	F1	<0.025	15	1.0131	-
		F2	0.080	8	1.0131	0.63
		F3	0.435	8	1.0131	3.4
		F4	0.748	20	1.0131	14
		F5	0.129	20	1.0131	2.6
		F6	0.176	25	1.0131	4.3
SAE-03	Start – Control C	F1	<0.025	15	1.0303	-
		F2	0.081	8	1.0303	0.63
		F3	0.453	8	1.0303	3.5
		F4	0.811	15	1.0303	11
		F5	0.174	20	1.0303	3.4
		F6	0.140	25	1.0303	3.4
SAE-04	Start – Biochar A	F1	<0.025	15	1.0294	-
		F2	0.068	8	1.0294	0.53
		F3	0.401	8	1.0294	3.1
		F4	0.941	15	1.0294	13
		F5	0.140	20	1.0294	2.7
		F6	0.129	25	1.0294	3.1
SAE-05	Start – Biochar B	F1	<0.025	15	1.0044	-
		F2	0.064	8	1.0044	0.51
		F3	0.438	8	1.0044	3.5

		F4	0.868	15	1.0044	12
		F5	0.153	20	1.0044	3.0
		F6	0.154	25	1.0044	3.8
SAE-06	Start – Biochar C	F1	<0.025	15	1.0016	-
		F2	0.071	8	1.0016	0.57
		F3	0.410	8	1.0016	3.3
		F4	0.803	15	1.0016	12
		F5	0.105	20	1.0016	2.1
		F6	0.136	25	1.0016	3.4
SAE-43	End – Control A	F1	<0.025	15	1.0507	-
		F2	0.073	8	1.0507	0.56
		F3	0.410	8	1.0507	3.1
		F4	0.907	15	1.0507	12
		F5	0.091	20	1.0507	1.7
		F6	0.204	25	1.0507	4.9
SAE-44	End – Control B	F1	<0.025	15	1.0735	-
		F2	0.076	8	1.0735	0.57
		F3	0.410	8	1.0735	3.1
		F4	0.763	15	1.0735	10
		F5	0.082	20	1.0735	1.5
		F6	0.267	25	1.0735	6.2
SAE-45	End – Control C	F1	<0.025	15	1.0821	-
		F2	0.075	8	1.0821	0.55
		F3	0.441	8	1.0821	3.3
		F4	0.727	15	1.0821	10
		F5	0.083	20	1.0821	1.5
		F6	0.278	25	1.0821	6.4
SAE-46	End – Biochar A	F1	<0.025	15	1.0293	-
		F2	0.075	8	1.0293	0.58
		F3	0.381	8	1.0293	2.9
		F4	0.682	15	1.0293	9.9
		F5	0.082	20	1.0293	1.6
		F6	0.200	25	1.0293	4.9

SAE-47	End – Biochar B	F1	<0.025	15	1.0135	-
		F2	0.067	8	1.0135	0.53
		F3	0.382	8	1.0135	3.0
		F4	0.822	15	1.0135	12
		F5	0.150	20	1.0135	3.0
		F6	0.129	25	1.0135	3.2
SAE-48	End – Biochar C	F1	<0.025	15	1.0155	-
		F2	0.072	8	1.0155	0.56
		F3	0.386	8	1.0155	3.0
		F4	0.651	15	1.0155	9.6
		F5	0.158	20	1.0155	3.1
		F6	0.122	25	1.0155	3.0

Sample Name	[Cd] ($\mu g \cdot g^{-1}$)
Blank	<0.6
Blank	<0.6
SS-2	1.4
SS-2	2.1
SS-2 Target	1.8
PGC13-095	7.7
PGC13-095-D	8.0
SAE-37	21.1
SAE-37-D	19.6
SAE-47	21.9
SAE-47-D	19.4

Control (ICV)	[Cd] (mg/L)
Control	3.9
Control Target	4.0
Control	0.41
Control Target	0.40
Control	3.8
Control Target	4.0
Control	0.42
Control Target	0.40
Control	4.2
Control Target	4.0
Control	0.39
Control Target	0.40

Table D-2. Total (*aqua regia*) Cd concentrations in the control and biochar treatment soils. Quality control and quality assurance data are included at the bottom of the table.

Sample Name	Treatment	[Cd] ($\mu g \cdot g^{-1}$)
SAE-07	Control A Start	22.9
SAE-08	Control B Start	20.0
SAE-09	Control C Start	22.1
SAE-43	Control A End	21.1
SAE-44	Control B End	21.2
SAE-45	Control C End	21.9
SAE-10	Biochar A Start	19.8
SAE-12	Biochar B Start	18.5
SAE-13	Biochar C Start	19.0
SAE-46	Biochar A End	18.1
SAE-47	Biochar B End	18.7
SAE-48	Biochar C End	15.0
Blank	-	<1.0
SS-2	-	1.9
SAE-04	-	20.3
SAE-04	-	19.3
SAE-047	-	17.7
SAE-047	-	19.7

Table D-3. Organic matter content of the control and biochar treatment soils at the start and end of the soil ageing experiment. Quality assurance data are included at the bottom of the table.

Sample Name	Treatment	Organic Matter (% LOI)
SAE-07	Control A Start	31.9
SAE-08	Control B Start	29.0
SAE-09	Control C Start	31.1
SAE-43	Control A End	27.7
SAE-44	Control B End	27.7
SAE-45	Control C End	26.7
SAE-10	Biochar A Start	33.6
SAE-12	Biochar B Start	36.0
SAE-13	Biochar C Start	35.3
SAE-46	Biochar A End	32.3
SAE-47	Biochar B End	32.2

SAE-48	Biochar C End	28.4
SAE-45	Control C End	26.9
SAE-45	Control C End	26.4
SAE-48	Biochar C End	28.4
SAE-48	Biochar C End	31.4

8.5 APPENDIX E

Additional Information

Quality assurance and quality control targets.

Table E-1. Target values for quality assurance and quality control values used in this thesis.

Table E-1. Inductively coupled plasma (ICP) target values for Cd in quality assurance and quality control (QA/QC) samples.

QA/QC Type	Target Cd Value ($\mu g \cdot g^{-1}$) (Laboratory Mean)	Warning Limits	Control Limits
Method Blank			
ICP-OES	<1.0	n/a	n/a
ICP-MS	<0.05	n/a	n/a
Certified Reference Material			
NIST-1570A (Spinach)	2.4	2.2-2.7	2.0-2.9
SS-2	1.8	1.5-2.1	1.3-2.3
Tort-2	25.3	22.2-28.4	20.6-30.0

QA/QC Type	RSD[a] (%)
Duplicate	70-130

[a] RSD = Relative