Evaluation of Cu and Pb Bioavailability from Compost Amended Soils

by

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Dedication

This thesis is dedicated to my daughter Shae to prove that the pursuit of knowledge is never a wasted endeavour and that success in life comes at its own pace.

It is also dedicated in memory of my Poppy, Winston Snow, whose unconditional love and support has allowed me to face any task I accept in life. The example he set for us regarding appreciation, acceptance and hard work is a guiding light to us all.

To my loving husband Dale, thank you for enduring through this long process. I know it demanded alot from you. You rose to the challenge and I am as happy as you to have now reached the conclusion at long last.

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Abstract

Land application of biosolid or industrial compost raises concerns regarding heavy metal accumulation in soils, plants and free-ranging livestock. A strip-split plot design evaluated two levels of sewage sludge (22/44 t ha^{-1}) and municipal solid waste (21/42 t ha^{-1}) compared to nitrogen fertilizer and an unfertilized control. Treatments were evaluated with three levels of limestone ($CaCO_3$ at 0, 3 and 6 t ha^{-1}) over two years corn seeded no-till. Swiss Chard cultivated in field soil samples within a greenhouse monitored heavy metal bioavailability. Compost amended soil was incorporated into chicken feed to simulate soil ingestion in a free-range production system. Compost application increased total soil Pb over time yet decreased both available soil and corn plant Cu levels over time. No heavy metal accumulation was observed in Swiss Chard or poultry tissue. Biosolid composts containing elevated Cu or Pb levels can be safely used for crop or free-range broiler production.

Key words: biosolid, corn yield, heavy metals, municipal solid waste, poultry, sewage sludge, swiss chard.

List of Abbreviations and Symbols Used

AAFC	Agriculture and Agri-Food Canada
ANOVA	analysis of variance
AOAC	Association of Analytical Communities International
BNQ	Bureau de Nomalisation du Québec
$CaCO_3$	calcium carbonate or limestone
cm	centimetre
CO_2	carbonate
Cu	copper
$CuSO_4$	copper sulfate
ºC	degrees Celsius
DM	dry matter
Fe	iron
GA	Georgia
GLM	general linear model
ha	hectare
HNO_3	nitric acid
ICAP	inductively coupled argon plasma
K	potassium
kg	kilogram
kg ha^{-1}	kilogram per hectare
kg m^{-2}	kilogram per square metre
L	litre
L ha^{-1}	litres per hectare
m	metre
m^2	square metre
MA	Massachusetts
MD	Maryland
Mg kg^{-1}	milligram per kilogram
ml	millilitre
mm	millimetre
Mo	molybdenum
MSW	municipal solid waste
N	nitrogen
NH_3	ammonia
NH_4 NO_3	ammonium nitrate
NJ	New Jersey
NO_3	nitrate
NS	Nova Scotia
NRC	National Research Council
ON	Ontario
P	phosphorus
Pb	lead
PE	Prince Edward Island
%	percent

ppm	part per million or mg kg^{-1}
QC	Québec
®	registered trademark
SAS	Statistical Analysis Software
SCC	Standards Council of Canada
SS	sewage sludge
t	tonne
t ha^{-1}	tonne per hectare
Zn	zinc

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Chapter 1: Introduction

In order for plants to grow and complete their life cycle, they must acquire not only macronutrients such as N, P, and K but also essential micronutrients such as Fe, Zn and Cu as well. Plants have evolved highly specific mechanisms to take up, distribute, and store these nutrients (Lasat 2000). In the use of waste composts the distinction between metal elements being incorporated for use as an essential micronutrient in the soil structure versus its addition as merely a contaminant, must be considered. Metal elements are moved across biological membranes by proteins with transport functions (Stenesh 1998). Also, sensitive mechanisms maintain intracellular concentration of metal ions within the physiological range for both plant and animal tissues. The mechanisms for uptake of nutrients in plants are usually selective, with the plants preferentially acquiring some ions over others (Wenzel and Hauschild 1998).

Metal ions possess a charge, and because of their charge, cannot move freely across the lipophilic structures of the cellular membranes. Therefore, ion transport into cells must be mediated by membrane proteins with transport functions, generically known as transporters (Lasat 2000). Ion uptake selectivity depends upon the structure and properties of membrane transporters. These characteristics allow transporters to recognize, bind, and mediate the transport of specific ions across membranes. Some transporters mediate the transport of divalent cations but do not recognize mono- or trivalent ions (Lasat 2000).

In common nonaccumulator plants, a metal such as Cu, being an essential micronutrient, does not accumulate past the metabolic needs (<10 mg·kg^{-1}) of the plant (Meharg 2005). In contrast, metal hyperaccumulator plants can accumulate exceptionally high amounts of metals, often in the thousands of mg·kg^{-1} (Prasad 2003). In some instances these hyperaccumlator plants are purposely located in areas that have a history of elevated levels of heavy metals in order to reduce the level of contamination (Prasad and Freitas 2003). When employed in this intentional manner, using plants to remove pollutants from the environment is known as phytoremediation (Raskin et al 1997). When this hyperaccumulation occurs in plants commonly used as livestock and human feed such as corn, the concern of toxicity risk is brought to public attention. It would be beneficial to the environment, urban populations, and agricultural producers if the true risk of heavy metal contamination in composted wastes was well-defined. A better understanding of how such elements are utilized in the ecosystem when taken up by plants or animals, will allow for a safe and well-defined role of waste composts in the future.

With the increase in urban populations comes a proportional increase in a variety of biosolid wastes. These wastes can be successfully recycled when composted and applied on agricultural soils to add valuable nutrients to the soil-plant ecosystem (Warman and Termeer 2005a). Composting of biosolids can help to stabilize the components and reduce the bioavailability of heavy metals such as Cu and Pb (Brown et al 2003). Because of the increased production of these new organic fertilizer sources, there are concerns regarding the bioavailability of heavy metals such as Cu and Pb to crops and livestock, which may come in contact with the composted material. Many questions concerning the presence of heavy metals arise when this type of material is used in agricultural systems. These questions revolve around the concern from the possible passage of contaminants from the soil to crops or livestock who may come in direct contact with soil, and ultimately to people. The bioavailability of soil-born Cu or Pb to crops and animals that are exposed to the soil where alternative compost products are applied to agricultural fields is the focus of this study. In small amounts, Cu is an essential trace element required for plant and animal growth (McDowell 2003, Adriano 2001). However excessive intake can have an adverse effect on growth and overall health (US National Committee for Geochemistry 1974) and therefore application to soil must be monitored when alternative compost wastes are used. Non-essential elements such as Pb found in composted wastes at any level are of concern because of the negative health effect it can have towards soil organisms, plants and animals when the compost wastes are applied as an agricultural amendment. For these reasons, this study serves an important role in determining the risk associated with the application of various composts within an agricultural setting to the safety of the end user.

The existing compost regulations in Canada are based on the study of total measurable inorganic heavy metals (Ge et al 2006). Along with consideration of the trace element content, the compost quality guidelines consider the maturity, foreign matter and pathogen content of the composted material. Table 1 illustrates the current maximum trace element concentration for agricultural compost and the associated category rating.

Table 1. Current Canadian compost quality guidelines for Cu and Pb content and the associated category rating

	Category A* (Type AA and A)	Category B** (Type B)
Trace Element	(mg kg^{-1} , air dried mass)	(mg kg^{-1} air dried mass)
Copper (Cu)	100	757***
Lead (Pb)	150	500

*BNQ and CCME standards

**BNQ, CCME and AAFC standards

***Only specified with BNQ, not CCME or AAFC

Chapter 2: A General Review of Cu and Pb Function

Some micronutrients are essential for optimal growth in plants and animals and must be present in soil ecosystems, or feed in the case of animals (Foth and Ellis 1996). Cu is an example of one essential micronutrient as it serves an important role in plant structure and function. Cu requirement varies with age and stage of development of the plant. Soil Cu nutrient sufficiency levels, also called critical values, for favorable corn production are recommended to be 5 – 20 mg kg^{-1} of soil (Soil and Water Quality: An Agenda for Agriculture 1993). Although typical agricultural soil Cu levels are usually less than 25 mg kg^{-1}, they have been found to be as high as 85 mg kg^{-1} without adversely affecting corn production (Ontario Ministry of the Environment 2001). The youngest emerging leaves in corn plants can turn yellow and the tips may die when soil Cu is at levels that cause deficiency (Richardson 1997).

Animals also possess a metabolic requirement for Cu and it is one of the nutrients considered in feed formulation (NRC Press 2000). The range provided by the National Research Council (NRC) for the dietary Cu requirement of various animals is 5 – 16 mg/kg, varying with body size and type of animal. There is a higher demand for Cu for ruminant species compared to monogastrics (NRC Press 2000). Surprisingly, ruminants are more sensitive to Cu toxicity, while non-ruminants in general have a high tolerance for Cu, especially if the diet contains adequate Zn and Fe. Toxic Cu levels in feed are reported to be as low as 25 mg/kg of feed for sheep and 40 mg/kg for cattle, or as high as 800 mg/kg for poultry. Recommended values are given as a guideline only as a specific minimum requirement. Cu cannot be administered with great accuracy, because Cu absorption and utilization in animals is affected by the presence of several other minerals and dietary factors. Zinc, Fe, Mo, inorganic sulfate and other nutrients can reduce Cu absorption (NRC Press 2000).

In animals, Cu is required for the proper function of a number of key systems in the body. The enzyme activity associated with iron metabolism, elastin and collagen formation, melanin production, and central nervous system structure are among a few (Shils et al 2006). Cu is essential for red blood cell formation by allowing Fe absorption from the small intestine and release of Fe in the tissue into the blood plasma. Cu is required for several aspects of growth, including bone formation by promoting structural integrity of bone collagen and for normal elastin formation in the cardiovascular system. Cu is also required in the nervous system as a component of cytochrome oxidase which is essential for normal myelination of brain cells and spinal cord. Overall immune system health is also dependent on Cu as indicated by a decrease in immune status in deficient animals. In addition to its use for growth and immune function, Cu is often added to livestock diets as an antimicrobial agent at doses greatly exceeding the nutritional requirement (Skrivan et al 2005).

There are other micronutrients present in the environment considered to be non-essential to growth, but tolerated at low levels. Some of these micronutrients can become harmful to plants and animals at elevated levels. Pb is an example of one such trace element that does not participate in any known beneficial biological function (NRC 2005). It can be found in agricultural soils at levels ranging from 0.1 mg/kg of soil to 20 mg/kg (Iskandar and Kirkham 2001), while Pb in phosphate rocks can be as high as 100 mg/kg (NRC 2005). The variation of total Pb content in non-contaminated soils is not very high as soils are an insignificant natural contributor of Pb. The majority of the element is present in contaminated soils is a consequence of human industrial processes (Iskandar and Kirkham 2001).

It is agreed that elevated levels of Pb in soil is hazardous to microbial, plant and animal life, however, there is some discrepancies regarding what level of Pb is considered enough to declare a soil contaminated. Government agencies have provided guidelines in order to identify soils considered contaminated with Pb. In Canada, the soil quality guideline for agricultural soils is listed at 70 mg/kg (Canadian Soil Quality Guidelines for the Protection of Environmental and Human Health 1999). The US Environmental Protection Agency (EPA) maximum allowable standard for Pb in bare residential soil is 400 mg/kg by dry weight, but uncontaminated soil levels are defined as those less than 50 mg/kg (EPA Ecological Soil Screening Levels for Lead 2005). According to the EPA guidelines, a value of 120 mg/kg is considered to be toxic to plants. The impairment of health or performance in plants or animals by exceeding tolerable levels Pb also depends upon the balance of other nutrients (NRC 2005). Contaminated soils are usually only considered to be truly contaminated when the Pb is provided in a highly available form (NRC 2005). Levels of Pb may be as low as 0.5 mg/kg when dietary calcium levels are low or may reach levels of 100 mg/kg when calcium levels are above normal values.

These inconsistent guidelines for Pb in soils require further clarification in order to better understand the true risk of Pb in soils towards plant and animal life.

Chapter 3: Literature Review

3.1 Alternative Agricultural Amendments

Composting of waste materials has been a practice for thousands of years. Over the last few decades as the agricultural revolution focused on the use of synthetic fertilizers as soil amendments, composting has become a less common practice (Abimbola and Olenyk 1998). Composting is an aerobic process that uses microbial populations under elevated temperatures to biodegrade organic compounds resulting in the nutrient rich product referred to as compost. According to Fares et al (2005), municipal compost may represent one of the sustainable and renewable organic products in arid zones that can be used to minimize the loss of soil carbon via CO_2 emission and at the same time enhance overall soil quality.

An increase in urban populations produces a proportional increase in a variety of biosolid and municipal wastes. Biosolid wastes refer to the semisolid material that is left from sewage treatment or industrial wastewater processes. Municipal wastes originate from residential organic material designated for landfill disposal. These wastes can be successfully recycled in a mixture of compost and used in an agricultural application to add valuable nutrients and organic matter to the soil-plant ecosystem (Warman and Termeer 2005b). Compost application has many benefits to plants and soil, when used in a modern field production system, as compost is both a soil amendment and a soil conditioner, adding nutrients as well as organic matter. Mkhabela and Warman (2005) found Municipal Solid Waste (MSW) compost supplied similar amounts of P as an inorganic fertilizer while also increasing soil pH, something inorganic fertilizer is not capable of doing. The process of composting also converts available N that would have been leached as NO_3^- into groundwater or released into the air as NH_3, into organic N in the biomass (Abimbola and Olenyk 1998). In a thorough review of MSW compost use in agricultural studies, Hargreaves et al (2008) indicates that provided a consistent and quality compost is produced, and that guidelines and standards are followed for input and uptake monitoring, MSW compost use should be encouraged for agriculture, municipalities, landscaping and gardening.

According to Overcash et al (2005), the goals of waste management are to protect human health and the ecological health of terrestrial and aquatic systems that receive a variety of wastes including animal wastes, agricultural wastes, biosolids, industrial wastes, and municipal and/or industrial effluents. The researchers state that understanding and managing the behaviour of organic chemicals introduced into the environment via waste disposal makes it possible to attain the goal of beneficial reuse of organic wastes, while protecting human health and the environment. Land treatment of organic wastes is a technology that has economical advantages for application where there is a desire to retain and enhance agricultural systems for sustainable production, while conserving water through waste reuse and protecting ground and surface water natural resources (Overcash et al 2005). Mkhabela and Warman (2005) indicated the use of industrial compost derived from material such as municipal solid waste assists in recycling of plant nutrients, and thereby, reducing environmental degradation associated with the disposal of such wastes to industrial facilities.

One of the concerns with the use of composted municipal and industrial wastes as a source of agricultural fertilizer has been the potential for contamination of soil with many heavy metals, including elements such as Pb and Cu (Cook and Beyea 1998). Concern also exists for any agricultural product or livestock which may come in contact with the compost amended soils. Composting these wastes can help to stabilize the components and reduce the bioavailability of heavy metals such as Pb (Brown et al 2003, Mondini et al 2003). According to Strawn and Sparks (2000), the increase in soil organic matter seen with the application of composted wastes to agricultural land provides an increase in functional groups that attract metals to form chemical bonds and making them unavailable to plants. For this reason, there exists a vital need to assess the true nature of the metal contamination in agricultural soils (Loska et al 2004). It is the bioavailable metal content in the soil that creates an impact on the soil quality and, subsequently, its use for crop production.

3.2 Issues with Compost Use

Despite potential increased metal contaminant loads, testing the initial compost source for total element levels would not be an appropriate indication of risk. A variety of plant-soil interactions can alter the true bioavailability of the metal element. One such variation suggested to occur is that many plants are resistant to hyperaccumulation of an element and therefore the risk to crop production would be minimized (Lasat 2002). Another adjustment is that plants produce allelopathic chemicals as a method for identifying plant-bacteria associations capable of degrading contaminants in the soil (Siciliano and Germida 1998) and further decrease risk to the plant. As previously indicated, the overall risk can be minimized prior to field application of industrial wastes by proper composting of the product. Warman and Termeer (2005b) found that when sewage sludge was applied in a composted form rather than straight sewage sludge (SS), Cu availability from soil was decreased. The researchers emphasize that it was still important to monitor heavy metal content of plant tissue after several years of sewage sludge applications to ensure that crops do not accumulate toxic levels. Zheliazkov and Warman (2004) also agree that mature composted sewage sludge and municipal solid waste which may contain high levels of heavy metals may be utilized as a soil amendment on agricultural crops without risk for phytotoxicity. Furthermore, these researchers feel that existing regulatory levels require revision. Also, the latter authors point out the need to consider additional factors when revising standards, such as metal accumulation from repeated applications, long term bioavailability, and environmental consequences. Hargreaves et al (2008) found in a review of current literature that despite an observed increase in total element concentrations with MSW compost additions, only a small portion of this amount is considered leachable and therefore bioavailable. Another point omitted with the existing regulations and recommendations is the landscape structure and soil type. Repeated applications of sewage sludge or municipal solid waste composts on flood plains create concerns for regional contamination through seasonal floodwaters (Nova Scotia Environment 2010).

Despite the concern for contamination, the positive aspects of composted waste products utilized as a field amendment must also be considered. Applications of compost were found to improve soil properties for several years after applications had ceased, while also providing nutrients and liming effects for the growing corn (Eghball et al 2004). To support the concept that biosolid composts can improve soil organic matter content by up to 55%, the researcher compared its use with an uncomposted biosolid amended soil. The field experiment Eghball et al (2004) conducted over a 4-year period involved hand applied compost to plots containing 6 corn rows. The compost was applied in late autumn after harvest and was incorporated into the soil through discing. Eghball et al (2004) indicated that applications of compost to agricultural land provide residual effects on crop production and soil properties. These residual effects of corn grain yield and N uptake were found to have lasted for at least one additional growing season, while the effect on soil properties, such as pH changes were longer lasting. The author indicated the residual effect may be caused by the small portion of N and other nutrients that are available to plants directly after application, and remaining portions becoming available over time. This helps to minimize such a detrimental environmental consequence as nitrate leaching that can sometimes be seen with use of conventional fertilizer only.

3.3 Cu and Pb in Plants

Often the characterization of metal toxicity found in ecosystems is based on total metal soil concentrations (Gorsuch et al 2006). This may not be sufficient to evaluate the true potential risk associated with the contaminated soils as the physical and chemical properties of the soil itself influences metal partioning and, therefore, the metal mobility, bioavailability, and potential toxicity to the environment (Daoust et al 2006). Metal contaminated acidic soils are seen as detrimental for plant species due to the increased metal bioavailability provided by low pH (Stehouwer and Macneal 2004). The researchers also indicate that too high a pH in biosolids may also be disadvantageous. During a three-year field experiment with alfalfa, the researchers found a decrease in Cu uptake by forage legumes caused by the alkaline soils and biosolids, creating a deficiency in the forages despite the substantial amounts of Cu in soil and biosolids.

Daoust et al (2006) also indicate that an alkaline soil pH combined with poor soil organic matter content can provide increased bioavailability where amplified metal contaminants exist. The researchers found increasing the dissolved organic carbon, such as through the dissolution of organic matter by an alkaline pH, contributed to increasing the Cu content in the soil solution. This appears to indicate the breaking down of organic matter content when pH levels are too high causes Cu to become more bioavailable. This study concluded that soil properties influenced the mobility and overall toxicity of Cu. Water-extracted Cu fraction increased with decreasing pH, decreasing organic matter content and clay content. An interaction between pH and organic matter was observed as the extracted Cu fraction increased with increasing dissolved organic C. In this study, the various soils, which were artificially amplified with 6000 mg kg^{-1} of Cu mixed in dry soil, were seen to be toxic to barley plants as growth was inhibited, varying with Cu content and soil properties. Sukreeyapongse et al (2002) also determined the existence of a pronounced increase in release rates of metals seen with decreasing pH. Research by

Alva et al (2000) found that plants accumulated significantly greater concentrations of Cu at higher soil pH than at lower soil pH (pH range of 5.7 – 6.5).

Another consideration when utilizing composted waste products as an agricultural field amendment is the method of application. Viator et al (2002) applied biosolid compost to poor quality soil at a rate of 44.8 Mg ha^{-1} for the production of sugarcane. While the method of application of this biosolid compost did not provide any significant differences, they concluded soil incorporation was preferred to within-row application. The subsoil incorporation practice did not decrease root surface area and, consequently, sugarcane yields as did the within-row application method. At the conclusion of their two-year trial, the biosolid compost application did not increase nutrient metal accumulation in sugarcane leaves beyond acceptable limits, and therefore, biosolid compost was determined to be beneficial for use in agricultural soils to provide long term fertility and lower off-site impacts compared to other methods of biosolid waste disposal.

Mkhabela and Warman (2005) found that composted waste products such as municipal solid waste (MSW) compost utilized as a soil amendment was a good source of P. Yet the study determined a low availability of compost-N that would mean an additional N source in the form of inorganic fertilizer would have to be supplemented to compost applications. In this study however, the experimental design utilized a small total number of plots, small plot size of only 4m by 4m with 4 rows of each crop per plot, and each plant species were grown in different locations each year of the two year trial. Improvements in soil fertility from the use of composted waste products may be better observed over a long term study and not merely viewed as a one time fertilizer source. One important aspect presented by these researchers was that the application of MSW compost significantly increased soil pH compared to the use of inorganic fertilizer.

The length of time since a composted organic waste product has been added to agricultural soil may play a significant role in determining its benefits. Daoust et al (2006) considered the influence of soil properties and age on Cu toxicity. They found that soil pH, organic matter, and clay content all played an important role. The age of organic soil portions provided a slight reduction in toxicity, while an increase in toxicity was determined in soils with low organic matter content. The study would suggest the improvement in soil organic matter content seen with the addition of organic waste allows for long term reduction in Cu toxicity. Overall, when composted waste products are used as an agricultural field amendment the potential risk to plants for metal toxicity is a combination of the physical and chemical properties of the soil itself.

3.4 Cu and Pb in Poultry

Cu is a trace element that is often added to poultry diets to act as an antimicrobial and contribute to animal growth. A general assumption has been made that Cu levels moderately higher than NRC requirements can be tolerated and even provide additional gains in body weight and feed consumption (Mondal et al 2010). Banks et al (2004) found an approximate level of Cu supplementation contrary to this assumption. They found that when levels of Cu exceeding 375 mg kg^{-1} are ingested by chickens, a negative response in body weight gain, feed consumption, and feed conversion was observed (Pesti and

Bakalli 1996). Banks et al (2004) found their experimental results supported this previous research as increased Cu supplementation resulted in reduced feed intake and body weight gains of chickens. The researchers suggest this may have been caused by feed intake regulation through Cu levels creating a possible toxic or corrosive response. The exact concentration of Cu in available form that can be tolerated by poultry before negative growth effects are seen has been broadly debated. Some research has indicated tolerated levels may be as high as 450 or 500 mg kg^{-1}. Banks et al (2004) also point out that the pH of Cu contaminated soil plays an important role in the level of Cu toxicity of poultry.

Another point to consider regarding the effects of increased Cu concentrations in poultry diets is the purported chelation with and accompanying reduced utilization of phytin P. In a study by Banks et al (2004) where male broilers were fed increased concentrations of $CuSO_4$ in combination with phytase and nonphytate phosphorus, there were linear reductions in the performance characteristics of body weight, body weight gain, feed consumption, feed conversion, tibia and toe ash weights, as well as apparent P retention as a percentage of total P. In this experiment, increased Cu supplementation did not reduce the efficacy of phytase but did decrease overall performance. The major storage of P in plant seed is in the form of phytate. It has the ability to bind to divalent and trivalent minerals such as Cu, with an increased binding affinity at a pH between 5 and 7. The phytate-mineral complex that results subsequently reduces the bioavailability of the mineral within the complex due to a decreased solubility at typical intestinal pH. Banks et al (2004) found that increased concentrations of Cu reduced feed intakes and body weight gain of chickens. The addition of phytase to the poultry diet to increase the utilization of phytin P causes a reduction in mineral solubility leading to a reduction in the ability of phytase to hydrolyze P from the phytin-mineral complex. Therefore, these researchers concluded supplementation of prophylactic concentrations of Cu from $CuSO_4$ had detrimental effects on the performance and P retention in young broilers.

The actual method for determining bioavailability of heavy metals that may be ingested by a species such as poultry needs to be considered. As it is not always suitable to use livestock species for heavy metal bioavailability, an alternative smaller scale method should be investigated. Juhasz et al (2003) described a method for determining the bioavilability of a heavy metal utilizing in vivo gastrointestinal experiments. An indicator species is fed heavy metal contaminated soil, and this external dose minus the fecal or non-bioavailable fraction provides the absorbed fraction or bioavailable portion. The bioavailabilty can then be presented as a percentage. The researchers indicate that studies to date have found agreement between element availability obtained from in vitro laboratory methods and from such *in-vivo* or live animal studies.

Should a livestock or poultry species be ingesting heavy metal contaminated compost products in a production system, there is concern for how the compost product might act to provide volume without nutrition. Cellulose is another feed additive sometimes used in research with poultry diets as a filler or to provide bulk in a control treatment. Addition of cellulose to a poultry diet decreases feed intake, feed efficiency, and body weight (Noy and Sklan 2002). This particular study considered the combined effect of feed protein and fat levels in 3 x 3 and 2 x 3 factorial experimental designs, respectively. Yet it appeared that it was the cellulose inclusion level which contributed to decreased feed intake, feed efficiency, and body weight, regardless of feed protein or fat levels. An interaction between cellulose and protein level was observed for feed intake and feed efficiency but not body weight. However, this study only reviewed the performance of chicks in the first week posthatch after which all were returned to a standard grower diet. The researchers felt that significant study has already been given to the effects of such factors in older broilers. It is interesting that in such trials considering cellulose inclusion in diets of older broilers actually increased feed intake, contradicting the concept that an increased intestinal "fill" effect would decrease feed intake, as was observed in the study of one week post hatch. The effect observed with the various treatments studied by Noy and Sklan (2002) was indicated as the portion of diet separate from yolk nutrient utilization, where the diets saturated intestinal uptake capabilities or influenced subsequent feed uptake. The exact proportion of nutrients from the diet versus yolk utilization was not indicated in this study and could raise questions about individual variability, as the specific trial utilized a small sample size. The concept of increased instead of decreased feed intake where a filler substance such as composted SS or MWS is included in poultry diets would suggest there may not be any risk for decreased growth performance in the birds due to a substitution effect. However, the issue of the inclusion of heavy metals in a SS or MSW compost that may be consumed by poultry would mean the birds showing increased feed intake could be at greater risk for increased heavy metal consumption. Luo et al (2005) found that birds receiving the highest treatment of supplemental copper (450 mg kg^{-1}) in the form of copper sulphate had lower daily feed intake and daily gains. This may suggest there is a limit to how much Cu a bird will consume and will not ingest the bioavailable form completely unrestricted.

Research has been conducted to evaluate the risk of tissue accumulation in animals that are ingesting heavy metals at concentrations higher than existing standards. In a study by Skrivan et al (2006), Cu was supplemented at multiple levels above the nutritional requirement of 9.2mg Cu kg^{-1} for laying hens. The results indicated that levels increased linearly in excreta, and though there was a significant increase in eggshell and liver, no liver sample exceeded a Cu content of 80 mg kg^{-1}. These researchers feel there is a marginal environmental risk to laying hens from excessive Cu supplementation. Therefore, one might argue that current tolerance levels should be increased for poultry and possibly other monogastric species. One concern might be the excessive Cu levels which are excreted through faecal matter and would then be utilized as an agricultural amendment. Despite the low mobility of Cu in the soil as a result of its high affinity to organic matter, an issue to be raised may be the possibility of run off conditions. Although considered a minimal risk, Cu originating from poultry excreta may cause contamination of surface and ground water where excessive surface soil is lost. Trace elements such as Cu were found to be readily soluble from poultry litter, with more than 70% in the cationic form which are relatively unstable complexes (Jackson et al 2003).

3.5 Issues with Compost Standards

Many countries have adopted guidelines or regulations regarding the use of compost in agricultural and other land (Hogg et al 2002). The Canadian guidelines for compost quality with respect to heavy metals are based on the combined recommendations of three organizations which are responsible for the development of standards and regulations for compost and composting (Fortin et al 2005). These recommendations are based on the independent criteria and business needs of each interested industry partner rather than controlled scientific studies. For this reason, there is much discussion on what is appropriate for the overall Canadian guidelines, and a requirement exists for continued, independent research on this issue.

The three organizations involved in compost standards and regulations are Agriculture & Agri-Food Canada (AAFC), the Canadian provincial and territorial governments, and the Standards Council of Canada (SCC) (through the *Bureau de normalisation du Québec* (BNQ)) (Fortin et al 2005). This collective responsibility reflects government regulatory requirements (of both AAFC and the provinces and territories) as well as voluntary industry initiatives (BNQ). Zheljazkov and Warman (2004) point to the need for more independent research to evaluate various industrial composts as soil amendments and nutrient sources for agricultural crops. For this reason, this study serves an important role in reviewing appropriate application rates of various composts within an agricultural setting and considering the end use safety of this application through determining the bioavailability of Cu and Pb.

The bioavailability of compost contaminants such as Cu and Pb is an important consideration in determining appropriate use. Juhasz et al (2003) describes bioavailability as a physiologically or environmental uptake process, the portion of the total available element or mineral that is absorbed by an organism, or the extent a contaminant is available for biological conversion. The researchers state another term to be considered is phytoavailability, which describes how an element or mineral is absorbed into plant tissue. Unlike organic contaminants, most metals do not undergo microbial or chemical degradation, thereby resulting in their accumulation in soils. The mobilization of metals in soils for plant uptake and leaching to groundwater can be minimized through chemical and biological immobilization. Immobilization of metals is obtained using a range of inorganic compounds, such as lime and phosphate compounds and organic compounds, such as biosolids (Juhasz et al 2003). The mechanisms proposed for the immobilization and consequent reduction in the phytoavailability of metals by the soil amendments include increased formation of organic and inorganic metal complexes, precipitation of metals, and reduction of metals from higher valency mobile forms to lower valency immobile forms (Juhasz et al 2003).

Bioavailability of compost-born contaminants varies with soil type including clay content, mineral, and organic matter composition (Naidu et al 2003). This concept must be exercised with caution and understood to be a generalization when reconsidering compost guidelines. Metal phytoavailability may vary with soil pH where decreasing pH decreases the binding capacity of metals but actual results may also vary with plant type and age of organic matter. Naidu et al (2003) found that long-term incubations of contaminant spiked soils, simulating field conditions, showed an exponential decline in contaminant bioavailability with aging.

Previous research has demonstrated the effects of using sewage sludge (SS) compost on the levels of some micronutrients in corn production (Rappaport et al 1988; Cripps et al 1992; Kiemnec et al 1990). The factors affecting the bioavailability of an element include the soil pH, the specific plant species, the growth stage, the biosolid source, the soil conditions, and the chemistry of the element (Warman and Termeer 2005a). Another type of compost gaining in popularity through increased usage is municipal solid waste (MSW). The application of MSW compost to agricultural land is regarded as a better alternative to disposing the material in landfills due to the associated environmental concerns such as eutrophication of waterways through phosphorus runoff (Mkhabela et al 2006). The compost product source is therefore another factor to consider regarding possible regulations and standards, as different sources might have specific needs and risk considerations.

Debate still exists between current researchers as to the true nature of risk depending upon type of compost, the rate of application, and the type of cropping systems utilized. Karathanasis et al (2005) makes the point that increases in land applications of biosolid wastes as soil amendments have raised concerns about potential toxic effects of associated metals on the environment. They indicate that the risk may be greater than anticipated considering a significant metal load may be associated with dispersed biosolid colloid particles. On the other hand, Warman and Termeer (2005a) suggest that composting the wastes stabilizes the components by increasing soil pH and providing increased surface area for which the metals can bind and reduces any negative side effects. A study by Zheljazkov and Warman (2004) found the increasing addition of municipal solid waste composts to soil reduced bioavailability of Cu and Zn as observed in the decrease of an assigned bioavailability factor. The researchers point out that the addition of organic matter rich compost improved the soil organic matter content, which can change the fractionation of a heavy metal, redistributing it to a less bioavailable form. It was suggested that with the break down of organic matter over time in field production systems there would be a redistribution of heavy metals such as Cu and Pb. The type of compost, the rate of application, and the type of cropping systems utilized may affect this redistribution and changes in mineral speciation. Therefore, long-term field experiments must be conducted in order to determine the fate of heavy metals in soils after compost application under different cropping systems.

Should the use of such alternative composts become more common in traditional crop production then the issue of maximum tolerable limits must be addressed. Bar-Tal et al (2004) considered the loading limits of sewage sludge compost application each year for growing wheat. When applied at rates of 3, 6, and 12 kg m^{-2}, biosolid compost increased total dry matter, grain production, and quantities of N, P, and K uptake, increasing with increasing compost amount. Land application of composted organic biosolid wastes is preferable to the use of raw material for reasons of hygiene, uniformity of product, and public acceptability. While composting allows for reduced bioavilability of heavy metals, Bar-Tal et al (2004) determined the composting procedure stabilizes available N as well thus reducing the value of the biolsolids as N fertilizers. Utilizing a greenhouse container experiment, the researchers found most of the N uptake from the composts occurred during the first 50-60 days when compared to commercial fertilizer. It should be taken into consideration that the quantity of organic N applied in the composts was 3 to 10 times greater than the amount of N taken up; thus the contribution of organic N to plant uptake increased progressively from the first to the last year. Therefore, in the long run, biosolid compost contributes to mineral N supply. The researchers suggest that dynamic nutrient transformations and uptake models could be an important tool used to manage compost applications. Reviewing the alternative compost products for their fertility value may be of more use when detailing maximum tolerable limits rather than merely restricting total use based on heavy metal components.

The continued and frequent use of alternative composts would also require that regulations be based on the understanding of the effect these applications may have on common crop species. Bioaccumulation of heavy metals such as Pb and Cu in plant species grown in fields amended with doses of sewage sludge still causes concern in the general public. Ortiz and Alcaniz (2006) determined heavy metal concentrations in orchardgrass (*Dactylis glomerata L.*) leaves were unrelated to total concentrations in soil studied, with the exception of Zn. Although many other research projects have also studied the bioavailability of heavy metals in soil, it is usually studied in acidic or neutral soils under laboratory or greenhouse conditions. These researchers sought the knowledge of metal bioavailability in the context of sewage sludge amended soils rich in carbonates.

They concluded the application of sewage sludge, even in high doses, did not have any negative effects on the availability of metals to the plant *Dactylis glomerata L.*.

Biosolids, such as sewage sludge and municipal solid waste, are often considered major sources of potential metal pollutants. While total metal loads applied to soils are important and regulations are often based on such, research has repeatedly shown that it is the metal solubility and bioavailability that is most significant (Basta et al 2005). The reactions occurring within the soil such as sorption and precipitation and metal speciation play important roles in determining the bioavailability of heavy metals. The residual metals found in biosolids create different reactions in the field compared to research soils artificially spiked with metal salts. Basta et al (2005) specifies it is the pH of the soil system that is the most important property governing metal bioavailability. The knowledge of the specific nature of the element such as form, solubility, and charge is also fundamental to understanding its end use and transport and subsequently, to prescribing appropriate management techniques (O'Connor et al 2005) and government standards.

The concern for the potential risk of contamination of heavy metals such as Cu when biosolids are applied to agricultural soils is not well understood over the long term. Oliver et al (2005) considered biosolid application and incubation over a seven-year period in various soil conditions. The concentration of bioavailable Cu remained relatively low and stable throughout the seven-year period of incubation despite an overall substantial reduction in organic carbon. The results indicated application of biosolids did not pose any significant risk in the short to medium term, with respect to Cu, and it seems unlikely biosolid Cu will pose a long-term hazard.

Whether for short or long term consideration it is usually the total metal concentration in compost that is determined. Nolan et al (2005) also considers the total metal concentrations in soils are a poor indicator of metal toxicity because metals exist in different solution and solid phase forms which affect bioavailability. This makes the implied risk associated with metal contamination in soils difficult to gauge. For this reason, regulations should move toward bioavailabilty based principles and overall fertility management in order to indicate factual risk (Prasad 2003).

3.6 Project Objectives

The objectives of this research were to:

1. Conduct long-term field experiments to determine the effect of Cu and Pb in soils on corn tissue after successive MSW and SS compost applications under no-till cropping systems.
2. Asses the bioavailability of Cu and Pb and their accumulation in plant tissue in both field (*Zea mays*) and greenhouse (*Beta vulgaris*) environments when exposed to biosolid composts as a soil amendment.
3. Examine bioavailability and accumulation of Cu in poultry tissues when field soil amended with biosolid compost is ingested by poultry.

The long-term goal of this project is to enhance the understanding of the bioavailability behaviour and long-term effects of compost-born Cu and Pb in agricultural soils on crop uptake and its impact on other organisms within the environment. It would be greatly beneficial to the environment, urban populations, and agricultural producers if waste compost found a safe and well defined role in the ecosystem. This project is intended to furthering our understanding of crop and livestock uptake of Cu and Pb in compost amended soils.

Chapter 4: Methodology

4.1 Field Experimental Design

The field experiment was conducted over two growing seasons (in 2005 and 2006) on dykeland soil near Truro, Nova Scotia, Canada. The field measuring approximately one hectare was surrounded by perennial forages and separated from surrounding fields by dykeland ditches. The dykeland soils utilized were classified as Acadia Association, a heavy textured silty loam with pockets of imperfect drainage (Webb et al 1991). Topography was level to gently undulating and the soil was stone free. The study was conducted on fields which had received the same MSW and SS compost treatments over the previous four years, at the same consistent application rates as this study. The established treatments allowed for an assessment of long-term field applications of the amendments. Each field season began with spraying the region to be seeded with Roundup® Weathermax herbicide at a rate of 1.67 L ha^{-1} to control weed growth in research plots. Two types of compost mechanically applied each spring were a municipal solid waste (MSW) and sewage sludge compost (SS) to plots 6 m long and 12 m wide with 3 m buffer zones between all plots. The MSW compost was collected from the Colchester Regional Composting Facility in Kemptown, Nova Scotia, and the SS compost from Fundy Compost in Brookfield, Nova Scotia. A general analysis of what is typically observed for MSW and SS composts utilized in this study is shown in Table 2.

Table 2. Typical MSW and SS compost compositions as utilized in the field study

	MSW Compost**	SS Compost***
Dry Matter %	70.7	41.1
Nitrogen %	1.22	0.59
Calcium %	2.62	0.52
Phosphorus %	0.32	0.28
Potassium %	0.34	0.06
Magnesium %	0.17	0.05
Sodium %	0.21	0.02
Iron mg kg^{-1}	2085	2290
Copper mg kg^{-1}	20.8	29.8
Zinc mg kg^{-1}	86.6	105.1

*Special Products Test Report, Nova Scotia Agriculture and Fisheries Quality Evaluation Division.
**Analysis on as received basis reported 6/16/2004 Truro, Nova Scotia.
***Analysis on as received basis reported 7/9/2004 Truro, Nova Scotia.

The experiment was a strip-split-plot factorial design with two factors organized in strips. The experiment was replicated in three blocks and was randomized within each of the three blocks. One factor included seven treatments consisting of two MSW compost rates, two SS compost rates, two N fertilizer rates and an unamended control that were applied each year. The low and high rates of the MSW and SS composts applied were 21 and 42 t ha^{-1} (MSW) and 22 and 44 t ha^{-1} (SS) on a dry weight basis. Nitrogen levels in the N treatments were provided by top dressing NH_4NO_3 at 120 kg N ha^{-1} for the low rate and 252 kg N ha^{-1} for the high rate after germination during each year. The second factor included three levels of powdered limestone ($CaCO_3$) for altered pH at zero (0 t ha^{-1}), low level (3 t ha^{-1}) and a high level (6 t ha^{-1}), supplied a month following seeding in a strip pattern. All amendments applied to the research field are shown in Table 3. The site was seeded each field season during late spring with Roundup Ready Dekalb hybrid seed corn (DKC27-12) using no-till planter (AGCO White model 8104 four row corn planter, Duluth, GA) at a rate of 79074 seeds per hectare (32 000 seeds/acre actual setting).

All plots were fertilized at the time of seeding with 100 kg ha^{-1} of 25-0-15. Approximately 4 weeks after seeding, another treatment of Roundup® Weathermax herbicide was applied at a rate of 1.67 L ha^{-1}. Once plots became established, buffer zones of 3m between plots containing established perennial grasses were mowed using a flail mower attachment. In the 2006 field season, the experimental plots were reseeded one month following the initial attempt, due to poor corn germination rates and damage caused by crows (*Corvus brachyrhynchos*).

Table 3. Description of field experiment treatments utilized in the study showing amendment types and associated application rates on a DM basis

Treatment Label	Amendment	Application Rate
1	MSW	Low 21 t ha^{-1}
2	MSW	High 42 t ha^{-1}
3	SS	Low 22 t ha^{-1}
4	SS	High44 t ha^{-1}
5	N (34-0-0)	Low 0.12 t ha^{-1}
6	N (34-0-0)	High 0.252 t ha^{-1}
7	Control	-
A	Lime	None
B	Lime	3 t ha^{-1}
C	Lime	6 t ha^{-1}

Mid-season and harvest composite soil samples were taken from each of the sixty-three plots within the experimental field. Soil cores were sampled at random locations within each plot to obtain the upper 15 cm of the soil substrate. Soil samples were kept refrigerated at 4º C until time of preparation for analysis. Composite tissue samples of the chopped corn plants were obtained at time of harvest towards the end of September. Utilizing a two-row corn harvester head equipped with a Processor (Dion 1224 forage harvester, Boisbriand, QC), each plot was gathered into a silage cart and a random sample technique was employed to gather a representative sample. Total plot yields were also determined at this time by recording gross silage cart weights after each subsequent plot was harvested. During the second harvest in 2006, an altered method for obtaining yields and composite corn samples by hand was utilized due to excessive wet field conditions, which disallowed large-scale equipment use. A total of 6 corn plants were cut by hand power tools from each plot, weighed whole and then fed through a three-row corn harvester from which sample material was obtained. Fresh yield for 2006 harvest was used for comparison between treatments only, due to the above mentioned field conditions. All tissue was oven dried at 70 ºC for a minimum of three days to determine dry matter yields and to prepare for trace element analysis. After drying, tissue samples were ground in a Wiley Mill (Standard Model #3, Arthur H. Thomas Co., Philidelphia, NJ) in order to pass through a 2.5 mm screen. Soil samples were air dried at room temperature and then ground with a mortar and pestle.

4.1.1 Statistical Analysis

Statistical analysis software SAS version 9.1 (SAS Version 9.1 2002-2003) was used to evaluate the significance of the differences between treatments and to determine any interactions of factors. When experimental results were analyzed the model assumption of normal distribution was met. The specific test PROC GLM was used to specify any degree of interaction and test hypotheses for the effects of a linear model. The procedure also created an output data set of residuals, which were subsequently plotted against predicted values. To check normality and constant variance assumptions, residuals were calculated from PROC GLM using the OUTPUT statement. Means values were considered significantly different when $P < 0.05$. Transformations were not required to meet ANOVA model assumptions of normal distribution of residuals. Yield results were analyzed for basic descriptive statistics to provide correlation and average totals.

4.2 Greenhouse Experimental Design

A greenhouse experiment was conducted at the NSAC Plant Science greenhouse in Truro, NS. The soil used was obtained from 45 of the plots in the field experiment, excluding 18 plots, which contained an inorganic N fertilizer treatment. The experiment was a split-strip-split-plot factorial design with two factors organized in strips. The corresponding plots for this container experiment consisted of four treatments containing two levels of SS compost, two treatments of MSW compost, and three factors of lime to serve as varying pH levels. Specific application rates were 21 and 42 t ha^{-1} of MSW compost and 22 and 44 t ha^{-1} SS compost. Lime rates were 0 t ha^{-1}, 3 t ha^{-1} and 6 t ha^{-1}. Unamended controls were also included. Treatments had been applied to field soil for a total of seven years prior to the greenhouse pot experiment. In the beginning of September 2006, two soil samples from the top 15 cm surface soil layer in each plot were removed as disks; each weighed out to 1 kg and placed in 3 L, 20.32 cm diameter plastic growing pots lined with paper disks in order to retain soil and limit leaching, and then placed on saucers. The two containers from each plot were divided into treatments of added N (0.36 g per pot of 34-0-0) and no added N. All pots were placed in a cold frame greenhouse at temperatures ranging from 6 to 20ºC and a lighting schedule of 18:6 (light:dark). At this time, the N treatment group received 0.36 g of 34-0-0 (NH_4NO_3) providing 110 kg ha^{-1} of fertilizer equivalent to 37.4 kg ha^{-1} of actual N. The cultivar Ford Hook Giant of Swiss chard (*Beta vulgaris* L.) (Veseys Seeds, York, PE) was sown in each pot at a density of 8 seeds per container (247 seeds per m^2). Each container was thinned after germination to 4 plants per pot and after several weeks to 2 plants per pot. All plants received no additional fertilizer throughout the trial, only regular applications of tap water. Analysis was not available for the typical tap water used.

Plants were harvested on day 70 after seeding by cutting above the soil line. Plant tissue was rinsed in tap water to remove any soil debris and placed in labeled paper sample bags. Fresh weights were obtained in order to determine yields. Soil samples from each pot were also taken at this time, placed in labeled plastic containers and refrigerated at 4ºC. All plant tissue was oven dried at 70ºC for a minimum of three days to determine dry matter yields and to prepare for trace element analysis. After drying, tissue samples were ground in a coffee grinder to a fine particle size. Soil samples were air dried at room temperature and then ground with a mortar and pestle.

4.2.1 Statistical Analysis

A two-factor ANOVA with replication was utilized to assess any effect of treatments on plant yields. Statistical analysis software SAS version 9.1 (SAS Version 9.1 2002-2003) was used to evaluate the significance of the differences between and any interactions of factors. When experiment results were analyzed the model assumption of normal distribution was met. The PROC GLM test was used to specify any degree of interaction and test hypotheses for the effects of a linear model regardless that a number of cells were missing. The procedure also created an output data set of residuals, which were subsequently plotted against predicted values. To check normality and constant variance assumptions, residuals were calculated from PROC GLM using the OUTPUT statement. Means values were considered significantly different when $P < 0.05$. Transformations were not required to meet the model assumptions of normal distribution of residuals. Yield results were analyzed for basic descriptive statistics to provide correlation and average totals. The UNIVARIATE procedure was calculated with the residuals of the model to check for normality.

4.3 Poultry Experimental Design

Care, handling, and sampling of the animals herein were approved by the Nova Scotia Agricultural College Animal Care and Use Committee. The poultry experiment was conducted in 2006 at the Nova Scotia Agricultural College Poultry Unit in Truro, NS. A total of 96 day old male broiler chicks were randomly allotted to 24 Peterseime cages (4 birds/cage) and raised to 1 kg size at 25 days. The experiment was a random block design. The birds were fed a standard starter ration *ad libitum* for the initial 7 days and then fed treatment diets with various levels of compost amended soil incorporated for the remainder of the trial to determine the bioavailability of Cu in the compost. Experimental diets were formulated to meet or exceed NRC specifications (1994) for poultry broilers, which included a basal Cu mineral supplement in the form of $CuSO_4$ at 8 mg kg^{-1}. The original compost source contained levels of Cu at 250 – 750 mg kg^{-1} and were incorporated at 6% DM to provide 0, 15, 30 and 40 mg kg^{-1} in the diet on a dry matter basis. A chemical control of inorganic Cu and an unamended control with a bulking agent of Alphaflox were also included. Treatments provided for inclusion of Cu at amounts additional to basal diet and are listed in Table 4. The estimated average volume of compost ingested per bird on a daily basis was 5.68 grams. According to NRC (1994), the concentration of Cu in broiler diets that is considered to be toxic is 250-500 mg kg^{-1} while the dietary requirement is 8 mg Cu kg^{-1}. All Cu treatments in this experiment were below toxic levels yet above nutritional requirement.

Table 4. Description of poultry experiment treatments showing increasing concentrations and sources of Cu in addition to the basal diet quantity of 8 mg kg^{-1}

Treatment	Compound	Cu levels provided
1	Compost	0 mg kg^{-1}
2	Compost	15 mg kg^{-1}
3	Compost	30 mg kg^{-1}
4	Compost	45 mg kg^{-1}
5	Inorganic $CuSO_4$	40 mg kg^{-1}
6	Cellulose Bulking Agent	0 mg kg^{-1}

To calculate growth performance, body weights were recorded for each cage in order to calculate growth performance and feed conversion ratios. Body weight measurements were taken at days 0, 6, 14 and 25. Weight of feed consumed for each cage was measured on days 0, 4, 7, 12, 19, 21, 23 and 25 in order to determine total feed consumption per treatment as well as feed to gain ratio. Fecal matter was sampled at day 12 and 25 from each cage from a dropping tray under the cages and was immediately frozen. Tissue and fecal samples were freeze dried for 24 hours and then ground using a standard coffee grinder. At day 25 all birds were sacrificed by cervical dislocation. Muscle and liver samples obtained from each cage of 4 birds were combined and immediately frozen. Prior to trace element analysis of the poultry tissue and fecal samples, 2 g of dried material was dissolved using a general nitric acid digestion procedure (modification of AOAC Official

Method 985.01 where the quantities of solute and solvents have been adjusted, as described on page 37). The concentrations of trace elements in the soil, plant and animal tissue were measured by inductively coupled plasma spectrometry (ICAP) at the PEI Analytical Laboratory in Charlottetown, PE.

4.3.1 Statistical Analysis

A two-factor ANOVA with replication was utilized to assess any effect of treatments on bird growth. Statistical analysis software SAS version 9.1 (SAS Version 9.1 2002-2003) was used to evaluate the significance of the differences between and any interactions of factors. When experiment results were analyzed the model assumption of normal distribution was met. The PROC MIXED test was used to specify any degree of interaction and test hypotheses for the effects of a linear model regardless that a number of cells were missing. The procedure also created an output data set of residuals, which were subsequently plotted against predicted values. To check normality and constant variance assumptions, residuals were calculated from the OUTPUT statement followed by the PROC UNIVARIATE and PLOT statements to illustrate normality. Means values were considered significantly different when $P < 0.05$. Transformations were required for liver, muscle and fecal2 Cu levels (transformed by ½, 1/3 and 1/3, respectively) in order to meet the model assumptions of normal distribution of residuals. Yield results were analyzed for basic descriptive statistics to provide correlation and average totals.

4.4 Analytical Procedures

All dried and ground plant, poultry, and soil samples were dissolved for element analyses following the hot nitric acid digestion open vessel procedure (modification of AOAC Official Method 985.01). Sample material was weighed out to 2±0.05 g and placed in a glass 250 ml Velp Scientifica acid digestion tube. A blank control was run every 20 samples for standardization. A 30 ml quantity of Trace Metal Grade HNO_3 (Fisher Scientific Co., Ottawa, ON) was pipetted into each tube underneath a fume hood. Each tube was placed in an open vessel digestion block (Tecator Digestion System 20, Hoganas, Sweden) and heated to 90 ºC for 45 minutes. After the initial 15 minutes, the tubes were washed with a 2% HNO_3 and deionized water solution to ensure complete sample saturation. The digestion block temperature was increased to 120 ºC after 45 minutes and remained at this temperature for the rest of the digestion procedure. Once the volume in each tube was digested to approximately 5 ml, it was removed from the digestion block and placed in a cooling rack. After cooling, each tube was rinsed three times with 2% HNO_3 solution and filtered through a fine porosity (Fisher brand Q2) filter paper into a 50 ml volumetric flask. Once the solution had filtered through, the filter papers were rinsed again with 2% HNO_3 solution. Every 20 samples a blank was run as a reference as well as a Cu standard and biological tissue standard (Reference Material 8414 Bovine Muscle, National Institute of Standard and Technology, Gaithersburg, MD). Each 50 ml volumetric flask was brought up to volume and poured into three appropriately labeled scintillation vials and then refrigerated until time of analysis on ICAP. The concentrations of trace elements in all samples were measured by inductively coupled plasma spectrometry (ICAP) model No. 61 (Thermo Jarrell Ash, Franklin, MA) at the PEI Analytical Laboratory in Charlottetown, PE.

Chapter 5: Results and Discussion

5.1 Field Experiment

5.1.1 Results

Although the experiment was repeated over two growing seasons, results cannot be statistically compared due to the varying harvest and sampling methods employed due to the extreme wet field conditions in 2006. The control plots mean yield was 0.85 kg DM · m^{-2} (8.5 tonnes $ha^{-1}$for 2005). The treatments in 2005 were significantly different than the control at the 95% confidence level with a p-value of < 0.01. Highest yields were observed for plots receiving MSW compost treatments where average yields were 1.08 and 1.21 kg m^{-2} for low and high application rates, respectively. The largest plant yields were subsequently both SS compost treatments followed by both nitrogen level treatments. Results for 2005 are shown in figure 3 below. As expected, the control treatments provided the lowest mean yields in 2005. This result is anticipated as these plots would have the minimal quantity of nutrients available for the growing season.

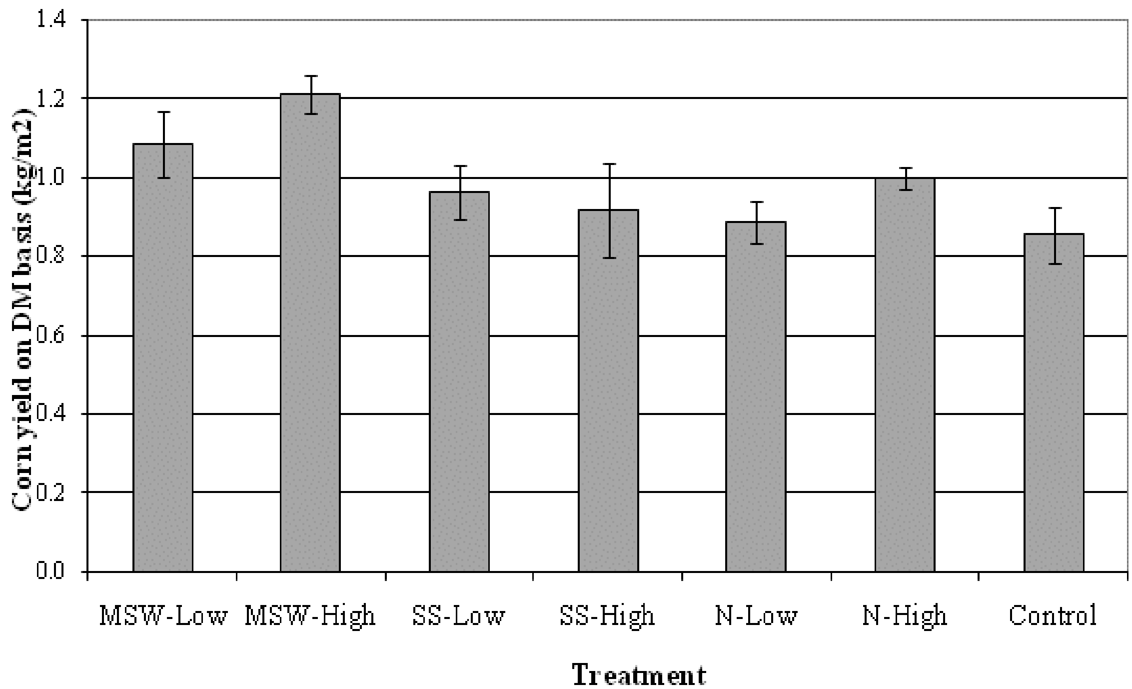

Figure 1. Corn yield response to amendment applications after first season in 2005 on Field 306. Average yield values shown on a DM basis per square metre with standard error of the treatment means included.

The 2006 growing season was year two of the experiment. At this stage the treatments applied to each block had been performed for 5 consecutive years in 2006, with the final two concerning this experiment and for the production of silage corn. Chemical analysis of the compost itself did not indicate there was any reduction in the quality of the compost as compared to previous years. The cold and wet spring conditions of 2006 resulted in slow and uneven crop emergence as the conversion of soil nitrogen to available forms by microorganisms would have been hampered when soils were saturated and colder in temperature. As shown in Table 5, average temperatures were higher during the 2006 growing season and precipitation amounts were less than normal. Despite this, during the month of September harvesting conditions were hampered due to the blockage of perimeter drainage ditches which allowed waters to spill over onto some plots. Field conditions were excessively wet in areas. This combined with dry soil conditions at time of plant emergence provided for poor crop growth.

Table 5. Actual versus expected climate averages during research periods

(July – Aug – Sept)	2005*	2006*	Expected Average**
Mean Max Temperature (ºC)	23.6	23.3	22.9
Average Temperature (ºC)	16.6	17.3	16.8
Total Rainfall (mm)	270.8	170.6	289.4

*http://climate.weatheroffice.ec.gc.ca/climateData/monthlydata_e.html
** Canadian Climate Normals, Environmental Canada, Debert, NS

With respect to altering soil pH by different lime application rates, there was only a marginal positive correlation of 24.5% for yields in 2005 in treatment 4. It appeared as though lime applications had negligible impact on corn yield. Wind drift was a consideration in top dressing powdered limestone to individual plots. Excessive wet plot conditions might have also played a role in negating the soil pH increase expected from lime applications.

In 2006 the soil amendment treatments were significantly different than the control at the 95% confidence level with a p-value of 0.02. Results are illustrated in Figure 4. As observed in the previous year there was a detected significant difference between compost treatments, but no interaction of compost and lime levels.

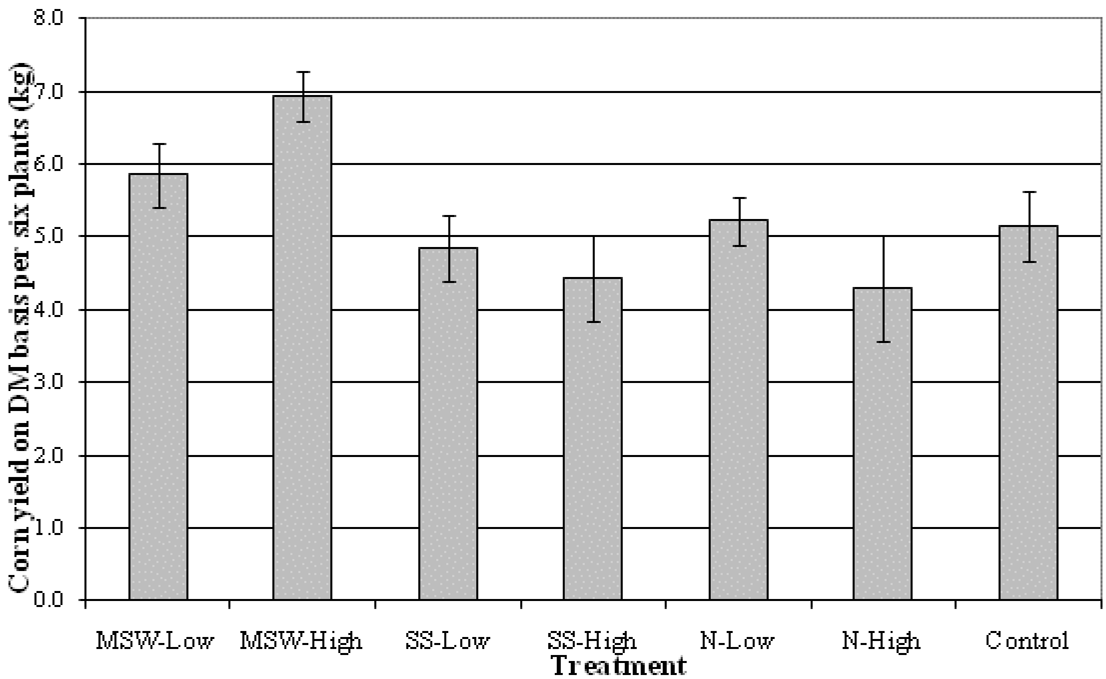

Figure 2. Corn yield response to amendment applications after second season in 2006 on Field 306. Average yield values shown on a DM basis per six plants from each plot with standard error of the treatment means included.

It should be noted that the apparently significant contrasts observed between the compost treatments and control seen in 2006 results were not consistent with the previous year's results. The control treatment did not result in the lowest yield averages at time of the 2006 harvest. These results were skewed for treatment 4 and 6 due to missing plot results where no plants where available for harvest. When the missing plots are excluded, the resulting treatment averages remain below the control group value. Therefore, the population differences observed were likely due to an anomaly related to weather conditions rather than being treatment related.

There was no strong correlation seen in 2006 yields to the various lime levels as shown in Table 6. Some variation was observed between replications. This again indicates variability in field conditions. As observed in the previous season, highest yields were seen with MSW compost treatments. There was variability in which treatments provided the subsequent high yields, however the lowest was witnessed in the Nitrogen High and SS compost High treatments. This would appear to indicate some other aspect affected plant growth and subsequent heavy metal uptake, such as the previously mentioned wet field conditions.

Table 6. Analysis of variance for the effect of treatment, replication and lime application rate on soil Cu and Pb uptake (mg kg^{-1}) at harvest, and plant tissue Cu and Pb (mg kg^{-1}) uptake at harvest

		Year							
		2005 Harvest						2006 Harvest	
Source of variation	DF	Soil Cu	Soil Pb	Plant Cu	Plant Pb	Soil Cu	Soil Pb	Plant Cu	Plant Pb
Treatment	6	**	NS†	NS	NS	**	**	NS	*
Lime Rate	2	NS	NS	NS	NS	NS	NS	NS	NS
Replication	2	NS	NS	**	**	*	*	NS	NS
Trt X Rep	12	**	NS	NS	**	*	*	NS	NS
Trt X Lime	12	NS	NS	NS	NS	NS	NS	NS	*
Lime X Rep	4	*	NS	NS	NS	NS	NS	NS	NS

† results not significant
* results significant at <0.05 probability level
** results significant at <0.01 probability level

In the first season in 2005 there was a significant response seen in field soil for the level of Cu as influenced by compost treatment in both the initial sampling period taken after seeding and the second period taken at harvest (Table 7 Observed average Cu response). A treatment effect was seen to alter soil Cu levels, as well as a treatment by blocking and treatment by lime rate interactions. During the second season significant differences were seen for both elements Cu and Pb in field soil. The initial sampling period indicated an effect of compost treatment on both elements (Table 9). A more detailed effect was observed during the second field soil sampling period as interactions of treatments and blocking indicated, along with treatment and block effects (Table 6). It was interesting that during the second season soil Pb levels very high throughout the season, exceeding previous years' values by approximately 10 times.

Table 7. Observed average Cu response in two year field experiment where soil and plant tissue Cu levels (mg kg^{-1}) were considered following application of composts

Treatment	Estimated Cu content applied (mg kg^{-1})	Year: 2005 Harvest		Year: 2006 Harvest	
		Soil Level	Plant Level	Soil Level	Plant Level
Values averaged across all lime treatments					
MSW Low*	20.7	22.53	661.79	10.38	7.89
MSW High	41.4	10.64	611.12	10.46	9.05
SS Low	29.8	11.04	7.27	11.90	8.91
SS High	59.6	12.48	9.85	13.17	7.40
N Low	0	9.69	9.63	10.61	8.13
N High	0	11.04	9.72	10.97	9.02
Control	0	9.48	326.25	10.10	9.50

*Abbreviation code list for treatments: MSW = Municipal Solid Waste, SS = Sewage Sludge, N = Nitrogen, Low = low application rates, High = high application rates.

Table 8. Observed average Pb response in two year field experiment where soil and plant tissue Pb levels (mg kg^{-1}) were considered following application of composts

	Year			
	2005 Harvest		2006 Harvest	
Treatment	Soil Level	Plant Level	Soil Level	Plant Level
	Values averaged across all lime treatments			
MSW Low*	8.06	1.69	254.78	1.02
MSW High	4.78	3.72	256.39	0.72
SS Low	4.39	0.50	292.79	0.24
SS High	4.79	1.36	325.80	0.45
N Low	3.91	1.05	260.36	0.22
N High	4.24	4.11	269.49	1.24
Control	3.56	0.79	249.72	2.61

*Abbreviation code list for treatments: MSW = Municipal Solid Waste, SS = Sewage Sludge, N = Nitrogen, Low = low application rates, High = high application rates.

Plant tissue analysis indicated a significant block effect for the values of Cu in the 2005 season. For Pb there was a significant compost treatment and block effect observed, as well as a compost treatment and block interaction. During the 2006 season plant tissue results indicated no effect for Cu levels but a significant effect for Pb by compost treatments and a treatment by lime rate effect (Table 6). It should be noted that although no significant response was observed in soil Pb levels for either years, the second year of the trial showed excessive level of Pb in all samples within the research field (Table 8). At the completion of the first year plant tissue accumulation of Pb appeared to be significantly different by treatment. However, following the poor growth response observed in the second year Pb levels were not strongly different by treatment or blocking group.

Table 9. Statistical results for Cu and Pb levels over two year trial period for both plant tissue and soil samples

Dependant Variable	F Value	Pr > F
Tissue Cu 2005	2.94	0.0099
Tissue Pb 2005	5.43	< 0.0001
Tissue Cu 2006	1.06	0.4564
Tissue Pb 2006	2.11	0.0545
Soil Cu 2005 after seeding	29.77	0.0023
Soil Pb 2005 after seeding	1.39	0.4152
Soil Cu 2006 at harvest	2.47	0.0121
Soil Pb 2006 at harvest	1.04	0.4740

The two field seasons' yield results could not be directly compared due to variation in sampling methods. During the initial harvest a whole plot sampling procedure was used. Weights of all plant material was chopped, collected and weighed. Due to excessive wet conditions during the second harvest in 2006 actual methods had to be altered to accommodate the inability to utilize tractor equipment on the field. At this time 6 plants were harvested by hand from each plot and were used to calculate yields. Also due to sporadic growth in plots there were several plots where no plants were available for harvest.

5.1.2 Discussion

The variability of corn yields observed as a response to nitrogen levels may have depended on rates of leaching and denitrification from the composts. Mamo et al (1999) identified that nitrogen leaching is greater with high compost application rates combined with commercial N fertilizer supplementation, compared to multiple smaller annual compost applications and reduced supplemental N fertilizer. It was also indicated that the biochemical properties of the compost mixture and its management strongly affected crop response and N leaching (Mamo and Gessesse, 1999). The experimental field in question during this study was exposed to stagnant water which might be concluded to have played a role in loss of available nitrogen in the composts. Although moisture content of the compost mixture has less of an effect on nitrogen loss than C:N ratio or substrate pH, excess water content above 60% can adversely affect nitrogen conservation (Weier et al, 1993) in addition to inhibiting corn growth from root stress due to lack of soil oxygen (Drew, 1990). A more appropriate approach to maximize the fertilization benefits of the composts would be to utilize a split application, with the second application being carried out as a side dressing once corn plants were established (Gardner and Rausser, 2002). It can be considered that the compost treatments provided adequate fertilization needed for corn production to achieve consistent yields, even though results observed may not have been maximized yields. It should also be noted that biosolid composts do have an inherent variability with each production lot and specific lot analysis would present a clearer picture of fertilization benefits.

Increased values observed for Cu in plant tissue brings forward the concern for contamination from metal filings in the harvesting equipment. Brass compounds in the corn harvesting equipment bushings present the potential for wear metals becoming mixed in with the corn tissue samples and contributing to total Cu levels.

Even though an important consideration in heavy metal bioavailability is the specific soil pH (Alva et al 2000), the method utilized in this experiment of hand applied top-dressing of powdered lime to manipulate the soil pH may not have allowed sufficient lime rates in the no-till system. Without incorporation into the soil the powdered limestone was affected by wind and water, and without sufficient time to allow the lime to take effect in the soil structure. A water based application of lime slurry or the addition with compost may provide a more consistent pH response within field plots. These techniques may reduce the lime variability by maximizing uniform application within plots and minimizing losses to air and surface water (Mkhabela et al, 2006).

The results of this study suggest that composts derived from SS and MSW which contain concentrations of heavy metals above accepted standards could be used as a topdressed soil amendment without toxic effects on an agricultural crop and without increasing the normal range of elements in the crop tissue. With the exception of results observed for MSW compost amendments in plant tissues for 2005, all soil samples showed higher concentrations than plant tissue samples for both years for the elements Cu and Pb (Table 9). The trend observed in Cu and Pb soil samples during the second and final year (2006) of the trial indicates increasing heavy metal content where high application levels were used (Table 10). Mean Pb values for soil samples for both SS and MSW compost amendments in 2006 were seen to be much higher compared to the previous year as shown in Table 8. However, the specific long term effects of the accumulation of heavy metals in agricultural soils following repeated SS and MSW compost applications needs to be carefully considered. The specific soil structure and topography should be taken into account when determining sound regulations for agricultural soil applications of SS and MSW compost. It is the type of soil, proposed crops and topography that can assist in understanding how the nutrient take up will be matched with rate of application (Barry et al 1995). Soils containing a low pH level, organic matter content or cation exchange capacity do not possess the ability to retain heavy metals and thus minimize the risk where compost is repeatedly applied. An understanding of the soil physical properties at the site of application may allow a better estimate of the nutrient and contaminant loading most appropriate to that site.

Table 10. Element analysis for high application levels of compost amendments

Analysis Variable	Mean Cu Values mg kg^{-1}	Mean Pb Values mg kg^{-1}
Tissue SS compost 2005*	9.85	1.37
Tissue MSW compost 2005	173.91	3.72
Tissue SS compost 2006	7.33	0.45
Tissue MSW compost 2006	9.07	0.72
SEM	41.3	0.74
Soil SS compost 2005 after seeding	16.23	13.07
Soil MSW compost 2005 after seeding	11.45	11.21
Soil SS compost 2006 at harvest	13.17	325.80
Soil MSW compost 2006 at harvest	10.45	256.39
SEM	1.27	81.8

*Abbreviation code list for treatments: MSW = Municipal Solid Waste, SS = Sewage Sludge

Table 11. Effect of treatment on soil Cu and Pb concentration (mg kg^{-1}) in corn plants at second year harvest (2006)

Compost Treatment	Soil Cu	Soil Pb
MSW 21 t ha^{-1}*	10.38	254.78
MSW 42 t ha^{-1}	10.45	256.39
SS 22 t ha^{-1}	11.90	292.78
SS 44 t ha^{-1}	13.17	325.80
N 0.12 t ha^{-1}	10.61	260.36
N 0.252 t ha^{-1}	10.97	269.49
Control	10.10	249.72
SEM	0.54	13.31
	P-value	P-value
Compost Treatment	P<0.0001	P<0.0001
Treatment x Rep	P<0.0001	P<0.0001

*Abbreviation code list for treatments: MSW = Municipal Solid Waste, SS = Sewage Sludge, N = Nitrogen

5.2 Greenhouse Experiment

5.2.1 Results

No correlation in yield levels to lime levels were observed, most treatments had a small insignificant association, only the control treatment showed a positive relationship with a 10.27 correlation factor. There was no observed difference between treatments ($P > 0.05$). There was no interaction between compost treatments and lime application for the yield of Swiss Chard. Not surprisingly application of compost negatively impacted Cu levels when nitrogen was also applied, since the group not receiving additional nitrogen fertilizer had decreased plant tissue growth. Specifically treatments 2 (MSW High) and 7 (Control) grew poorest due to the lack of additional nitrogen. The control treatment (7) is as would be expected as the field plot soil would not have been receiving comparable fertilization quantities over the two year experiment period.

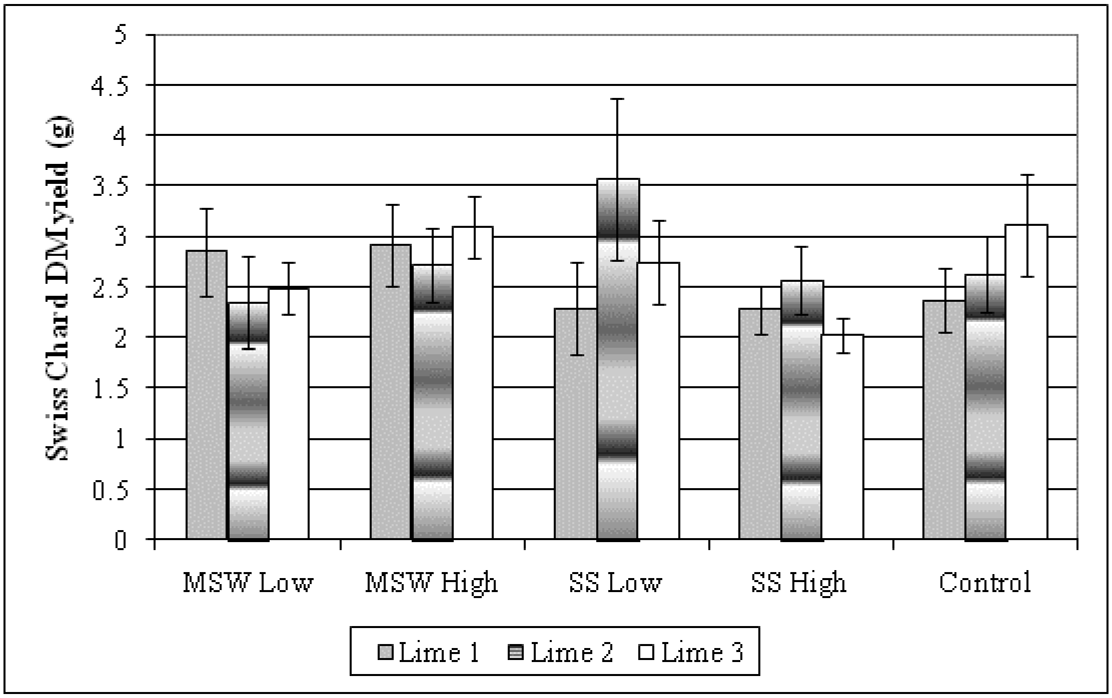

Figure 3. Yield results for Swiss Chard grown in greenhouse experiment with field soil containing different compost amendments. Results for each treatment group are compared to lime application levels and are given on a DM basis (g) with standard error of the treatment means included.

When added nitrogen is considered, there was a difference observed between the two groups in this container experiment ($P < 0.0001$). Again there is no observed effect between compost treatment groups. SS compost at a high level had decreased yields in this container experiment as well as the corresponding field experiment. Treatment 3 (SS Low) showed the greatest variance in yields for both nitrogen levels, as several containers contained larger plant growth. The following graph (Figure 4) clearly illustrates that plant growth was negatively affected when no supplementary nitrogen was added.

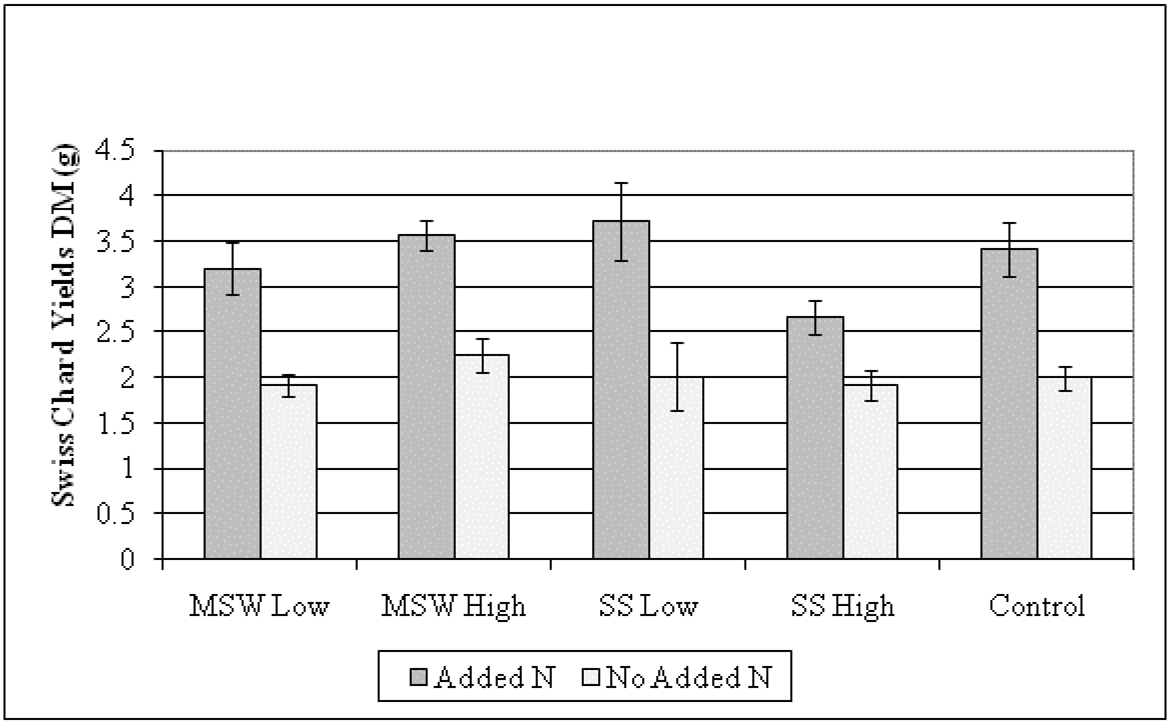

Figure 4. Greenhouse Swiss Chard yield results show treatments responded to different nitrogen application levels. Average yield values are shown on a DM basis with standard error of the treatment means included.

Plant tissue Cu levels were shown to be significantly different within the model assumptions, with a treatment as well as a blocking effect, indicating a difference between compost treatments. However, there were no interactions between lime levels, compost treatment and blocking. Plant tissue Pb levels were unaffected by compost or liming treatments, with mean Pb levels of 0.51 mg kg^{-1}. The soil used in the greenhouse experiment was the same for both blocks (added nitrogen and no added nitrogen), with each block laid out the entire length of the greenhouse. Cu levels were significantly different between compost treatments ($P< 0.05$), where again there was a block and treatment effect, but no interactions between compost treatments and liming rates. Tests of the hypotheses using the Type III MS for compost treatment by blocking interaction as an error term showed a significant effect for compost treatment and for lime levels for both Cu and Pb levels found in the sampled soil. Mean soil Cu and Pb levels were 11.2 mg kg^{-1} and 3.2 mg kg^{-1}, respectively. Following the trial, mean Cu levels were 13.3 and Pb 6.95 mg kg^{-1}. Cu levels showed only a significant effect for compost treatments at the end of the trial, with a trend for increased lime level application providing potential increased soil pH. Similar results were seen with Pb levels, where only a significant difference between treatments was observed at the end of the trial period. Mean overall tissue levels were 7.04 mg kg^{-1} for Cu, and 0.51 mg kg^{-1} for Pb.

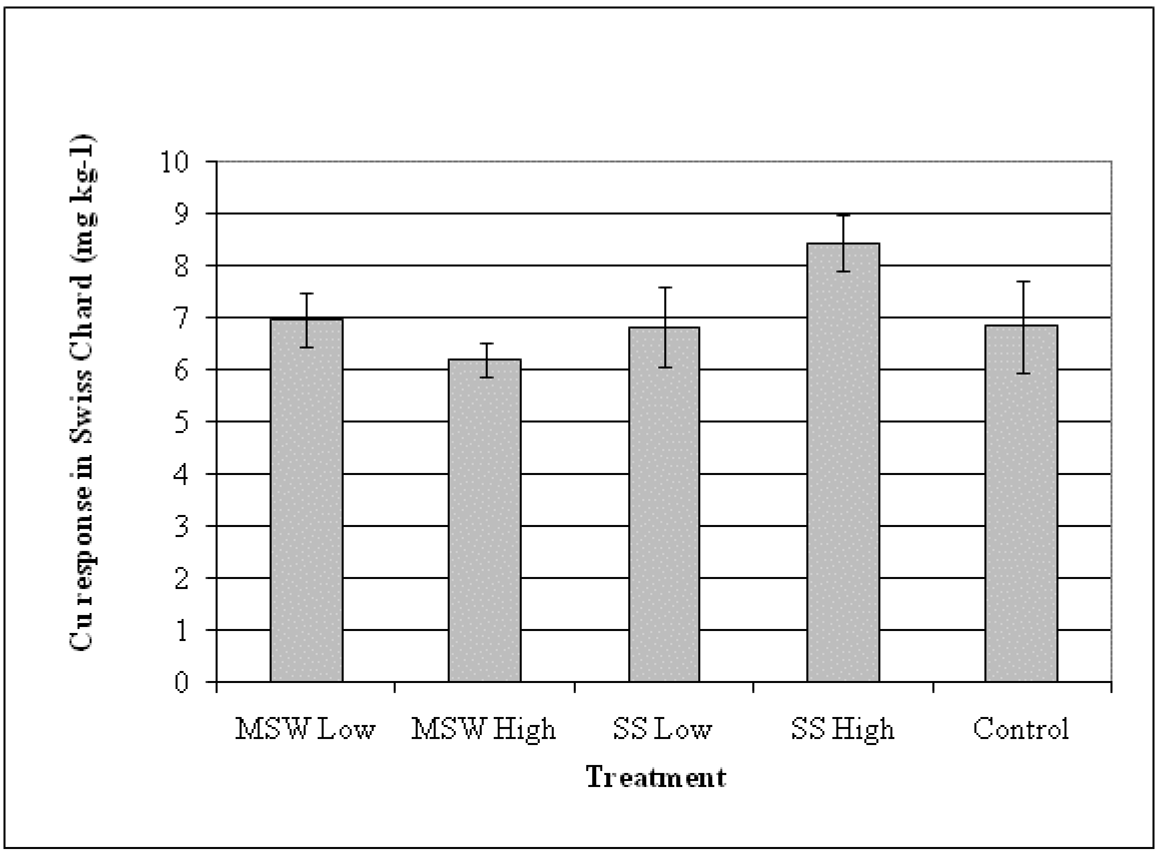

Figure 5. Applications of compost treatments to field soil affected Swiss Chard tissue Cu concentrations when grown in a greenhouse experiment. Averaged Cu concentrations (mg kg^{-1}) for both nitrogen rates are shown with corresponding standard error of the treatment means included.

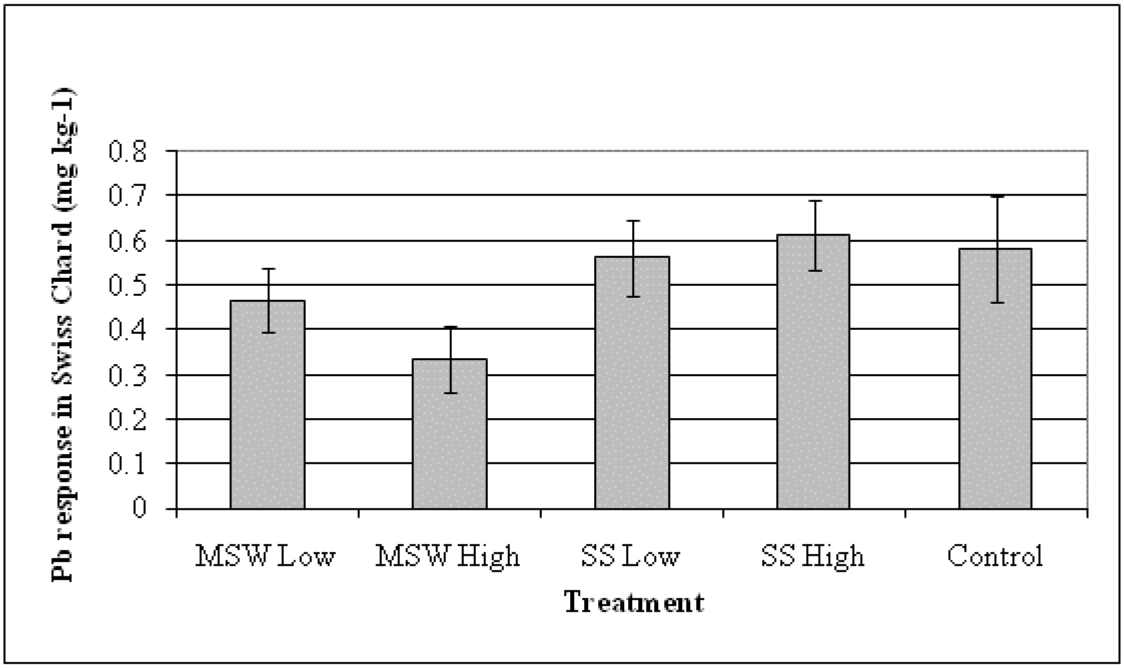

Figure 6. Applications of compost treatment to field soil affected Swiss Chard tissue Pb concentrations when grown in a greenhouse experiment. Pb concentrations (mg kg^{-1}) are shown with corresponding standard error of the treatment means included.

5.2.2 Discussion

According to USDA Database for Standard Reference (2005) for raw Swiss chard, the expected Cu levels are 1.79 mg kg^{-1}. When considered on a DM basis and assuming raw Swiss chard contains 8% DM the expected Cu content would be 22 mg kg^{-1}. The experimental results were less than this expected value. A similar food product to Swiss chard is spinach. The NIST standard reference material lists Cu content at 12.2 mg kg^{-1}. There is no standard reference for Pb levels as it is considered a contaminant only with no nutrient value in an agricultural crop. The NIST standard reference material for spinach does list Pb at 0.20 mg kg^{-1}. Assuming similar expected values should be observed in Swiss chard, the experiment results showed elevated levels in all treatment groups, including control. Overall, both Cu and Pb levels observed in treatment groups were not significantly different from those of the control group, although a trend for decreased Pb levels where compost was used was noted.

The results suggest that composts derived from SS and MSW with similar concentrations of heavy metals above accepted standards can be used as a soil amendment without risking accumulation of toxic effects on an agricultural crop and without increasing the normal range of elements in the crop tissue. However, the specific long term effects of the accumulation of heavy metals in agricultural soils following repeated SS and MSW compost applications needs to be carefully considered. Additionally the specific soil structure and topography need to be taken into account when determining sound regulations for agricultural soil applications of SS and MSW compost. An understanding of the soil physical properties at the site of application may allow a better estimate of the nutrient and contaminant loading most appropriate to that site.

5.3 Poultry Experiment

5.3.1 Results

In many animals it is the liver which functions as a filter for wastes and therefore would be considered an appropriate location to test for accumulation of heavy metals. Following completion of the experiment ICAP results it was determined Cu levels in poultry tissues were not influenced by compost treatment. The level of blocking relative to height above floor relates to bird body weight in that cages lower to the floor showed a higher weekly average bird weight than the cages found closer to the ceiling in the room. However, the effect was not as pronounced as the trial progressed and birds matured. Throughout the length of the experiment the lowest bird body weights were from treatments 1 (0 mg kg^{-1} Cu compost) and 6 (cellulose). These results are shown in Figure 7. Cu level in poultry muscle was not affected by compost treatments, which may confirm suggestions (Demirbas 1999) that poultry consuming a diet containing levels of heavy metals higher than current standards is appropriate for food consumption.

Following the initial fecal sample collection one week after starting feed treatments, treatment 3 (30 mg kg^{-1} Cu), 4 (45 mg kg^{-1} Cu) and 5 (45 mg kg^{-1} $CuSO_4$) showed a slight increase in Cu levels compared to remaining treatments (Table 11).

Table 12. Element analysis for poultry fecal samples indicating increasing Cu concentrations (mg kg^{-1}) at initial (Day 12) and final (Day 25) sampling period

Treatments	Initial Fecal Sample Mean Cu Values mg kg^{-1}	Final Fecal Sample Mean Cu Values mg kg^{-1}
1 – 0 mg kg^{-1} Cu	36.87	75.81
2 – 15 mg kg^{-1} Cu	59.64	97.04
3 – 30 mg kg^{-1} Cu	80.54	109.08
4 – 45 mg kg^{-1} Cu	80.98	127.52
5 – $CuSO_4$ control	87.06	179.57
6 – cellulose control	42.98	86.09

The initial fecal sample collection after one week of the treatment diets showed that treatment 1 and 6 were each significantly different from treatments 2, 3, 4 and 5 at $P<0.05$. The final fecal sample collection following completion of the experiment determined that treatments 1 and 6 were only significantly different from treatment 5

(P<0.05). It was treatment 5 that contained a pure $CuSO_4$ addition at similar inclusion levels as treatment 3. However, more Cu was excreted from this positive chemical control compared to the composted sources. Bird growth by day 25 was improved for treatment groups containing the highest included levels of Cu.

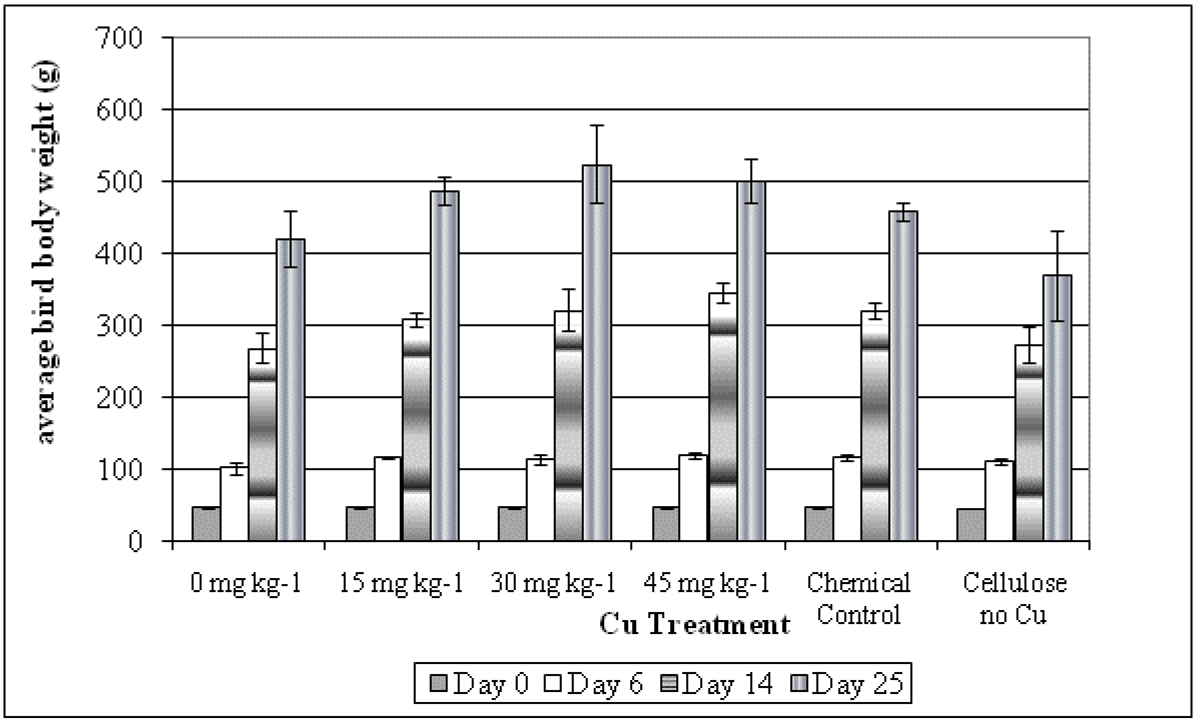

Figure 7. Poultry growth observed in response to additions of supplemental Cu over a period of 25 days, and compared to two control treatments. Bars show standard error of the treatment means.

5.3.2 Discussion

Lowest body weights were for birds in treatment groups 1 and 6, where no additional Cu was provided in the bird diets. Treatment 1 was a compost addition with 0 mg kg^{-1} Cu and treatment 6 was a cellulose bulking agent with no additional Cu as well. These results reinforce the concept that Cu supports animal development, even at levels above the NRC recommendations. Cu levels for intestinal samples are probably not the most appropriate comparison since these collected samples contained intestinal tissue as well as intestinal contents. It would be difficult to assume the proportion of Cu located in the fecal matter alone and not the quantity that would be found in the intestinal lining tissue. The Cu results from fecal matter samples reflected the quality of Cu supplemented in the diet. This would suggest that the risk of Cu hyperaccumulation in poultry tissue is low under these circumstances and the element may be bio-unavailable. The differences observed in the second fecal samples from treatment 1 (0 mg kg^{-1} Cu compost) and 5 (40 mg kg^{-1} $CuSO_4$ chemical control) when compared to treatment 6 (0 mg kg^{-1} Cu cellulose filler) indicate there was a different utilization of Cu depending on its source. This does not appear to support the notion that all Cu was bound to organic matter once composted and therefore bio-unavailable to the birds. This experiment demonstrates that birds not only tolerate higher than currently recommended Cu levels in their diets, but also appear to benefit in regards to increased growth. Ewing et al (1998) also supports the notion that there is a difference in element utilization depending on the source as their study found copper citrate better utilized than copper sulphate as seen with improved weight gain and feed conversion ratio.

Considerations for future investigations could include designing alternative experimental methods to deal with the problem of compost material distribution within the feed ration. One might question whether the birds had selectively excluded any clumps of compost. The use of intubation techniques to ensure birds are equally consuming the test compost material would remove these doubts. Conversely, the current experimental methods reflect real world results since in a free-range poultry production system the birds would display natural animal behaviours that include selectively excluding any large clumps of compost from their diet. This may be a factor to further decrease any potential risk to the birds themselves and therefore, the use of resulting meat products for human consumption.

Chapter 6: Conclusion

6.1 Preamble

The use of alternative agricultural soil amendments such as MSW and SS composts show potential to provide adequate nutrients for crop production and production of forages for poultry in a free range production system. The concerns for heavy metal bioavailability appear to be minimized in SS and MSW composted products. The heavy metals found in the composted products appear to be bound to organic material when appropriate soil pH levels are maintained for crop production.

After studying the bioavailability and effects of compost-born Cu and Pb applied to agricultural soils on crop and poultry uptake, this research indicates MSW and SS composts with elevated levels of Cu and Pb have the potential to safely be incorporated into crop and animal production and could therefore prove to be economically valuable to agricultural systems. As well, future use of these materials may provide the added benefit of providing more definition to the content of municipal and industrial composted wastes.

Where MSW and SS composts were applied to a no-till corn production system the effects of the industrial composts on soil and plant Cu and Pb levels were reviewed. Variances from added nutrients and varying pH levels seemed to have little effect. Poultry experiments assessed Cu bioavailability when compost amended soils were included in broiler diets. The incorporation of Cu in poultry tissues was not affected by compost ingestion although levels in fecal matter increased proportionately with increased feed consumption. Further monitoring of heavy metal levels in poultry manure would be needed should the material be reapplied to agricultural soils. This research could be used for predicting crop and livestock uptake of Cu and Pb following industrial compost application on agricultural soils and aid in fine-tuning appropriate compost quality guidelines.

6.2 Objectives

The goal of this research was to enhance the understanding of the bioavailability behaviour and long-term effects of compost-born Cu and Pb in agricultural soils on crop uptake and its impact on other organisms within the environment. Reviewing the specific objectives of this research we find they were met.

1. Successive applications of compost showed low bioavailabilty of Cu and Pb and that combined with correct soil conditions compost applications provide valuable nutrients in long-term field experiments under no-till cropping systems.
2. Minimal concerns exist for Cu and Pb bioavailability and accumulation in plant tissue in both field (*Zea mays*) and greenhouse (*Beta vulgaris*) environments when exposed to soils receiving successive applications of biosolid composts under no-till cropping systems.

3. No accumulation of Cu in poultry tissues was observed when composted biosolid soil was ingested by growing poultry in a controlled environment. The Cu excreted in poultry feces did proportionally increase with intake of composted biosolid soil indicating low bioavailability from the source.

6.3 Summary of Findings

The field experiment showed that successive applications of MSW and SS compost used under no-till cropping systems appear to be appropriate agricultural amendments for growing corn. The effect of utilizing a composted waste product with high Cu and Pb content for crop production does not appear to pose a significant risk for plant hyperaccumulation.

In the greenhouse experiment heavy metals such as Cu and Pb that may be found in soils do not appear to have a strong bioavailability to accumulate in certain plant tissue. This conclusion was observed in both the field environment with the traditional livestock crop of corn and in the greenhouse environment when Swiss chard was grown. The exposure to biosolid composts containing elevated levels of Cu and Pb when used as a soil amendment allowed for adequate plant growth, and did not cause increased levels to be found in plant tissues.

When biosolid compost with high Cu content is ingested by young broilers as part of daily feed there does not appear to be any accumulation of the element in poultry tissues.

The bioavailability is considered to be relatively low as shown by intake levels corresponding to fecal matter Cu content observed during the poultry experiment.

6.4 Future Direction

It can be concluded that there is potential for use of alternative wastes as agricultural amendments in the Maritime region. Once properly composted, MSW and SS wastes used as an agricultural soil amendment have decreased bioavailability of heavy metals as indicated by the lack of accumulation in plant or animal tissues.

This study has focused on the soil, plant and animal interaction with heavy metal laden composts. Another aspect of the agricultural ecosystem that should be considered for potential effect is the water sources in the region. As observed with the effects of seasonal flood waters on the experiment field, all agricultural amendments can be further contaminated by water born heavy metals following periods of saturation. Upstream scrap yards which contain Pb batteries and old rail yards where unknown chemical contamination exist, may be of concern following periods of flooding.

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