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Site- and Species-Specific Patterns of Metal Bioavailability in Edible Plants

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Abstract

Differences in metal uptake between plant species and soil types were compared to assess the safe use of mildly contaminated soils for the growth of edible food crops. Accumulation of metals in five plant species grown in each of three field soils and a commercial soil were evaluated in a controlled environment room. Metal bioavailability varied more with plant species than with type of soil. Among a number of physical and chemical soil properties that were determined, high metal content and low percent organic matter were the best predictors of increased metal bioavailability. Contamination levels of metals measured in soil and vegetable samples were used to calculate bioconcentration factors and hazard quotients. The results indicated significant differences between plant species. The most metal-accumulating species was carrot and the most mobile element was cadmium. Some hazard quotients exceeded the threshold value of 1, even in soils considered uncontaminated by current guidelines. Overall, these results reinforce the need to include soil characteristics when setting threshold guidelines for metal content of agricultural soils and indicate the need for species-specific planting guidelines.

Keywords: cadmium, copper, lead, zinc, plant uptake, risk assessment, hazard quotient

Introduction

Metal contamination in soils

Some metals (e.g., Cu, Zn, Mn) are required at low concentrations in plants for structurally catalytic components of proteins and enzymes; however, high concentrations of both essential and non-essential metals can cause toxicity (Vanassche and Clijsters 1990). Elevated amounts of metals in soils arise from natural ore bodies, and from anthropogenic sources such as industrial production, mining, sewage sludge usage and fertilizer application (reviewed in Ross 1994). Such contamination can affect humans directly through inadvertent consumption of soil or, more commonly, ingestion of crops grown under contaminated conditions. For example, elevated concentrations of Cd in soils used to grow rice (*Oryza sativa* L.) led to human renal tubular dysfunction among Asian families (Kasuya 2000).

Soil metal regulation

Recognizing the need to reduce risk to human consumers, several countries have established permissible concentrations for many potentially toxic metals in agricultural soils. These agricultural threshold limits, including those set by the Canadian Council of the Ministers of the Environment (CCME 2006), are based on the concentration of metals in the bulk soil (total metal). However, some metal ions are tightly bound within the soil and only a fraction of the total metal in a soil is bioaccessible (available for uptake), and only a portion of the bioaccessible pool is bioavailable (actually taken up) (Peijnenburg and Jager 2003). Because of this, current limits to metal content in agricultural soil may overestimate the potential for elevated concentrations of metals in plants (Rieuwerts et al. 1998). There is a general consensus that total soil metal is not an adequate predictor of metal bioavailability and is not useful in assessing potential risk from soil contamination (Tack and Verloo 1995; Sauvé et al. 1999, 2000).

While the mechanisms of metal uptake are not entirely clear, it is believed that metals are taken up in their ionic form via active and passive transport (Clemens et al. 2002). Adsorption processes in the soil largely control the release of metal ions into soil solution, hence they influence the bioaccessibility of metals to plants (Bradl 2004). Thus, factors influencing metal adsorption and distribution regulate the availability of metals to plants (reviewed in McBride 1989). Fine textured soils tend to decrease metal bioaccessibility due to increased adsorption of metals to exchange sites on clay colloids (Brazauskiene et al. 2008). Metal solubility generally increases as soil pH decreases (Evans et al. 1995) and both conductivity and CEC are positively correlated with metal bioaccessibility (Shuman 1999). These physicochemical factors, which greatly affect the bioaccessibility of metals, should be factored into the development of more realistic agricultural threshold limits (Jung and Thornton 1996). Because thorough physical and chemical analyses are costly and time-consuming, there is a need for risk assessment models that use a reduced set of physical and chemical variables to predict metal uptake by plants.

Screening level risk assessment

At the screening level, the potential for a substance to present a health hazard can be estimated by assessing the amounts of known hazards in that substance. A number of approaches have been taken to assess the risk to humans of eating food produced on contaminated soils. One such method is the calculation of a biotic concentration factor (BCF), which is the ratio of the concentration of metal accumulated in the plant to the concentration of the metal in the soil (reviewed in Antunes et al. 2006). An index of the translocation of metal from root to shoot can be calculated by dividing the BCF for aboveground tissue by the BCF for root tissue. Plants (or edible tissues) with a higher BCF present a greater potential hazard to consumers.

Alternatively, one can compare the absolute amount of a potential hazard in food to the amount of that hazard that is known to cause adverse health effects. The Codex Alimentarius Commission (CAC), which is a joint Commission of the World Health Organization and the Food and Agriculture Organization, has established international standards for both organic and inorganic contaminants in food and animal feed (CAC 2007). The CAC used a combination of scientific information (e.g., toxicological data, estimates of typical consumptions patterns, and identification of susceptible groups) and political considerations (e.g., fair trade, barriers to international trade and technological or processing limitations) to establish a "Codex limit" for many pesticides and metals. Food that contains concentrations higher than the Codex limit are not permitted on the international market (CAC 2007).

Another approach to screening for a potential risk of consumption involves the calculation of a 'hazard quotient' (HQ; Pierzynski et al. 2000), which is a measure of the amount of a metal consumed relative to the threshold of toxicity of that metal for humans. If the HQ is below 1, there is no potential discernable risk to consuming the food; if the HQ is above 1, then the consumer is potentially at risk of suffering metal toxicity. Sipter et al. (2008) used HQ to assess the risk of consuming vegetables grown in contaminated gardens in Hungary. Although the concentrations of As, Cd and Zn exceeded the limits for agricultural soil, the corresponding HQs were below 0.05, indicating that none of the six vegetables tested accumulated metals to a potentially dangerous level.

Influence of plant species and plant tissue on metal bioavailability

Current models of risk assessment and tolerance thresholds for agricultural soils focus on metal-specific guidelines; however, two plant species grown in the same soil do not necessarily pose the same risk to human consumers. Different plant species have varying abilities to accumulate metal ions, both overall and within specific tissue types (Alexander et al. 2006). For example, spinach leaves have higher concentrations of Mn than do spinach roots, yet within radish the highest concentrations of Mn are found in the roots (Intawongse and Dean 2006). Moreover, plant leaves often have higher metal concentrations than stems; thus, leafy vegetables grown on contaminated soils may pose the greatest risk (Qadir et al. 2000, Harrison 2001). Cultivars within a species may also vary in metal uptake (Ingwersen and Streck 2005). In addition, different metals have varying bioavailability. For example, in vegetables grown in metal-contaminated soil, Mn and Zn were readily bioavailable whereas Cu, Cd and Pb were less bioavailable (Intawongse and Dean 2006).

A number of physiological variables influence the amount of metal taken up and translocated within a plant (reviewed in Clemens et al. 2002). To date, studies of the movement of metals from soil to plants have not revealed any predictable patterns and have varied with respect to the metal in question, the plant species used, or the plant tissue tested (Sinha et al. 2006). Because it is difficult to generalize patterns of metal uptake and accumulation from one species, or cultivar, to another, and different life stages of a single species may behave differently (Page et al. 2006), it understandable that models of risk assessment do not take plant species into consideration. However, as more species-specific information is gathered, it may possible to place crops into a number of categories and subsequently establish planting guidelines that better reflect the interaction between soil, type of crop and metal accumulation.

Objectives

In this study, the bioavailability of metals for five edible food crops were determined in order to assess the need for species-specific planting guidelines for soils with low to moderate levels of metal contamination. For four metals (Cd, Pb, Zn and Cu), the potential health risks to humans consuming the vegetables were estimated.

Materials and methods

Soil preparation

Soils were obtained from the topsoil layer (25 cm) of an agricultural site (A) and a forested site (F) near Salaberry-de-Valleyfield, Québec (45°16'N, 74°06'W). A third soil (L) was obtained from an agricultural site northeast of London, Ontario (43°7'N, 81°26'W). These three sites were chosen because they have elevated concentrations of a number of metals, including Cd and Pb soils A and F (Ge and Hendershot, 2005) and Cd in soil L (Shute and Macfie, 2006). Soil F was not meant to represent a soil in which crops would normally be grown, it was chosen to increase the range of concentrations of metals in the experimental soils. We collected only one soil sample from each location because we were more interested in having four distinct soil types than in trying to characterize or represent the soil heterogeneity within each field site. A commercial potting soil, Promix (P), was used for comparative purposes. All soil samples were air-dried and sieved to $\leq$2 mm as per Metals in the Human Environment-Strategic Network (MITHE-SN) guidelines (MITHE-SN 2007).

Metal content of the soil

Acid digestion for metal content followed the United States Environmental Protection Agency (US EPA) test method SW-846 (US EPA 2005). This procedure extracts all metals except those tightly bound in silicate minerals, which are not bioaccessible under natural conditions. For three replicates of each soil, 1.0 g soil was digested in 1 ml OmniTrace® nitric acid (EMD Chemicals Inc., Gibbstown, USA) in a glass test tube capped with a glass marble.

Samples were digested at 100^{o}C until the vapours were clear (approx. 3 h). After cooling to room temperature, samples were filtered (qualitative paper #413, VWR International, Mississauga, Canada) then brought to a final volume of 50 ml with distilled water. Controls of distilled water, HNO_3, and Montana Soil (Standard Reference Material 2711, National Institute of Standards and Technology, Gaithersburg, USA) were similarly processed and analyzed. Digested samples were stored at 4^{o}C until analyzed using Inductively Coupled Plasma-Atomic Emission Spectroscopy (ICP-AES; Perkin Elmer Optima-3300 DV ICP-AES, RF Generator Power-1300 Watts, Gas Flow Rate- 15 L min^{-1}, Auxillary Flow Rate- 0.5 L min^{-1}). The detection limit for each element was calculated to be equal to three times the standard deviation of readings for the nitric acid blanks.

Soil characterization

All soils were analyzed in triplicate for general soil properties. Soil conductivity was measured after equilibration for 30 min in deionized water at a 10:1 liquid: soil ratio with a Hanna Instruments 8033 electrode (Hanna Instruments, Laval, Canada) calibrated at 25ºC and 1413 μS cm^{-1}. The supernatants of these solutions were then measured for pH using a glass electrode calibrated at pH 4 and 10. Soil texture was determined using a sedimentation method adapted from Diaz-Zorita et al. (2002), which involved physical fractionation of sand, silt and clay particles suspended in 1% sodium pyrophosphate. Organic matter content was measured via loss on ignition at 500ºC for 12 h (Heiri et al. 2001).

Pot trials

Five crop species, carrot (*Daucus carota* L.), radish (*Raphanus sativus* L.), lettuce (*Lactuca sativa* L.), soybean (*Glycine max* (L) Merr.) and wheat (*Triticum aestivum* L.), were potted in each of the four sieved soils, one plant per 10 cm diameter pot, and placed in a greenhouse with natural lighting, supplemented by halide lamps on a 16 h photoperiod at a minimum temperature of 25 ± 2ºC. Pots were watered daily to 75% field capacity with deionized water. Six mL of Hoagland's nutrient solution (Hoagland and Arnon, 1950) was administered to all emerged seedlings twice a week for the duration of their growth period. Upon maturation of the edible tissues (approx. days to maturity: carrot, 65; radish, 28; lettuce, 45; soybean, 80; wheat, 65), plants were harvested. Roots of carrot and radish were rinsed in distilled water for 5 min, followed by a wash in 1 mM $CaSO_4 \cdot 2H_20$ for 30 min to remove metals adsorbed to the root surface (Taylor et al. 1998), followed by a rinse in distilled water for 5 min. Lettuce leaves were rinsed in deionized water and blotted dry. Wheat grain and soybeans were untreated. All plant tissues were dried to a constant weight in a 60^{o}C oven.

Plant tissue analysis

Edible tissues were analyzed for total metal content following US EPA test method SW-846 (US EPA 2005) in the same manner as for soil samples, with 0.1 g dry tissue being digested in 1 ml OmniTrace® nitric acid. Controls of distilled water, HNO_3, and tomato leaves (Standard Reference Material 1573a, National Institute of Standards and Technology, Gaithersburg, USA) were similarly processed and analyzed. The metal content of digested plant tissues was determined using ICP-AES, as described above for soil.

To compare the amounts of Cd, Pb, Zn and Cu accumulated by the edible tissue across soil types, bioconcentration factors (BCF) were calculated using equation 1 (modified from Antunes et al. 2006):

[1] $$BCF = M_e / M_s$$

Where M_e = mean concentration of the metal in the edible tissue (mg/kg dry weight) and M_s = mean concentration of the metal in the soil (mg/kg dry soil).

Statistical analysis

Soil metal content and general soil properties were analyzed by one-way ANOVA. For all ANOVA where significant ($p<0.05$) main effects were detected, Tukey's test of multiple comparisons was applied. Plant tissue metal concentrations were compared among species and between soils by means of multiple analyses of variance (MANOVA). Normality and correlation tests were undertaken prior to data testing. All statistics were performed using SAS Version 16.0.

Screening level risk assessment

In terms of risk to human health, ingestion of vegetables grown under contaminated conditions was the only exposure pathway considered in this study. The average daily dose (ADD) was calculated using equation 2 (modified from Sipter et al. 2008):

[2] $$ADD = (C_{veg} \times Y_{veg}) / BW$$

Where C_{veg} = concentration of the metal in the fresh edible tissue (mg/kg fresh weight), Y_{veg} = average amount of the vegetable consumed (kg/day), and BW = average bodyweight (kg). The ADD was calculated separately for Cd, Pb, Zn and Cu, as well as for males and females due to differences in average bodyweight (69.4 kg for females, 82.6 kg for males). Average consumption values were obtained from Canada's Food Guide (Health Canada 2008).

The non-carcinogenic risk of vegetable consumption was characterized using a hazard quotient (HQ), which is the ratio of the ADD to the reference dose (RfD) for each metal (Pierzynski et al. 2000). A reference dose corresponds to the maximum amount of a metal that can safely be consumed per day, per kg body weight. Reference doses were obtained from the Integrated Risk Information System (US EPA 2006): 0.001 mg/kg/day for Cd; 0.035 mg/kg/day for Pb; 0.3 mg/kg/day for Zn; and 0.04 mg/kg/day for Cu. If the HQ is greater than 1, then the ADD of that particular metal exceeds the RfD, indicating that there is a potential risk associated with consumption of food containing that metal.

Results

Soil characterization

The commercial potting soil (P) and the agricultural soil from London (L) had higher pH than the two soils from Québec (A and F) (Table 1). Soil P, which contains peat, had 2.5 to 4 times more organic matter when compared to the three field soils (Table 1). According to the United States Department of Agriculture Soil Texture Triangle (USDA 2008) which is based on the percentages of sand, silt and clay, soils P, L, A and F are classified as sandy loam, clay loam, silt loam and loamy sand, respectively. Conductivity in the four soils ranged from 83 $\mu S\ cm^{-1}$ in A to 397 $\mu S\ cm^{-1}$ in P (Table 1).

Concentrations of Ba, Co, Cr, Mo, Ni, and Se did not differ among the four types of soil ($p = 0.238$ to 0.843, data not shown). The concentrations of these six elements were below the Canadian Soil Quality Guidelines established by the Canadian Council of Ministers of the Environment (in mg/kg; Ba 750; Co 40; Cr 64; Mo 5; Ni 50; Se 1;CCME, 2006). The concentration (mean $\pm$ SE) of Al was highest in soil F ($38.2 \pm 0.4 \times 10^3$ mg/kg), and lowest in soil P ($15.7 \pm 0.4 \times 10^3$ mg/kg; $p < 0.001$). Soil F contained the highest concentration of Mn (1068 ± 26 mg/kg); the lowest Mn was in soil P (260 ± 11 mg/kg; $p < 0.001$). Acceptable limits for Al and Mn in agricultural soils have not been established.

The concentrations of Cd, Cu, Pb and Zn were highest in soil F, and approached or exceeded the Canadian Soil Quality Guidelines (Table 1). The only soil that met the guideline for Zn was soil P (Table 1). Because the concentrations of Cd, Cu, Pb and/or Zn exceeded the CCME (2006) guidelines in at least one of the soil types, these four metals were chosen for screening level risk assessment.

Metal content of vegetable samples

Multiple analyses of variance (MANOVA), including all 12 elements (Al, Ba, Cd, Co, Cr, Cu, Fe, Mn, Mo, Pb, Se and Zn) measured in the soil, showed significant site effects on the mean vectors of the edible tissue metal data (Wilks' lambda = 0.63; $F_{(3,48)} = 9.06$; $p<0.0001$). Vegetables grown in soils A and F had higher concentrations of metals than did those grown in the other two soils. Multiple analyses of variance also detected significant species effects on the mean vectors of the edible tissue metal data (Wilks' lambda = 0.17; $F_{(12,128)} = 9.75$; $p>0.0001$). The metal content in edible tissues increased in the order of soybean < wheat < lettuce < radish < carrot.

The results of vegetable analyses for Cd, Pb, Zn and Cu are shown in Table 2. The soil types are presented in order of increasing metal content (P<L<A<F), a pattern which was consistent across all metals. The concentrations of metals in the vegetables generally increased with increasing metal in the soil. Soybeans germinated in soil F, but the seedlings did not survive past 4 days. A second set of seeds was sown, but these seedlings also died.

Although soil F was the only soil with concentrations of Cd above the CCME (2006) limit (Table 1), concentrations of Cd in carrot, radish and lettuce grown in both soils A and F were well above the Codex limit (CAC 2007) for human consumption. Wheat and soybean grown in soil L also exceeded the limit for Cd. Although all soils were well below the CCME (2006) limit for Pb in agricultural soil, carrot and radish grown in each of the field soils were at or above the Codex limit (CAC 2007) for consumption. Concentrations of Pb in wheat and soybean were below the detection limit. The concentrations of Zn in carrot, radish and lettuce grown in soils A and F were an order of magnitude higher than the concentrations of Zn in wheat and soybean grown in those soils. Concentrations of Zn in radish and soybean grown in soil P were higher than expected, given the low concentration of Zn in this soil. The results for Cu are very interesting. Although the mean concentrations for Cu in soils P, L and A ranged from 21 to 26 mg/kg, respectively (Table 1), the concentrations of Cu in carrot, wheat and soybean grown in these soils ranged over 3 orders of magnitude. Codex limits for Zn and Cu in food have not been established as they are essential micronutrients.

In general, the magnitude of BCF was Pb < Zn < Cu < Cd (Fig. 1). The very high BCF values for carrot, radish and lettuce grown in soil P are a result of Cd being 0.3 to 1.3 mg/kg in the plant tissues and the concentration of Cd in soil P being below the detection limit. To calculate these BCFS, one half the detection limit for Cd (0.001 mg/kg) was used for the soil concentration. In soil P, a very high proportion of the Cd must have been bioaccessible. Among the field soils, the only BCF values for Cd that were above 1 were for carrot and radish grown in soil L (Fig. 1A). The BCF for Cd did not vary among vegetables in soil L, but carrot concentrated Cd to a greater extent than did both radish and lettuce in each of soils A and F. While all BCFs for Pb were below 0.1, the highest BCF for Pb was for carrot grown in soil A (Fig. 1B). No Pb accumulated in seeds of wheat or soybean grown in any soil type. For Zn, all of the BCFs were below 0.12; the highest value was for carrot grown in soil A (Fig 1C). The BCFs for Cu were below 0.5 for all vegetables in each of the field soils (Fig. 1D). For Cu, the highest BCFs were recorded for wheat grown in the three field soils.

Screening level risk evaluation to human health

The results of the human health screening level risk assessment for females are shown in Fig. 2. The data for males were similar, but due to their higher average body mass the HQ values for males were 95% those of the females (data not shown). In terms of Cd (Fig. 2A), consumption of neither wheat nor soybean would pose a risk (all HQ below 0.1); however, carrot, radish and lettuce grown in soils A and F, and radish grown in soil L had HQs well above 1 (range: 2.1 to 37). No vegetable presented a risk with respect to consuming an unacceptable amount of Zn; the highest HQ was only 0.3 (Fig. 2B). Carrot and radish grown in soil A, and carrot radish and lettuce grown in soil F had Zn HQ values ranging from 2 to 8 (Fig. 2C). For Cu, only wheat grown in soil A had an HQ above 1 (Fig. 2D).

Discussion

Soil characterization

The pH and percent organic matter in the field soils are typical of agricultural soils in eastern Canada (Gregorich et al. 1995). Given the lower pH of soils A and F, one might expect metals in these soils to be more bioaccessibile (Evans et al. 1995, Peijnenburg and Jager 2003, Sinha et al. 2006); however, these two soil also have a lower conductivity, which could result in reduced metal bioavailability (Shuman 1999). Soil L had a higher proportion of clay and a higher pH relative to soils A and F, thus plants grown in soil L might be expected to have lower metal content. To examine metal bioavailability in more detail, two variables were considered: the influence of soil type and the influence of plant species.

The influence of soil type

Generally, metal content in the vegetables increased with increasing metal in the soil and with decreasing soil pH, although this pattern was not true for some plant/soil combinations. No consistent relationship between metal type, BCF and soil type was observed, which is in contrast with Alam et al. (2003) who found BCF values for As decreased with increasing soil metal.

If pH were the dominant variable in terms of metal bioavailability, then the BCFs should be highest in soil A and second highest in soil F. Although this pattern of BCF was not observed for all metals within any one plant species, nor for any one metal across plant species, the soil with the lowest pH (A) did yield the highest BCF for Cd, Pb and Zn in carrot, for Zn in radish and for Cu in wheat. Furthermore, metals such as Cd and Cu tend to precipitate out of the soil solution at pH values ranging from 6-8 (Kamnev and van der Lelie 2000, Ismail et al. 2005), suggesting that soils L and P should have a smaller proportion of these metals available for uptake. However, in radish the highest BCF for Pb was seen for plants grown in soil P and wheat grown in soil L had the second highest BCF for Cu.

Soil texture, conductivity and OM could also contribute to a difference in bioavailability of metal in the soils. Of the three field soils, soil F had the lowest clay content, the highest OM content, and a mid-range conductivity, all of which should result in decreased metal bioavailability (Ali et al. 2004). However, as was seen for pH, no clear relationship between these variables and metal uptake within a plant species, or across plant species, was seen.

Of course, it is reasonable to believe that some combination of physicochemical characteristics determines metal bioaccessibility within a soil, and different conditions may differentially affect different metals. It could be that the range of available data was too narrow or an insufficient number of variables were measured in this study to clearly identify the relationship between soil type and metal accumulation in the plants. Nonetheless, if the physicochemical characteristics of a soil lead to increased bioaccessibilty of at least one metal, one might expect many plants grown in that soil to accumulate that metal. This was not seen.

For example, in soil L, only carrot had high BCFs for Cd and Pb and wheat had the highest BCF for Cu. Clearly, species-specific variables are as important, or more important, than soil characteristics in terms of regulating metal bioavailability.

Notwithstanding the above, it is important to note that BCF values obtained from potted plant experiments may overestimate BCFs relative to plants grown in the field because metal bioaccessibility is often higher in soils that have been dried and stored (reviewed in Bartlett and James 1980). In addition, plants in our study were grown at 25ºC, which may have resulted in increased metal bioaccessibility relative to plants grown in cooler field soil (Antoniadis and Alloway 2001). On the other hand, some of the larger wheat and soybean in our experiment became pot-bound. Had they been permitted to explore a larger volume of soil, they may have accumulated higher concentrations of metals.

The influence of species

The relationship between soil characteristics and vegetable species is also illustrated by the BCFs. Overall, carrot showed the highest potential for Cd, Pb and Zn uptake, whereas wheat accumulated Cu to greater extents than the other vegetables did. The high BCFs for carrot are surprising since Intawongse and Dean (2006) found that concentrations of Cd Cu, Mn and Zn were higher in carrot leaves than in carrot roots. Others have reported that roots generally contain elevated concentrations of metals than do shoots due to retention of metals in the root (reviewed in Clemens et al. 2002). Radish is also a root crop and, while concentrations of Cd and Zn were higher in radish than in the non-root crops, the concentrations of Zn and Cu were sometimes as high, or higher, in wheat and soybean than they were in radish. In our study, lettuce accumulated relatively low concentrations of each metal. This is in contrast to many other reports in which leafy vegetables (especially lettuce and spinach) were shown to accumulate metals to a greater extent than some other vegetable types (Qadir et al. 2000, Harrison 2001, Alexander et al. 2006).

Within each soil, accumulation of metals varied among plant species. The differences in the BCFs for each metal among the five vegetables suggest that plant species differ in uptake and translocation abilities. In addition, because no one vegetable consistently took up any one metal from each soil, net bioavailability must be the result of plant/soil interactions. Our results are in agreement with Jung and Thornton (1996), Alexander et al. (2006), Intawongse and Dean (2006) and Sipter et al. (2008) who found that plant metal concentrations differed with plant species and the pattern among species varied between different elements.

Screening level risk assessment

As expected, all vegetables grown in soils P and L had very low HQs; these soils were below the CCME (2006) limits for Cd, Pb and Cu, and soil L was only slightly above the limit for Zn. In addition, each soil was well below the CCME (2006) limit for Pb and no vegetable accumulated Pb to a potentially unsafe level. It is also not surprising that carrot, radish and lettuce grown in soil F had high HQs for Cd and Zn; that soil had four times the CCME (2006) limit for Cd and 30 times the limit for Zn. Similarly, soil A had 6 times the limit for Zn, and each of carrot, radish and lettuce had Zn HQs above 1. Muchuweti et al. (2006) reported unsafe concentrations of Cd, Cu, Pb and Zn in six dietary crops that were grown in soils that exceeded the United Kingdom regulations for these metals. In general, soils that exceed quality guidelines offer the potential for a contaminated crop.

However, elevated concentrations of metal in a soil do not necessarily result in high HQs; for example, the HQs for Zn were low for vegetables grown soil L, which was 10% over the CCME (2006) limit for Zn. Sipter et al. (2008) also reported non-toxic concentrations of metals in vegetables that were grown in contaminated soil. At the same time, growth in an apparently "uncontaminated" soil can result in a potentially unsafe crop. For example, Cd in soil A was well below the CCME (2006) limit, yet the HQs for Cd in these vegetables ranged from 2 to over 30.

Our HQ calculations were based on the assumption that consumers ate only one type of vegetable in their diet and the vegetable was eaten raw. This was intended to represent the worst case scenario of eating produce from these soils. In reality, most people eat a mixed vegetable diet and some methods of vegetable preparation (e.g., boiling) may leach metals prior to consumption. Nonetheless, our results indicate that soil use guidelines should not be based solely on the concentrations of metals in soil but also other soil properties and on crop species. For example, guidelines might include a list of 'low accumulating' crops that might be suitable for growth on mildly contaminated soils (Alexander et al. 2006).

Conclusions

No generalized pattern of metal uptake was observed either between soil types or between plant species. That is to say that no single soil type or plant species was observed to hold the highest influence on metal uptake; rather, metal uptake was observed to vary depending on soil, plant species, and metal in question. This indicates the need for a more thorough investigation of the influences of plant/soil interactions on metal uptake, and integration of the findings into risk assessment models.

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Table 1. Selected physicochemical properties for each of the four soils.

Soil	pH*	OM (%)	Sand (%)	Silt (%)	Clay (%)	Conductivity (μS/cm)	Cd (mg/kg)	Cu (mg/kg)	Pb (mg/kg)	Zn (mg/kg)
P	6.5±0.01a	28.8±0.6a	70±5a	25±2c	5±1b	396.7±2.1a	ND	21.4±5.0b	4.2±1.1c	135±11c
L	6.8±0.1a	6.8±0.1c	24±3b	48±2b	28±5a	250.0±3.2b	0.3±0.1b	23.4±1.2b	10.5±0.3b	227±9c
A	5.1±0.1c	7.0±0.01c	22±2b	69±4a	9±3b	83.2±2.2d	0.8±0.1b	25.8±0.7b	14.9±2.0b	1211±61b
F	5.8±0.1b	11.6±0.1b	80±3a	16±4c	4±2b	198.3±4.6c	6.3±0.3a	45.6±1.1a	40.1±0.7a	6807±131a
CCME limit†							1.4	63	70	200

***** Within each column, means sharing the same letter are not significantly different (one-way ANOVA followed by Tukey's test, (p<0.05). Values are mean ± SE, n=3.

ND = not detected (detection limits in μg/kg: Cd, 1.5; Cu, 1.5; Pb, 15; Zn, 5)

† The Canadian Council of Ministers of the Environment limits for agricultural soil are presented for comparison (CCME 2006).

Table 2. Concentrations of metals in edible tissues of five plants species grown in each of the four soils.

	Concentration of metal (mg/kg dry weight)				
Vegetable	Soil Type P	L	A	F	Codex limit*
	Cd				
Carrot	0.7 ± 0.3b	0.8 ± 0.2b	38.6 ± 19.9a	42.5 ± 11.1a	1
Radish	1.3 ± 0.8b	1.9 ± 0.7b	11.0 ± 3.8b	13.3 ± 1.5a	1
Lettuce	0.3 ± 0.01b	1.6 ± 0.3b	3.7 ± 3.4b	8.2 ± 1.4a	2
Wheat	ND	0.5 ± 0.02a	0.06 ± 0.06c	0.2 ± 0.004b	0.22
Soybean	ND	0.3 ± 0.01a	ND	- -	0.11
	Pb				
Carrot	0.3 ± 0.2b	1.0 ± 0.5b	12.2 ± 3.1a	6.5 ± 2.2ab	1
Radish	0.9 ± 0.9a	2.5 ± 1.3a	3.8 ± 1.3a	3.9 ± 2.2a	1
Lettuce	1.9 ± 0.2a	0.7 ± 0.7a	2.6 ± 1.3a	2.8 ± 1.6a	3
Wheat	ND	ND	ND	ND	0.22
Soybean	ND	ND	ND	- -	0.22
	Zn				
Carrot	41.0 ± 10.2a	25.9 ± 4.3a	1401 ± 199a	2737 ± 734a	
Radish	52.0 ± 7.7c	23.2 ± 6.8c	955 ± 5b	1890 ± 560a	
Lettuce	24.9 ± 8.2b	30.1 ± 7.2b	372 ± 20b	1350 ± 76a	
Wheat	16.8 ± 0.6b	22.6 ± 1.8b	32.6 ± 14.4b	168 ± 11.3a	
Soybean	56.5 ± 0.4a	39.8 ± 3.1b	42.3 ± 0.8b	- -	
	Cu				
Carrot	6.5 ± 1.3c	8.4 ± 4.3c	35.6 ± 3.7a	21.2 ± 2.6b	
Radish	5.2 ± 1.6b	9.1 ± 3.5b	19.7 ± 7.8b	27.9 ± 2.7a	
Lettuce	3.0 ± 1.2a	4.6 ± 0.4a	4.6 ± 0.6a	7.1 ± 2.8a	
Wheat	0.7 ± 0.7b	60.4 ± 3.0a	122 ± 54a	83.2 ± 1.0a	
Soybean	4.4 ± 0.8c	41.9 ± 2.2a	32.9 ± 0.8b	- -	

* The Codex Alimentarius Commission standards (CAC 2007) for metal content in the vegetables are presented for comparison. These Codex limits have been converted from mg/kg fresh weight to mg/kg dry weight using a wet:dry ratio of 10:1 for root and leaf tissues, and a wet:dry ratio of 10:9 for seeds; these values correspond to average in-lab measurements before and after drying. Codex limits have not yet been established for Zn and Cu.

Note:

Within each row, means sharing the same letter are not significantly different (one-way ANOVA followed by Tukey's test, $p<0.05$)

Values are mean ± SE, n=3.

ND = not detected (detection limits in μg/kg: Cd, 1.5; Cu, 1.5; Pb, 15; Zn, 5)

- - = soybean did not survive in soil F.

Figure captions

Figure 1. Bioconcentration factors (BCFs) of vegetables grown in the four soils. BCF = M_e/M_s (concentration of metal in the edible tissue/ concentration of metal in the soil). A (Cd), B (Pb), C (Zn), D (Cu). Soil Type: P, Promix; L, London Agricultural Soil; A, Québec Agricultural Soil; F Québec Forest Soil.

Figure 2. Hazard quotients (HQ) of vegetables grown in the four soils. HQ is an estimate of the risk associated with consumption of each vegetable (a ratio of the amount of metal consumed relative to the maximum amount of metal deemed to be non-hazardous). A (Cd), B (Pb), C (Zn), D (Cu). Soil Type: P, Promix; L, London Agricultural Soil; A, Québec Agricultural Soil; F Québec Forest Soil.