

## MITHE-SN ANNUAL SYMPOSIUM

### P.I. Platform Abstracts

**Project Title:**

(S2) Controls of bioaccessibility of trace elements in soils

**Investigators:**

Hendershot, W., McGill University

**Summary:**

The overall objective of S2 is to determine the mechanisms that control the movement of trace elements from soils to plants. Part of the work is focused on the ecotoxicological effects of high levels of soil trace metals on plant growth while another part looks at the movement of metals and metalloids into food crops. Over the last few years Yong Hong Wu developed a terrestrial biotic ligand model (BLM) based on results obtained for multiple metal mixtures (Cd, Cu, Ni, Zn) in hydroponic culture as part of her PhD research. This work is continuing in a more complex system that includes both soil and plants by Dina Schwertfeger as part of her PhD. In a similar vein, Akiko Sato has studied cerium (Ce) in both hydroponic and soil culture experiments since it appears to be the rare earth element most likely to result in toxicity in soils, based on results from the aquatic literature. In general, the results supports the idea that a terrestrial BLM is a useful tool in evaluating the competition for different cations ( $M^{2+}$ ,  $Ca^{2+}$ ,  $H^+$ ) at the soil solution – root interface.

During 2007, research on urban gardens in Montreal, Hamilton and London was begun with the leadership of Étienne Foucher. Due to historical contamination of cities by a wide range of human activities, they now represent a complex matrix of soils with different edaphic conditions and trace element concentrations in a relatively small geographical area. Since one of the primary goals of the <Soils and Plants> theme is to identify the possible pathways of contamination of the human food chain, urban gardens provide an experimental framework where food production on contaminated soils can be examined efficiently. Although the field experiment conducted in 2007 is, in some ways, poorly controlled from an experimental design perspective, it does answer some fundamental questions: Is there a link between soil trace element contamination and high concentrations of trace elements in the food produced? Are there significant differences between different plant types? Can research conducted on soils deemed too contaminated by Montreal's public health officials help them to decide whether the ban on vegetable production is warranted?

Research results from these sub-projects are presented as posters by my students and you are encouraged to read their abstracts and visit them at the poster session.

**Project Title:**

(S3) The role of speciation in the accumulation of Tl and Se

**Investigator(s):**

<sup>1</sup>Renkema, H., <sup>2</sup>Arthur, L., <sup>3</sup>Harskamp, J., <sup>3</sup>Kersbergen, L., <sup>3</sup>Kikkert, J., <sup>3</sup>Koopmans, A.,  
<sup>4</sup>O'Donnell, M., <sup>1</sup>Hale, B. and <sup>3</sup>Berkelaar, E.

<sup>1</sup>University of Guelph, Guelph, ON

<sup>2</sup>Ontario Ministry of Agriculture, Food and Rural Affairs, Guelph, ON

<sup>3</sup>Redeemer University College, Ancaster, ON

<sup>4</sup>McMaster University, Hamilton, ON

**Summary:**

An important route of exposure to trace elements that may be toxic, such as thallium (Tl) or selenium (Se) is via foodstuffs. Therefore, an important aspect of assessing the risk of trace elements to humans is to develop models that enable predictions of the amount of trace elements taken up by plants grown on agricultural soils. In light of this, a number of projects are ongoing.

The goals of **Project I (Tl and plants)** are i) to investigate the nature of the competition between Tl(I) and K for accumulation by durum wheat and spring canola.; ii) to investigate Michaelis-Menton kinetics for Tl and K uptake by durum wheat and spring canola; and iii) to measure accumulation of Tl by durum wheat and spring canola grown in soil with varying levels of Tl and K. Two posters, "*Can thallium accumulation by plants be predicted from potassium accumulation?*" by Renkema *et al.* and "*The influence of competing ions on Se and Tl accumulation by durum wheat and spring canola*" by Kikkert *et al.* will be presented at the MITHE symposium. The goals of **Project II (Se and plants)** are i) to measure accumulation of Se by durum wheat and spring canola grown in soil with varying levels of  $\text{SeO}_3^{2-}$ ,  $\text{SeO}_4^{2-}$ , and  $\text{SO}_4^{2-}$ ; and ii) to investigate effects of transpiration on Se uptake and translocation. Results will be presented in posters titled "*The influence of competing ions on Se and Tl accumulation by durum wheat and spring canola*" by Kikkert *et al.* and "*Soils & Plants Project S3: The role of speciation in the accumulation of Tl and Se*" by Renkema *et al.* The goal of **Project III (Soil Cd BLM)** is to develop and validate a tBLM for soybean based on data collected from field-grown soybean. The goal of **Project IV (Diffusion)** is to determine the importance of diffusion in the process of trace metal accumulation by plants using Cd as a model metal. Wheat and canola plants were exposed to 1 or 5  $\mu\text{M}$  Cd at a pH of 6.0 in exposure solutions containing only  $\text{Cd}^{2+}$  species (all Cd was 'free') or in solutions where the  $\{\text{Cd}^{2+}\}$  was buffered with EDTA.  $\{\text{Cd}^{2+}\}$  were measured with a Cd-microelectrode at various sites near the root surface so that rates of Cd flux could be determined. Results will be presented in a poster titled "*Soils & Plants Project S3: The role of speciation in the accumulation of Tl and Se*" by Renkema *et al.*

**Project Title:**

(S4) The impact of microbial activity and trace metal speciation in the rhizosphere on metal uptake by plants: A synthesis of project S4

**Investigator(s):**

<sup>1</sup>Courchesne, F., <sup>1</sup>Cloutier-Hurteau, B., <sup>2</sup>Hendershot, W., <sup>3</sup>Macfie, S., <sup>1</sup>Sauvé, S. and <sup>1</sup>Turmel, M.C.

<sup>1</sup>Université de Montréal, Montréal, Canada

<sup>2</sup>McGill University, Montréal, Canada

<sup>3</sup>University of Western Ontario, London, Canada

**Summary:**

Three interconnected projects focusing on the links between trace metals in soils and their uptake by plants were run. The objective of the first project is to determine the impact of microbial activity and of trace metal speciation in the rhizosphere (poster by Cloutier-Hurteau *et al.*) on the uptake of metals by edible plants. This exploratory experiment was conducted with wheat in a growth chamber (poster by Turmel *et al.*). The second project derives from the first and is more methodological in nature. It looks at the potential of Rhizon microlysimeters for the *in situ* sampling of the rhizosphere solution. Our work with water extracts indeed indicated that a more direct access to the rhizosphere solution was needed to improve the measurement of trace metal in the rhizosphere and thus, the assessment of the exposure of plant roots to metals. Because a reliable sampling method does not exist for that purpose, it needs to be developed. The third project expands on the first and on work performed by Hendershot's group. It aims at developing functions representing the links between metal contents in soils and in plants for a range of edible plants grown for human consumption. The first phase of the project was run in 2007 and consisted of a field experiment involving the measurement of 19 trace elements in soils and in vegetables (bean, broccoli, lettuce, radish, Swiss chard, tomatoes) grown in 28 urban gardens of Montréal (22), London (3) and Hamilton (3) (poster by Gingras *et al.*). This large project was run in close collaboration with W Hendershot, S Macfie and E Berkelaar. A second phase to this project using extensive growth chamber experiments will be initiated in 2008. The results showed that DOC concentrations, water-soluble metals (Al, Cd, Cu, Ni, Pb, and Zn) and microbial biomasses were significantly higher in the rhizosphere than in the bulk soil of the wheat experiment. Metal speciation in the rhizosphere also differed from that of the bulk although the trends varied between metals with, for example, Cu being more preferentially bound to organic ligands than Al. Statistical analyses further showed that microbial activity had a stronger impact on the speciation of dissolved metals in the rhizosphere than in the bulk soil. This reflected the absolute microbial enrichment of the rhizosphere and, as suggested by molecular profiling (PCR-DGGE) analyses, the presence at the soil-root interface of microbial assemblages that differ from those of the bulk soil. The assays with Rhizon samplers showed that the device had no impact on pH and on the concentrations of major ions. As for trace metals, microlysimeters had a slight impact on Cd and Zn concentrations but they retained dissolved Cu and Pb. New assays are now being conducted with soils to confirm these findings. As for the experiment conducted in urban gardens, the chemical analyses of plant and soil samples were completed in December 2007. Final results are not yet available but some links between metal contents in soils and plants were established using multivariate statistical analyses. These analyses will be critical to select soils and vegetables for the second phase of this project.

**Project Title:**

(S5) Metal accumulation depends more on plant species than on soil characteristics.

**Investigator(s):**

<sup>1</sup>Macfie, S., <sup>2</sup>Foucher, E., <sup>3</sup>Courchesne, F., <sup>3</sup>Turmel, M-C. and <sup>2</sup>Hendershot, W.

<sup>1</sup>Department of Biology, University of Western Ontario

<sup>2</sup>Natural Resource Sciences, McGill University

<sup>3</sup>Département de géographie, Université de Montréal

**Summary:**

Six edible plant species (bean, broccoli, lettuce, radish, swiss chard and tomato) were grown in each of 27 urban gardens in Montréal, Hamilton and London as part of a collaborative project involving Groups S2, S3, S4 and S5. The aims of this study were (1) to determine the extent to which metal uptake varied with plant species and (2) to improve our ability to predict metal accumulation in edible plants based on the characteristics of the soil. This particular sub-set of the study involved an investigation of the relationships between concentrations of metals in edible plant parts and the metal content of the soil. In no case was there a positive linear relationship between concentration in the plant and concentration in the soil. A few garden soils contained concentrations of Cu, Pb and/or Zn that were above the CCME limits for agricultural soil; however, this was not reflected in the corresponding plant tissues. Interestingly, plants with the highest concentrations of Cu, Pb and Zn were grown in the soils with the lowest concentrations of the corresponding metal. Even in gardens with very low concentrations of metals in the soil, concentrations of Cd and Pb in most plant species were above the CODEX alimentarius limit for human consumption.

Different plant species responded differently to a number of metals. Across the range of garden soils, concentrations of Cu were 20-50% lower in radish than in the other crops. Swiss chard had the highest concentrations of Cr, Mn and Pb at most sites whereas concentrations of Cd were highest in lettuce. Plant/soil interactions were particularly interesting for gardens in London and Hamilton; swiss chard and/or lettuce grown at these sites had particularly high concentrations of Cd, Cr, Cu, Mn, Ni and Pb despite having soils with low concentrations of most metals.

Overall, these results indicate an important need for species-specific planting guidelines into consideration when setting acceptable limits of metal content for agricultural soils.

**Project Title:**

(S6) Chemical forms and release of trace elements in soils and soil solutions

**Investigator(s):**

<sup>3</sup>Sauvé, S., <sup>1</sup>Wallschlaeger, D., <sup>2</sup>Hendershot, W., <sup>3</sup>Courchesne, F.

<sup>1</sup>Department of Environmental Sciences, Trent University

<sup>2</sup>Department of Natural Resource Sciences, McGill University

<sup>3</sup>Department of Geography, Université de Montreal

**Summary:**

We have completed our efforts on the initial modelling and validation of an alternate approach for modelling metal competition for sorption on soil surfaces focusing on acquiring and using free metal speciation data. This model is currently limited to interactions among Cu, Cd, Ca and H in mineral soils for pH higher than 5.

We have collected and completed the analysis of a series of manure and composts to analyze for trace elements and specifically for arsenic in chicken manure. We have recently redone part of the analysis to obtain valid data for the low concentrations samples. We sometime observe concentration of As compatible with Roxarsone-treated feeds (which is normally not allowed in Canada). Later work on As in soils will focus on the soil physicochemical properties which do influence As speciation and solubility (as identified when we finalize the interpretation of our dataset – we suspect sulfates, phosphate, organic matter and iron oxides).

## P.I. Poster Abstracts

### Project Title:

(S1) Impact of long- and short-term fertilization and management practices on trace element dynamics in crops and soils

### Investigator(s):

<sup>1</sup>Grant, C.A., <sup>2</sup>Flaten, D., <sup>3</sup>Sauvé, S. <sup>2</sup>Tenuta, M. and <sup>1</sup>Malhi, S.

<sup>1</sup>Agriculture and Agri-Food Canada

<sup>2</sup>University of Manitoba

<sup>3</sup> Université de Montréal

### Summary:

Field studies were conducted for six years at seven locations across the Canadian prairies to evaluate the impact of application of monoammonium phosphate fertilizer varying in Cd concentration on Cd concentration in durum wheat (*Triticum turgidum* L.) and flax (*Linum usitatissimum* L.). Cadmium concentration in the seed of both crops increased with application of P fertilizer, even when the fertilizer contained only trace concentrations of Cd. However, crop Cd concentration was higher when the fertilizer added contained higher Cd concentrations, particularly when rate of fertilizer application was also high. The impact of Cd concentration in the fertilizer became more apparent with repeated applications over time with the effect being proportional to the total Cd applied. Cadmium concentration in crops and the effect of fertilizer application varied with soil characteristics.

Effects of fertilizer management, tillage system and crop sequence were also investigated in field studies conducted over four years at two locations near Brandon, Manitoba. Tillage system and source of P fertilizer had little effect on Cd accumulation in crops. However, concentration and accumulation of Cd was higher and Zn lower in durum wheat grown after canola (*Brassica napus* L.) than after barley (*Hordeum vulgare* L.) or flax. Preceding crop may influence Cd accumulation through release of Cd from decomposing crop residue, rhizosphere effects, or mycorrhizal colonization.

Cadmium accumulation in crops may be reduced by selection of crop sequence that reduces the availability of Cd to the following crop. However, the risk of Cd accumulation in crops will increase with long-term application of Cd-containing fertilizers. Risk of Cd accumulation can be reduced by reducing the Cd concentration of fertilizer and by improving P use efficiency to minimize fertilizer application rate.

**Project Title:**

(S1) Effect of short- and long-term applications of manure and fertilizer on AMF colonization and Cd concentration in sunflower (*Helianthus annuus* L.)

**Investigator(s):**

<sup>1</sup>Liu, A., <sup>1</sup>Bittman, S., <sup>1</sup>Forge, T.A., <sup>1</sup>Hunt, D.E., <sup>1</sup>Kowalenko, C.G., and <sup>2</sup>Grant, C.A.

<sup>1</sup>Agriculture and Agri-Food Canada, Agassiz, BC

<sup>2</sup>Agriculture and Agri-Food Canada, Brandon, MB

**Summary:**

Reduction of Cd levels is important for improving food quality of some crops. One of the sources of Cd in agricultural soils is fertilizer, especially P fertilizer. This study compared growth and Cd uptake by sunflower (*Helianthus annuus* L.) grown in soils receiving two fertilizer sources, manure vs. inorganic fertilizer, at the same rate of P (30 kg ha<sup>-1</sup>). The project included two studies. The first, a greenhouse study, used soils collected from a replicated field trial that had received 11 years of: 1) no fertilizer (control), 2) inorganic fertilizers, and 3 and 4) dairy manure at two rates. This experiment included treatments that modified root colonization by arbuscular mycorrhizal fungi (AMF), using fumigation or fumigation and re-inoculation with AMF. The second study compared a single application of dairy slurry manure and inorganic fertilizers in the field. Sunflower shoots and roots were sampled at the 6- and 10-leaf stages. Total plant dry weight, grain yield and Cd concentration in grain were determined at crop maturity.

In the greenhouse study, root colonization by AMF was not influenced by fertilizer source but was affected by the fumigation/ inoculation treatments. Grain Cd concentration was not associated with AM colonization level. In contrast, Cd concentration in grain was significantly lower in plants growing on soils that had received manure compared to inorganic fertilizer. In the field study, there was also a significant reduction in Cd concentration with manure. The ratio of Cd to P was similar in the fertilizer and manure materials used in this study, so both treatments received the same dose of Cd. In both field and greenhouse studies, the reduced Cd concentration in the grain of plants receiving manure may be explained by dilution due to more growth, although there was no difference in biomass of the 'Low manure' and fertilizer treatment in the greenhouse trial of 2007. Growing Cd accumulating crops on soil with a history of manure may be an interesting option for farmers wanting to reduce Cd levels. However, further work is needed to verify and explain these results.

**Project Title:**

Growth, mycorrhizal colonization and Cd uptake by sunflower (*Helianthus annuus* L as influenced by tillage and fallow

**Investigator(s):**

<sup>1</sup>Liu, A., <sup>1</sup>Bittman, S., <sup>1</sup>Forge, T.A., <sup>1</sup>Hunt, D.E., <sup>1</sup>Kowalenko, C.G., and <sup>2</sup>Grant, C.A.

<sup>1</sup>Agriculture and Agri-Food Canada, Agassiz, BC

<sup>2</sup>Agriculture and Agri-Food Canada, Brandon, MB

**Summary:**

The movement of cadmium (Cd) into the food chain is a concern for human health. Colonization of crop roots by arbuscular mycorrhizal fungi (AMF), which appears to be negatively correlated with transportation of Cd into plant tissue, is reduced by intensive tillage and summer fallow. Phosphorus fertilizer can be a source of Cd and inhibit AMF development. The objective of this study was to assess the effects of summer fallow, long-term tillage practices and P fertilization, on AMF colonization, growth and Cd uptake by sunflower (*Helianthus annuus* L.). In the tillage trial, the following tillage treatments were applied (since 1998): no-till [Z], aeration [A], plough and disk [PD], roto-till [R]. The ‘previous fallow’ trial was arranged in a split plot design (main plot, fallow vs. corn in previous year; subplot, 0, 30 and 45 kg P ha<sup>-1</sup>). Both experiments were conducted in 2005 and 2006. Sunflower shoots and roots were sampled at the 6-leaf stage, 10-leaf stage and before flowering to measure percentage of root colonized by AMF, shoot dry weight and root dry weight. Total shoot dry weight, grain yield and Cd concentration in grain were also determined at crop maturity.

In general, previous fallow and intensive tillage (R and PD) reduced root colonization and early growth of sunflower shoots, but did not affect whole plant or grain yield at maturity compared to previous corn and limited tillage (A and Z). Fertilization with P reduced AMF colonization but increased root and shoot dry weight. These results are consistent with studies on corn. Despite the effects on AMF, the tillage and fallow treatments generally did not produce significant effects in grain Cd concentration or uptake. Also, grain Cd concentration was not affected by P application. Since two different methods used to reduce AMF colonization failed to affect Cd concentrations, we conclude a limited role for AMF colonization in Cd uptake in sunflowers. However, we cannot exclude other possible explanations for the data that would preserve a role for AMF in Cd uptake.

## Student Poster Abstracts

### Project Title:

(S4) Comparing WHAM 6 and MINEQL+ 4.5 for the chemical speciation of  $\text{Cu}^{2+}$  in the rhizosphere of forest soils

### Investigator(s):

Cloutier-Hurteau, B., Sauvé, S. and Courchesne, F.  
Université de Montréal, Montréal, QC

### Summary:

The soil-root interface is a key site of metal transfers from the soil to plants. The speciation of metals in the soil solution of this microenvironment is thus of prime interest to increase the reliability of ecological risk assessment. Unfortunately, few studies have evaluated the speciation of metals in the rhizosphere due to technical difficulties. In this context, the geochemical modeling of speciation could give useful information regarding the fate of metals in the rhizosphere. However, models used to calculate speciation integrate soil processes in different ways and they are not always adapted to the specific conditions of the rhizosphere solution. Yet, to our knowledge, no comparative study has evaluated the relative accuracy of speciation models in the rhizosphere. Therefore, the aim of this work is to evaluate the performance of two speciation models, WHAM 6 and MINEQL+ 4.5, in calculating the free Cu ion ( $\text{Cu}^{2+}$ ) activity on bulk and rhizospheric soil samples.

The comparison of speciation models was made on 18 bulk and 18 rhizospheric soil samples collected in two contrasted Canadian forested areas located near industrial facilities. On these soils, reference  $\text{Cu}^{2+}$  activity was measured by an ion-selective electrode (ISE) in water extracts. The modeling of Cu speciation was kept comparable between models and was performed using pH, dissolved organic carbon (DOC), major ions, and total dissolved Al, Ca, Cu, Mg, and Zn concentrations as input data. Four scenarios representing the DOC composition using fulvic, humic and acetic acids were derived from the literature and used in the modeling exercise. Different scenarios were used to contrast DOC composition between soil components (rhizosphere vs bulk) and soil pH levels (acidic vs neutral to alkaline). The performance of the modeling, for both models, is evaluated by comparing the calculation of  $\text{Cu}^{2+}$  activity with the measured values obtained by ISE.

The model MINEQL+ 4.5 provided good predictions of  $\text{Cu}^{2+}$  activities, while predictions from WHAM 6 were poor because they overestimated Cu complexation with DOC. Modeling with WHAM 6 could be improved by adjusting the proportion of inert DOC and its composition, but it remained weaker than predictions with MINEQL+ 4.5. These results suggested that the discrepancies between speciation models were attributed to differences in the binding capacity of humic substances with Cu, where WHAM 6 appeared to be too aggressive. Therefore, we concluded that chemical interactions occurring between Cu and DOC were key factors for an accurate simulation of Cu speciation, especially in rhizospheric forest soils, where high variation of the DOC concentration and composition are observed.

**Project Title:**

Trace element content in vegetables grown in urban gardens

**Investigator(s):**

Foucher, É., Hendershot, W. and Lalande, H.

**Summary:**

Concerns for the safety of urban gardeners and their families resulted in “Santé publique” closing 9 community gardens in Montreal in 2007. The decision to stop vegetable production was based on soil chemical analyses and estimates of uptake into the vegetables. This study was initiated to determine the actual levels of trace elements in different vegetables grown on urban gardens.

Vegetables grown in urban soils can accumulate trace elements in their tissues and may represent a risk to the health of their consumers. The amount of a trace element accumulating in the edible part of a plant depends both on soil properties and on plant species. This study was conducted to evaluate the differences in trace-element uptake patterns between 6 species of commonly grown vegetables. Radish, lettuce, Swiss chard, broccoli, green beans and tomatoes were grown in 24 collective or community gardens located in Montréal, Hamilton and London. Six additional samples were bought at food markets and grocery stores near Montreal for comparison. Soil and vegetable tissues were digested using hot nitric acid. Concentrations for a range of trace elements were determined using ICP-MS. A difference in trace element content was observed between different vegetables. As predicted by the literature, leafy vegetables proved to accumulate more trace elements than radish, broccoli, green beans and tomatoes. Trace-element content was generally higher in vegetables grown in urban gardens than in vegetables from the market but the trend is not consistent for all elements; tomatoes from the urban gardens, for instance, contained on average less Cd than tomatoes bought from the market. All vegetables were under the limits given by the Commission of the European Communities in regards of Pb and Cd. In the gardens where vegetables have accumulated relatively high quantities of trace elements, or there is concern as to the safety of vegetables, exposure can be considerably reduced by avoiding the production and consumption of leafy vegetable such as lettuce and Swiss chard.

**Project Title:**

(S4) Vegetable growth experiment in urban gardens

**Investigator(s):**

<sup>1</sup>Gingras, N., <sup>1</sup>Cloutier-Hurteau, B., <sup>1</sup>Courchesne, F., <sup>2</sup>Foucher, E., <sup>2</sup>Hendershot, W., <sup>2</sup>Lalande, H., <sup>3</sup>Macfie, S. and <sup>1</sup>Turmel, M.C.

<sup>1</sup>Université de Montréal, Montréal, QC

<sup>2</sup>McGill University, Montréal, QC

<sup>3</sup>University of Western Ontario, London, ON

**Summary:**

This project aims at developing functions to represent the links existing between metal contents in soils and in edible plants grown for human consumption. This large project was run in close collaboration with the research groups of W Hendershot, S Macfie and E Berkelaar. The first phase of the project was conducted during the summer and fall of 2007 and consisted of a field experiment involving the measurement of 21 trace elements in soils and in vegetables grown in 28 urban garden plots. The second phase will be based on extensive growth chamber experiments and will be initiated in the spring of 2008 to investigate specific relationships between metal contents in plants and in soils. To this end, multivariate statistical analyses were first performed on the urban garden data to select the vegetables, soils and metals that will be the focus of the second phase. Here we present the experimental conditions for the urban garden experiment and the preliminary results of the statistical analyses.

The urban garden growth experiment was conducted in 28 gardens plots located in Montréal (22), London (3) and Hamilton (3). The six vegetables (bean, broccoli, lettuce, radish, Swiss chard, tomatoes) were planted in each plot in June, when the soils were sampled, and harvested in August 2007. After harvest, the edible part of each plant was collected, dried at 50°C and grinded using a coffee mill. The soils materials and plant parts were dissolved in HNO<sub>3</sub>. The extracts were analyzed in triplicate for Ag, As, Ba, Cd, Ce, Co, Cr, Cu, Lu, Mn, Mo, Ni, Pb, Pt, Rb, Se, Sr, Tl, V, Y and Zn using an ICP-MS. The multivariate statistical analyses of chemical data were run with the CANOCO 4.5 software for Windows<sup>®</sup> and included principal component analysis (PCA) and partial redundancy analyses (RDA).

The chemical analyses of plant and soils were completed in December 2007. Preliminary PCA results for metals in soils allowed the identification of three main elemental clusters: i) Mn-Y-Lu-Ce, ii) Zn-Cd-Mo-Ag-Sr and iii) Cr-Ba-Se-Pb-Ni-Cu-Tl-V-Rb-Co-As. The elements Pt and Nb were not correlated with other metals. In general, metal concentrations in soils increased with organic matter content and pH values. The RDA results for vegetable data indicated that associations existed between i) peeled and entire radish, ii) lettuce and Swiss chard and iii) bean and tomato. The leaf vegetables showed the highest elemental concentrations, notably for Mn, Rb, Sr and Zn. The RDA performed with plant to soil concentration ratio identified the same vegetable groups. In this case, the leaf vegetables were strongly enriched in Cd and Rb. The RDA further indicated that spatial variability explained only a small fraction of metal distribution in plants and soils.

**Project Title:**

(S3) The influence of competing ions on Se and Tl accumulation by durum wheat and spring canola grown in soil

**Investigator(s):**

Kikkert, J. and Berkelaar, E.

Redeemer University College, Ancaster, ON

**Summary:**

An important route of exposure to trace elements such as thallium (Tl) or selenium (Se) that may be toxic to humans, is via foodstuffs. We have previously shown that for plants in hydroponic solutions, the pairs  $Tl^+$  and  $K^+$ , and  $SeO_4^{2-}$  and  $SO_4^{2-}$  compete with one another for uptake. The objectives of the current study are to measure accumulation of Tl and Se in plants grown in soil containing various concentrations of Tl and K, and Se (as  $SeO_4^{2-}$  or  $SeO_3^{2-}$ ) and  $SO_4^{2-}$ .

Soil was gathered, homogenized, and separated into 18 containers. Soil was then spiked with Tl (0, 0.1, 0.5, or 5 ppm) with or without additional K (0 or 150 ppm) or Se (0, 0.1, 1, or 5 ppm as either  $SeO_3^{2-}$  or  $SeO_4^{2-}$ ) with or without additional  $SO_4^{2-}$  (0 or 50 ppm; only in the  $SeO_4^{2-}$  - spiked soil). Soils were allowed to age for one month and then placed in pots and seeded with durum wheat (*Triticum turgidum* cv 'Kyle') or spring canola (*Brassica napus* var 'Hyola 401'). Plants were grown outside and watered as needed with either deionized water or a dilute fertilizer solution. Canola plants were harvested at four times, and wheat twice, during the growing season, with the last harvest being at the mature stage.

Both species were successfully grown to maturity, and soil and plant samples have been collected and dried, although analysis of these samples has not yet been completed. Plants growing in the high Se soil (containing  $SeO_4^{2-}$  but not  $SeO_3^{2-}$ ) had stunted growth, especially if  $SO_4^{2-}$  levels were low. This may have been due to either less  $SeO_4^{2-}$  accumulation by plants, or due to an increased ability to cope with higher internal Se levels when more  $SO_4^{2-}$  is available.

**Project Title:**

(S5) The role of organic matter in bioavailability of metals in urban garden soils

**Investigator(s):**

Murray, H. and Macfie, S.M.

Department of Biology, University of Western Ontario, London, ON

**Summary:**

Very few studies have combined the effects of plant species, metal speciation and soil characteristics on the bioavailability of metals. The aim of this study is to determine the effects of a number of soil properties on metal uptake in edible crops. The goal is to assess the need for soil-specific and/or plant-specific guidelines for the acceptability of soils for agriculture. Five urban garden soils were analyzed for metal content, pH, conductivity, texture, cation exchange capacity and organic matter content. Concentrations of Pb were more than 2 times the CCME (Canadian Council of Ministers of the Environment) limit in two of the soils; one of these contained 6 times the limit for Cu while the other contained a slight excess of Zn. In the third soil, Cd was 1.6 times the CCME limit. Concentrations of all measured elements in the remaining two soils were below the CCME limits. All soils were similar in pH and texture. Conductivity values were between 405  $\mu$ S/cm-1330  $\mu$ S/cm while organic matter content ranged from 6.94%-18.19%. In addition to determining the relationships between soil characteristics and the uptake of metals into crops, the organic matter in these soils will be manipulated to better assess the importance of this variable to metal bioavailability. While this project alone will not result in a complete model for the uptake of metals by plants, it will supplement the research efforts of other members of the Soils and Plants group.

**Project Title:**

(S3) Can thallium accumulation by plants be predicted from potassium accumulation?

**Investigator(s):**

<sup>1</sup>Renkema, H., <sup>2</sup>Koopmans, A., <sup>2</sup>Berkelaar, E. and <sup>1</sup>Hale, B.

<sup>1</sup>University of Guelph, Guelph, ON

<sup>2</sup>Redeemer University College, Ancaster, ON

**Summary:**

As a potassium analog, Tl(I) is readily accumulated by plants; although plant consumption is the primary route of human exposure to Tl, models to predict plant uptake of this metal are not well developed. The competition between  $Tl^+$  and  $K^+$  for uptake and possible similarities of  $Tl^+$  and  $K^+$  uptake kinetics by durum wheat (*Triticum turgidum*) and spring canola (*Brassica napus*, a known hyperaccumulator of Tl) and were investigated to determine if  $Tl^+$  uptake by plants could be predicted based on  $K^+$  uptake. To determine competition, wheat and canola plants were exposed to 0.1 or 10  $\mu M$  Tl in a 1:1 to 1:10 000 concentration ratio with K for 0-300 min, and to determine uptake kinetics, plants were exposed to 1-75  $\mu M$  Tl(I) or 5-250  $\mu M$  K for 0-150 min. Root and shoot Tl concentration were determined and  $K^+$  uptake during exposure was measured by solution-K depletion. For the competition, increasing K concentration decreased  $Tl^+$  uptake by both wheat and canola.  $Tl^+$  uptake by wheat was reduced at a much lower concentration of K than canola – K in excess of 1-10  $\mu M$  reduced  $Tl^+$  uptake by wheat while K in excess of 1000  $\mu M$  was required to reduce  $Tl^+$  uptake by canola. For the kinetics,  $Tl^+$  uptake by canola occurred at greater rate than  $K^+$  uptake by canola or wheat or  $Tl^+$  uptake by wheat; accumulation of Tl by wheat and K by wheat and canola occurred at similar rates. Accumulation of Tl by wheat could not be modeled using Michaelis-Menten (M-M) kinetics, while for canola the  $K_M$  was 54  $\mu M$   $Tl^+$  and  $I_{max}$  was 0.45  $\mu g$  Tl g root  $DW^{-1} h^{-1}$ . For  $K^+$  uptake by wheat,  $K_M$  was 88  $\mu M$   $K^+$  and  $I_{max}$  0.19  $\mu g$  K g root  $DW^{-1} h^{-1}$ , and for  $K^+$  uptake by canola,  $K_M$  was 140  $\mu M$   $K^+$  and  $I_{max}$  0.30  $\mu g$  K g root  $DW^{-1} h^{-1}$ . Although there was no significant difference between  $K_M$  values (perhaps due to the large se values), the uptake of  $Tl^+$  by canola did occur at a significantly greater rate than the uptake of  $K^+$  by canola or wheat or the uptake of  $Tl^+$  by wheat. Wheat accumulates Tl and K at similar rates and the two ions compete about equally, so Tl uptake by wheat may be predictable from models for K uptake. However, because canola accumulates Tl in preference to K, models for K accumulation would underpredict Tl accumulation.

**Project Title:**

(S2) Controls of bioaccessibility of trace elements in soils

**Investigator(s):**

Sato, A. and Hendershot, W.

Natural Resource Sciences, McGill University, Montréal, QC

**Summary:**

Cerium is one of the most abundant rare earth elements (REE) with a concentration in soils equal to or greater than Ni and an estimated average concentration in the Earth's crust of  $66 \mu\text{g g}^{-1}$ .

Although not considered highly toxic in aquatic ecosystems, it has been shown to be more toxic to *Hyalella azteca* than Ni or Zn. The REE are not considered to be essential plant nutrients but they are being used in China as fertilizers and have been shown to increase growth in some studies. The purpose of this study is to investigate the effect of Ce on growth of *Cucumis sativa* and establish a dose-response relationship.

A 10-day spiking-leaching experiment was done to prepare soils for the pot experiment in the greenhouse. The soil samples were spiked with Ce for 5 days and then leached with water containing nutrients for another 5 days in order to remove excess dissolved metal. The total Ce concentrations at the end were 235, 480, 1360, 5100, 16900, 19000  $\text{mg kg}^{-1}$ . Seeds were germinated on a Petri dish in darkness for 2 days and the seedlings were transplanted to the pots each containing 500 g of soil. The plants were grown for 14 days.

The results showed that Ce inhibited plant growth measured as root, stem and leaf lengths. Root growth was most severely affected, then the stem and the leaf. Bioaccumulation of Ce was also observed in *Cucumis sativa*, however, most Ce was found in the root and much less Ce was translocated to the leaf. Further study is underway to examine the extractability of Ce from the spiked soils and the bioavailability of different forms of Ce to *Cucumis sativa*.

**Project Title:**

Differences in copper bioavailability and phytotoxicity in leached and non-leached copper-spiked test soils

**Investigator(s):**

Schwertfeger, D. and Hendershot, W.

Natural Resource Sciences, McGill University, Montreal, QC

**Summary:**

Recently, the standard method for performing soil trace metal ecotoxicity tests used in the development of critical threshold values has been put into question. Traditional soil spiking procedures do not allow excess dissolved metal to leach from the soil and may result in a greater proportion of dissolved metal than would occur in contaminated field soils that are continually leached by rain, snowmelt or irrigation. Metal additions may also displace exchangeable acidity thereby reducing soil solution pH in response to the added metal. Likewise, metal additions may displace other elements bound to the soil solid surfaces thereby increasing the ionic strength of spiked samples. These changes are known to affect soil metal bioavailability and are considered artifacts of the soil spiking procedure. These artifacts may contribute to the differences in ecotoxicity observed between laboratory spiked and contaminated field soils.

We developed a method to leach spiked samples such that more environmentally relevant test soils may be prepared. Preliminary tests using Cu showed that the leaching procedure resulted in more consistent pH values among spiked samples than soil samples prepared following the traditional soil-spiking procedure. Electrical conductivity (EC) values were also found to be more consistent, as well as more environmentally relevant, among spiked samples when the leaching procedure was used.

Phyto-toxicity tests were conducted with barley using a series of leached and non-leached Cu-spiked soil samples. The experiment was performed twice using a different test soil each time. Inhibition of barley root growth was less severe in leached samples (i.e. higher IC<sub>20</sub> value) than non-leached samples for Test Soil #2. The shift in IC<sub>20</sub> values were most likely the result of a lower concentration of dissolved Cu in the leached samples. For Test Soil #1, however, the IC<sub>20</sub> values were not significantly affected by leaching. It is hypothesized that the greater amount of dissolved Cu in the non-leached samples was counter balanced by a greater amount of other dissolved ions (i.e. Ca and H<sup>+</sup>), which compete for binding sites on the root surface. Further analyses are currently underway which should help determine the mechanisms controlling bioavailability at the soil/solution interface and root surface when dissolved metals are added to soils.

**Project Title:**

(S4) Influence of microbial activity and trace metal speciation in the rhizosphere on metal uptake by wheat

**Investigator(s):**

Turmel, M.C. and Courchesne, F.

Université de Montréal, Montréal, QC

**Summary:**

Few studies have investigated the links existing between microbial activity in the rhizosphere, metal speciation and metal uptake by edible plants. Our approach was to conduct a growth experiment using metal-contaminated soil materials that had previously been manipulated to generate two initial levels of microbial activity. The specific objectives of the study were: 1) to contrast the microbial and chemical properties of the rhizosphere (Rz) and bulk (Bk) components of manipulated soils and 2) to establish the links between the microbial or chemical properties of the Rz and the uptake of trace metals (Al, As, Cd, Ce, Co, Cr, Cu, Mn, Ni, Pb, Tl and Zn) by wheat.

The soil samples were collected close to a zinc plant in an abandoned agricultural field and under an adjacent forest stand. Soils were sampled, air-dried in the laboratory, sieved at 2 mm and homogenised. The experiment was performed in a growth chamber with a special dwarf cultivar of spring wheat (*Triticum aestivum* cv. USU-Perigee). The pots contained an inside (rhizobag around plants roots) and an outside compartment separated by a 25- $\mu$ m nylon mesh. Before the experiment, soil materials were sterilized using gamma rays and a 90% sterile soil was produced by mixing 90% of fully sterilised soil with 10% of intact soil. Wheat was grown in sterilized and intact soils, either agricultural or forested. Treatments were replicated four times. The experiment ran for eight weeks until the plants reached maturity. At the end of the experiment, the Rz material contained in the rhizobag was separated from the Bk material surrounding the rhizobag. In each Rz and Bk samples, microbial (microbial biomass C and N, dehydrogenase, urease, arylsulfatase and acid phosphatase activity and bacteria profiling by PCR-DGGE) and chemical properties (H<sub>2</sub>O, EDTA and hot acid extractable metals) were measured. The wheat parts (roots, shoots and seeds) were analysed for total trace metal content.

Non-parametric statistical analyses showed that microbial activity and chemical properties differed between Bk and Rz materials for both soils and treatments. For example, arylsulfatase and dehydrogenase were higher in the intact than in the treated Rz soil. Moreover, water soluble Al, Cu, Zn and Pb were higher in the sterilised than in the intact Rz soil. The profiling of bacterial diversity with the PCR-DGGE method also showed differences between soil compartments (Bk vs Rz) and treatments (intact vs sterilised soil) as revealed by the Jacard similarity index. We further ran multivariate analyses such as redundancy analyses. They suggested that differences in dissolved metal speciation existed between Bk and Rz soils for Al, Cu and Zn, and that these differences were associated to microbial variables like arylsulfatase or dehydrogenase activity, DGGE bands or microbial biomass. Analyses also indicated that microbial activity and soluble trace metals in the Rz could account for part of the metals taken up by wheat.