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Methods to Predict and Reduce Trace Metal Levels in Lettuce Grown on Contaminated Urban Soils

(Méthodes pour prédire et réduire les métaux traces dans la laitue cultivée sur des sols contaminés)

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March, 1998

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements of the degree of Master of Science.

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Preface

The research presented in this thesis is the work of the candidate. Although Prof. W.H. Hendershot and Dr. N. Cook are co-authors on papers to be submitted for publication, the role of Prof. Hendershot was strictly that of supervision, whereas Dr. Cook provided technical and editorial assistance to the project. In keeping with the 'Guidelines for Thesis Preparation', the following paragraphs are reproduced in full:

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Abstract

Methods to Predict and Reduce Trace Metal Levels in Lettuce Grown on Contaminated Urban Soils

The work in this thesis demonstrates whether resins are better than conventional soil extractants at predicting plant metal concentrations, and whether low-cost soil treatments can effectively reduce metal content and concentrations in lettuce leaves.

Researchers have shown that ion exchange resins can simulate root metal uptake behaviour by acting as ion sinks. Chemical extractants on the other hand, generally do not behave in this manner. Thus, we would expect resins (in either bead or membrane form) to predict plant metal concentrations better than conventional extractants. For the first study, a procedure using anion exchange resin membranes (AEM) treated with either EDTA or DTPA chelators was chosen, since previous studies showed this method to be effective at predicting plant metal uptake, and practical for routine laboratory use. In addition, several conventional extractants were selected for comparison.

The results showed that the resin membranes (in either DTPA or EDTA form) did not predict plant metal concentrations better than the other extractants when elements were considered singly or simultaneously. For instance, DTPA and Na acetate extractants, as well as the $HNO_3/HClO_4$ digestion method best predicted Pb concentrations in lettuce. Regressions showed that over 85% of the variability in Pb was explained by these chemicals, whereas 81% and 72% of the variability was explained by the AEM/EDTA and AEM/DTPA methods respectively. In addition, the DTPA, EDTA, HCl, and H_2O extractants, as well as the membrane treated with EDTA best predicted plant Cu concentrations (yielding r^2 values between 0.62 and 0.77), while the digestion procedure explained 64% of the variability in plant Zn concentrations. When all three metals are considered simultaneously, the AEM-EDTA method predicted bioconcentrations better than AEM-DTPA, however it was not the best predictor overall. Sodium acetate and DTPA extractants worked just as well as the AEM-EDTA procedure,

while the acid digestion method best estimated lettuce metal concentrations.

For the second part of the thesis, a study was undertaken to test the effectiveness of various food processing byproducts to stabilize metal contaminants in soils, and compare the results with those of a Na-based aluminosilicate (zeolite). Previous research has shown that food waste materials are effective at removing metals from wastewaters, but they have not been tested in metal-polluted soils. In addition, these byproducts are abundant and inexpensive. Hence, the rationale was to determine if such low-cost techniques can be adopted for soil stabilization.

Results showed that the waste materials tested (wheat shorts, peanut skins, and filter press mud), were generally ineffective at reducing lettuce metal uptake. Significant decreases in plant metal concentrations were found; however, these were mostly due to dilution effects. In addition, significant reductions in plant Ca and Mg concentrations were observed which were mostly a result of dilution as well. In comparison, the zeolites were not more effective. No significant decreases in plant metal concentrations and uptake were found, however reductions in plant nutrient concentrations were observed on some zeolite-treated soils.

The results also showed that some food waste materials had significant positive impacts on lettuce growth, and on soil organic carbon and available P contents. Hence, these low-cost treatments could be used as fertilizers.

Résumé

Méthodes pour prédire et réduire les métaux traces dans la laitue cultivée sur des sols contaminés

Dans cette thèse, nous comparons l'efficacité des résines d'échange ionique et celle des extractions de sol conventionnelles pour prédire les concentrations de métaux dans des plantes. Aussi, nous étudions la faisabilité d'utiliser des traitements de sol économiques pour réduire le montant et la concentration de métaux traces dans les feuilles de laitue.

Plusieurs chercheurs ont trouvé que des résines peuvent simuler l'action des racines de plantes en fonctionnant comme un réservoir d'ions. Les extractions chimiques ne fonctionnent pas de cette manière. Donc, on prévoit que les résines (en forme de membrane ou de billes), seront plus fiables pour prédire les concentrations de métaux dans les plantes que les extractions. Pour la première étude, des membranes de résines d'échange anionique traitées avec EDTA ou DTPA ont été utilisées en accord avec des recherches démontrant que la méthode est efficace et pratique dans le laboratoire. En plus, diverses extractions chimiques du sol ont été choisies pour fins de comparaison.

On a trouvé que les membranes (traitées avec EDTA ou DTPA) n'ont pas mieux prédit les bioconcentrations de métaux que les extractions chimiques. Par exemple, les extractions au DTPA, acétate de Na, et la méthode de digestion, $HNO_3/HClO_4$, ont bien prédit le Pb dans la laitue. Les résultats de régressions montrent que ces produits expliquent plus de 85% de la variabilité en Pb, tandis que les membranes traitées avec EDTA et DTPA ont expliqué 81% et 72% de la variabilité respectivement. En plus, les extractions DTPA, EDTA, HCl, H_2O, et les membranes traitées avec EDTA ont expliqué de 62% jusqu'à 77% de la variabilité en Cu dans les feuilles de plantes, tandis que $HNO_3/HClO_4$ a expliqué 64% de la variabilité en Zn. Si on considère tous les métaux ensemble (Pb, Cu, et Zn), les membranes traitées avec EDTA ont prédit les bioconcentrations de métaux de meilleure façon que celles traitées avec DTPA, mais aussi bien que les extractions de sol avec DTPA et acétate de Na. En plus, la méthode de

digestion a mieux prédit les bioconcentrations de métaux que toutes les autres techniques essayées.

Pour la deuxième partie de la thèse, on a determiné l'efficacité de résidus agricoles pour stabiliser les métaux dans les sols, et on a comparé ces résultats avec ceux des zéolites (de Na). D'autres chercheurs ont trouvé que ces produits peuvent être très efficaces pour décontaminer des eaux, mais aucun essai n'a été testé dans les sols. En plus, ces produits sont pas cher et très abondants. Donc, on voulait savoir si des traitements économiques peuvent être utilisés pour stabiliser des métaux dans les sols contaminés.

On a trouvé qu'aucun résidu testé n'a effectivement réduit la quantité de métaux dans les plantes. Bien que les concentrations de métaux dans la laitue aient été réduites significativement, ces réductions résultaient de la dilution. En plus, les concentrations de Ca et Mg dans laitue ont été réduit à cause de la dilution aussi. En comparaison, les zéolites n'étaient pas plus efficaces. Il n'y avait pas de réduction significative dans le montant et la concentration de métaux dans les plantes, mais les concentrations de Ca et Mg dans la laitue ont été réduites sur quelques sols traités.

Les résultats montrent aussi que les résidus agricoles ont significativement amélioré la croissance des plantes, le montant de matières organique dans les sols, et la disponibilité de P. Donc, ces produits économiques peuvent être utilisés pour fertiliser les sols.

Acknowledgments

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Finally, the author would like to express his deepest thanks to Anita Tallini and family for providing the much-needed moral support throughout the course of the project.

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Introduction

The problem of heavy metal pollution in our environment is becoming widespread and the major reason for this is the increasing application of metal-laden products such as fertilizers, chemicals, and sewage sludge from the agricultural sector, as well as increasing emissions of metals from both the automotive and mining industries (Tiller, 1989). Apart from these anthropogenic sources, metal emissions into the soil, water, and atmosphere have also been shown to originate from windblown dusts, volcanoes, forest fires, and soil formation processes (Tiller, 1989).

Regardless of the source of pollution, heavy metals produce toxic effects on living organisms if high concentrations are bioaccumulated (Tiller, 1989). For instance metals may inhibit enzymatic activities in micro-organisms and decrease the diversity of soil life (Tyler, 1981). In addition several soil biological processes such as N and P mineralization, cellulose degradation, and N_2 fixation, as well as seed germination, crop quality and yield can be seriously affected by excess metal concentrations (Tiller, 1989; Bell & Teramura, 1991; Nwosu et al., 1995). Of even greater importance is the potentially toxic effects metals can have on humans exposed to contaminated crops.

Thus, there is a need for a method that can successfully predict metal concentrations so that we can better gauge toxicity to plants and other organisms at higher levels of the food chain. In addition if remedial action becomes necessary, metal stabilization methods may regulate the plant toxicity problem at much lower cost than more sophisticated metal removal strategies.

The focus of the thesis is to compare the effectiveness of different methods at predicting plant metal concentrations, and to assess the ability of various soil treatments to reduce these concentrations.

CHAPTER ONE

Literature Review

Predicting Metal Content in Plants: Chemical Extractants Vs. Ion Exchange Resins

So far in Canada, the extent of metal contamination has been assessed by determining the total concentration of heavy metals in soils (CCME, 1991). Although this provides a good idea as to the state of contamination of the soil, it does not tell us how much of the metals are available for uptake by organisms. Methods that predict availability or uptake are therefore necessary for assessing potential toxicity of metals to soil life. Only then can measures be taken to reduce soil metal levels if they are high enough to impede the normal functioning of the organism.

According to Sposito (1989) the bioavailable fraction of metals in soils refers to those elements that can readily ionize, diffuse through soil solution and to the rooting system of the plant, and affect plant growth once absorbed. Thus the availability of elements in soils may be controlled by five distinct processes (McBride, 1994):

(i) <u>desorption or dissolution</u> which refers to the ability of metals to desorb from soil colloids and to be dissolved from solids into the soil. The rate in which these processes occur is dependent on the elements and solids involved.

(ii) <u>diffusion and convection</u> which refers to the ability of elements to move through soil pores.

iii) <u>sorption or precipitation at new sites</u> which can reduce the ability of plants to take up elements especially in clayey or humic soils.

(iv) <u>absorption of elements by roots</u> may involve both active and passive phenomena and is dependent on the concentration of the chemical near the rooting zone. The availability of elements can be controlled to some extent by the root which has the capacity to mobilize metals by releasing protons and chelating agents.

(v) translocation in plants which refers to the transfer of elements from roots to plant tops and may be influenced by soil chemical factors such as pH, phosphate, and base cation concentrations, as well as environmental factors.

These processes are best illustrated in the following figure.

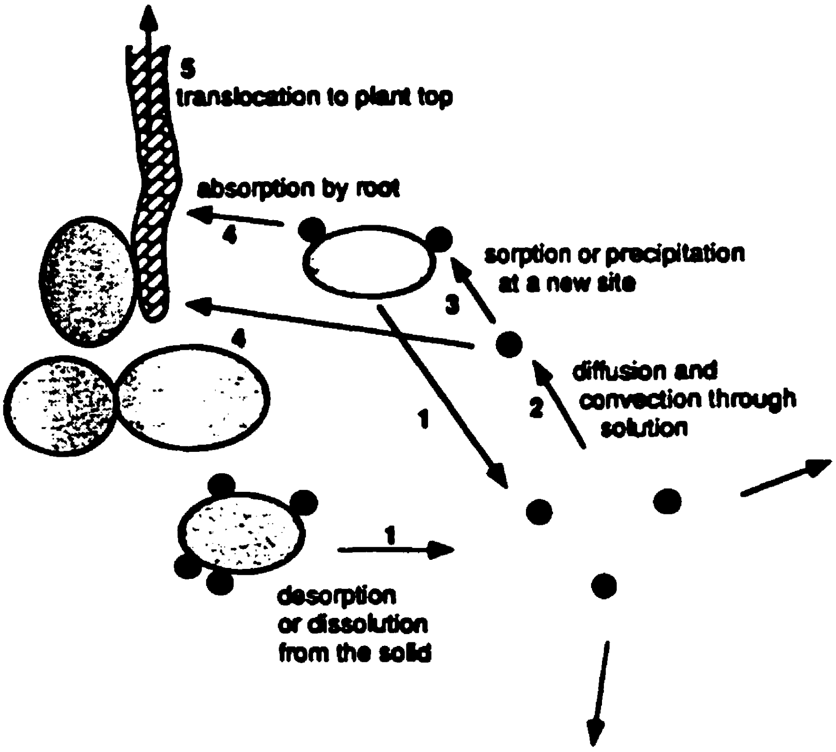

Figure 1.1: The five processes controlling element availability to plants (McBride, 1994).

Desorption/dissolution, diffusion/convection, and sorption/precipitation describe the extent of element mobility in the soil and consequently the degree to which the metal is made available to plants. Thus, such processes should be taken into account by a soil test designed to measure metal bioavailability (McBride, 1994). Assessing the relative mobility of an element and consequently predicting plant metal uptake can be best accomplished by analyzing the soil solution since it is from the liquid phase of the soil that nutrients and metals are likely to be taken up by plants (Kabata-Pendias and Pendias, 1992). The difficulty with this approach, according to Sposito and Bingham (1981), "lies with the need to separate the appropriate solution, to know which ionic species to measure, and to analyze the low concentrations involved." Despite these problems, soil solution analysis may still be the ideal method for predicting metal uptake in plants (Tiller, 1989). For instance, Jopony and Young (1993) measured the Pb concentration in soil solution by equilibrating the soil as a suspension in a 0.01 M Ca^{2+} electrolyte solution under aerobic conditions for a 14-day period. Analysis of the final filtrate for Pb showed good correlation with Pb uptake by red fescue (grass species) over a wide range of soil pH and total Pb concentrations. Although this approach seems quite promising, very little research has focused on soil solution analysis of metals.

By far the most widely used approach to predict plant metal uptake has been to employ chemical extractants. The main goal of using extractants according to Tiller (1989) was to find statistical relationships between element levels in plants and specific chemical-extractable fractions in soils. This approach has proven to be very practical for the purpose of crop management whereby various solutions of dilute acids, chelating agents, and salts have been employed for soil metal testing (Tiller, 1989).

A dilemma that researchers face when attempting to predict metal uptake in plants with chemical extractants is deciding which procedure to use. One reason for this is that chemicals can remove metals associated with different soil fractions, and since we do not know exactly which fractions plants are removing metals from, predicting metal uptake using any one extractant can be difficult.

It is well-known that metals in soil may be associated with various fractions (Tessier et al., 1979; Beckett, 1989). These include: the soluble fraction which is composed of uncomplexed metal ions and soluble organo-metallic and inorgano-metallic complexes; the exchangeable fraction in which the metals may be associated with clays, organic matter or amorphous materials by weak electrostatic forces; sites on some clay minerals and Fe, Al, and Mn oxides in which elements are held relatively strongly by covalent or coordinate forces; insoluble organic materials which include living or dead cells and organic secretions from organisms onto which cations are complexed or chelated; iron, aluminum or manganese oxide fraction in which metals may be adsorbed onto or occluded within the crystalline structures; as well as metal precipitates involving carbonates, sulfides, phosphates and hydroxides (Beckett, 1989).

Various extractants have been developed and used to remove metals from these fractions. The success of these chemicals to predict plant metal uptake is based on correlation studies and is dependent on the element and crop in question. For instance DTPA (pH 7.3) which is supposed to extract metals in the exchangeable, organically bound and precipitated fractions (Schalscha et al., 1982), showed good correlation between extracted Zn and Zn taken up by bermudagrass, sorghum and oats, but showed low correlation between DTPA-extractable Mn, Pb, and Ni and the amounts of these elements taken up by the three crops (Taylor et al., 1993). The same study also showed that Pb extracted by 0.1 M HCl (which removes metals from the exchangeable fractions and metals adsorbed onto oxides, carbonates and decomposing organic matter - Luoma and Jenne, 1976), correlated well with Pb taken up by oats ($r=0.99$), but poorly with Pb taken up by bermudagrass ($r=-0.13$). An earlier study carried out by the same authors using the same extractants showed that only DTPA-extractable Zn correlated well with Zn uptake by all legume crops used, whereas significant correlations between DTPA-extractable Fe, Pb, and Cu, and crop uptake depended on the cover crop used (Taylor et al., 1992). Results from this study also showed no significant correlations between DTPA-extractable Mn and Ni and uptake of these metals by any of the legumes used. Metals extracted with 0.1 M HCl also showed that extractable Mn did not correlate significantly with Mn uptake by any of the crops, and that significance of correlation between HCl-extractable Fe, Zn, Pb, and Cu and crop uptake was dependent upon the type of legume in question. In addition, Roca and Pomares (1991) showed that EDTA-extractable Zn, Cu, and Ni significantly correlated with uptake of these three elements with potato leaves, whereas none of these metals extracted by EDTA showed significant correlation with corn leaf uptake. Differences in correlations were also noticed between plant parts. For instance, the authors demonstrated that EDTA-extractable Zn and Cd correlated significantly with uptake by the wrapper leaves of lettuce, whereas only EDTA-extractable Ni was significantly correlated with uptake by the edible leaves of this crop. Similarly, although EDTA-extractable Zn, Cu, Cd, Ni, Pb, and Cr showed no significant correlation with uptake of these elements by corn leaves, uptake of Ni and Pb by corn grain did correlate well with extractable Ni and Pb.

It is obvious from these results that it is difficult to choose one chemical extractant to predict metal uptake in plants. Clearly, the effectiveness of an extractant is dependent on the element, plant, and plant part in question. This lack of universality in predicting plant metal uptake is partly due to the fact that extractants were designed for specific elements or soil conditions. For instance, the DTPA soil test was originally developed to predict Fe, Mn, Cu, and Zn uptake in crops grown on calcareous soils (Lindsay and Norvell, 1978; O'Connor, 1988). This chemical extracts labile elements and correlates well with plant micronutrient concentrations, provided the soil metal levels are neither too high nor too low. Although the soil test has been shown to reliably reflect metal loadings to soil, it was not designed to successfully predict heavy metal uptake in plants. This is because plants do not necessarily respond to increased metal loadings to soil (O'Connor, 1988). Thus good correlations between DTPA-extractable metals and plant metal uptake as observed in some studies (Mulchi et al., 1992; Tsadilas et al., 1995) may very well be coincidental.

Another reason why chemical extractants are not universally effective in predicting metal uptake may be because procedures are not applied properly. There are numerous variations in soil test methods carried out by researchers. For instance, differences between studies in reagent concentrations and pH, soil:solution ratios, and suspension shaking time are quite common (Tiller, 1989). Consequently this can affect results and conclusions drawn from these results. In addition, conditions that would seem unimportant to the extraction procedure may often go ignored and consequently unspecified in the publication of results. For example, the effects of acid ammonium oxalate extractant (which releases metals associated with Fe and Al oxides) have been shown to depend on the lighting conditions (example: dark conditions → less dissolution of oxides; light conditions → greater dissolution) (de Endredy, 1963), yet many studies do not specify this parameter (Beckett, 1989).

Another possible explanation for the lack of universality of various chemical extractants may be due to the fact that extractants do not effectively mimic root behaviour. Plant roots are known to extract metals from the soil solution as well as metals released from clay minerals by root exudates (such as H^+ ions and chelating agents) (Ross, 1994). Although some extractants have been shown to remove metals from both these fractions, the extent to which the metals are removed by chemicals may not be comparable with the amounts extracted by plants. Thus the ideal extractant would be the one that extracts metals from the same soil fractions that plants extract metals from, and to the same extent.

The difficulty in using any one extractant to predict metal uptake in different plant species is evident, and with growing environmental concern, the need to find a technique that can quickly and effectively perform this task is becoming increasingly important. Over recent years, interest in using synthetic ion exchange resins to study soils, aqueous systems, or other media has increased (Skogley and Dobermann, 1996). Much of this research has focused on predicting nutrient uptake in plants (Acquaye et al., 1972; Sibbeson, 1977; Binkley and Matson, 1983; Abrams and Jarrell, 1992; Qian et al., 1992; Mclaughlin et al., 1994), and results generally indicate that the resin technique is superior to conventional chemical extractants. For instance, Qian et al. (1992) developed a method for simultaneously extracting plant available N, P, S, and K using anion and cation exchange membranes and found that P and K uptake by canola plants was more closely correlated with membrane-extractable P and K than with $NaHCO_3$-extractable P and K. In addition, N and S uptake by canola plants correlated better with membrane-extractable NO_3 and SO_4 than with $CaCl_2$-extractable NO_3 and SO_4.

Studies employing ion exchange resins and/or membranes to predict trace metal uptake by plants also showed that resins were more effective at predicting metal uptake than conventional extractants (Hamilton and Westermann, 1991; Jing and Logan, 1991; Lee and Zheng, 1993; Lee and Zheng, 1994). For instance, Lee and Zheng (1993) demonstrated that wheat uptake of Cd correlated far better with chelex resin membrane-extractable Cd (r=0.97) than with Cd extracted by any other chemical used (0.1 M HCl, r=0.59; 0.01 M $CaCl_2$, r= 0.84; DTPA (pH 7.3), r=0.59; DTPA (pH 5.3), r=0.61).

According to Abrams and Jarrell (1992), part of the reason why resins work more effectively than chemical reagents is that resins can act as ion sinks which integrate soil element concentrations and diffusion rates. Since plants also behave as ion sinks (Skogley, 1992), the resin technique may provide a more accurate measure of assessing element bioavailability and plant metal uptake.

The following is a brief discussion of the equilibrium and kinetic processes involving resins, some of the properties of resins, and their use in the soil and environmental fields.

Physical chemistry of exchange equilibria

Ion exchange reactions are reversible processes with resins. For instance a resin can be converted from one form to another by washing the resin with an excess of electrolyte solution. Thus one can convert the H^+ form of the resin to the Na^+ form by flushing the resin with an excess Na salt solution. The general process of ion exchange is indicated in the following reaction

$$\mathbf{RA + B^+_{(aq)} \rightarrow RB + A^+_{(aq)}}$$

where R is the cation exchange resin, and A and B are ions associated with the resin (Kitchener, 1957). As written, the A form of the resin is shown to be completely converted to the B form. This can occur if A^+ ions are being continuously removed from solution or if there is an excess of B^+ ions in solution. If however a quantity of RA encounters only a limited quantity of B^+ ions in solution, an equilibrium is eventually set up whereby the resin contains both A and B forms. This is represented in the following expression (Kitchener, 1957):

$$\mathbf{RA + B^+_{(aq)} \leftrightarrow RB + A^+_{(aq)}}$$

The position of the equilibrium will depend on the proportion of RA relative to the B ion, as well as on the affinity of both ions for the resin. In addition the nature of the resin employed and the presence of other substances in solution can affect the position of the equilibrium (Kitchener, 1957). These factors indicate that resins exhibit some degree of specificity for one element relative to another which is an important property for many resin applications.

Kinetics of ion exchange

The rate of exchange processes involving resins is governed by the rate at which ions move to and from the liquid surrounding the resin bead (a process known as film diffusion), and the rate in which ions move to and from the interior of the resin grain (a process referred to as particle diffusion) (Kitchener, 1957). Both of these processes are dependent upon various factors such as the resin particle size, mixing of the resin with solution, the concentration of ions in solution, temperature, ion size, and the degree of cross-linking within the resin bead (Kitchener, 1957).

Properties of Resins

Styrene is the basic unit in the manufacture of most ion exchange resins. This compound is polymerized with other styrene molecules to produce a polystyrene chain. It is onto these long chain-like structures that divinylbenzene molecules are added for cross-linkages, and the extent to which the cross-linkage molecules are added can influence the physical and chemical properties of the resins (Skogley and Dobermann, 1996). The most common resins used in soil studies are the macroporous ion exchange resins which are spherical in shape and referred to as resin beads (Figure 1.2).

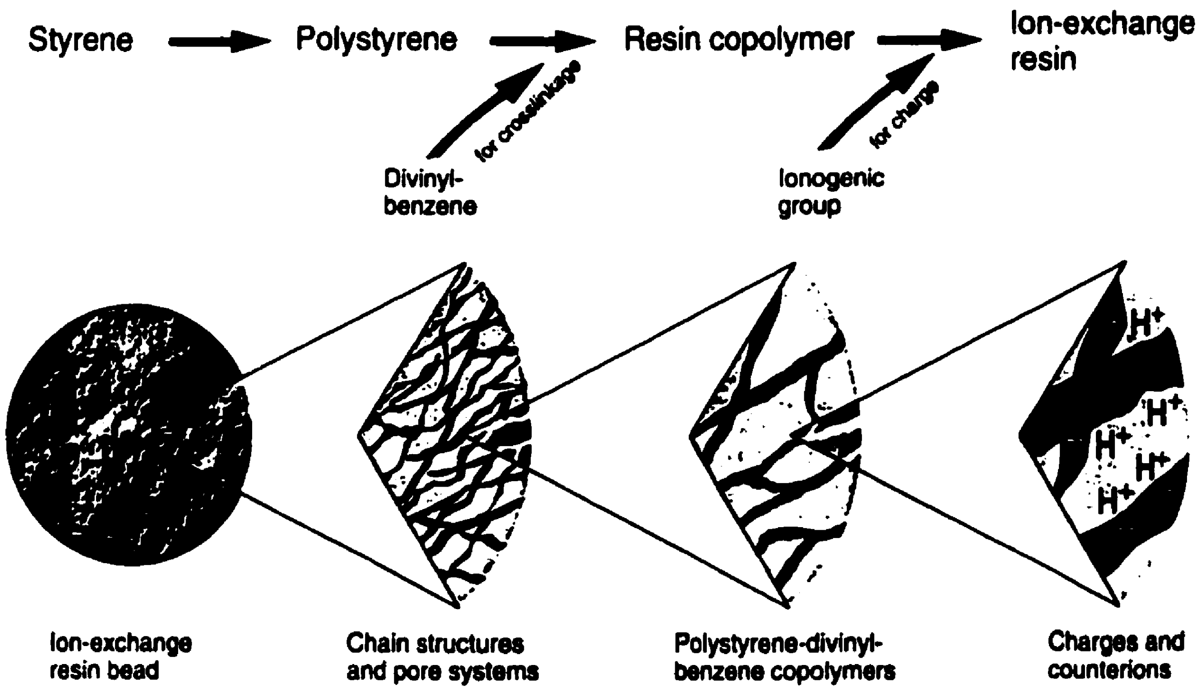

Figure 1.2: Ion exchange resin formation sequence and properties of a macroporous resin bead (Skogley and Dobermann, 1996).

Attached to the resin during its manufacture are the active (or ionogenic) groups which primarily determine the chemical properties of the resins (such as exchange capacity). Ionogenic groups such as sulfonic acid, carboxylic acid, and hydroxides are found on cation exchangers, whereas trimethylamine or dimethyl-ß-hydroxyethylamine groups are found on anion exchangers (Skogley and Dobermann, 1996). Thus the cation exchangers have acidic properties (strong or weak), whereas anion exchange resins are either strongly basic or weakly basic. The strongly acidic cation exchangers saturated with H^+ ions have a high affinity for major cations or trace metal cations and thus can be used for assessing cation mobility and bioavailability (Skogley and Dobermann, 1996). Weakly acidic cation exchange resins have a high affinity for H^+ ions which can be displaced only when nutrient or metal cation concentrations in solution are high enough to overcome affinity forces between the resin and H^+ ion (Skogley & Dobermann, 1996). This type of exchanger can be used in situations involving the supply of nutrients for plant uptake (Skogley and Dobermann, 1996).

Uses of ion exchange resins in the soil and environmental fields

Commercial applications of ion exchange resins have included preparation of pure water, extraction and concentration of uranium from low-grade ores, separation of rare earth and trans-uranic elements, and removal of salts from sea water or brackish water supplies (Kitchener, 1957). In the soil and environmental fields, resins have either been used as an ion source providing various nutrient ions to the soil medium for plant uptake, or as an ion sink whereby target ions are removed from the soil solution in exchange for the counterions on the resins (Skogley and Dobermann, 1996). This latter application has received considerable attention in research studies involved with predicting nutrient and metal bioavailability. Such studies have employed both diffusion-sensitive and batch methodologies which will be briefly discussed.

According to Skogley and Dobermann (1996), diffusion-sensitive methodologies (such as *in situ* resin techniques) integrate two major factors which determine nutrient and metal bioavailability. These are: i) the solution ion concentrations around the rooting zone and ii) the element diffusion rate to the rooting area. Several diffusion-sensitive methods for assessing bioavailability have been investigated. For instance, Binkley and Matson (1983) used resin bags for *in situ* measurement of N mobility in soils. Abrams and Jarrell (1992) and Savic et al., (1986) applied ion exchange membranes in situ for assessing soil P bioavailability, determining P diffusion rates, and mapping nutrient patterns in the rooting zone. Also, Skogley (1994) used diffusion-sensitive mixed-bed resin capsules for simultaneously accumulating positively charged and negatively charged ions from soil and water media.

Alternatively, batch system resin methods have also been used in many studies. Such methods involve mixing soil, water, and resin together and shaking the suspension for some period of time (Skogley and Dobermann, 1996). This approach has been applied for analyzing anions (anion batch systems), cations (cation batch system), and both anions and cations in soils (universal mixed-bed batch system) (Qian et al., 1992; van Raij, 1994; Skogley and Dobermann, 1996). Although such extraction methods do not provide any estimates of element diffusion, they are analogous to root uptake behaviour since the resins operate as semi-infinite sinks (Abrams and Jarrell, 1992).

Due to the reported effectiveness of resins to predict availability and metal uptake, efforts were taken to further test of a resin procedure. Thus, the purpose of the first study was to provide additional evidence regarding the effectiveness of resins compared to conventional extractants. Also, since many of the studies comparing resins with chemical reagents have been tested on artificially contaminated ("spiked") soil systems (Acquaye et al., 1972; Hamilton and Westermann, 1991; Qian et al., 1992; Lee and Zheng, 1993; 1994), this research was carried out using urban soils subjected to long-term metal contamination.

Remediating Contaminated Soil

Several measures can be taken to deal with metal-polluted soils once a site assessment has confirmed that a piece of land is contaminated. These include: transfer of the excavated material to a landfill area; removal of the contaminants by various physical, chemical, or biological methods; or stabilization of the metals in order to restrict their movement in the soil profile. In addition to this, measures should always be taken to minimize or prevent further contamination of the site by targeting the source of the problem. The following is a brief discussion of the abovementioned remediation strategies.

Excavation and Landfilling

This method provides a simple and quick approach to dealing with the problem of soil contamination, but may be complicated by various factors. These include the high costs of fill material and transporting contaminants, the difficulties in finding a landfill area that accepts highly contaminated soils, and other environmental problems resulting from this approach (such as traffic movement, noise, and atmospheric and surface water pollution) (Smith, 1987). In addition, metals may be mobile enough to move away from excavation boundaries and underneath adjacent buildings which can not be dug under. As a result, it may be necessary to prop up buildings, protect rivers and streams, temporarily lower groundwater levels, and monitor the working environment and neighbourhood (Smith, 1987). In addition, if soil is deposited elsewhere, there is the possibility of an environmental problem occurring at the landfill site. In spite of these drawbacks, excavation followed by landfilling can provide a suitable solution when the level of contamination is restricted to well-defined shallow boundaries.

Removal of Contaminants

As an alternative to landfilling, several strategies can be implemented that remove metal contaminants from the soil profile. These include physical (solvent leaching, gravity separation, and particle sizing), chemical (neutralization, chemical oxidation, and chemical reduction), thermal, and biological techniques.

Physical methods such as solvent leaching or extraction procedures make use of extracting agents such as ethylenediaminetetraacetic acid (EDTA) or diethylenetriaminepentaacetic acid (DTPA), acidic solutions, and chelating resins to remove metal contaminants from soil. Various studies have demonstrated the effectiveness of such chemicals. For instance, Hong and Pintauro (1996) showed that EGTA (ethylene glycol-(aminoethylether)-N,N,N',N'-tetraacetic acid) was very effective in removing Cd from kaolin in the presence of adsorbed alkaline earth metals. It was shown that the chelator specifically removed Cd, extracting over 80% from the clay component, while approximately 90% of Ca and Mg was still adsorbed. In a previous study, Hong and Pintauro (1995) also showed that EGTA and DCyTA (1,2-diaminocyclohexane N,N,N',N'-tetraacetic acid) were effective in removing Cd from kaolin over a wide range of pH (2.5-12.5). This demonstrates the potential of these compounds to clean up soils with varied properties. Sengupta and Sengupta (1993) showed that highly porous PTFE (polytetrafluoroethylene) membranes treated with chelating resins were very effective at removing Cu ions from sludges that had high concentrations of metal-complexing ligands (such as oxalate). Mazidji et al. (1992) also demonstrated that chelating resins and ion exchange resins were useful at removing Cd, Cu, and Zn ions from solution and consequently reducing the toxicity of these metals to bacteria. For instance, reductions in Cu toxicity to *Photobacterium phosphoreum* were from 100%-6% and 98%-3% for the chelating resin and ion exchange resin respectively. In addition, the authors showed that both resins were effective under varied pH conditions. Microtox assays demonstrated reductions in Cu toxicity from 100% to about 6% when either resin material was subjected to solution pH values of 5 to 8.

Despite the effectiveness of chelating agents and resins, one serious drawback of implementing them for large scale soil clean up, is their high cost. For instance, Metal Sorb-7 (a high molecular weight chelating compound containing various functional groups) and Duolite CS-346 (a macroporous chelating resin) cost $2.32/kg and $11.00/kg respectively, whereas stabilizing agents such as lime or Bentonite clays are much cheaper alternatives ($0.198/kg and $0.297/kg respectively) (Czupyrna et al., 1989).

Other physical methods which separate the metal-containing fractions on the basis of specific gravity, particle size, and settling velocity can also be used when the contamination is present in specific fractions of the soil (namely the fine, coarse, or organic). This approach can also be expensive when set-up, plant maintenance and operating costs are factored in (Smith, 1987).

Thermal techniques involve the application of heat to soils in order to break down or volatilize contaminants. This approach has been widely used for removing hydrocarbons from soils, but may also be applied for metals. For instance steam stripping involves the injection of steam into soils to facilitate the evaporation of contaminants (such as Hg, As, or Se) (Smith, 1987). A disadvantage with this method is that toxic gases leaving the soil must also be captured and treated to prevent contamination of other environmental matrices. In addition, the resulting increases in soil temperature can significantly alter the properties of the soil and possibly render it biologically inert.

Chemical oxidation and reduction procedures can facilitate metal removal from soil. For instance oxidizing Cr^{3+} to Cr^{6+} will increase the solubility of the metal and consequently improve its extraction through solvent leaching techniques. In addition, reducing Cr^{6+} to Cr^{3+} with $FeSO_4$ will enhance the precipitation of chromium and consequently reduce its concentration in soil solution. These reactions normally require long and close contact between the soil and the chemical agents. In addition an excess of treatment chemical is necessary to ensure completion of the redox reactions (Smith, 1987). As a result, implementing such techniques to remove metals can be slow and costly.

As an alternative to the above-mentioned physical and chemical methods, biological remediation provides a more natural approach to soil decontamination. Plants have been widely studied for this purpose. For instance *Thlaspi caerulescens* was shown to hyperaccumulate large amounts of Zn and Cd when grown on plots treated with metal-contaminated sewage sludge (Moffat, 1995). In addition, *Silene vulgaris* accumulated significant quantities of Pb and Zn when grown on mining dump material (Sieghardt, 1990). Other plants such as *Alyssum argentum*, *Thlaspi alpinum*, and *Stackhousia tryonii* were shown to hyperaccumulate Ni (up to 3-4% of leaf dry weight), and can potentially be used to clean up metal-laden soils (Reeves, 1995). In addition, plants can add aesthetic value to the contaminated land.

In spite of this, there are drawbacks with using vegetation for soil clean up. For instance, regular maintenance (such as watering and fertilizing soils) must be considered. Also, costs can be high since chelating agents are often required to facilitate metal transport to roots. In addition, dealing with the contaminated plants once harvested can be problematic.

Removal of metal contaminants from the soil can provide a permanent solution to the environmental problem, however there are various limitations associated with some of the discussed methods. For instance, physical techniques such as solvent leaching can treat sandy soils, but are less effective with clayey or organic soils. Also leaching methods are generally effective on soils with single contaminants rather than a range of metals (Smith, 1987). In addition, using chelating resins or oxidizing and reducing agents for large scale cleanups can be costly, while thermal methods can destroy soil properties and render a soil unsuitable for use without appropriate treatment following remediation.

Stabilization of Contaminants

Rather than removing metals from a soil profile, a cheaper, less labor-intensive, and less destructive approach to dealing with the contamination, would be to add treatments (that restrict metal movement and availability) directly in the soil without having to remove them at a later date. Such treatments 'stabilize' metals by altering soil properties (such as pH, available P content, and cation exchange capacity) that influence metal mobility.

Several compounds have been studied for their effectiveness at metal stabilization. For instance, Czupyrna et al., (1989) showed that a synthetic aluminosilicate (Valfor 200 zeolite) was effective at immobilizing Cd, Ni, Cu, and Zn when these elements were present singly or together in solution. Compared to cation exchange and chelating resins (which are generally used for metal removal), the authors showed that the immobilization efficiency (k), defined as the quantity of metals adsorbed divided by the metals initially present in solution, was higher for the aluminosilicate than for these other compounds. For all metals tested, the k value of Valfor 200 was 0.20, whereas for Amberlite IR-124 (a cation exchange resin) and Amberlite

IRC-718 (a chelating resin), k values were 0.05 and 0.04 respectively.

Gworek (1992a) also showed that synthetic aluminosilicates were effective at immobilizing metal contaminants. Zeolites added to Pb contaminated soils decreased Pb content in lettuce leaves, grasses, and oats by 49-73%, 47-77%, and 58-68% respectively. Gworek (1992b) further demonstrated that Cd concentrations were lower in plants grown on zeolite-treated soils than in untreated soils. Cadmium levels in lettuce, ryegrass leaves, and ryegrass roots were reduced by 85.8%, 73.9%, and 51.8% respectively compared to controls.

The effectiveness of aluminosilicates to stabilize metals and consequently reduce plant metal uptake is largely due to its high cation exchange capacity. Ions such as Na and Ca (which are normally associated with zeolites), can be exchanged for metal ion contaminants in soil solution. Additionally, these base cations can elevate pH levels when released in solution, and subsequently increase the number of negatively charged sites on soil colloids. As a result metal adsorption on these sites will increase.

Apart from their reported effectiveness, synthetic aluminosilicates are cheaper than some chemicals used for metal removal. For instance, Valfor 200 cost $0.551/kg, compared to Amberlite IR-124 and Amberlite IRC-718 which cost $6.61/kg and $11.00/kg respectively (Czupyrna et al., 1989). The authors also determined that the cost to clean up soil, spiked with Cr, Cd, and Ni at 30 meq/kg of total metal, with Valfor 200 (and $FeSO_4$ to reduce Cr^{6+}) was estimated to be in the range of $23.9/ton-$33.8/ton. In comparison, the costs of transporting and disposing contaminated soil in a landfill site was estimated at $150/ton-$250/ton depending on the distance to the landfill area.

In addition to zeolites, other compounds such as lime, K_2HPO_4, hydrous manganese oxides, and peat have been used for stabilizing metals in soils. Pierzynski and Schwab (1993) demonstrated that limestone was effective at decreasing KNO_3-extractable Zn in soils and reducing Zn and Cd concentrations in soybean tissue. This may be explained by the fact that lime raises soil pH which increases metal sorption on colloidal surfaces. In addition, limestone was found to increase soybean growth. Czupyrna et al. (1989) also showed that hydrated lime was effective at immobilizing Cd, Ni, Cu, and Zn when these metals were present singly or together in solution, and that its immobilization efficiency (k=0.74) was greater than that of some chelating resins, namely Duolite CS-346 (k=0.21) and Dowex XFS-4195 (k=0.19). Also at $0.198/kg, lime is much cheaper than the lesser effective resin material (both of which cost $11.00/kg) (Czupyrna et al., 1989).

Reduction in plant metal uptake by increasing available P content in soils has been demonstrated with the use of K_2HPO_4 and Thomas phosphate basic slag (TBS). Pierzynski and Schwab (1993) showed that K_2HPO_4 significantly reduced KNO_3-extractable Zn and soybean tissue Zn concentrations. Mench et al. (1994) also demonstrated that TBS (made of steel metallurgy waste material) lowered Pb bioavailability and plant tissue Pb concentrations. In both studies, the precipitation of metal phosphates may likely explain the reduction in plant uptake.

Also, hydrous manganese oxides (HMO) have been effectively used for metal stabilization. Mench et al. (1994) showed that HMO decreased Cd levels in tobacco and ryegrass, as well as Pb concentrations in ryegrass shoots. In addition, Pb and Cd levels in the water-soluble fractions of HMO-treated soils were significantly lowered compared to controls. The effectiveness of this treatment could be explained by its low point of zero charge (PZC) which is 1.5-2.0 (Mench et al., 1994). At soil pH values higher than the PZC, the HMO becomes negatively charged and its cation exchange capacity increases. Thus even under acidic conditions, HMO have a high adsortion capacity. Also, since Cd and Mn ions compete with each other for plant uptake sites (Kabata-Pendias and Pendias, 1992), the reduction in Cd uptake may be explained by the addition of Mn to the soil from the HMO treatment.

Organic treatments have also been demonstrated to be effective at metal stabilization by increasing soil cation exchange capacity. Leaching tests on soil-peat mixtures showed that peat had a high adsorption capacity for Pb which could be explained by the polyacidic functional groups associated with the humic content of the treatment (Majid et al., 1996). The authors calculated the adsorption maximum to be 150.4 mg Pb/g peat, whereas for soil it was 12.6 mg/g. Majid et al., (1996) also showed that Pb adsorption on peat was rapid (reaction was complete in 5 min.) and that the total Pb fixation in soil occurred at a peat loading of 20% (wt/wt).

Although many of the above-mentioned stabilization products provide an effective and lower cost alternative to metal removal (or landfilling) strategies, applying these materials on a large scale can still be expensive.

Over the past twenty years or so, studies have been carried out using lower cost food waste material to remove metal ions from contaminated aqueous systems. Many of these products including rice hulls and bran, corn cob meal, peanut skins and hulls, and soybean hulls are abundant and relatively inexpensive (Randall et al., 1975; Henderson et al., 1977; Henderson et al., 1977; Randall et al., 1978; Suemitsu et al., 1986; Okieimen and Okundaye, 1989; Marshall et al., 1993; Roy et al., 1993; Marshall and Champagne, 1995). For instance, rice-milling byproducts such as hulls and bran are produced in large quantities every year (1.4 billion kg and 640 million kg respectively) and cost between $0.00 and $0.077/kg. Other waste products such as corncob meal, peanut hulls, and soybean hulls are also abundant and cost about $0.008/kg, $0.012/kg, and $0.08/kg respectively. These materials are far cheaper than some of the previously discussed stabilization products (lime→$0.198/kg, Valfor 200 zeolite→$0.551/kg), and therefore applying them on a large scale would be more cost-efficient.

In addition to their low cost and abundance, several research studies have demonstrated these products to be effective at remediating metal-contaminated waters. For instance, Roy et al, (1993) demonstrated that rice hulls effectively removed Sr, Cd, and Pb from solution (adsorbing 94%, 98%, and 99% of these metals respectively), as well as over 99% of $Cr_2O_7^{2-}$ and $H_2AsO_4^-$ from contaminated water. Marshall et al., (1993) showed that defatted extrusion stabilized rice bran removed over 82% of Cr and Cu, and about 94% of Zn from solution. Zinc removal compared favorably with Amberlite IRC-718 chelating resin which adsorbed 100% of this metal. A more recent study by Marshall and Champagne (1995), also showed that defatted extrusion stabilized rice bran effectively removed Cu and Zn from metal plating wastewater solution and compared well with the Amberlite resin. For instance 95-100% Zn and 79-100% Cu was removed by the byproduct, compared to Zn and Cu removal of 98-100% and 87-100% respectively by the resin. In addition, the authors showed that soybean hulls were just as effective as the resin at adsorbing Cu from wastewater (82.6-99.8% removal by the hulls compared to 87.2-100% removal by Amberlite IRC-718).

Tannin-containing waste products such as peanut skins, are also highly efficient materials for metal ion removal. Randall et al., (1975) showed that wastewater Cu concentrations as high as 200-1000 ppm can be reduced to less than 0.5 ppm by pouring the solution through a column packed with peanut skins. Also, equilibrium experiments carried out by Randall et al., (1978) showed that peanut skins significantly decreased the metal content of solutions. For instance, Cu, Zn, Pb, and Cd levels decreased from 50 to 0.42 ppm, 21.8 to 1.6 ppm, 100 to 0.7 ppm, and 100 to 7.8 ppm respectively, when a metal-containing solution was shaken for several hours with this treatment.

The effectiveness of these food waste products can be explained in part by the organic compounds they contain. For example, tannic compounds in peanut skins contain phenolic groups which directly participate in ion exchange. Thus, two hydrogen ions on adjacent hydroxyl groups are exchanged for one Cu ion in solution (Randall et al., 1975). Also, the lignocellulosic fraction of soybean hulls, which makes up 86.2% of hulls and consists of metal-binding compounds like lignin, cellulose, and hemicellulose, may explain the high adsorption capacity of this treatment (Marshall and Champagne, 1995). In addition, phytic acids in rice bran contain several hydroxyl groups which can participate in charge-charge interactions with metals (Marshall et al., 1993). Also, phytic acids are a principal storage component of P in cereal grains (Turecki et al., 1994), and therefore precipitation of metal phosphates may occur when bran products are added to metal-contaminated systems.

Because these food waste materials are effective at remediating metal-contaminated waters, are widespread, relatively inexpensive (compared to zeolites or lime), and have not been tested on soil systems, measures should be taken to demonstrate their stabilization potential in soils. Therefore, the purpose of the second part of the thesis was to test the effect of food waste products on metal uptake and concentrations in plants grown on contaminated urban soils, and compare the results with those of a commercially available zeolite. In addition, the changes in soil properties resulting from treatment addition were measured in order to explain plant metal uptake and concentration effects.

CHAPTER TWO

Using Anion Exchange Resin Membranes and Chemical Extractants to Predict Metal Concentrations in Lettuce Grown on Urban Soils

Using Anion Exchange Resin Membranes and Chemical Extractants to Predict Metal Concentrations in Lettuce Grown on Urban Soils

Abstract

Ion exchange resins have been shown to simulate root metal uptake behaviour by acting as ion sinks when in contact with soil. Thus they have been considered more suitable for predicting bioavailable metal compared with selective chemical extractants.

This study focusses on comparing the effectiveness of several chemical extractants with anion exchange membranes treated with either DTPA or EDTA at predicting Pb, Cu, and Zn concentrations in lettuce plants (*Lactuca sativa* cv. Buttercrunch) grown on contaminated urban soils. Results demonstrated that DTPA, $HNO_3/HClO_4$, and Na acetate were the best predictors of Pb concentration in lettuce leaves, explaining 88%, 86%, and 85% respectively of the variability. The DTPA and EDTA chelators, as well as the membrane treated with EDTA, the HCl, and H_2O extractants best predicted Cu concentrations yielding r^2 values of 0.77, 0.72, 0.67, 0.69, and 0.62 respectively. Results also showed that the $HNO_3/HClO_4$ digestion procedure best predicted Zn concentrations in lettuce explaining 64% of the variability.

For all metals analyzed, the anion exchange membrane treated with EDTA predicted bioconcentrations better than the membrane treated with DTPA. Results also showed that the membrane techniques did not predict plant metal levels better than the other methods tested. The Na acetate and DTPA extractions worked just as well as the membrane procedure, and the $HNO_3/HClO_4$ digestion was demonstrated to be the best predictor overall.

Key Words : Anion exchange membrane, chemical extractants, metal concentrations, urban soils, *Lactuca sativa.*

Introduction

Metal contamination has been assessed by determining the total concentration of heavy metals in soils (CCME, 1991). Although this measurement may indicate the level of contamination in the soil, it does not tell us how much of the metals are available for uptake by organisms. Therefore, alternative methods that effectively predict availability or plant metal uptake are required for assessing potential toxicity to soil organisms. This would provide a sound scientific basis for identifying soils that require remediation if bioavailable metals are high enough to adversely affect organisms in direct contact with the contaminants and those at higher levels of the food chain.

Chemical extraction procedures have been widely used to predict metal uptake in plants. The main goal of using extractants, according to Tiller (1989), is to find statistical relationships between element levels in plants and specific chemical-extractable fractions in soils. This approach has proven to be useful for the purpose of crop management whereby various solutions of dilute acids, chelating agents, and salts have been widely employed for soil metal testing (Tiller, 1989).

A problem researchers face with chemical extraction procedures is deciding which extractant to use to predict plant metal uptake. This is because different chemicals can remove metals associated with different fractions of the soil. For instance, $CaCl_2$ extracts metals found in the soluble and exchangeable fractions (McLaren & Crawford, 1973; Iyengar et al., 1981), DTPA (pH= 7.3) is believed to extract the exchangeable and organically bound trace metals, and dissolves "precipitates" (Schalscha et al., 1982), while Na acetate (pH= 5.0) specifically targets the carbonate fraction (Tessier et al., 1979). Since we do not know exactly which soil fractions plants are removing metals from, predicting metal uptake using any one of these or other extractants can be troublesome. In addition the difficulty in choosing an extractant is compounded by their lack of universality in predicting metal uptake. For instance numerous studies have demonstrated that the effectiveness of an extractant to correlate with plant uptake may be dependent upon the metal, plant, and/or plant part in question (Roca & Pomares, 1991; Taylor et al., 1992; Taylor et al., 1993).

Studies in which ion exchange resins and/or membranes have been used to predict element uptake in plants have increased over recent years. Most of this research has focused primarily on predicting nutrient uptake in crops (Abrams & Jarrell, 1992; Qian et al., 1992; McLaughlin et al., 1994), and results generally indicate that the resin method is a better predictor than conventional extractants. Other studies correlating resin membrane extractable metals with plant uptake have also shown the resin technique to be superior to chemical extractants (Hamilton & Westermann, 1991; Jing & Logan, 1991; Lee & Zheng, 1993; Lee & Zheng, 1994). For instance Lee & Zheng (1993) demonstrated that wheat uptake of Cd correlated better with chelex resin membrane extractable Cd ($r = 0.97$) than with Cd extracted by any other chemical used (0.1 N HCl, $r = 0.59$; 0.01 M $CaCl_2$, $r = 0.84$; DTPA (pH 7.3), $r = 0.59$; DTPA (pH 5.3), $r = 0.61$). Liang & Schoenau (1995) used anion exchange membranes (AEM) treated with DTPA to show that AEM/DTPA extractable Cd correlated better with Cd uptake in oats compared with DTPA extractable Cd. Tejowulan (1994) using the same technique demonstrated that AEM/DTPA extractable Cu, Zn, and Mn from several Indonesian soils correlated better with canola uptake of these elements than DTPA extractable Cu, Zn, and Mn. He also showed that the anion exchange membrane treated with EDTA better predicted canola uptake of Cu, Zn, and Mn than an EDTA extraction procedure.

According to Abrams & Jarrell (1992), part of the reason why resins work more effectively than chemical extractants is that resins (like plants) can act as ion sinks which integrate soil element concentrations and diffusion rates. Unlike resins, chemical extractants generally do not behave in this manner.

In addition, Tejowulan (1994) demonstrated that anion exchange resin membranes in either DTPA or EDTA forms are practical for routine use in the laboratory. For instance, the structure of the membrane is strong enough to facilitate handling; results obtained are reproducible; and the membranes are reusable.

Based on these advantages, the anion exchange membrane procedure was chosen for this study. In addition several chemical extractants were used. The purpose of this project was to provide further evidence as to whether the resin membrane method is more, less, or equally effective at predicting plant metal concentrations than conventional extractants.

Materials and Methods

Soil Sampling

Four soils sampled within the Montreal urban area were used for this study (one from a city-owned property, two from railway sites, and one from a military base). Two of the soils had total Pb concentrations above 1000 mg/kg which exceeds the acceptable level for commercial land use in Canada (CCME, 1991). Two had total Cu concentrations greater than 150 mg/kg (which is higher than acceptable levels for agricultural land use), and one soil had a total Cu content of 500 mg/kg (which is above standards for commercial land use). Regarding Zn contamination, one soil had levels above 500 mg/kg surpassing standards for residential land use, and another soil had concentrations greater than 600 mg/kg which exceeds acceptable levels for agricultural land in Canada. In addition, a forest soil (St. Bernard) which had Pb, Cu, and Zn concentrations well below the acceptable limits of these metals for residential land use was used as a control.

All soils were homogenized and sieved to pass 2 mm before using them for the greenhouse experiment. In addition, a sample of each of the homogenized and sieved soils was air dried and ground to pass 1-mm and 0.5-mm sieves for soil analyses.

Greenhouse Experiment

Seven hundred grams of each of the 5 soils (< 2 mm) were placed in 125 mm pots. The experiment was replicated 5 times. Calcium carbonate was added to the control soils (20 g/kg dry soil) so that the pH would be comparable to the contaminated soils. All soils were left to incubate in the greenhouse for 30 days, after which they were fertilized with 10 ml of 0.1 M KH_2PO_4, 0.06 M KNO_3, and 0.1 M NH_4NO_3, and sown with five lettuce seeds (*Lactuca sativa* cv. Buttercrunch). This crop was chosen as a result of its easy maintenance, propensity to accumulate metals, and direct relevance to humans. Fertility rates were based on the pH and available nutrient content of the soils, and were determined using a fertilizer recommendation program (Fornier, 1995). Three other fertilizations were carried out during the course of the experiment (26 days, 33 days, and 38 days after seeding) but only KNO_3 and NH_4NO_3 were applied at 0.17 M and 0.04 M concentrations respectively. Additional phosphate fertilizer was not added due to its potential effects on metal precipitation. Seedlings were thinned to one over the first 36 days. The experiment continued for an additional 34 days (until harvest). At harvest time roots were cut off and discarded, while leaves were thoroughly washed with distilled water, dried, and kept for analysis.

The conditions in the greenhouse were maintained at 40 - 50 % relative humidity, 20°C day temperature, 15°C night temperature, and 16 h daylight. Plants were watered daily with distilled water to maintain soil at approximately 25% water content.

Laboratory Experiment

Plant Analysis

All plant tissue samples were oven dried for 48 h at 64°C. Lettuce leaves were hand-crushed and digested using an HNO_3/H_2O_2 digestion procedure (Mench et al., 1994). An inter-laboratory audit sample (V-8) containing Pb, Cu, and Zn concentrations of 1.30 ± 0.97 mg/kg dry tissue, 10.2 ± 1.2 mg/kg, and 25.2 ± 4.6 mg/kg respectively (after outlying values were removed for Pb and Zn - Koteles et al., 1992) was measured as having 0.82 ± 0.11 mg/kg, 9.18 ± 0.62 mg/kg, and 22.2 ± 1.6 mg/kg of Pb, Cu, and Zn respectively. All digests were analyzed for these elements using a 2380 Perkin Elmer Flame Atomic Absorption Spectrophotometer (AAS) or a Varian Spectra 300 Graphite Furnace AAS (with Zeeman background correction) when metal concentrations were below detectable levels for the flame.

Soil Analysis

The soils were analyzed for physical and chemical properties including pH (1:2 soil/water ratio - Hendershot et al., 1993a), electrical conductivity (1:2 soil/water ratio - Janzen, 1993), cation exchange capacity (Hendershot et al., 1993b), organic C content (Nelson & Sommers, 1982), and particle size (McKeague, 1976). Several chemical extractions were also carried out on the soils and analyzed for Pb, Cu, and Zn. Methods used include the following:

i) 0.1 N HCl (Norvell, 1984)

ii) 0.01 M EDTA (pH 8.6) (Trierweiler & Lindsay, 1969)

iii) 1.0 M Na acetate (pH 5.0) (Tessier et al., 1979)

iv) 0.005 M DTPA (pH 7.3) (Liang & Karamanos, 1993)

v) An H_2O extraction method in which 15 g of 2-mm sieved soil and 30 ml of deionized H_2O were shaken in a 50 ml centrifuge tube for 16 h on a reciprocal shaker. Following 20 min of centrifuging at 600 *g*, the supernatant was filtered through a 0.45 μm nylon syringe filter and the filtrate was analyzed for metals.

vi) An $HNO_3/HClO_4$ digestion procedure (Cook, 1998) was carried out whereby 1 g of soil (ground to pass 0.5 mm) was digested with 15 ml of concentrated HNO_3 (trace metal grade) for 16 h at room temperature and then at 150°C (in a block digester) for about 3 h. Following a brief cooling period, 5 ml of concentrated $HClO_4$ (trace metal grade) was added to each tube, and samples were further digested at 180°C for 3-4 h. An inter-laboratory audit soil sample (ECSS #7) reported to contain 15.0 ± 2.0 mg Pb/kg, 28.0 ± 0.4 mg Cu/kg, and 101 ± 6 mg Zn/kg (Sheldrick and Wang, 1995) was digested as well, and metal concentrations obtained were 20.2 ± 0.6 mg Pb/kg, 20.5 ± 0.6 mg Cu/kg, and 91.6 ± 1.3 mg Zn/kg.

In addition to the above mentioned chemical extractants, two anion exchange membrane procedures were used. The method is described in detail in Tejowulan (1994), but is summarized below:

Positively charged anion exchange membranes (BDH product # 551642S) were cut into strips of 3 cm x 6 cm, and soaked in a solution of 0.01 M DTPA chelator (disodium salt) for a 16 h period. DTPA is negatively charged and will therefore bind to the resin membrane, however according to Tejowulan (1994) enough negative charges on the chelator will be available for metal binding as well. To ensure complete saturation of the membranes with DTPA before the extraction was carried out, the membranes were further soaked in the chelator solution for an additional hour by shaking on a reciprocal shaker.

For the extraction procedure, the anion exchange membrane strips (with bound chelator) were placed in a 125 ml plastic bottle holding 5 g of soil (< 2 mm) and 100 ml of deionized, distilled water. The membranes (with soil and water) were shaken for 1 h on a reciprocal shaker at low speed (about 120 strokes/min), then rinsed with water and placed in a 50 ml centrifuge tube containing 20 ml of 1.0 N HCl eluant. The membrane strips were shaken in the eluant solution for 1 h to displace the metals bound to the chelator surface. The metals retrieved in the acidic solution were analyzed on flame AAS, while the membranes were regenerated with 0.01 M DTPA and kept for future use.

A second membrane procedure was carried out as described above, except a 0.01 M EDTA (disodium salt) chelator solution was used instead of DTPA.

Statistical Analysis

In addition to the data obtained from the five soils in this study, the lettuce Cu data from another study was also used (Sauvé et al., 1996) to increase the sample size for more powerful correlation and regression analyses. Also the lettuce Pb and Zn data from Cook (1998) was used for the same purpose. Both of these studies utilized the same urban soils for their experiments, and greenhouse conditions in these studies were similar to the conditions in our research. Chemical and membrane extractions on these soils were carried out as part of this study. For all the extraction data (membrane and chemical extractions), values were *log* (10) transformed in order to normalize the data. The plant metal concentration values were normally distributed and therefore not transformed. All statistical analyses were carried out using Systat software version 5.0 (Wilkinson, 1992). The level of statistical significance is represented by * for $p<0.05$, ** for $p<0.01$, *** for

$p<0.005$, **** for $p<0.001$, and ^{ns} when not significant ($p>0.05$).

Results and Discussion

The physical and chemical properties of the urban soils used in this study are presented in Table 2.1. The pH of the soils were are around 8, except for the control which had a pH of 5.58. Their alkaline nature may be explained by the presence of ash material (resulting from coal burning) or calcite. The hydrolysis of mineral oxides contained in ash would likely form mineral hydroxides which could explain the high pH values observed. The acidic properties of the control (St. Bernard soil, pH = 5.58) may be explained by the presence of organic acids resulting from the decomposition of the forest leaf litter. The texture of the soils used were mostly sandy loam while one soil was a loamy sand, one a silty loam and another was a loam. The cation exchange capacity (CEC), electrical conductivity (EC), and organic carbon content of the soils were more variable with ranges of 6.3 to 51.9 cmol(+)/kg, 257 to 9650 µS/cm, and 13.2 to 108 mg/g respectively.

The lettuce bioaccumulation data are shown in Table 2.2. The Pb data for lettuce grown on five of the soils are excluded because the replicates were highly variable. The results show that Zn concentrations were an order of magnitude greater than Cu and Pb concentrations in the plant tissues for all soils tested. This may be due to higher levels of Zn in some soils compared to Pb or Cu.

The concentrations of metals extracted are presented in Tables 2.3, 2.4, and 2.5 for Pb, Cu, and Zn respectively. Water was found to be the weakest extractant for all three elements. This was expected since H_2O extracts metals from the soluble fraction of soils in which only a tiny proportion of the total metal content is usually found. The extractant removed between 0.006 to 0.58 mg Cu/kg, 0.0 to 3.5 mg Pb/kg, and 0.02 to 0.27 mg Zn/kg. The $HNO_3/HClO_4$ digestion which estimates the total concentration of metal was the strongest extractant. Concentrations of 23.2 to 3198 mg/kg were found for Cu, 44.7

to 2170 mg/kg for Pb, and 122 to 3090 mg/kg for Zn. The anion exchange membrane (AEM) treated with either EDTA or DTPA removed lower amounts of metals than the two chelators (DTPA and EDTA) and the more acidic extractants (HCl, Na acetate pH 5.0, and $HNO_3/HClO_4$), but they removed greater amounts of Pb, Cu, and Zn than H_2O. The extractants can be classified in order of decreasing strength. For Zn our results showed that:

$HNO_3/HClO_4$> HCl > Na acetate> EDTA> DTPA> AEM/EDTA> AEM/DTPA> H_2O;
for Cu:
$HNO_3/HClO_4$> EDTA> HCl> DTPA> Na acetate> AEM/DTPA> AEM/EDTA> H_2O;
and for Pb:
$HNO_3/HClO_4$> HCl> EDTA> Na acetate> DTPA> AEM/DTPA> AEM/EDTA> H_2O.

Regression plots for the lettuce and *log* transformed extractable metal data for Pb, Cu, and Zn are shown in Figures 2.1 to 2.3. In general, good regressions were obtained with most extractants for Pb (Figures 2.1 a-h). The AEM/DTPA, AEM/EDTA, DTPA, EDTA, and Na acetate extractions as well as the $HNO_3/HClO_4$ digestion explained 72%, 81%, 88%, 81%, 85%, and 86% respectively of the variability of Pb in lettuce. Only H_2O and HCl extractants explained a low proportion of the variance of Pb (59% and 42% respectively), and HCl alone was not significant at the 0.05 level ($p < 0.1$). The fact that the resin membrane extractions produced good results, significant at the probability levels of 0.01 and 0.005 for AEM/DTPA and AEM/EDTA respectively, could be due to their ability to simulate root uptake behaviour by acting as ion sinks. The membranes extract metals from the soil solution as metal cations are being replenished by the dissolution of or desorption from soil solid phases. This may be the way plants remove elements from the soil (Lee and Zheng, 1993). Results also showed that other extractants, such as DTPA, Na acetate, and $HNO_3/HClO_4$ explained a larger proportion of the variance in Pb than the two membrane-chelator extractions. The reason for this may be that the membrane extractions were carried out for only 1 h. This may not have been long enough for the soil to replenish the solution with metals as the cations are removed by the membranes. Therefore the membranes may not be accumulating sufficient amounts of metals to reflect actual differences in bioavailable metal among soils.

A study using anion exchange membranes treated with DTPA to predict Pb concentrations in lettuce yielded better results ($r^2 = 0.984$; $p < 0.05$; $n = 4$) (Liang and Schoenau, 1995). In the study, an in-situ method was used, where the AEM/DTPA was inserted into a soil saturated with water. The heavy metal concentration gradient established in the soil with this method may be similar to that of a plant-soil system. This may explain why 98% of the variability was accounted for. It is worth noting that the results were found to be significant at the 0.05 level (compared with $p < 0.01$ for results obtained in our study), despite a higher r^2 value. This demonstrates the effect of sample size on the significance level of results. Thus, studies using a larger number of samples would be useful to confirm whether the AEM/chelator in-situ method is better than the membrane extraction procedure at predicting Pb concentrations in lettuce.

Regression plots of plant Cu and extractable Cu are shown in Figures 2.2 a-h. Results show that the AEM/DTPA, Na acetate, and $HNO_3/HClO_4$ methods explained 61%, 59%, and 61% respectively of the variability in lettuce Cu concentrations and produced results significant at the 0.005 level. On the other hand, AEM/EDTA, DTPA, EDTA, H_2O, and HCl extractions yielded better regressions, explaining 67%, 77%, 72%, 62%, and 69% respectively of the variance, and produced results significant at the 0.001 level.

Results obtained with the membrane-chelator complexes, DTPA, and EDTA extractions were comparable with those produced from a previous study which used these extractants to predict Cu uptake in canola plants grown on several Saskatchewan soils (Tejowulan, 1994). This study showed that the DTPA and EDTA extractions which explained 74% and 64% respectively of the variability in Cu uptake in canola were better at predicting uptake than the membrane-DTPA or membrane-EDTA extractions which explained 59% and 61% respectively of the variance. Results obtained in our study showed this pattern as well. In a similar study conducted by Tejuwulan (1994) using these same extractants on Indonesian soils, the reverse pattern was found. That is, membranes treated with either DTPA or EDTA were better at predicting Cu uptake in canola than either DTPA or EDTA extractions. The lower pH, organic matter content, and carbonate content of soils in humid climates compared with dryer climates could influence metal availability and possibly explain the observed results. Further studies using soils with more varied properties (such as pH and organic carbon content) are warranted to confirm whether membrane-chelator extractants predict Cu levels in plants more effectively than conventional extractants such as DTPA or EDTA.

Regressions obtained for Zn are presented in Figures 2.3 a-h. The r^2 values here were generally lower than those obtained for Pb and Cu. The AEM/DTPA extraction explained 23% of the variance in Zn concentration, AEM/EDTA explained 52%, the DTPA extraction - 38%, EDTA - 44%, H_2O - 48%, HCl - 56%, Na acetate - 49%, and the $HNO_3/HClO_4$ digestion method accounted for 64 % of the variability. Despite these low values, results were significant at either the 0.05, 0.01, 0.005, or 0.001 levels for all extractants (except AEM/DTPA which had a value of $p < 0.1$).

The overall performance of the extractants to predict metal concentrations in lettuce tissue is shown in Table 2.6. The DTPA, $HNO_3/HClO_4$, and Na acetate extractions were the best predictors of Pb bioconcentration; DTPA, EDTA, HCl, AEM/EDTA, and H_2O best predicted plant Cu levels; and the digestion procedure best explained Zn variability in lettuce compared to the other extractants tested. In each case, the membrane extractions did not outperform the chemical extractants, however significant results were obtained for all lettuce concentration - membrane extractable metal relationships except for one. If we look at the ability of the extractants to simultaneously predict Pb, Cu, and Zn concentrations in lettuce tissues, AEM/EDTA performed just as well as the DTPA and Na acetate extractants, and was surpassed only by the $HNO_3/HClO_4$ digestion procedure.

Summary and Conclusions

The purpose of this study was to compare the effectiveness of ion exchange membrane methods with various conventional extractants to predict metal concentrations in lettuce. We found that the membrane-chelator extractants gave significant results for most of the regressions, and that the anion exchange membrane treated with EDTA was an overall better predictor of Pb, Cu, and Zn than the one treated with DTPA. However, the results show that the membrane extraction was not the best method for predicting metal concentrations. The Na acetate and DTPA procedures worked just as well as the membrane technique, and the $HNO_3/HClO_4$ method proved to be the best predictor overall. This was contrary to what we expected. Possible reasons for this may be suggested. Perhaps a longer extraction time might have improved correlations between membrane - extractable metals and plant metal concentrations, since the soils would have a better chance to equilibrate. Further studies showing the effect of extraction time on predictability would be necessary to test this hypothesis. The properties of the soils may also influence the relationships observed. The soils used in this study had a limited range in pH (mostly around 8). So, certain extractants such as DTPA (which was developed to assess micronutrient status in high pH, calcareous soils - Lindsay and Norvell (1978)), would be expected to yield better correlations under these conditions than other extractants. This was observed in the case of Cu.

Another reason why the resin membranes did not perform better than expected may be due to the soil sample size used for the statistical analysis. In the case of the Cu and Zn data, 13 observations were used to assess the significance of the correlations, and even fewer data points were used for Pb ($n = 8$). A larger sample size including soils with a wider range of physical and chemical properties could give more conclusive information regarding the predictive ability of the various methods. Finally, our study focused on predicting Pb, Cu, and Zn concentrations in only one response variable (i.e., lettuce). Better conclusions can be drawn on the overall effectiveness of the membranes and extractants if other plant types and metals were tested for.

In spite of the above mentioned factors (which should be taken into consideration in future studies), the AEM-chelator extraction has the potential to be a useful tool for soil metal testing. In addition to its effectiveness in predicting metal concentrations in lettuce, Tejowulan (1994) mentioned some practical advantages to using this method: The membranes can simultaneously extract several metal cations; the AEM strips are reusable and as a result, long-term costs of the membranes can be kept low. (This is in contrast to conventional extracting solutions which are discarded after a single use); and the physical structure of the resin membranes is strong enough to make their handling easy.

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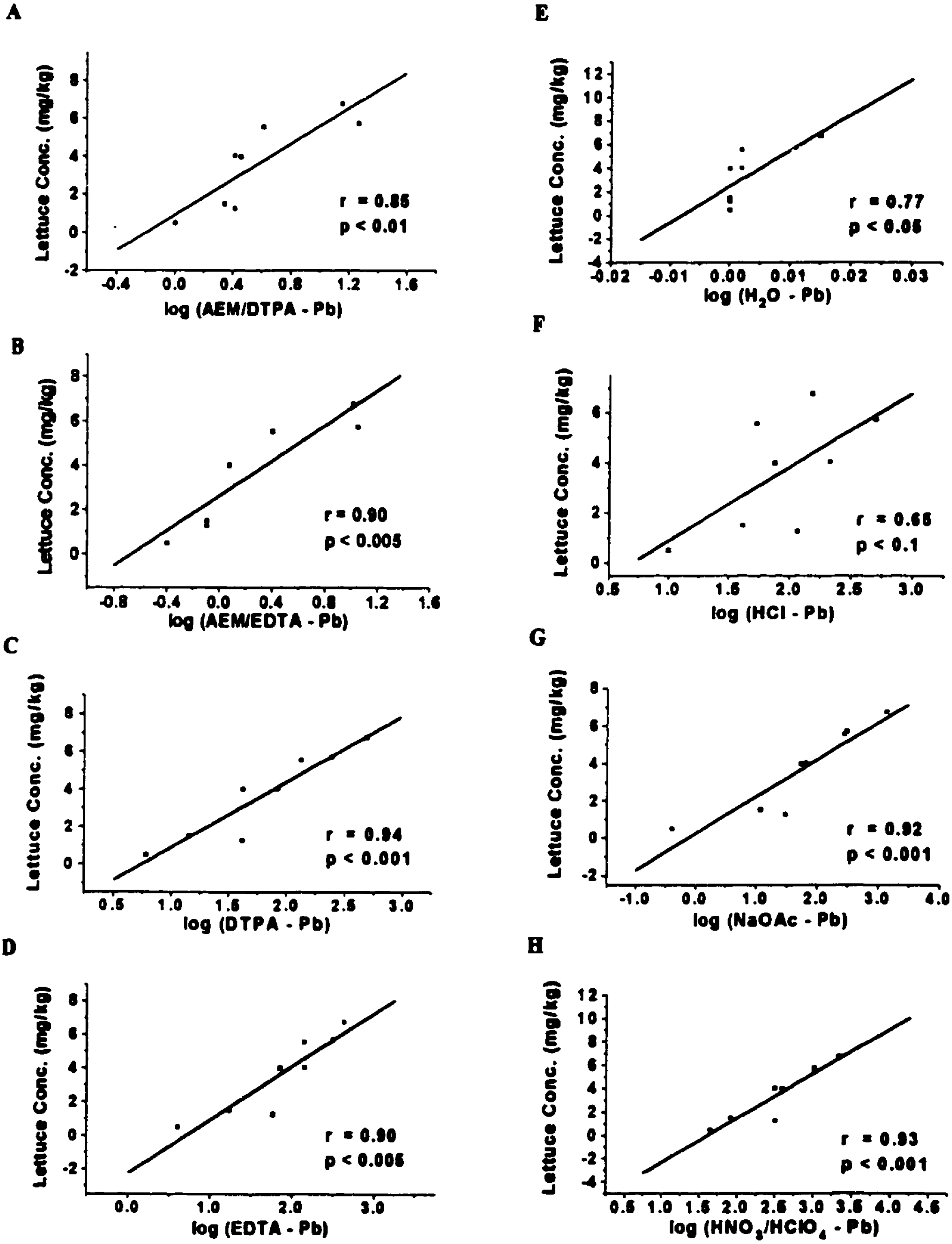

Figure 2.1: Lead concentrations in lettuce (mg/kg) grown in the greenhouse as related to soil Pb extraction concentrations (mg/kg).

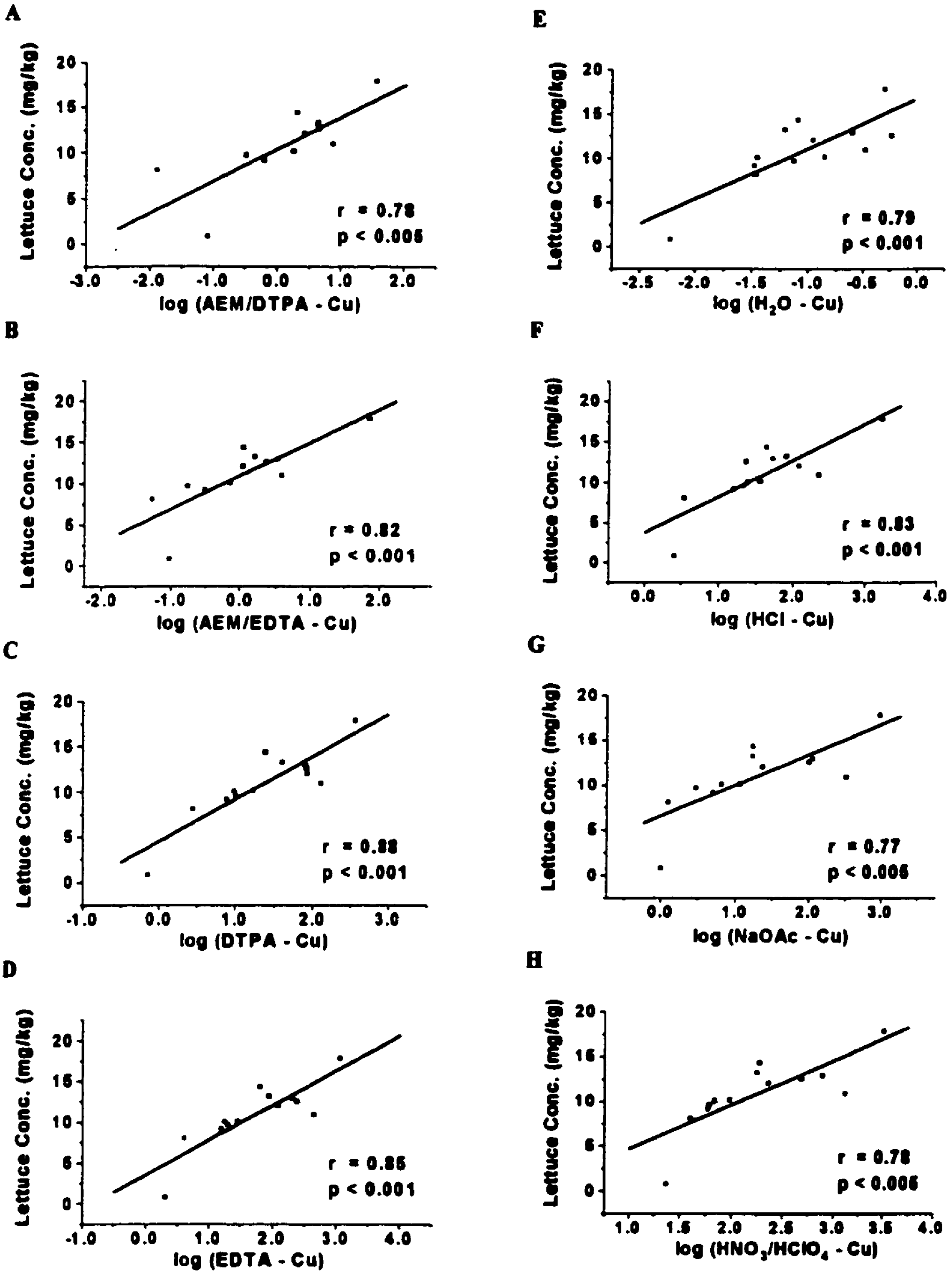

Figure 2.2: Copper concentrations in lettuce (mg/kg) grown in the greenhouse as related to soil Cu extraction concentrations (mg/kg).

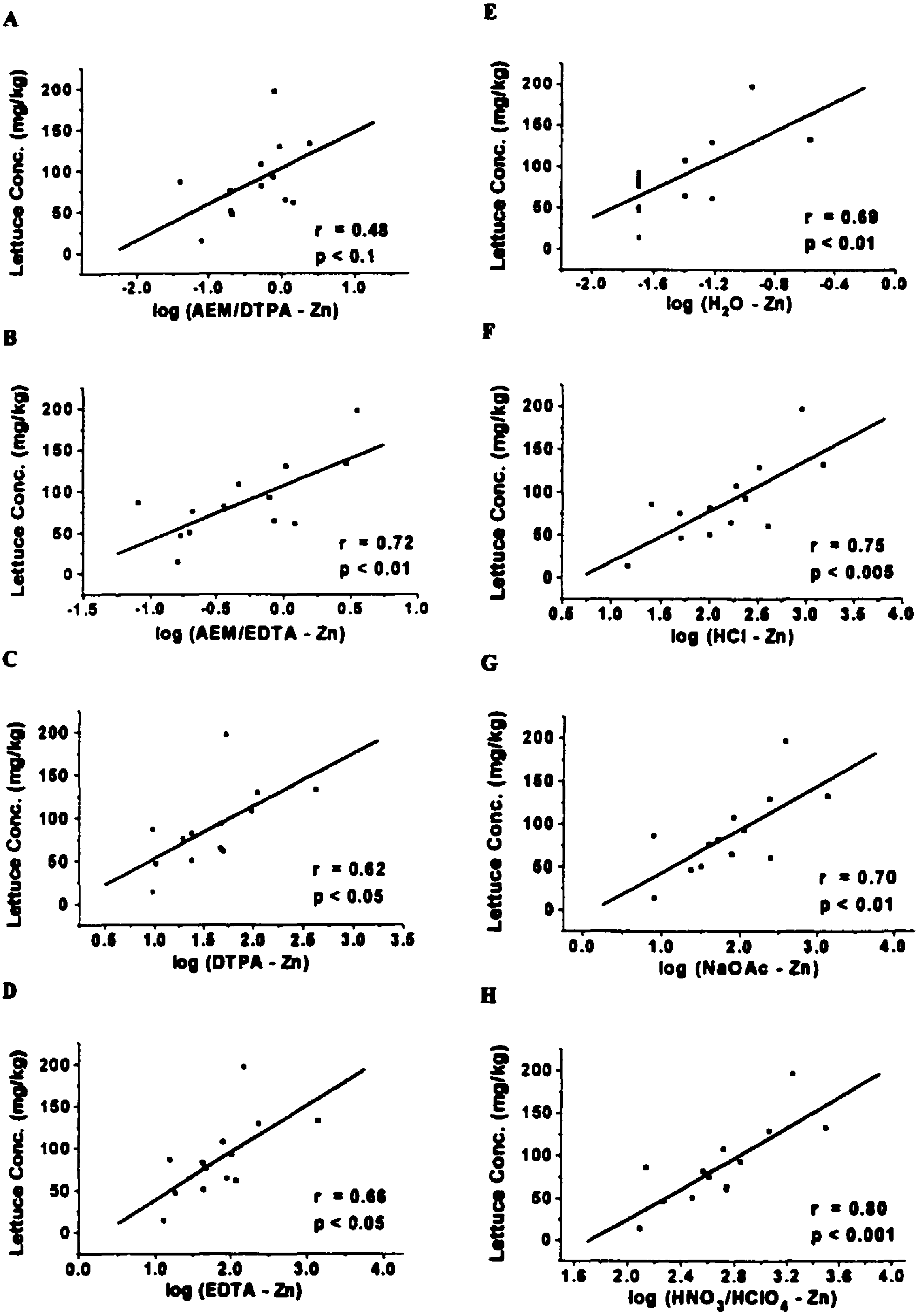

Figure 2.3: Zinc concentrations in lettuce (mg/kg) grown in the greenhouse as related to soil Zn extraction concentrations (mg/kg).

Table 2.1: Selected soil physical and chemical properties.

Soil	pH (H_2O)	CEC (cmol(+)/kg) [2]	EC (µS/cm)	Organic C (mg/g) [1]	Texture
1*	7.34	51.9	2210	108	silty loam
2*	8.29	16.7	1800	21.1	loam
3*	7.97	6.8	9670	53.4	sandy loam
4*	7.84	13.2	628	53.2	sandy loam
5*	8.03	12.8	644	28.4	sandy loam
6*	8.49	14.7	869	31.1	sandy loam
7*	8.90	6.3	724	13.2	sandy loam
8*	7.94	10.0	635	27.1	sandy loam
9	5.58	35.2	266	77.4	sandy loam
10	7.93	13.7	398	42.3	loamy sand
11	7.99	12.3	257	24.0	sandy loam
12	8.01	12.4	368	14.8	sandy loam
13	7.97	12.2	513	17.0	sandy loam

[1] Mean of 2 replicate samples.

[2] Mean of 3 replicate samples.

* Soil data obtained from other research studies

N.B. Soil 9 = control

Table 2.2: Concentrations of Pb, Cu, and Zn in leaves of lettuce grown in the greenhouse on contaminated urban soils.

Soil	Pb (mg/kg dry tissue)	Cu (mg/kg dry tissue)	Zn (mg/kg dry tissue)
1*	*hv*	8.1 ± 3.1	86.6 ± 6.1
2*	4.0 ± 1.2	9.7 ± 2.1	50.8 ± 14.5
3*	*hv*	17.9 ± 4.5	197 ± 29
4*	*hv*	12.1 ± 3.3	108 ± 32
5*	5.6 ± 2.3	10.9 ± 2.4	133 ± 13
6*	*hv*	12.6 ± 2.2	130 ± 27
7*	*hv*	10.2 ± 2.1	82.2 ± 11.5
8*	1.5 ± 0.4	10.1 ± 1.0	61.4 ± 6.7
9	0.5 ± 0.2	0.8 ± 0.7	14.4 ± 3.2
10	1.3 ± 1.1	13.2 ± 3.3	64.9 ± 14.6
11	5.7 ± 0.7	9.2 ± 3.2	46.8 ± 12.4
12	4.0 ± 1.0	14.4 ± 3.9	93.1 ± 7.4
13	6.8 ± 1.3	12.9 ± 1.0	76.0 ± 8.3

hv Plant replicates for these soils were highly variable

* Plant data obtained from other research studies

Table 2.3: Chemical extraction data for Pb.

Soil	H_2O (x10 $^{-2}$ mg/kg)	AEM/ EDTA (mg/kg)	AEM/ DTPA (mg/kg)	DTPA (mg/kg)	Na Acetate (mg/kg)	EDTA (mg/kg)	HCl (mg/kg)	HNO_3/ $HClO_4$ (mg/kg)
2*	0.5 ± 0.2	1.2 ± 0.0	1.6 ± 0.0	83.3 ± 2.0	64.8 ± 4.8	142 ± 0	212 ± 14	314 ± 6
5*	0.5 ± 0.1	2.5 ± 0.2	3.1 ± 0.2	133 ± 35	276 ± 83	140 ± 0	53.3 ± 3.0	1038 ± 307
8*	0.0 ± 0.0	0.8 ± 0.0	1.2 ± 0.0	14.3 ± 1.8	11.7 ± 1.6	17.3 ± 1.1	41.2 ± 1.7	82.5 ± 1.5
9	0.0 ± 0.0	0.4 ± 0.0	0.0 ± 0.0	6.0 ± 0.0	0.4 ± 0.0	4.0 ± 0.0	10.0 ± 0.0	44.7 ± 2.6
10	0.1 ± 0.0	0.8 ± 0.0	1.6 ± 0.0	41.2 ± 4.2	29.3 ± 4.6	57.9 ± 2.0	115 ± 2	315 ± 18
11	2.6 ± 0.3	11.3 ± 0.5	17.4 ± 0.9	241 ± 3	303 ± 34	311 ± 11	502 ± 64	1027 ± 84
12	0.0 ± 0.0	1.2 ± 0.0	1.9 ± 0.2	41.9 ± 0.0	53.4 ± 4.6	71.3 ± 2.3	75 ± 11	391 ± 28
13	3.5 ± 0.1	10.4 ± 1.1	13.2 ± 5.0	486 ± 57	1321 ± 79	424 ± 12	151 ± 4	2170 ± 255

Mean and standard deviations of 3 replicate samples.
* Soils from other research studies

Table 2.4: Chemical extraction data for Cu.

Soil	H_2O ($\times 10^{-2}$ mg/kg)	AEM/ EDTA ($\times 10^{-1}$ mg/kg)	AEM/ DTPA ($\times 10^{-1}$ mg/kg)	Na Acetate (mg/kg)	DTPA (mg/kg)	HCl (mg/kg)	EDTA (mg/kg)	HNO_3/ $HClO_4$ (mg/kg)
1*	3.4 ± 0.5	0.5 ± 0.0	0.1 ± 0.2	0.2 ± 0.0	2.7 ± 0.1	3.4 ± 0.1	4.0 ± 0.0	39.9 ± 0.0
2*	7.4 ± 0.3	1.7 ± 0.0	3.3 ± 0.6	1.9 ± 0.2	10.1 ± 0.3	21.7 ± 0.7	20.0 ± 0.0	60.4 ± 1.5
3*	50.0 ± 2.2	718 ± 29	362 ± 39	942 ± 70	361 ± 4	1723 ± 36	1132 ± 12	3198 ± 141
4*	11.0 ± 0.9	11.0 ± 1.4	26.3 ± 2.9	21.8 ± 1.8	83.8 ± 2.8	118 ± 6	121 ± 5	312 ± 29
5*	33.0 ± 2.1	38.0 ± 3.2	75.3 ± 5.1	317 ± 24	126 ± 2	221 ± 18	433 ± 6	1296 ± 302
6*	58.0 ± 8.8	23.1 ± 1.1	44.6 ± 4.0	97.7 ± 8.5	82.6 ± 1.5	23.0 ± 3.8	237 ± 33	479 ± 20
7*	14.0 ± 0.1	7.1 ± 1.2	17.9 ± 2.4	10.5 ± 0.3	17.0 ± 1.1	35.5 ± 2.8	28.6 ± 1.2	95.5 ± 2.1
8*	3.5 ± 0.0	7.2 ± 0.8	17.7 ± 0.8	5.4 ± 0.1	9.6 ± 0.5	24.0 ± 0.8	18.0 ± 0.0	67.9 ± 1.0
9	0.6 ± 0.0	0.9 ± 0.4	0.8 ± 0.0	0.0 ± 0.0	0.7 ± 0.1	2.5 ± 0.0	2.0 ± 0.0	23.2 ± 1.5
10	6.1 ± 0.2	16.1 ± 1.8	43.2 ± 1.8	16.0 ± 0.0	39.4 ± 0.8	79.9 ± 1.1	85.2 ± 1.1	176 ± 4
11	3.3 ± 0.4	3.1 ± 0.2	6.2 ± 0.3	4.0 ± 0.0	7.7 ± 0.1	15.5 ± 0.7	16.0 ± 0.0	58.5 ± 0.4
12	8.0 ± 1.0	11.2 ± 0.8	20.3 ± 4.2	16.0 ± 0.0	24.1 ± 0.0	43.2 ± 3.6	63.3 ± 6.1	186 ± 5
13	25.0 ± 0.4	33.6 ± 1.2	42.9 ± 8.1	109 ± 17	80.4 ± 3.3	52.8 ± 5.6	198 ± 5	773 ± 39

Mean and standard deviations of 3 replicate samples.

* Soils from other research studies

Table 2.5: Chemical extraction data for Zn.

Soil	H_2O (x 10^{-2} mg/kg)	AEM/ DTPA (x 10^{-1} mg/kg)	AEM/ EDTA (x 10^{-1} mg/kg)	DTPA (mg/kg)	EDTA (mg/kg)	Na Acetate (mg/kg)	HCl (mg/kg)	HNO_3/ $HClO_4$ (mg/kg)
1*	2.0 ± 0.0	0.4 ± 0.0	0.8 ± 0.0	9.4 ± 0.1	15.3 ± 1.2	7.9 ± 0.1	25.4 ± 0.6	137 ± 10
2*	2.0 ± 0.0	2.0 ± 0.4	2.0 ± 0.4	24.0 ± 0.5	42.6 ± 0.0	31.6 ± 0.2	99.9 ± 0.1	302 ± 6
3*	11.0 ± 1.0	7.9 ± 0.6	35.1 ± 1.2	53.0 ± 1.0	148 ± 3	385 ± 8	900 ± 50	1734 ± 42
4*	4.0 ± 0.0	5.2 ± 0.4	4.7 ± 0.5	95.7 ± 1.6	78.6 ± 0.1	81.4 ± 4.4	187 ± 17	519 ± 25
5*	27.0 ± 1.2	23.9 ± 1.7	29.3 ± 0.7	416 ± 3	1351 ± 41	1344 ±109	1515 ±164	3090 ± 47
6*	6.0 ± 0.0	9.3 ± 0.5	1.0 ± 0.8	109 ± 4	228 ± 10	238 ± 24	325 ± 1	1145 ± 31
7*	2.0 ± 0.0	5.3 ± 0.2	3.6 ± 0.4	24.0 ± 0.6	42.6 ± 0.0	52.4 ± 4.7	99.9 ± 0.1	366 ± 49
8*	6.0 ± 0.0	14.5 ± 1.2	12.1 ± 1.7	49.9 ± 3.6	116 ± 3	247 ± 22	400 ± 44	542 ± 15
9	2.0 ± 0.0	0.8 ± 0.0	1.6 ± 0.0	9.4 ± 1.1	12.7 ± 0.0	8.0 ± 0.0	14.8 ± 0.8	122 ± 12
10	4.0 ± 0.0	11.3 ± 0.5	8.5 ± 0.2	46.8 ± 1.0	87.2 ± 1.1	77.2 ± 4.7	166 ± 14	548 ± 1
11	2.0 ± 0.0	2.1 ± 0.2	1.7 ± 0.2	10.2 ± 0.1	18.0 ± 1.2	23.7 ± 0.1	51.0 ± 2.3	183 ± 6
12	2.0 ± 0.0	7.7 ± 0.9	7.9 ± 0.2	47.2 ± 0.3	103 ± 3	112 ± 0	233 ± 15	696 ± 2
13	2.0 ± 0.0	2.0 ± 0.4	2.1 ± 0.2	19.4 ± 0.9	47.3 ± 1.1	40.0 ± 0.0	49.9 ± 0.0	401 ± 7

Mean and standard deviations of 3 replicate samples.
* Soils from other research studies.

Table 2.6 : Regressions of lettuce metal concentrations and extractable metals

Extractant (x) (mg/kg)	Regression Equation (conc. in mg/kg)	r^2 Value	Standard Error	Sample Size (n)
AEM/DTPA	Pb conc. = $4.7 \cdot \log(x_{Pb}) + 0.96$	0.72^{**}	1.3	8
	Cu conc. = $3.5 \cdot \log(x_{Cu}) + 10$	0.61^{***}	2.6	13
	Zn conc. = $44 \cdot \log(x_{Zn}) + 104$	0.23^{ns}	43	13
AEM/EDTA	Pb conc. = $4.0 \cdot \log(x_{Pb}) + 2.7$	0.81^{***}	1.1	8
	Cu conc. = $4.0 \cdot \log(x_{Cu}) + 11$	0.67^{****}	2.4	13
	Zn conc. = $66 \cdot \log(x_{Zn}) + 108$	0.52^{**}	34	13
DTPA	Pb conc. = $3.5 \cdot \log(x_{Pb}) - 2.5$	0.88^{****}	0.89	8
	Cu conc. = $4.7 \cdot \log(x_{Cu}) + 4.5$	0.77^{****}	2.0	13
	Zn conc. = $61 \cdot \log(x_{Zn}) - 7.5$	0.38^{*}	38	13
EDTA	Pb conc. = $3.2 \cdot \log(x_{Pb}) - 2.2$	0.81^{***}	1.1	8
	Cu conc. = $4.3 \cdot \log(x_{Cu}) + 3.6$	0.72^{****}	2.2	13
	Zn conc. = $56 \cdot \log(x_{Zn}) - 15$	0.44^{*}	37	13
H_2O	Pb conc. = $307 \cdot \log(x_{Pb}) + 2.5$	0.59^{*}	1.6	8
	Cu conc. = $5.7 \cdot \log(x_{Cu}) + 17$	0.62^{****}	2.5	13
	Zn conc. = $88 \cdot \log(x_{Zn}) + 215$	0.48^{**}	35	13
HCl	Pb conc. = $2.9 \cdot \log(x_{Pb}) - 2.0$	0.42^{ns}	1.9	8
	Cu conc. = $4.5 \cdot \log(x_{Cu}) + 3.8$	0.69^{****}	2.3	13
	Zn conc. = $60 \cdot \log(x_{Zn}) - 41$	0.56^{***}	32	13
Na acetate	Pb conc. = $2.0 \cdot \log(x_{Pb}) + 0.26$	0.85^{****}	1.0	8
	Cu conc. = $3.4 \cdot \log(x_{Cu}) + 6.6$	0.59^{***}	2.6	13
	Zn conc. = $51 \cdot \log(x_{Zn}) - 6.6$	0.49^{**}	35	13
$HNO_3/HClO_4$	Pb conc. = $3.8 \cdot \log(x_{Pb}) - 6.1$	0.86^{****}	0.95	8
	Cu conc. = $4.9 \cdot \log(x_{Cu}) - 0.14$	0.61^{***}	2.6	13
	Zn conc. = $90 \cdot \log(x_{Zn}) - 154$	0.64^{****}	29	13

Statistical significance is represented by: * for 0.05; ** for 0.01; *** for 0.005; **** for 0.001; and ^{ns} when not significant.

Connecting Text

The previous chapter discussed the effectiveness of treated anion exchange membranes and several other extractants to predict plant metal concentrations. Such tests are necessary for finding techniques that can accurately assess soil toxicity to plants. Thus, if plant metal concentrations are predicted to be higher than the critical limits, this could justify remediating soils.

In such cases, several measures can be taken to deal with contaminated soils, including landfilling and metal-removal strategies. However, both of these approaches are relatively expensive to implement. Alternatively, metal-stabilization techniques can reduce the availability of contaminants to organisms and are cheaper to implement.

Over the years, various low-cost food processing waste materials have been effectively used to remove metals from wastewaters, but they have not been tested with soils. Such materials have properties that can increase metal stability. Hence, the following chapter discusses the ability of some byproducts to reduce metal content and concentrations in plants grown on urban soils.

CHAPTER THREE

The Effects of Food Processing Waste Materials and Zeolites on Metal Concentrations and Uptake in Lettuce Grown on Urban Soils

The Effects of Food Processing Waste Materials and Zeolites on Metal Concentrations and Uptake in Lettuce Grown on Urban Soils

Abstract

Over the past two decades, research has shown that food processing waste materials can effectively remove metals from contaminated wastewaters, yet no attention has been given to their stabilizing effects in soils, despite the low cost and availability of these byproducts. Thus, the focus of this study was to determine the effects of food waste materials (such as wheat shorts, peanut skins, and filter press mud) on plant metal concentrations and uptake, and to compare the results obtained with those of a commercially-available zeolite.

Our results showed that the byproducts tested were generally ineffective at reducing Pb, Cu, and Zn uptake in lettuce. Although, significant decreases in plant metal concentrations were observed, these were due to the diluting effects of increased plant biomass. In addition, significant reductions in lettuce Ca and Mg concentrations were found on some soils which were mostly attributed to dilution effects as well. In comparison, the Na-based zeolites did not significantly reduce plant metal concentrations and uptake which contrasts with the results of previous studies. Decreases in lettuce Ca and Mg concentrations were observed on some zeolite-treated soils, and plant Mg fell below concentrations suitable for optimum growth. Hence, nutrient deficiency problems might occur in plants grown on these soils treated with zeolites.

The food waste materials (wheat shorts and to a lesser extent peanut skins), also had significant positive effects on plant growth. In addition, soil properties such as organic carbon and/or available P significantly increased as a result of treatment addition, which probably explains the increases in biomass observed.

Key Words: Wheat shorts, peanut skins, filter press mud, zeolites, metal concentrations, metal uptake, urban soils, *Lactuca sativa*

Introduction

Abandoned railway, lead smelting, and battery recycling sites, as well as agricultural areas receiving pesticides, phosphate fertilizers, sewage sludge, and contaminated irrigation water can significantly increase trace metal levels in soils (Kabata-Pendias and Adriano, 1995). Other sources such as windblown dusts, forest fires, volcanoes, and soil formation processes can also contribute to the metal contamination problem in soils (Tiller, 1989). These metals may leach into groundwater or make their way up the food chain (plants→higher organisms) given time and the appropriate soil conditions.

Several measures can be taken to deal with metal-polluted soils if levels are high enough to cause toxicity to organisms or present a risk to human health. These include excavation and landfilling, removal of metal contaminants, or stabilization of metals in the soil matrix (Smith, 1987). The former two methods may provide a more permanent solution to the problem, but are complicated by various factors, one of which is their high costs. Alternatively, stabilization methods are cheaper, less labor-intensive, and less destructive than landfilling or metal-removal strategies, and function by altering soil properties that influence metal mobility.

It is well-known that soil properties (such as pH, metal oxides, electrical conductivity (EC), cation exchange capacity (CEC), organic carbon (OC), available phosphorus (P), and clay content) can influence the mobility and the availability of metals for plant uptake (Blume & Brümmer, 1991; Domergue & Védy, 1992; Calmano et al., 1993; He & Singh, 1993; Jurinak & Tanji, 1993; Chlopecka et al., 1996; and Yin et al., 1996). For instance, an increase in the soil OC content increases the number of functional groups that metal ions can bind to. At high pH, the negative charge of organic and inorganic colloids increases, since H^+ ions are released from their functional groups. This results in an increase in metal adsorption. In addition, a high available P content in soils reduces the mobility of metals by forming insoluble metal phosphate precipitates, while an increase in the soil EC level decreases metal ion activity in solution (Lindsay, 1979) and increases cation competition for plant uptake sites. As a result of these changes in soil properties, the amount of metal taken up (expressed as wt/plant) and the concentration of metal (expressed as wt/wt) should decrease in plant tissues. In addition, soil treatments may affect nutrient levels (Ca, Mg, etc.) in plants which can have antagonistic effects on metal uptake (Kabata-Pendias and Pendias, 1992).

Several compounds have been studied for their effectiveness at metal stabilization, for example hydrous manganese oxides (Mench et al., 1994), limestone (Pierzynski & Schwab, 1993), K_2HPO_4 (Pierzynski & Schwab, 1993), peat (Majid et al., 1996), and zeolites (Czupyrna et al., 1989; Gworek, 1992a,b). Czupyrna et al. (1989) showed that Valfor 200 zeolite effectively immobilized Cd, Ni, Cu, and Zn when these elements were present singly or in combination in solution. The zeolite has a high cation exchange capacity which largely explains its immobilization effectiveness. In addition, the base cation associated with the zeolite (Na) can increase pH levels of solution and subsequently reduce metal availability. Mench et al. (1994) demonstrated that hydrous manganese oxide addition to soils decreased Cd levels in tobacco and ryegrass, as well as Pb concentrations in ryegrass shoots. In addition, leaching studies by Majid et al. (1996) using soil-peat mixtures, showed that Pb adsorption on peat was rapid, and total metal fixation in soil occurred at a peat loading of 20% (wt/wt). In both of the latter studies, increases in the soil metal binding capacity are believed to be responsible for the observed results.

Although these treatments were demonstrated to be successful and are cheaper than landfilling or metal-removal strategies, applying these materials on a large scale can still be costly.

As an alternative to these stabilizing products, lower cost food waste material such as soybean hulls (Marshall & Champagne, 1995), corncobs (Henderson et al., 1977), rice hulls & bran (Marshall et al., 1993; Roy et. al., 1993), and peanut skins (Randall et al., 1975; Randall et al., 1978) have been used to reduce metal mobility in aquatic systems. These products are abundant and relatively inexpensive. For instance rice-milling byproducts such as hulls and bran are produced in large quantities every year (1.4 billion kg and 640 million kg respectively) and cost between $0.00 and $0.077/kg. This contrasts with Valfor 200 which costs $0.551/kg. In addition, these products were shown to be effective at remediating metal-contaminated wastewaters. Roy et al. (1993) demonstrated that rice hulls removed 94%, 98%, and 99% of Sr, Cd, and Pb respectively, as well as over 99% of $Cr_2O_7^{2-}$ and $H_2AsO_4^-$ from contaminated water. Marshall et al. (1993) showed that defatted extrusion stabilized rice bran removed 82.5% of Cr, 82.4% of Cu, and 93.7% of Zn from solution. Zinc removal compared favorably with Amberlite chelating resin which adsorbed 100% of this metal. Randall et al. (1975) showed that tannin-containing compounds such as peanut skins effectively decreased wastewater Cu concentrations from 200-1000 ppm to less than 0.5 ppm.

The effectiveness of these byproducts could be explained in part by the organic compounds in these materials. For instance, phytic acids in bran products contain hydroxyl groups which increase the exchange capacity of the material. In addition, phytic acids store considerable amounts of P (Turecki et al., 1994). Thus insoluble metal phosphates may form when bran products are added to metal-contaminated systems. Tannins in peanut skins contain phenolic groups which can participate in ion exchange; two hydrogen ions on adjacent functional groups are exchanged for one Cu ion in solution (Randall et al., 1975).

Although these materials have only been tested in metal-contaminated waters, their effectiveness, abundance, and low cost, makes it worthwhile to test some of these products in soil systems as well. The objective of this study was to treat contaminated urban soils with food processing waste materials and determine their effects on plant metal concentrations, uptake, plant biomass and soil properties. In addition stepwise multiple regression analysis was carried out to assess which soil properties and plant factors explained plant metal concentrations, uptake, and biomass.

Materials and Methods

Soil sampling and analysis

Four soils sampled within the Montreal urban area were used for this study. One was obtained from city-owned property, two from railway sites, and another from a military base. In addition, a forest soil (St. Bernard) was used as a control. All soils were homogenized and sieved to pass 2 mm before using them for the greenhouse experiment. In addition, a sample of each of the homogenized and sieved soils was air dried and ground to pass 1-mm and 0.5-mm sieves for soil analyses. The soils were analyzed for various physical and chemical properties including pH (1:2 soil/water ratio - Hendershot et al., 1993a), electrical conductivity (1:2 soil/water ratio - Janzen, 1993), cation exchange capacity (Hendershot et al., 1993b), organic C content (Nelson & Sommers, 1982), and particle size (McKeague, 1976).

In addition, the total metal concentrations of the soils were determined by an $HNO_3/HClO_4$ digestion procedure (Cook, 1998). A 1 g soil sample (ground to pass 0.5 mm) was digested with 15 ml of concentrated HNO_3 (trace metal grade) for 16 h at room temperature and then at 150°C (in a block digester) for about 3 h. Following a brief cooling period, 5 ml of concentrated $HClO_4$ (trace metal grade) was added to each tube, and samples were further digested at 180°C for 3-4 h. An inter-laboratory audit soil sample (ECSS #7) reported to contain 15.0 ± 2.0 mg Pb/kg, 28.0 ± 0.4 mg Cu/kg, and 101 ± 6 mg Zn/kg (Sheldrick and Wang, 1995) was digested as well, and metal concentrations obtained were 20.2 ± 0.6 mg Pb/kg, 20.5 ± 0.6 mg Cu/kg, and 91.6 ± 1.3 mg Zn/kg.

Treatments used and analyses carried out

Four treatments were used in this study. Filter press mud, which is a precipitate formed during the purification step of the sugar refining process, was obtained from Lantic Sugar Ltd. Wheat shorts were obtained from Robin Hood Multifoods Inc.; shorts make up the outer layers (pericarp) of the wheat kernel which are removed during the milling process. Peanut skins were provided by Kraft Canada Inc.; the reddish-brown seed cover is rich in tannins and is removed prior to peanut processing. The Valfor 100 zeolite used for this study was a synthetic aluminosilicate with Na as the exchange cation. The zeolite was used for comparison purposes and was provided by the PQ Corporation.

All treatments were analyzed for pH, EC, and OC content by the methods cited in the previous section. In addition Mehlich III extractions were carried out to determine the available P, Ca, and Mg concentrations of the treatments (Mehlich, 1984). The total P content of the treatments was assessed using an HNO_3/H_2O_2 digestion procedure (Mench et al., 1994), and the digests were analyzed colorimetrically. Samples of these digests were also used to determine the Pb, Cu, and Zn concentrations of the treatments.

Greenhouse experiment

Seven hundred grams of each of the 5 soils (< 2 mm) were placed in 125 mm pots. Calcium carbonate was added to the control soils (20 g/kg dry soil) so that the pH would be comparable to the contaminated soils. Each of the four treatments was mixed in with each soil. The filter press mud, wheat shorts, and peanut skins were added at 50 g/kg dry soil, whereas the zeolites were added at 10 g/kg dry soil. Also, no treatment was added to one set of soils, thus creating a control group. The experiment was replicated 5 times generating 125 potted soils ((4 treatments + 1 no treatment) x 5 soils x 5 blocks) which were arranged in a randomized complete block design (RCBD model). All soils were left to incubate with the treatments in the greenhouse for 30 days, after which a soil sample was taken from each pot and kept for analysis of pH, organic carbon content, electrical conductivity, and available P content (using the methods cited above). Results from these analyses would later be used for ANOVA and multiple regression analyses. The remaining soil was fertilized with 10 ml of 0.1 M KH_2PO_4, 0.06 M KNO_3, and 0.1 M NH_4NO_3, and sown with five lettuce seeds (*Lactuca sativa* cv. Buttercrunch). This crop was chosen as a result of its easy maintenance, propensity to accumulate metals, and direct relevance to humans. Fertilizer application rates were based on the pH and available nutrient content of the soils, and were determined using a fertilizer recommendation program (Fournier, 1995). Three other fertilizations were carried out during the course of the experiment (26 days, 33 days, and 38 days after seeding) with 10 ml of solution containing KNO_3 and NH_4NO_3 (at 0.17 M and 0.04 M concentrations respectively). Additional phosphate fertilizer was not added due to its potential effects on metal precipitation. Seedlings were thinned to one over the first 36 days. The experiment continued for an additional 34 days (until harvest). At harvest time roots were cut off and discarded, while leaves were thoroughly washed with distilled water, dried, and kept for analysis.

The conditions in the greenhouse were maintained at 40 - 50 % relative humidity, 20°C day temperature, 15°C night temperature, and 16 h daylight. Plants were watered daily with distilled water to maintain soils at approximately 25% water content.

Plant analysis

All plant tissue samples were oven dried for 48 h at 64°C. Lettuce leaves were weighed to determine biomass, hand-crushed, and digested using an HNO_3/H_2O_2 digestion procedure (Mench et al., 1994), and analyzed for Pb, Cu, Zn, Ca, and Mg. All digests were analyzed for these elements using a 2380 Perkin Elmer Flame Atomic Absorption Spectrophotometer (AAS) or a Varian Spectra 300 Graphite Furnace AAS (with Zeeman background correction) when metal concentrations were below detectable levels for the flame. An inter-laboratory audit sample (V-8) containing 1.30 ± 0.97 mg Pb/kg dry tissue, 10.2 ± 1.2 mg Cu/kg, 25.2 ± 4.6 mg Zn/kg, 12700 ± 903 mg Ca/kg, and 2140 ± 126 mg Mg/kg (after outlying values were removed for Pb and Zn - Koteles et al., 1992), was also digested and measured as having element concentrations of 0.82 ± 0.11 mg Pb/kg, 9.18 ± 0.62 mg Cu/kg, 22.2 ± 1.6 mg Zn/kg, 13700 ± 1380 mg Ca/kg, and 2130 ± 76 mg Mg/kg

Statistical analysis

Analysis of variance (ANOVA) revealed no significant differences among blocks. Consequently blocks were treated as replicates. ANOVA tests were further carried out to assess treatment effects on soil pH, available P content, organic carbon content, and electrical conductivity for each soil. In addition, ANOVA was used to determine if significant differences in plant metal and nutrient concentrations, biomass, and plant metal and nutrient uptake existed among the treated soils. Uptake values were calculated by multiplying the element concentrations in plants by their biomass. To determine which treatments produced significantly different responses compared to the controls (untreated soils), either Tukey or Scheffé tests were carried out (depending on whether or not the same number of replicates were being compared between groups). All statistical analyses were carried out using Systat software version 5.0 (Wilkinson, 1992), and the level of significance between treatment and control groups was defined by * for $p<0.05$, ** for $p<0.01$, *** for $p<0.005$, and **** for $p<0.001$.

Stepwise multiple regression analyses were also conducted to determine which plant factors and soil properties significantly affected plant metal concentrations, uptake, and biomass. Several variables needed to be *log* (10) transformed before the analyses were carried out. These included: organic carbon, available P, electrical conductivity, Pb concentration, Pb uptake, Cu uptake, Ca uptake, and Mg uptake. The other variables (pH, Cu concentration, Zn concentration, Zn uptake, Ca concentration, Mg concentration, and biomass) were left as is since they were normally distributed.

Results

Physical and chemical properties of soils and treatments

The physical and chemical properties of the soils are presented in Table 3.1. Most of the soils are sandy loam except for one (which is a loamy sand). The alkaline nature of the urban soils B-E may be due to the presence of calcite or the application of ash material (as a result of coal burning activities), whereas the low pH of the forest soil A (control) may be explained by the presence of humic acids resulting from leaf litter decomposition. The decomposed material may also explain the high OC content and CEC observed in this soil.

Some properties of the treatments are presented in Table 3.2. The pH of the zeolites is significantly greater than the pH of the other treatments due to the high Na content associated with the aluminosilicates (99.9% of CEC). The EC, available Mg, and OC content of the wheat shorts and peanut skins are higher than the other two treatments. Also the amount of available P is highest in the wheat short and FPM treatments, whereas the available Ca contents of the FPM and peanut skins are greater than the wheat shorts and zeolites.

Metal concentrations of soils and treatments

The total metal concentrations in soils, based on an $HNO_3/HClO_4$ digestion procedure, are shown in Table 3.3. The results indicate that the control has the lowest concentrations of Pb, Cu, and Zn which are well below the residential and agricultural criteria for soil contamination. Soils C and E have the highest Pb concentrations compared to the other soils and are in excess of the commercial standards for soil quality, whereas soil D is just above agricultural standards for Pb concentration. Soils B and D are above the agricultural criteria for Cu contamination, and soil E is above commercial standards. Results also show Zn levels greater than residential standards for soil B and higher than agricultural criteria for soil D.

Table 3.4 shows that the total metal concentrations in all treatment samples are low. The total Pb concentrations are below detection limits of the flame AAS for all treatments, whereas Cu concentrations in the FPM and zeolites are below the limit of detection.

Treatment effects on plant nutrient and metal concentrations

The effect of the treatments on plant nutrient concentrations are shown in Tables 3.5 and 3.6. In general, the treatments decreased Ca and Mg concentrations in lettuce tissue with significant decreases seen for some soil-treatment combinations for each element. For instance, wheat shorts significantly decreased Ca concentrations in plants grown on soils D and E, and Mg concentrations in plants grown on soils C, D, and E. FPM significantly reduced Ca and Mg concentrations in plants grown on soils D and E. Plants on peanut skin-treated soils had significantly lower levels of Ca in soils B, C, and D compared to controls, and significantly lower levels of Mg in soils C, D, and E compared to controls. Also, lettuce grown on zeolite-treated soils B, C, and E had significantly lower concentrations of Ca and Mg than control plants.

The effects of the treatments on the trace metal concentrations in lettuce are shown in Tables 3.7-3.9. No significant differences in plant Pb concentrations were found between treated soils and controls. The exception was in peanut skin-treated soil D in which plant Pb concentrations were significantly lower than controls. Significant differences in Cu concentrations were obtained between controls and plants grown on wheat short-treated soils C, D, and E. Also, lettuce plants grown on FPM-treated soil C contained significantly lower Cu concentrations than controls. Significantly lower Zn concentrations were observed in lettuce plants grown on wheat short-treated soils C, D, and E compared to controls, and in plants grown on peanut skin-treated soil E. Also significantly higher Zn concentrations were obtained in plants grown on wheat short-treated soil A compared to controls.

Treatment effects on biomass

Results show that a significantly higher biomass was obtained for plants grown on wheat short-treated soils compared to controls. This was seen for all soils tested (Table 3.10). Results also illustrate that plants grown on soil D (receiving peanut skin treatment) were of significantly higher biomass than the control plants.

Treatment effects on plant nutrient and metal uptake

Tables 3.11 and 3.12 respectively demonstrate the treatment effects on Ca and Mg uptake in lettuce plants. Uptake patterns among the various soil-treatment combinations are similar for both nutrients, however results show that more Ca is taken up than Mg. Results also demonstrate that plants grown on wheat short-treated soils had significantly higher Ca levels (for soils A-D) compared to controls, and a significantly higher Mg content (for soils A-C) compared to controls. Significantly greater Ca and Mg levels were also obtained in plants grown on peanut skin-treated soil D compared to the forest soil.

Data in Tables 3.13, 3.14, and 3.15, respectively show the treatment effects on Pb, Cu, and Zn uptake in plants. Results indicate no significant treatment effects on plant Pb uptake. In contrast, significant increases in Cu and Zn uptake were seen in plants grown on wheat short-treated soils A and B compared to controls, and in peanut skin-treated soil D.

Treatment effects on soil properties

The effects of the treatments on soil chemical properties are shown in Tables 3.16-3.19. The wheat shorts and FPM significantly increased the available P content of the soils (Table 3.16) which is consistent with the high available P content of these two treatments. The elevated P levels in wheat shorts is due to the phytic acids in this treatment which store significant quantities of this element (Lehrfeld and Wu, 1991; Turecki et al., 1994). Despite the high total P content of the peanut skins, most of it is in an unavailable form (Table 3.2). This explains why the available P content of the soils treated with this material did not differ significantly from the controls. Results also show that zeolites significantly decreased available P content in some of the soils.

Table 3.17 shows the EC levels of the differently treated soils. The EC of the wheat short-treated soils is significantly greater than the controls. This is consistent with the high EC observed in the wheat shorts and may be explained by the high Mg content of this treatment (Table 3.2). Although the significant increase in EC in the zeolite-treated soils may be explained by the excess in Na provided by the treatment, the results are inconsistent with the low EC value obtained for zeolites as shown in Table 3.2. This may be due to zeolite degradation in the soils which could have caused the release of Na and the high EC.

The wheat shorts, peanut skins, and zeolites have a significant effect on the pH of the soils (Table 3.18). Both the wheat shorts and peanut skins decreased the pH of the soils compared to the controls which may be explained by the release of hydrogen ions from the phytic acids (of wheat shorts) and from the tannic compounds (of peanut skins). Although the decrease in pH was statistically significant, little effect on plant metal uptake would be expected since the pH of the wheat short and peanut skin treated soils was only 0.05 to 0.44 units lower than the controls and would have little effect on metal solubility. The significant increase in pH in the zeolite-treated soils is consistent with the high pH observed for this treatment (Table 3.2) and may be explained by its high base cation content. Also, the results showed that the pH of the FPM-treated soils was not lower than the pH of the controls, despite the acidic nature of the FPM treatment (pH=4.59).

The effects of the treatments on the OC content of the soils is reflected in Table 3.19. Both the wheat short and peanut skin-treated soils have significantly higher OC contents than the control soils. This is consistent with the high OC content of these treatments (Table 3.2). The OC contents of the FPM and zeolite-treated soils did not differ significantly from the controls which is consistent with the low OC content of these treatments.

Factors affecting plant metal concentrations, uptake, and biomass

Stepwise multiple regression procedures yielded the following statistical models when log OC (mg/g), log available P (mg/kg), log EC (µS/cm), pH, plant Ca_{conc} (mg/kg), and plant Mg_{conc} (mg/kg) were assessed for their effects on plant metal concentrations (mg/kg):

(3.1) $\log Pb_{conc} = -1.52 \log OC - 0.0001\ Mg_{conc} + 2.84$
$(R^2 = 0.601,\ p<0.001,\ n=105)$

(3.2) $Cu_{conc} = -7.44 \log OC - 6.07 \log \text{available } P - 0.0001\ Ca_{conc} + 0.004\ Mg_{conc} + 20.6$
$(R^2 = 0.727,\ p<0.001,\ n=105)$

(3.3) $Zn_{conc} = -40.1 \log OC - 28.0 \log \text{available } P + 0.018\ Mg_{conc} - 0.001\ Ca_{conc} + 112$
$(R^2 = 0.631,\ p<0.001,\ n=100)$

Results indicate that the soil OC content is a predominant factor in predicting Pb, Cu, and Zn concentrations in lettuce tissue. A negative relationship is seen in all three regressions which is consistent with the theory that organic matter reduces metal availability via cation exchange or complexation reactions. The variable was found to be highly significant ($p<0.001$) in all three equations. The available P content of the soil is another important predictor of plant Cu and Zn concentrations. Negative relationships were obtained in equations 3.2 and 3.3 which is consistent with the fact that phosphates can precipitate metals and consequently reduce plant metal concentrations. The variable was highly significant in both regressions ($p<0.001$).

Calcium and/or Mg concentrations in the plant tissues were also found to be significant at predicting lettuce Pb, Cu, and Zn concentrations. The variables were significant at either $p<0.05$, $p<0.005$, or $p<0.001$ levels. Positive relationships between Cu and Mg concentrations and Zn and Mg concentrations suggest synergism between the nutrient and these two metals, whereas the negative relationships observed between Mg and Pb, Ca and Cu, and Ca and Zn concentrations suggest antagonism between these nutrient-metal combinations.

Stepwise regression analysis produced the following equations when the factors log OC (mg/g), log EC (µS/cm), log available P (mg/kg), pH, log Ca_{upt} (µg) and log Mg_{upt} (µg) were analyzed for their impact on lettuce metal uptake (µg):

(3.4) $\log Pb_{upt} = -1.16 \log OC + 1.17 \log Ca_{upt} + 0.393\ pH - 5.90$

$(R^2 = 0.659, p<0.001, n=101)$

(3.5) $\log Cu_{upt} = -0.79 \log OC - 0.47 \log \text{available } P + 1.64 \log Mg_{upt} - 0.51 \log Ca_{upt} - 0.99$

$(R^2 = 0.824, p<0.001, n=100)$

(3.6) $Zn_{upt} = 131 \log Mg_{upt} + 32.9\ pH - 633$

$(R^2 = 0.529, p<0.001, n=101)$

Results show that the soil OC content was a highly significant factor for predicting Pb and Cu uptake in lettuce ($p<0.001$ for regressions 3.4 and 3.5). Results also demonstrate that available P is important for predicting Cu uptake ($p<0.001$), but not for assessing Pb and Zn uptake in lettuce. Relationships between Cu and Mg, and Zn and Mg uptake in plants were significant ($p<0.001$ in both cases) and positive. Results also show a significant negative relationship between Ca and Cu uptake in plants ($p<0.05$) and a significant positive association between Ca and Pb uptake ($p<0.001$). In addition, regression models 3.4 and 3.6 show that pH has a significant positive impact on Pb and Zn uptake, yielding p-values less than 0.001 and 0.005 respectively. This means that raising soil pH (from above neutral conditions to over pH 9) increases plant metal uptake, which is contrary to what is usually observed. However, our results are consistent with those of Davies (1992) who also found a positive relationship between pH and Zn uptake in radish leaves, when plants were exposed to soil pH values of 4.6 to 7.1.

Stepwise regression analysis yielded the following equation when all soil and plant factors (log OC (mg/g), log available P (mg/kg), log EC (µS/cm), pH, plant Ca_{conc} (mg/kg), plant Mg_{conc} (mg/kg), log plant Pb_{conc} (mg/kg), plant Cu_{conc} (mg/kg), and plant Zn_{conc} (mg/kg)) were assessed for their effects on plant growth (g):

(3.7) $\text{Biomass} = 1.79 \log \text{available P} - 0.0001\ Ca_{conc} + 1.17 \log EC - 1.73$
$(R^2 = 0.328,\ p<0.001,\ n=100)$

All variables in the model gave significant p-values (0.001, 0.001, and 0.05 for log available P, Ca_{conc}, and log EC respectively), however the constant term was not significant ($p<0.5$). As a result the analysis was repeated without the constant in the model and the following regression was obtained:

(3.8) $\text{Biomass} = 0.90 \log OC + 1.86 \log \text{available P} - 0.0001\ Ca_{conc}$
$(R^2 = 0.775,\ p<0.001,\ n=103)$

In this model, all variables were highly significant ($p<0.001$ level), and the variability in biomass was also better explained. The regression shows that both organic carbon and available P contents in soils have positive impacts on biomass and that plant Ca concentration is negatively related to growth. The model also shows that plant metal concentrations do not significantly affect biomass which is contrary to what was expected. This may be because plant metal concentrations were not high enough to cause significant decreases in lettuce growth.

Discussion

Effects of wheat short addition to soils

Results showed that wheat shorts were not successful at decreasing Pb concentrations, but did significantly lower Cu and Zn concentrations in lettuce plants grown on wheat short treated soils C, D, and E. Although control plants on these soils had metal concentrations below critical levels (9.2-14.4 mg/kg Cu and 47-93.1 mg/kg Zn for controls compared with critical levels of 20-100 mg/kg for Cu and 100-400 mg/kg for Zn - Kabata-Pendias and Pendias, 1992), plant metal concentrations on the treated soils were reduced even further. For instance, Cu concentrations ranged from 3.0 to 8.5 mg/kg, whereas Zn concentrations were between 21.7 and 51 mg/kg. Our results also show that reductions in plant metal concentrations were not due to decreases in metal uptake. In fact, Cu and Zn uptake was not significantly changed. Since biomass was found to be significantly greater in plants grown on treated soils C, D, and E compared to controls, the conclusion is that the metals were diluted in the plant tissues. This increase in biomass could be due to significant increases in available P and OC contents of the treated soils, since both of these properties were shown to positively affect growth in regression model 3.8. Thus, the organic matter could have increased soil porosity which would enhance root penetration, and increase nutrient absorption in plants.

Results also showed significant increases in Cu uptake and biomass on wheat short treated soils A and B, while no change in plant Cu concentrations occurred on these soils. This implies that plant Cu uptake was sufficient to keep pace with crop growth, and that the treatment had no effect on the bioavailability of this metal on these soils. Similar results were also seen for Zn on treated soil B. In addition, results demonstrated an increase in Zn bioavailability on treated soil A, since plant Zn concentrations, uptake, and biomass significantly increased compared to controls. Thus, the increase in Zn uptake was greater than the increase in plant growth. Despite this, there was no danger of Zn toxicity to lettuce, since concentrations did not go beyond critical levels.

A possible reason for the increase in Zn uptake on soils A and B, could be due to the significant increase in Mg uptake on these soils (attributed to the high Mg content of the wheat shorts - Table 3.2). Kabata-Pendias and Pendias (1992) noted that synergistic associations between these two elements exist within plants which is confirmed in our regression analysis (equation 3.6). Hence, Mg may facilitate the uptake of Zn.

Results also illustrate that wheat shorts significantly decreased plant Ca and Mg concentrations on some soils as a result of dilution. Although nutrient concentrations did not fall below levels considered adequate for growth (approximately 5000 mg Ca/kg tissue and 2000 mg Mg/kg tissue - Salisbury and Ross, 1985), lettuce tissues on some of the treated soils had Mg concentrations close to this limit. Hence, Mg deficiency could occur if biomass increased further.

In other wheat short treated soils, no significant effects on lettuce Ca and Mg concentrations were observed, which means that the increases in Ca and Mg uptake and plant biomass occurred at similar rates. Thus, the availability of the nutrients to lettuce crops was not affected in these soils by the treatment.

Effect of peanut skin addition to soils

Results showed that peanut skins were generally ineffective at lowering Pb, Cu, and Zn concentrations and uptake in lettuce. However, significant decreases in Zn concentrations were found on soil E. This was due to a dilution effect since Zn uptake did not decrease on this soil. In addition, plants grown on treated soil D had significantly lower Pb concentrations than controls, which was also due to a dilution effect since biomass increased on this soil while Pb uptake was not significantly changed. This increase in biomass may be explained by the increased OC content of this soil. Significant increases in Cu and Zn uptake were also observed on soil D, however this did not affect plant Cu and Zn concentrations since biomass increased proportionately to uptake. Hence, peanut skins did not affect metal availability to plants on this soil.

The reduction in lettuce Ca and Mg concentrations on some peanut skin-treated soils was mostly a result of dilution. Thus, rate of growth exceeded the rate in which Ca and Mg ions were transported to lettuce tissues. In one case however (soil C), plant Ca and Mg concentrations were reduced as a result of a decrease in Ca and Mg availability to lettuce crops since biomass remained unchanged on this soil. Hence, peanut skins could reduce the transport of nutrients to the plants. Like the wheat short treated soils, plant Ca and Mg concentrations on the peanut skin treated soils were not below 'adequate' levels. However, lettuce Mg concentrations on some treated soils were close to the limit.

Effects of filter press mud addition to soils

The addition of FPM had no effect on Pb and Zn concentrations and uptake in lettuce. Similar results were seen for Cu on most soils except soil C, where significant reductions in plant Cu concentrations were observed.

Results also show that plant Ca and Mg concentrations significantly decreased on FPM-treated soils D and E, but remained above levels considered adequate for growth. The reduction in nutrient concentrations is due to dilution since Ca and Mg uptake did not decrease on these soils.

Effect of zeolite addition to soils

In comparison to the food processing byproducts, the Na-based zeolite was not more effective at reducing plant metal concentrations and uptake. In fact, no effect on plant Pb, Cu, and Zn concentrations and uptake were observed on zeolite-treated soils. This contrasts with studies carried out by Gworek (1992a,b) who reported significant decreases in Pb and Cd concentrations in lettuce grown on zeolite-treated soils. It should be noted however, that since biomass and uptake results were not reported in these studies, it is difficult to determine whether reductions in metal concentrations were due to dilution effects or significant decreases in metal uptake.

Results also showed significant decreases in plant Ca and Mg concentrations on treated soils B, C, and E, and plant Mg concentrations were below levels considered adequate for growth. Hence, the zeolites may pose a potential threat to plants grown on these soils.

Summary and Conclusions

Results from the present study indicated that the food processing waste products (wheat shorts, peanut skins, and filter press mud), were generally ineffective at reducing metal uptake in lettuce. Significant decreases in plant metal concentrations were observed on some treated soils, but these were mostly due to the diluting effects of increased plant growth. Results also showed significant decreases in crop Ca and Mg concentrations on some of the treated soils, though the concentrations were either at or above levels considered adequate for growth. In most cases these reductions were due to dilution effects, however decreases in Ca and Mg bioavailability were observed on one peanut skin treated soil.

In comparison, the Na-zeolite was not effective at reducing metal concentrations and uptake in lettuce, but did decrease plant Ca and Mg concentrations on some of the amended soils. Also, the zeolites lowered plant Mg concentrations below 'adequate' levels on some soils.

The results also showed that some food waste materials (such as wheat shorts and to a lesser extent peanut skins) did have significant positive effects on lettuce growth. In addition, soil OC and/or available P contents were significantly increased by wheat short, peanut skin, and filter press mud treatments. Thus, soil fertility and crop yield may be significantly improved by the application of these inexpensive treatments.

In conclusion, since none of the food processing byproducts significantly decreased metal availability to plants, their potential use for large scale metal stabilization would be minimal. However, they may be used as fertilizers in situations where organic or phosphate amendments are necessary. Additional experiments are required to determine this. Also, further studies using other crops (and/or testing other metals) would further clarify the effectiveness of these treatments as 'metal-stabilizers'. In addition, tests on other byproducts (such as rice bran and hulls, corncob meal, and soybean hulls) should be carried out to determine the potential of these low-cost products to stabilize metals in soil systems.

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Table 3.1: Selected physical and chemical properties of urban soils.

Soil	pH (H_2O)[1] (1:2)	EC (µS/cm)[1] (1:2)	Organic C[1] (mg/g)	CEC[2] (cmol(+)/kg)	Texture
A	5.58	266	77.4	35.2	sandy loam
B	7.93	398	42.3	13.7	loamy sand
C	7.99	257	24.0	12.3	sandy loam
D	8.01	368	14.8	12.4	sandy loam
E	7.97	513	17.0	12.2	sandy loam

[1] Mean of 2 replicate samples.
[2] Mean of 3 replicate samples.

Table 3.2: Chemical properties of treatments.

Treatment	pH (H_2O) (1:10)	EC (µS/cm) (1:10)	Org. C (mg/g)	Total P (mg/kg)	Av. P (mg/kg)	Av. Ca (mg/kg)	Av. Mg (mg/kg)
Wheat Shorts	6.45	1710	507	8740	864	415	3750
Peanut Skins	6.01	940	584	3600	261	1910	1490
Filter Press Mud	4.59	567	45.8	810	718	2130	100
Na-based Zeolites	10.67	555	0	0	0	0	0

Mean of 2 replicate samples are presented.

Table 3.3: Total metal concentrations in urban soils as determined by an $HNO_3/HClO_4$ digestion procedure including the CCME (1991) soil quality standards.

Soil	Pb (mg/kg)	Cu (mg/kg)	Zn (mg/kg)
A	44.7 ± 2.6	23.2 ± 1.5	122 ± 12
B	315 ± 18	176 ± 4	548 ± 1
C	1030 ± 84	58.5 ± 0.4	183 ± 6
D	391 ± 28	186 ± 5	696 ± 2
E	2170 ± 255	773 ± 39	401 ± 7
Residential	500	100	500
Agricultural	375	150	600
Commercial	1000	500	1500

N.B. Means and standard deviations of 3 replicate samples are presented.

Table 3.4: Total metal concentrations in treatments determined by HNO_3 acid digestion.

Treatment	Pb (mg/kg)	Cu (mg/kg)	Zn (mg/kg)
Wheat shorts	ND	19.90 ± 0.03	99.5 ± 0.1
Peanut Skins	ND	40.3 ± 0.1	50 ± 14
Filter Press Mud	ND	ND	49.3 ± 0.4
Na-Zeolite	ND	ND	49.4 ± 0.6

ND Below detection limit for flame AAS (0.19 mg/l for Pb, 0.08 mg/l for Cu, 0.01 mg/l for Zn).

N.B. Means and standard deviations of 2 replicate samples are presented.

Table 3.5: Ca concentrations in lettuce grown on treated urban soils.

Treatment	Soil A (x 10^3 mg/kg)	Soil B (x 10^3 mg/kg)	Soil C (x 10^3 mg/kg)	Soil D (x 10^3 mg/kg)	Soil E (x 10^3 mg/kg)
Control	17.0 ± 5.7	14.1 ± 2.1	14.1 ± 1.9	30.6 ± 4.2	14.8 ± 1.5
Wheat shorts	18.1 ± 6.5	13.7 ± 1.6	11.8 ± 0.6	$12.8 \pm 0.6^{****}$	$10.1 \pm 1.4^{****}$
FPM	15.1 ± 2.5	11.1 ± 1.6	13.0 ± 1.4	$16.7 \pm 4.5^{****}$	$12.0 \pm 0.8^{*}$
Peanut skins	N/A	$10.8 \pm 1.9^{*}$	$10.8 \pm 1.2^{*}$	$11.4 \pm 1.0^{****}$	12.6 ± 2.0
Na-based zeolites	10.4 ± 2.5	$6.6 \pm 1.0^{****}$	$6.9 \pm 0.5^{****}$	N/A	$6.2 \pm 0.4^{****}$

Statistical significance between treated soil and control is represented by:
* for $p < 0.05$; ** for $p < 0.01$; *** for $p < 0.005$; and **** for $p < 0.001$.
N.B. Means and standard deviations of 4 or 5 replicate samples are presented.

Table 3.6: Mg concentrations in lettuce grown on treated urban soils.

Treatment	Soil A (x 10^3 mg/kg)	Soil B (x 10^3 mg/kg)	Soil C (x 10^3 mg/kg)	Soil D (x 10^3 mg/kg)	Soil E (x 10^3 mg/kg)
Control	2.6 ± 0.2	3.2 ± 0.4	3.1 ± 0.7	5.2 ± 0.3	3.7 ± 0.2
Wheat shorts	3.4 ± 0.9	4.1 ± 0.5	$2.1 \pm 0.2^{*}$	$2.5 \pm 0.4^{****}$	$1.9 \pm 0.1^{****}$
Filter press mud	2.9 ± 0.6	3.0 ± 0.3	2.5 ± 0.5	$4.1 \pm 0.5^{**}$	$2.9 \pm 0.7^{*}$
Peanut skins	N/A	3.7 ± 1.2	$2.0 \pm 0.2^{*}$	$2.4 \pm 0.3^{****}$	$2.3 \pm 0.3^{****}$
Na-based zeolites	1.9 ± 0.4	$1.8 \pm 0.2^{*}$	$1.3 \pm 0.1^{****}$	N/A	$1.6 \pm 0.2^{****}$

Statistical significance between treated soil and control is represented by:
* for $p < 0.05$; ** for $p < 0.01$; *** for $p < 0.005$; and **** for $p < 0.001$.
N.B. Means and standard deviations of 4 or 5 replicate samples are presented.

Table 3.7: Pb concentrations in lettuce grown on treated urban soils.

Treatment	Soil A (mg/kg)	Soil B (mg/kg)	Soil C (mg/kg)	Soil D (mg/kg)	Soil E (mg/kg)
Control	0.5 ± 0.2	1.3 ± 1.1	5.7 ± 0.7	4.0 ± 1.0	6.8 ± 1.3
Wheat shorts	0.9 ± 1.1	0.6 ± 0.2	4.7 ± 1.4	2.0 ± 0.9	6.1 ± 1.2
Filter press mud	0.4 ± 0.2	1.2 ± 0.6	4.7 ± 1.3	2.7 ± 1.9	6.5 ± 1.4
Peanut skins	N/A	0.8 ± 0.2	3.9 ± 1.5	1.2 ± 0.3*	5.4 ± 1.4
Na-based zeolites	1.9 ± 2.3	1.2 ± 0.5	6.5 ± 3.9	N/A	9.2 ± 1.8

Statistical significance between treated soil and control is represented by:
* for $p < 0.05$; ** for $p < 0.01$; *** for $p < 0.005$; and **** for $p < 0.001$.
N.B. Means and standard deviations of 4 or 5 replicate samples are presented.

Table 3.8: Cu concentrations in lettuce grown on treated urban soils.

Treatment	Soil A (mg/kg)	Soil B (mg/kg)	Soil C (mg/kg)	Soil D (mg/kg)	Soil E (mg/kg)
Control	0.8 ± 0.7	13.2 ± 3.3	9.2 ± 3.2	14.4 ± 3.9	12.9 ± 1.0
Wheat shorts	1.2 ± 0.5	9.9 ± 1.0	3.0 ± 0.2***	8.5 ± 2.1*	8.3 ± 0.8*
Filter press mud	1.5 ± 0.5	13.8 ± 1.7	4.7 ± 2.2*	13.4 ± 2.8	11.7 ± 3.9
Peanut skins	N/A	18.3 ± 4.8	5.8 ± 2.5	8.7 ± 2.1	11.0 ± 2.1
Na-based zeolites	1.1 ± 0.9	11.6 ± 2.2	5.3 ± 1.5	N/A	14.6 ± 2.3

Statistical significance between treated soil and control is represented by:
* for $p < 0.05$; ** for $p < 0.01$; *** for $p < 0.005$; and **** for $p < 0.001$.
N.B. Means and standard deviations of 4 or 5 replicate samples are presented.

Table 3.9: Zn concentrations in lettuce grown on treated urban soils.

Treatment	Soil A (mg/kg)	Soil B (mg/kg)	Soil C (mg/kg)	Soil D (mg/kg)	Soil E (mg/kg)
Control	14.4 ± 3.2	65 ± 15	47 ± 12	93.1 ± 7.4	76.0 ± 8.3
Wheat shorts	29.6 ± 7.3**	50.5 ± 6.5	21.7 ± 3.4**	47 ± 11*	51 ± 10*
Filter press mud	20.8 ± 3.7	71 ± 10	35.3 ± 8.3	88 ± 21	57 ± 14
Peanut skins	N/A	86 ± 25	38 ± 14	74 ± 32	51.6 ± 6.8*
Na-based zeolites	11.6 ± 5.4	71 ± 14	33.6 ± 7.9	N/A	85.3 ± 8.7

Statistical significance between treated soil and control is represented by:
* for $p < 0.05$; ** for $p < 0.01$; *** for $p < 0.005$; and **** for $p < 0.001$.
N.B. Means and standard deviations of 4 or 5 replicate samples are presented.

Table 3.10: Dry weight of lettuce plants grown on treated urban soils.

Treatment	Soil A (g)	Soil B (g)	Soil C (g)	Soil D (g)	Soil E (g)
Control	1.5 ± 1.7	1.1 ± 0.3	1.8 ± 0.9	0.2 ± 0.0	1.0 ± 0.4
Wheat shorts	5.6 ± 0.9***	3.5 ± 1.4***	5.2 ± 1.5****	2.0 ± 0.8**	3.0 ± 1.4*
Filter press mud	1.5 ± 0.5	1.5 ± 0.1	2.7 ± 0.8	0.8 ± 0.4	2.1 ± 0.7
Peanut skins	N/A	1.8 ± 0.4	1.7 ± 0.8	3.1 ± 0.9****	2.4 ± 0.9
Na-based zeolites	0.6 ± 0.4	1.2 ± 0.5	2.4 ± 0.8	N/A	2.0 ± 0.8

Statistical significance between treated soil and control is represented by:
* for $p < 0.05$; ** for $p < 0.01$; *** for $p < 0.005$; and **** for $p < 0.001$.
N.B. Means and standard deviations of 4 or 5 replicate samples are presented.

Table 3.11: Ca uptake in lettuce grown on treated urban soils.

Treatment	Soil A (x 10^3 µg)	Soil B (x 10^3 µg)	Soil C (x 10^3 µg)	Soil D (x 10^3 µg)	Soil E (x 10^3 µg)
Control	20.4±20.6	15.2 ± 2.3	24.4 ± 9.7	4.88±0.62	14.5 ± 5.5
Wheat shorts	85.2±11.9****	47.1±19.6***	61.6±19.3***	27.8±10.0*	32.3 ± 18.7
Filter press mud	21.7 ± 8.6	16.1 ± 2.7	35.0±10.0	12.5 ± 4.3	25.5 ± 7.7
Peanut skins	N/A	18.5 ± 3.4	18.8 ± 9.5	36.3±12.6***	31.3 ± 15.6
Na-based zeolites	5.60± 3.39	8.25±4.83	18.6 ± 5.0	N/A	12.5 ± 5.5

Statistical significance between treated soil and control is represented by:
* for $p < 0.05$; ** for $p < 0.01$; *** for $p < 0.005$; and **** for $p < 0.001$.
N.B. Means and standard deviations of 4 or 5 replicate samples are presented.

Table 3.12: Mg uptake in lettuce grown on treated urban soils.

Treatment	Soil A (x 10^3µg)	Soil B (x 10^3µg)	Soil C (x 10^3µg)	Soil D (x 10^3µg)	Soil E (x 10^3µg)
Control	3.96±4.46	3.56± 1.02	5.32±2.17	0.83±0.08	3.68± 1.40
Wheat shorts	18.4 ± 5.8***	14.3 ± 6.5***	11.0 ± 3.94*	5.03±2.18	5.79± 2.50
Filter press mud	4.55±1.99	4.71± 0.35	6.61±1.95	3.43±1.88	6.31± 1.43
Peanut skins	N/A	6.18± 1.00	3.73±1.93	7.47±2.59***	5.52± 2.63
Na-based zeolites	1.04±0.68	2.17± 0.97	3.60±0.81	N/A	3.12± 1.00

Statistical significance between treated soil and control is represented by:
* for $p < 0.05$; ** for $p < 0.01$; *** for $p < 0.005$; and **** for $p < 0.001$.
N.B. Means and standard deviations of 4 or 5 replicate samples are presented.

Table 3.13: Pb uptake in lettuce grown on treated urban soils.

Treatment	Soil A (µg)	Soil B (µg)	Soil C (µg)	Soil D (µg)	Soil E (µg)
Control	0.69 ± 0.70	1.44 ± 1.37	10.4 ± 5.6	0.64 ± 0.19	6.43±2.08
Wheat shorts	2.85 ± 3.69	2.05 ± 1.30	24.0 ± 9.1	3.78 ± 1.79	17.9 ± 7.8
Filter press mud	0.48 ± 0.23	1.89 ± 1.04	12.8 ± 5.8	2.63 ± 2.47	13.8 ± 7.9
Peanut skins	N/A	1.35 ± 0.29	6.22±2.22	3.95 ± 1.80	13.6 ± 7.1
Na-based zeolites	0.54 ± 0.66	1.43± 0.93	16.3±10.5	N/A	18.7 ± 9.3

Statistical significance between treated soil and control is represented by:
* for $p < 0.05$; ** for $p < 0.01$; *** for $p < 0.005$; and **** for $p < 0.001$.
N.B. Means and standard deviations of 4 or 5 replicate samples are presented.

Table 3.14: Cu uptake in lettuce grown on treated urban soils.

Treatment	Soil A (µg)	Soil B (µg)	Soil C (µg)	Soil D (µg)	Soil E (µg)
Control	1.14 ± 1.30	14.7 ± 5.3	15.7 ± 8.6	2.32 ± 0.76	12.6 ± 4.7
Wheat shorts	7.60 ± 2.25****	34.3±15.0*	15.3 ± 4.2	16.0 ± 5.1	24.4 ± 9.9
Filter press mud	2.13 ± 1.21	20.8 ± 1.8	12.5 ± 6.5	11.4 ± 6.4	25.0 ± 6.3
Peanut skins	N/A	30.6 ± 4.5	9.26±5.12	27.4 ± 10.5***	25.8±10.2
Na-based zeolites	0.69 ± 0.77	13.8 ± 5.3	13.3 ± 6.4	N/A	28.8±13.0

Statistical significance between treated soil and control is represented by:
* for $p < 0.05$; ** for $p < 0.01$; *** for $p < 0.005$; and **** for $p < 0.001$.
N.B. Means and standard deviations of 4 or 5 replicate samples are presented.

Table 3.15: Zn uptake in lettuce grown on treated urban soils.

Treatment	Soil A (µg)	Soil B (µg)	Soil C (µg)	Soil D (µg)	Soil E (µg)
Control	22.7 ± 28.1	71.2± 23.0	84.5±41.2	14.9 ± 1.7	72.6 ±21.2
Wheat shorts	178 ± 42****	170 ± 62*	113 ± 36	88.5±30.7	128 ± 42
Filter press mud	30.7 ± 11.0	103 ± 10	90.1±12.8	63.4±35.0	129 ± 29
Peanut skins	N/A	143 ± 20	62.6±30.2	224 ± 80****	123 ± 71
Na-based zeolites	6.85 ± 5.67	90.1 ±53.7	81.4±28.2	N/A	163 ± 49

Statistical significance between treated soil and control is represented by:
* for $p < 0.05$; ** for $p < 0.01$; *** for $p < 0.005$; and **** for $p < 0.001$.
N.B. Means and standard deviations of 4 or 5 replicate samples are presented.

Table 3.16: Available phosphorus content of urban soils treated for 30 days under greenhouse conditions.

Treatment	Soil A (mg/kg)	Soil B (mg/kg)	Soil C (mg/kg)	Soil D (mg/kg)	Soil E (mg/kg)
Control	32.4 ± 3.0	3.08±0.46	15.9 ± 0.9	17.6 ± 1.0	10.2 ± 0.5
Wheat shorts	54.9 ± 2.9****	40.2 ± 2.8****	57.2 ± 7.0****	70.8±25.0****	25.0 ± 5.9****
FPM	38.3 ± 1.4**	6.65±0.47**	23.6 ± 2.3*	27.5 ± 1.7	18.5± 0.7***
Peanut skins	26.5 ± 2.3**	5.13±1.08	16.2 ± 1.5	17.5 ± 1.5	9.92±0.95
Na-based zeolites	15.7 ± 1.7****	1.08±0.42	8.57±0.37*	10.1 ± 0.6	4.81±0.32*

Statistical significance between treated soil and control is represented by:
* for p < 0.05; ** for p < 0.01; *** for p < 0.005; and **** for p < 0.001.
N.B. Means and standard deviations of 4 or 5 replicate samples are presented.

Table 3.17: Electrical conductivity of urban soils treated for 30 days under greenhouse conditions.

Treatment	Soil A (µS/cm)	Soil B (µS/cm)	Soil C (µS/cm)	Soil D (µS/cm)	Soil E (µS/cm)
Control	793 ± 92	415 ± 44	314 ± 63	337 ± 40	496 ± 59
Wheat shorts	2320 ± 343****	1130 ± 105****	448 ± 66*	579 ± 37****	591 ± 66
FPM	657 ± 107	398 ± 44	270 ± 21	344 ± 49	448 ± 40
Peanut skins	837 ± 286	620 ± 398	365±114	386 ± 31	483 ± 18
Na-based zeolites	1190 ± 183	815 ± 62*	596 ± 35****	643 ± 58****	743 ± 68****

Statistical significance between treated soil and control is represented by:
* for p < 0.05; ** for p < 0.01; *** for p < 0.005; and **** for p < 0.001.
N.B. Means and standard deviations of 5 replicate samples are presented.

Table 3.18: pH of urban soils treated for 30 days under greenhouse conditions.

Treatment	Soil A	Soil B	Soil C	Soil D	Soil E
Control	7.41±0.05	7.90±0.05	7.93±0.12	8.07±0.04	7.88±1.00
Wheat shorts	7.11±0.07****	7.46±0.11****	7.66±0.14***	7.73±0.04****	7.83±0.11
FPM	7.48±0.03	7.94±0.05	7.99±0.09	7.97±0.07	7.94±0.06
Peanut skins	7.21±0.12***	7.71±0.10**	7.68±0.03***	7.77±0.05****	7.76±0.04
Na-based zeolites	7.61±0.09**	8.81±0.03****	9.03±0.05****	9.14±0.05****	9.07±0.03****

Statistical significance between treated soil and control is represented by:
* for $p < 0.05$; ** for $p < 0.01$; *** for $p < 0.005$; and **** for $p < 0.001$.
N.B. Means and standard deviations of 5 replicate samples are presented.

Table 3.19: Organic carbon content of urban soils treated for 30 days under greenhouse conditions.

Treatment	Soil A (mg/g)	Soil B (mg/g)	Soil C (mg/g)	Soil D (mg/g)	Soil E (mg/g)
Control	73.3 ± 4.0	40.5 ± 0.9	17.2 ± 0.6	13.0 ± 0.3	15.2 ± 0.9
Wheat shorts	83.5 ± 3.0****	47.5 ± 2.2****	24.6 ± 1.3****	21.2 ± 0.4****	23.8 ± 1.0****
Filter press mud	72.0 ± 3.4	39.0 ± 2.5	16.8 ± 0.6	13.7 ± 0.9	15.8 ± 1.1
Peanut skins	88.2 ± 0.4****	54.4 ± 2.1****	31.4 ± 2.2****	28.5 ± 3.1****	33.3 ± 3.4****
Na-based zeolites	79.1 ± 3.5	37.6 ± 1.7	17.5 ± 1.1	12.6 ± 0.7	14.7 ± 0.7

Statistical significance between treated soil and control is represented by:
* for $p < 0.05$; ** for $p < 0.01$; *** for $p < 0.005$; and **** for $p < 0.001$.
N.B. Means and standard deviations of 5 replicate samples are presented.

Conclusions

The objective of this thesis was to compare the effectiveness of various methods to predict plant metal concentrations, and assess the ability of low-cost treatments to reduce metal uptake and concentrations in lettuce.

In the first study, the results showed that the anion exchange resin membranes treated with EDTA predicted plant metal concentrations better than the membranes treated with DTPA. However, the AEM-EDTA method was not the best predictor of Pb, Cu, and Zn in lettuce, despite the fact that resins simulate root metal uptake better than conventional extractants. The results showed that Na acetate and DTPA extractants predicted metal concentrations just as well as the membrane-EDTA technique, while the $HNO_3/HClO_4$ digestion procedure best estimated plant metal concentrations. This contrasts with what we expected. Possible explanations can be provided for the results obtained. For instance, the extraction time of the soil-membrane-water mixture may have been too short. A longer extraction time might have improved correlations between membrane-extractable metals and plant tissue levels, since the soils would have a better chance to replenish the soil solution with metals. In addition, a larger number of soils with more varied physical and chemical properties could give more conclusive information regarding the predictive ability of the membranes and other methods. These hypotheses require further testing.

Despite this, the AEM-EDTA technique was effective at predicting the concentrations of several metals in lettuce. In addition, the durability and reusability of these membranes, as demonstrated by Tejowulan (1994), makes them practical for routine laboratory use.

For the second study, the food processing byproducts (wheat shorts, peanut skins, and filter press mud) showed little effectiveness at reducing metal uptake in lettuce. In fact, uptake increased on some of the treated soils. Significant reductions in plant metal concentrations were found, but these resulted from dilution. Thus, the rate of lettuce growth exceeded the rate of metal uptake. Results also showed significant decreases in

Ca and Mg concentrations in lettuce which were mostly due to dilution effects as well. In comparison, the zeolites were ineffective at reducing plant metal uptake and concentrations, but did decrease Ca and Mg bioconcentrations. In addition, lettuce Mg concentrations were reduced below levels adequate for growth on some zeolite-treated soils.

Although the food processing waste materials showed little potential at stabilizing metal contaminants in soils, they may have a use as fertilizers. The results showed that soil organic carbon and available P contents, as well as crop yield increased on some of the treated soils. Hence, wheat shorts, filter press mud, or peanut skins may be used where organic and/or phosphate amendments are necessary. Further testing for this is required. In addition, other low-cost byproducts (such as rice bran, corncob meal, and soybean hulls) should be tested to determine their potential at stabilizing metals in soil systems.

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