

**THE EFFECTS OF LONG-TERM AGING ON THE DISTRIBUTION AND BEHAVIOR  
OF HEAVY METALS IN THE SOIL: SOLUBILITY, MOBILITY, BIOAVAILABILITY, AND  
TOXICITY**

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# THE EFFECTS OF LONG-TERM AGING ON THE DISTRIBUTION AND BEHAVIOR OF HEAVY METALS IN THE SOIL: SOLUBILITY, MOBILITY, BIOAVAILABILITY, AND TOXICITY

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Sewage sludge is frequently applied as an agricultural fertilizer. However, this sludge often contains toxic metals, which contaminate the soil and create a risk for agriculture, as well as for humans, animals and plants in the surrounding environment. This dissertation analyzes the effects of long-term aging on the distribution and behavior of heavy metals (Cd, Cu, Pb, Zn, and Ni) in the soil, including solubility, mobility, bioavailability, and thus, toxicity. In 1978, a heavy application of metal-contaminated sewage sludge occurred at the Cornell Orchards in Ithaca, NY. This study examines the field site 40 years after this single application and compares it to a nearby site where sludge was not applied. The physical-chemical characteristics of the soil are measured by inductively coupled plasma optical emission spectroscopy (ICP-OES), and toxic metal availability is assessed using a sequential extraction procedure. The leaching of metals from the soil is estimated based on an experiment designed to simulate natural rainfall-driven leaching conditions. The bioavailability and plant uptake of the metals are determined by growing soybeans in sludge-contaminated and control soil samples. This dissertation also presents several new analytical methods that were developed in the course of the study, including the measurement of silver concentration using ICP-OES and the sequential extraction of metals from specific phases in the soil. This dissertation adds to our understanding of toxic metal solubility in the soil and proposes management methods to limit deleterious effects on soil productivity and environmental quality.

## BIOGRAPHICAL SKETCH

Tatyana (Smetanina) Dokuchayeva was born in the U.S.S.R. in the year when Nikita Krushchev became famous for his shoe-banging incident. Both of her parents were engineers and survivors of World War II and Stalin's Repressions. Neither parent ever wanted to speak about the past, since the persecutions of family members surviving both Nazi concentration camps and Soviet gulags were still part of the family's recent history, especially for Tatyana's beloved father.

Tatyana became interested in science when she was a high school student and fell in love with chemistry. One attractive aspect of chemistry was that, unlike history, literature and other humanities, it did not require the memorization of Lenin's studies. Chemistry, physics, and mathematics were the only core subjects that did not include Soviet propaganda and Marxism.

In 1984, Tatyana graduated with a master's in chemistry from Kyiv University. The same year, Tatyana had an addition to her new family, Anna. Tatyana would occasionally bring Anna by her lab and show her how she could make solutions turn different colors. For Anna, chemistry was like magic.

After obtaining her master's degree, Tatyana worked as an environmental scientist and led a project analyzing the biochemical properties of drinking water for the Boryspil International Airport in Kiev. Then came the fall of the Soviet Union. The 1990s were a chaotic and arduous time in the former Soviet states, and the only industry that seemed to be booming was oil and gas. Thus, Tatyana worked to analyze the chemical compositions of marsh gas and natural gas using gas chromatography and other methods.

In 1999, an opportunity arose to immigrate to the United States. This was the country that her beloved grandfather had dreamed to immigrate to when the Soviets first came in 1917. Tatyana's grandfather had tried, but he did not make it onto the ship. However, if he had, he

would have died with the ship's crew a few days later, and Tatyana would not have been here today. Now, 80 years later, Tatyana immigrated with her daughter, Anna, to Ithaca, NY, fulfilling her grandfather's dream.

Tatyana was determined to join the Cornell community and continue to submerge herself in the field of chemistry. Tatyana understood that scientific progress requires collaboration, hard work, learning new skills, and also a new language. In April 2000, Tatyana joined Cornell as a Research Support Specialist in the College of Human Ecology, where she studied pesticides, dye degradation and contaminants in residential floor dust. She collaborated with various visiting professors and also conducted research on the physical and chemical characteristics of textile fabrics. In 2006, Tatyana joined the Cornell Nutrient Analysis Laboratory, where she led the certification of laboratory environmental analyses for soil and non-potable water. After a long journey, Tatyana was back where she began, working with water and soil. Since then, she has conducted research and chemical analysis on wastewater, water, soil and plant tissues and has developed new analytical methods in the course of her work.

When Tatyana was recently presented with the opportunity to pursue her PhD, she jumped at the chance. She had originally planned to pursue a PhD soon after her master's, but then came the collapse of the Soviet Union and everything else that followed. Sometimes, life brings challenges and curtails our paths. These challenges teach us and give us new opportunities and new beginnings. Tatyana would like to continue working in a university environment, contributing to fundamental research and developing new methods that will aid our environment and society. Who would have thought that in your fifties, life is still just beginning?

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## TABLE OF CONTENTS

<b>BIOGRAPHICAL SKETCH</b>	<b>III</b>
<b>ACKNOWLEDGEMENTS</b>	<b>V</b>
<b>LIST OF TABLES</b>	<b>VIII</b>
<b>LIST OF FIGURES</b>	<b>X</b>
<b>CHAPTER 1: INTRODUCTION</b>	<b>1</b>
CAUSES OF TOXIC METAL CONTAMINATION IN SOILS	1
POSSIBLE EFFECTS OF TOXIC METAL CONTAMINATION IN SOILS	4
REGULATION OF SEWAGE SLUDGE DISPOSAL	4
REASONS FOR CONCERN	6
PRIOR STUDIES OF THE LONG-TERM FATE OF TOXIC METALS IN SOIL	7
AIMS OF THIS STUDY	8
REFERENCES FOR CHAPTER 1	10
<b>CHAPTER 2: PHYSICAL-CHEMICAL CHARACTERISTICS OF A SOIL AMENDED WITH SEWAGE SLUDGE 40 YEARS AGO</b>	<b>12</b>
INTRODUCTION	12
MATERIALS AND METHODS	13
RESULTS	15
DISCUSSION AND CONCLUSION	21
REFERENCES FOR CHAPTER 2	24
<b>CHAPTER 3: CHARACTERIZATION OF TOXIC METAL AVAILABILITY IN A LONG-TERM SEWAGE SLUDGE-AMENDED SOIL USING SEQUENTIAL EXTRACTION</b>	<b>26</b>
INTRODUCTION	26
MATERIALS AND METHODS	29
RESULTS	30
DISCUSSION AND CONCLUSION	42
REFERENCES FOR CHAPTER 3	44
<b>CHAPTER 4: LONG TERM LEACHING OF TOXIC METALS IN A SEWAGE SLUDGE-AMENDED SOIL</b>	<b>46</b>
INTRODUCTION	46

MATERIALS AND METHODS	48
RESULTS AND DISCUSSION	49
REFERENCES FOR CHAPTER 4	56
<b>CHAPTER 5: PLANT UPTAKE OF TOXIC METALS FROM A CONTAMINATED SOIL 40 YEARS AFTER SEWAGE SLUDGE APPLICATION</b>	<b>59</b>
INTRODUCTION	59
MATERIALS AND METHODS	61
RESULTS AND DISCUSSION	64
CONCLUSION	76
REFERENCES FOR CHAPTER 5	78
<b>CHAPTER 6: METHODS FOR MEASURING TRACE SILVER IN SLUDGE, PLANT TISSUE AND SOIL SAMPLES</b>	<b>81</b>
INTRODUCTION	81
MATERIALS AND METHODS	83
RESULTS	85
DISCUSSION	86
REFERENCES FOR CHAPTER 6	90
<b>CHAPTER 7: CONCLUSION</b>	<b>92</b>
<b>APPENDIX</b>	<b>94</b>
METHODS	94
REFERENCES FOR APPENDIX	117



## LIST OF TABLES

<b>Table 1.1.</b> Soil concentrations of available P, S, total N, total C, and organic matter in a sludge-amended and unamended soil previously determined by the Cornell Nutrient Analysis Laboratory (CNAL).	3
<b>Table 1.2.</b> Ceiling Concentrations, Pollutant Concentration, and Annual Pollutant Loading Rates established EPA regulations enacted in 1993 (e-CFR: TITLE 40).	6
<b>Table 2.1.</b> pH, organic matter content, and texture analysis of sludge soil and control soil.	15
<b>Table 2.2.</b> Total concentration of metals, sulfur and phosphorus in sludge soil (S1) & control soil (C1).	20
<b>Table 2.3.</b> Concentration of trace elements and organic matter in sludge soil measured in 2015 and 1997.	23
<b>Table 3.1.</b> Results of the Sequential Extraction Method applied to sludge soil (S1) and control soil (C1) from the Cornell Orchard Site. Sludge soil is shown in red, and control soil is shown in black. Values are the mean concentration (mg/kg) of elements in soil.	31
<b>Table 3.2.</b> Extraction Fe, Mn, P, S in sequential extraction test.	33
<b>Table 4.1.</b> Results of the soil leaching test. The mean concentration (mg/L) of metals and sulfur in the water from the leaching test for each soil sample.	50
<b>Table 4.2.</b> Kd values (L/kg) of toxic metals in sludge soil collected in 2017 and 1997 (McBride et al., 1998) at the Orchard site where sludge had been applied in 1978.	52

<b>Table 5.1.</b> Heavy metal content in fescue from the control site (C1) and in Goldenrod and Bull Thistle from the sewage sludge (S1) sites. Mean concentration (mg/kg) of elements in plants.	68
<b>Table 5.2.</b> Elements content in fescue grass grown in pots.	71
<b>Table 5.3.</b> Elements content in soybeans grown in pots.	75
<b>Table 6.1.</b> Results of the analysis of silver in sludge.	88
<b>Table 6.2.</b> Results of the analysis of silver in soil.	89

## LIST OF FIGURES

<b>Figure 2.1.</b> Sludge soil (S1) (right) and control soil (C1) (left) from the same field.	14
<b>Figure 2.2.</b> Results for soil total nitrogen and carbon (n = 12 for each).	17
<b>Figure 2.3.</b> Total concentration of sulfur and phosphorus in control soil (C1) and soil-sludge	18
<b>Figure 3.1.</b> Flow diagram of sequential extraction.	29
<b>Figure 3.2.</b> Results from the first three steps of sequential extraction of Cd and Zn	35
<b>Figure 3.3.</b> Extractable and non-extractable fractions of Cd and Zn (after digestion of the soil) from sludge-soil (S1) and control soil (C1) samples.	36
<b>Figure 3.4.</b> Results from the first three steps of sequential extractions Cu for control soil and sludge soil.	37
<b>Figure 3.5.</b> Extractable and non-extractable fractions of Cu (after digestion of the soil) from sludge-soil (S1) and control soil (C1) samples.	38
<b>Figure 3.6.</b> Results from the first three steps of sequential extractions of Ni.	39
<b>Figure 3.7.</b> Extractable and non-extractable fractions of Ni (after digestion of the soil) from sludge-soil (S1) and control soil (C1) samples.	40
<b>Figure 3.8.</b> Results from the first three steps of sequential extractions of Pb.	41
<b>Figure 3.9.</b> Extractable and non-extractable fractions of Pb (after digestion of the soil) from sludge-soil (S1) and control soil (C1) samples.	42
<b>Figure 4.1.</b> Buchner funnels and pump used in the leaching experiment.	49
<b>Figure 4.2.</b> Calculated downward velocity of metals (cm/year) in the sludge soil.	54
<b>Figure 5.1.</b> Images of plant species adapted to the soil in the field on the control and sludge soil sites.	60
<b>Figure 5.2.</b> Root systems of various plants.	66

<b>Figure 5.3.</b> Taproot system of bull thistle.	67
<b>Figure 5.4.</b> Grass growing in the two soils C1 (right) and S1 (left), 5 days after emergence.	69
<b>Figure 5.5.</b> Grass growing in the two soils C1 (right) and S1 (left), 10 days after seedling emergence.	70
<b>Figure 5.6.</b> Soybeans growing in sludge-soil (S1) 6 weeks after seedling emergence	74
<b>Figure 5.7.</b> Soybeans growing in agricultural soil (A1) 6 weeks after seedling emergence.	75

“A nation that destroys its soils destroys itself.”

- Franklin Roosevelt, U.S. President, 1937.

“Le microbe n’est rien, le terrain est tout.” (The microbe is nothing, the terrain is everything.)

- Louis Pasteur

## **Chapter 1: Introduction**

Over the years while working at the laboratory, I have spoken with many farmers, doctors, government organizations, schoolteachers, and community gardeners who often ask me the same questions: “Is my soil toxic? Will it affect my plants? How long will it take for my soil to recover from toxic metals?” This led me to start studying the behavior of toxic metals in contaminated soil to try to get a better understanding of their fate over time. Over the course of my work, I learned that many projects I worked on involved soil that was polluted due to the use of industrial waste as a fertilizer. I became interested in understanding whether the use of such waste in agriculture creates any problems for the environment and how long it might take for soil that has been contaminated with toxic metals to recover.

### **Causes of toxic metal contamination in soils**

Generally, toxic metals or metalloids can accumulate in soils as a result of industrial activities (e.g., mine tailings, sewage sludge, coal burning, accidental spills) or agricultural processes (e.g., fertilizer or pesticide application, animal waste, wastewater irrigation) (Wuana et al., 2011). Common contaminants include lead, chromium, arsenic, zinc, cadmium, copper, mercury and nickel (Evanko et al., 1997).

Unlike organic contaminants, they do not degrade (Kirpichtchikova et al., 2006), and their total concentration in soils persists for decades at least (Adriano et al., 2013). Since toxic metals remain in soil for a long time, they can inhibit microbial activity and therefore impact the biodegradation of organic co-contaminants (Maslin et al., 2000). Because of these serious impacts of toxic metals on soil health and possible risks for humans exposed to these soils, government agencies spend \$26 million per year to clean soil and prevent future contamination (Superfund | US EPA). The Environmental Protection Agency (EPA) estimates that tens of thousands of contaminated sites, which may cause environmental harm still remain in the United States.

Originally, the utilization of sewage sludge as agricultural fertilizer began due to its nutrient and organic matter content and because it also solved the urgent problem of waste disposal (Häni et al., 1996). Table 1.1 compares the nutrient profiles of two soils, one sampled in 2015 from the Cornell Orchards which had been treated with sewage sludge from a municipal wastewater treatment process in 1978 (“sludge soil”), and the other from a typical agricultural field sampled at the Musgrave Research Farm in Aurora, NY.

**Table 1.1.** Soil concentrations of available P, S, total N, total C, and organic matter in a sludge-amended and unamended soil previously determined by the Cornell Nutrient Analysis Laboratory (CNAL) in 2015.

<b>SOIL</b>	Available Phosphorus (mg/kg)	Available Sulfur (mg/kg)	Total Nitrogen (%)	Total Carbon (%)	Organic Matter (%)
Soil from sludge site 40 years after application	33	240	0.4	4.8	7
Agricultural soil from Musgrave Research Farm	5	12	0.3	2.8	3.5

Although the level of nitrogen in the two soils is similar, the sludge soil contains much higher levels of available phosphorus (33 mg/kg) and sulfur (240mg/kg), as well as higher levels of total carbon and organic matter—even 40 years after the application of sewage sludge. Thus the “half-life” of certain nutrients in the soil, particularly phosphorus and organic matter, is quite long and the environmental cost of building up soil levels of nutrients and non-essential or toxic elements must be considered when evaluating the costs and benefits of using sewage sludge as an inexpensive fertilizer. In addition to N, P and organic matter, sewage sludges have elevated concentrations of toxic metals such as Pb, Cr, As, Zn, Cd, Cu, Hg, Ni, and Ag. Although the levels of certain toxic metals in most municipal sewage sludges have been reduced since the early 1990’s when the EPA 503 rule was promulgated and regulated 8 or 9 metals, these metal concentrations in sludges usually exceed soil concentrations by a wide margin (CFR Title 40). Therefore, long-term sewage sludge application on farm fields is likely to gradually raise soil concentrations of metals such as Cd, Zn, Cu and Pb in particular.

## **Possible effects of toxic metal contamination in soils**

Toxic metal contamination of soils creates risks for agriculture, as well as for humans, animals and plants in the surrounding environment. With regard to agriculture, toxic metals may impact the quality of produce or animal feed grown on contaminated land, and at some level of contamination, may become toxic to crop plants and reduce yield.

The effects of heavy metals on both the environment and human health are becoming an increasing concern (Bolan et al., 2014). Heavy metal contamination in the food chain can lead to widespread poisonings, such as in Minamata, Japan during the 1950s. In this case, hundreds of people were affected by a devastating neurological disease resulting from the ingestion of fish contaminated with methylmercury from industrial wastewater (Knopf et al., 2010). More recently, high heavy metal concentrations in soils have become a concern in a number of countries. For instance, As accumulation in food crops presents a problem in many Asian countries, where millions of people may be at risk from As poisoning (Bhattacharya et al., 2012). In another example, accumulation of Cd in grazing livestock resulting from the use of phosphorus fertilizers can make some animal products unsuitable for human consumption (Loganathan et al., 2008). Similarly, there have been concerns about urban development of horticultural sites which contained toxic levels of metals such as As, Cu, Cd and Pb in soils resulting from excessive use of fungicides, herbicides, and fertilizers that are rich in these metals (Pietrzak et al., 2011).

## **Regulation of sewage sludge disposal**

The U.S. EPA has established regulations for sewage sludge disposal on farmland, including regulations around As, Cd, Cr, Cu, Pb, Hg, Mo, Ni, Se, and Zn concentrations in sludges and loadings in soil. Prior to the early 1970s there was no law regulating sludge disposal (National Research Council et al., 1996). In 1972, Congress directed the EPA to regulate disposal of sludge that enters navigable waters by enacting Section 405(a) of the Federal Water Pollution Control Act (Federal Water Pollution Control Act, 1948). In 1977,



Congress amended the law by adding new sections and requiring the EPA to develop regulations for the use and disposal of sewage sludge on land and in water (Federal Water Pollution Control Act, 1948). These guidelines required the EPA to “identify alternatives for sludge use and disposal” (Wuana et al., 2011), “specify what factors must be accounted for in determining the methods and practices applicable to each of the identified uses” (Evanko et al., 1997), and “identify concentrations of pollutants that would interfere with each use” (Kirpichtchikova et al., 2006). Once again, the law was amended to establish a timetable and technical standards for sewage sludge use and disposal (Federal Water Pollution Control Act, 1948). Congress directed the EPA to identify the presence of toxic pollutants in sewage sludge which may affect public health and the environment, and to establish acceptable practices to manage and limit these pollutants. Finally, in 1993, the EPA promulgated the final regulations for the disposal of sludge (CFR: Title 40). The new regulations provided ceiling concentration limits, pollutant concentrations limits and annual pollutant loading rates for various metals (see Table 1.2).

**Table 1.2.** Ceiling Concentrations, Pollutant Concentration, and Annual Pollutant Loading Rates established EPA regulations enacted in 1993 (CFR Title 40).

<b>Pollutant</b>	<b>Ceiling Concentration Limits (mg/kg)</b>	<b>Pollutant Concentration Limits (mg/kg)</b>	<b>Annual Pollutant Loading Rates (kg/ha/yr)</b>
Arsenic	75	41	2
Cadmium	85	39	1.9
Chromium <sup>1</sup>	3,000	1,200	150
Copper	4,300	1,500	75
Lead	840	300	15
Mercury	57	17	0.85
Molybdenum <sup>1</sup>	75	18	0.9
Nickel	420	420	21
Selenium	100	36	5
Zinc	7,500	2,800	140

<sup>1</sup>Above limits for chromium and molybdenum (except for ceiling concentration) have been deleted, and the pollutant concentration limits have been revised for selenium.

### Reasons for concern

As mentioned above, remediation of toxic metal-contaminated soils, particularly the removal of these metals using chemical or biological methods, is very difficult and expensive. Thus, preventing heavy metal pollution of agricultural soils is critical. Once metals are introduced into the environment (e.g., through the use of industrial waste as fertilizer), they will remain for a long time. Unlike organic matter that degrades in soil, metals do not. With the exception of mercury and selenium, which can be transformed and volatilized by microorganisms, most metals remain in a form that is unlikely to be very mobile either by leaching or volatilization. Therefore, fertilizing crops with fertilizer derived from industrial waste can make the land less suitable for agriculture (USDA, 2000).

Since toxic metals in soil may directly impact the agricultural industry and its economic wellbeing and productivity, it is vital to understand the behavior of toxic metals in soil after applying biosolids waste as fertilizer 40 years ago.

## **Prior studies of the long-term fate of toxic metals in soil**

There have been a number of studies conducted on how toxic metals in sewage sludges behave in soil over the long term (e.g., McBride, 1995; McBride, 1994; McBride et al., 2004; Kim et al., 2006; McBride et al., 1999). McBride (1995) summarized the understanding of toxic metal behavior in soils based on various published reports of land applications of sewage sludges (a.k.a., biosolids). In 1995, the application of biosolids in agriculture was not completely accepted in the scientific community as a viable disposal option, and there were concerns about the fate and phytoavailability of trace metals, particularly cadmium, contained in the biosolids over time. McBride (1995) described the reported behavior of metals in sludged soils, their solubility, uptake by plants, and induced deficiency of certain micronutrients in plants caused by excesses of these metals. The study considered various hypotheses as to recovery of an ecosystem after a long-term application of sewage sludge in agriculture. This study and further treatises by McBride provided several potential scenarios of the behavior of toxic metals in soil regarding the solubility of metals and soil-plant relationships in sludged soils up to 40 years after cessation of sludge application.

Another earlier study described the long-term fate of toxic metals in sites where little or no tillage had occurred (Brown et al., 1997). This study examined the distribution of metals to a depth of 1 meter under conditions of varying pH. The study analyzed the movement of Zn, Cu, Pb, and Mn to determine whether leaching depended on organic matter content or pH of the soil. The study found that the movement of metals was correlated with an increased level of dissolved organic matter in the soil, likely as a result of increased pH due to the application of lime-stabilized amendments. This is an important result, revealing that soil pH is a highly important variable in determining how strongly metals in various biosolids are retained by soil.

Other studies have described how sewage sludge is used as an agricultural fertilizer in the European Union (Wuana et al., 2011; Kirchmann et al., 2017). As in the US, the application of the sludge in agriculture was used as a solution to the urgent problem of waste disposal, with

the result that ecosystem changes were observed after long-term exposure and application of sludge.

One study investigated the impact of growing maize in soil polluted with biosolids, where biosolids had been applied for over 100 years. The study showed a high concentration of organic matter with a high concentration of phosphorus in the soil, which progressively released metals with lability decreasing in the order:  $\text{Cu} > \text{Cd} > \text{Zn} = \text{Ni} > \text{Pb}$ . In spite of the high concentration of phosphorus, which decreased the lability of Cd and Pb, maize tissue was shown to contain Cd, Pb, Cu, Zn, and Ni. The maize grown in this area (around 70 ha) is not suitable for consumption anymore, and instead this maize is being used for ethanol production (Mossa et al., 2020).

## **Aims of this study**

This dissertation investigates the long-term behavior of toxic metals, including their solubility, mobility, and bioavailability in soil which was amended with a one-time heavy application of sewage sludge forty years ago. To get a better understanding of the fate and environmental effects of toxic metals in soil locally (Ithaca, NY), I studied a plot at the Cornell Orchards, which has had a history of sewage sludge application. In 1978, a small area of the Cornell Orchards received a single heavy application of high-lime sewage sludge (pH near 7) from Syracuse, NY. The details and history of the application of this sludge are described in Chapter 2. As a consequence of this single heavy loading, “the nominal loadings of Cu, Cd, Zn, and Ni were about 50% of the allowable cumulative loadings under EPA Part 503 Sludge Rule” (Richards et al., 1998). Areas within the application site (“contaminated area”), exceeded the allowable loading significantly, since the sludge was not spread evenly across the field.

A portion of the field in which sludge was not applied was used as a control in this study. Presently, 40 years after the initial application of sewage sludge, there are only weeds and sod grass growing on the contaminated area and the control area.

This dissertation examines and compares the various properties of the sludged and the control soil. In Chapter 2, I examine the physical-chemical characteristics and total metal content of the soils. Chapter 3 uses a sequential extraction method to measure metal bonding strength and infer metal speciation or form in the soil, and Chapter 4 uses a leaching test to measure mobility and estimate the rate of toxic metal loss from the surface soil. Chapter 5 examines the effect of the soils on plant growth and phytotoxicity. Finally, Chapter 6 describes new methods for accurately measuring trace concentrations of silver in plant and soil samples.

The studies described in this thesis provide a more complete understanding of toxic metal solubility in soils and suggest management methods that may be used to limit deleterious effects on soil productivity and environmental quality.

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# Chapter 2: Physical-chemical Characteristics of a Soil Amended with Sewage Sludge 40 Years Ago

## Introduction

Many empirical studies over decades have led to an understanding of soil properties that are critical to soil health, that is, the functional capability and quality of soil. Inorganic nutrients are important for maintaining crop productivity. Nitrogen in both inorganic and organic forms in soil is ultimately made available to roots for healthy plant growth, and organic carbon in the soil is released into the atmosphere by mineralization in a dynamic balance with photosynthesis that helps to stabilize the atmospheric concentration of CO<sub>2</sub>. Organic matter is frequently strongly correlated to most other indicators of soil quality or health, but by itself does not adequately reflect the breadth of ecological processes occurring in soil. Nevertheless, soil organic matter is a key component of productivity that contributes to the minimization of synthetic inputs and a healthy food supply, while also providing benefits toward environmental protection. Soil pH is another critical parameter that indicates potential for macro- and micro-nutrient availability and microbial processes (Smith et al., 1997). However, excessive accumulation of certain micronutrients or toxic metals, as well as macronutrients such as phosphorus, can be a threat to a healthy food supply and environmental protection.

The measurement of soil properties can provide guidance for choosing management methods needed to limit deleterious effects of toxic metals (including excessive concentrations of micronutrients such as copper, zinc and molybdenum) on soil productivity and environmental quality. However, the ultimate fate of metals added to soil can only be determined through long-term measurements in the field. Physical, chemical and biological processes in soils may significantly impact and modify toxic metal solubility and bioavailability over time. The concentrations of bioavailable trace metals in soil are governed by a variety of reactions including complexation with organic and inorganic ligands, ion exchange and adsorption, precipitation and dissolution of solids, and acid-base equilibria (Mattigod et al., 1981). Soil



organic matter and pH play a key role in modifying metal availability to plant roots by the formation of soluble and insoluble metal-organic complexes, thereby controlling free metal cation concentrations in soil solution. It is generally accepted that free (uncomplexed) metal cations in soil solution are the most bioavailable and potentially toxic forms of metals such as Pb, Zn, and Cu. (John et al., 1995; McBride, 1995).

In this chapter, in order to investigate the long-term fate of toxic metals in a soil amended with a metal-contaminated organic-rich waste product, I examine the physical and chemical properties of a soil that received a heavy application of municipal sewage sludge 40 years earlier, and compare its properties to that of a control soil from the same field site. Implications of the changed characteristics of the soil with regards to soil and ecosystem health are discussed.

## **Materials and Methods**

### **History of the sludge application site**

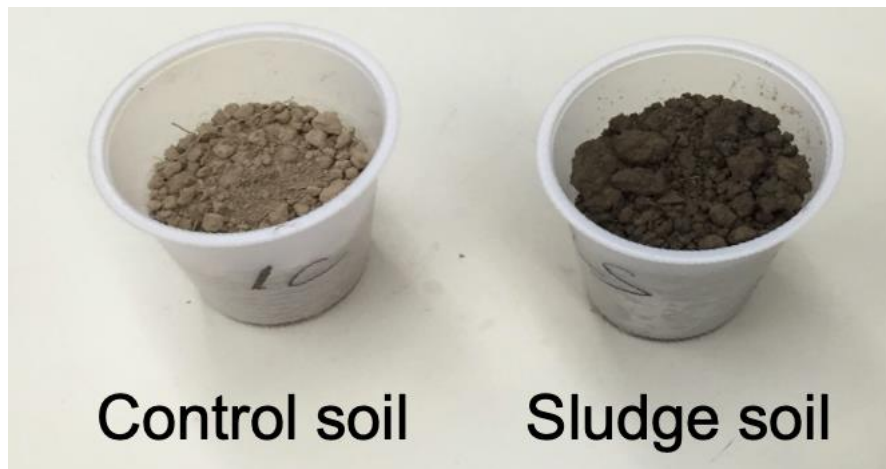
In mid-April, 1977, 100 tons of sewage sludge (pH 6.5, 49.8% moisture) was applied to 0.4 acres of subsoil (approximately 208 dry metric tons/ha) at Cornell Orchards. The sludge had been obtained from the Ley Creek Sewage Treatment Plant in Syracuse, NY. This plant received the effluents discharged by about 100 industries, as well as domestic wastes. The industrial activities represented included welding, plating, foundry, printing, laundering, fat rendering, and manufacture of bearings, die castings, gears, tools, steel and electrical products, china, paper board, chemicals, wood preservatives, beverages, dairy, and other food products. Wastewater entering this facility was treated to produce an anaerobically digested, waste activated sludge. No lime or other chemicals were added during the treatment process. The sludge transported to the Cornell Orchard from Ley Creek had been produced a year earlier, after which it was stockpiled in the field and allowed to weather to facilitate removal of excess soluble salts and decomposition of possible phytotoxic organic constituents. The sludge had a fertilizer equivalent of 0.7-1.0-1.4 (N-P-K) and contained 68.4% ash (Heffron et al., 1980). After

the sludge application, the site was cropped between 1979 and 1983 with the addition of some commercial fertilizer. After the soil was limed in 1985 and deep plowed, it remained fallow for much of the subsequent period and was planted to sod grass in 1986 (McBride et al., 1997).

### **Collection and analysis of soil samples**

In 2015, soil samples were collected from the surface soil (0-30 cm deep) at the Cornell Orchards (the “Orchard”) in Ithaca, NY. Two types of samples were collected: soil from the site that had been amended with municipal sludge (“sludge soil”, S1), and soil from a nearby area of the field that had not received sludge treatment (“control soil”, C1). The Orchard soil is a silty-clay loam, Hudson series, classified as fine, illitic, mesic Glossaquic Hapludalf (McBride et al., 1997). All soil samples were thoroughly homogenized and passed through a 2mm sieve. As shown in Figure 2.1 from visual inspection, the Sludge soil had higher organic matter content and better structure.

**Figure 2.1** Sludge soil (S1) (right) and control soil (C1) (left) from the same field.



12 replicates were collected for each soil type. The samples were analyzed for organic matter content by loss on ignition (LOI). pH was measured by electrode using a deionized water: soil ratio of 2:1. The texture of the soil was determined using the standard pipette method. The total nitrogen and carbon in the samples were analyzed by combustion analysis using a CN-

Analyzer by Elementar Americas, Inc. The total concentrations of trace elements in the samples were measured by digesting soils with nitric acid, hydrofluoric acid, and perchloric acid using a hotblock and analyzing the digests with ICP-OES (Spectro Analytical Instrumentations, Inc.). All methods are described more fully in the Appendix.

## Results

The sludge soil contains an unusually high concentration of organic matter (7.4%) compared to typical agricultural soils of the region. As shown in Table 2.1, the levels of organic matter and pH of the sludge soil (S1) are both much higher than those of the control (C1) soil. The higher pH of S1 may reflect a persistent buffering of pH in the near-neutral range by the heavy sewage sludge amendment. Although high-N amendments to agricultural soils are considered to be acidifying over the long term, this effect was not observed as a result of the sewage sludge application at the Orchard site.

**Table 2.1.** pH, organic matter content, and texture analysis of sludge soil and control soil.

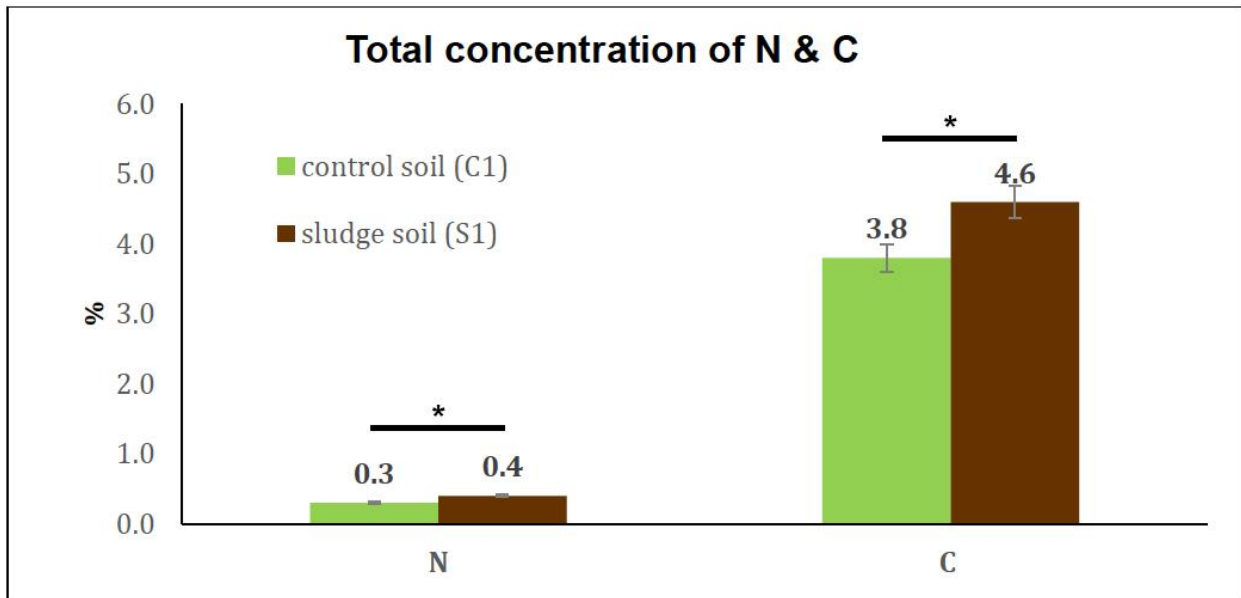
<b>Sample</b>	<b>pH</b>	<b>Organic matter (%)</b>	<b>Sand (%)</b>	<b>Silt (%)</b>	<b>Clay (%)</b>
<b>C1</b>	5.1	2.8	3.0	75.2	21.8
<b>S1</b>	6.7	7.4	12.0	62.6	25.3

The high level of organic matter in the sludge soil suggests that some sludge residual organic matter was resistant to decomposition and had not yet degraded at the time the samples were taken in 2015, nearly 40 years after the sewage sludge application. Generally, organic matter levels in soils amended with organic materials such as plant residue and livestock manure are observed to return to values near the level of organic matter originally in the soil within a relatively few years. Sewage sludge organic matter may be more resistant to decomposition than these forms of organic residues, as the half-life of sewage sludge organic carbon decomposition has been estimated to be around ten (10) years (Bell et al., 1991). The

present S1 study site's organic matter levels are still at three times the level of the surrounding C1 soil after 40 years, possibly because the degradation rate of soil organic matter is decreased by the high concentrations of toxic metals present in this soil (McBride et al., 1997). The large difference in pH between sludge soil (near neutral) and control soil (near 5) is worth noting because pH is a critical factor in controlling the solubility of potentially toxic metals; more acidic soils have a higher solubility of toxic metals and a larger fraction of the dissolved metals in the more toxic free ionic form.

The texture of the sludge soil and control soil are found to be different by the pipette method, possibly due in part to particles added to the soil from the sewage sludge. However, the high organic matter of S1 could have contributed to greater aggregation of particles, causing an artificially high estimate of coarse particles (sand fraction). Organic-rich soil amendments such as sludge and compost also influence other physical properties of soil, especially bulk density and porosity. Soil structure is analyzed by the size of stable aggregated particles. Soils with higher porosity often have larger particles similar in size to sand (Pagliai et al., 1981).

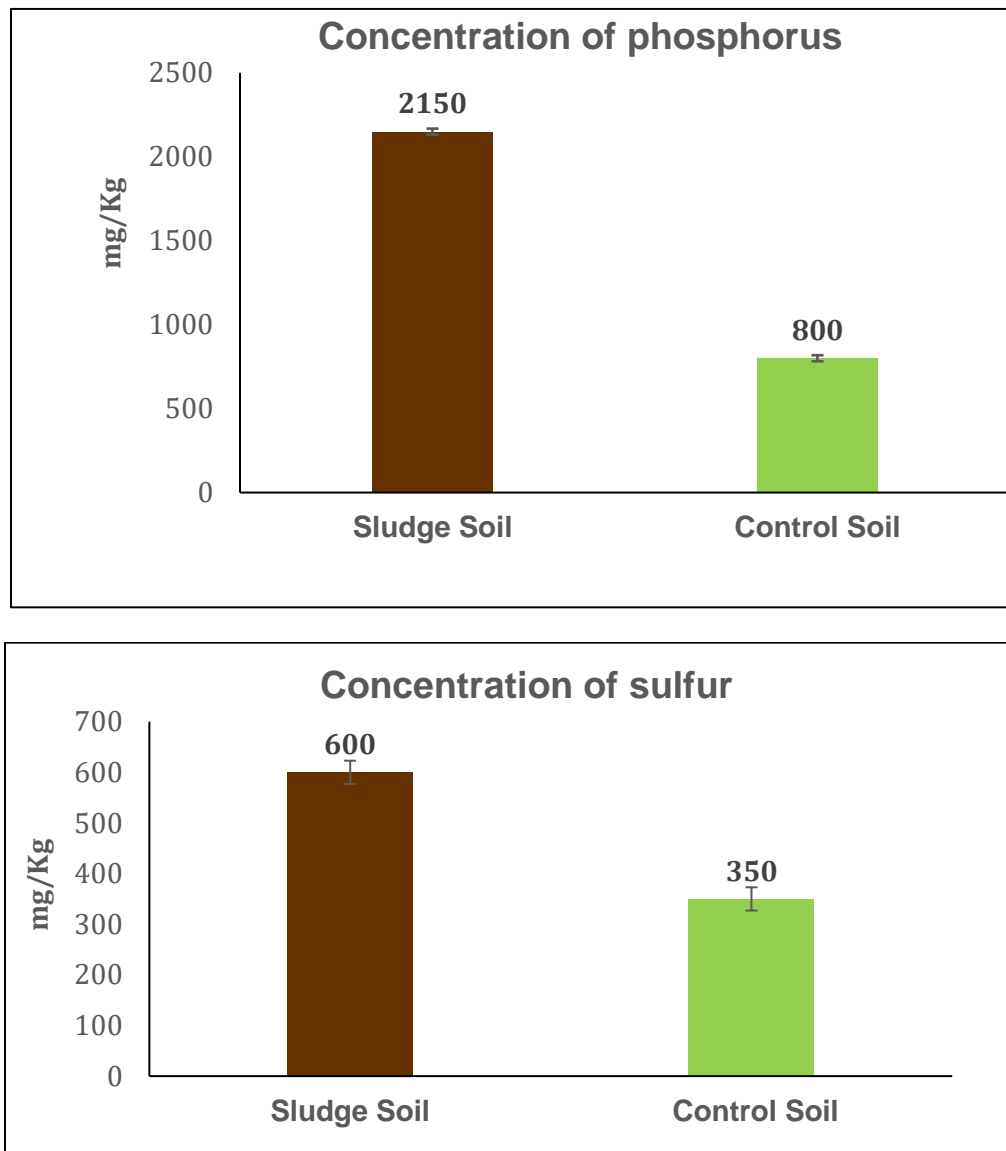
**Figure 2.2.** Results for soil total nitrogen and carbon (n = 12 for each). Error bars show standard deviation. Asterisks represent significance according to Student's t-test at  $p < 0.001$ .



The results of the test for total nitrogen and carbon (Figure 2.2) showed that sludge soil contains a slightly higher level of nitrogen. Control soil (C1) contained 0.3% nitrogen, and sludge soil (S1) contained 0.4% nitrogen. The concentration of carbon is 3.6% in control soil (C1) and 4.6% in sludge soil (S1). (This study did not distinguish between organic and inorganic carbon.) These higher N and C concentrations in S1 are expected from the higher level of residual organic matter.

Figure 2.3 shows the total concentrations of sulfur and phosphorus measured in sludge soil and control soil.

**Figure 2.3.** Total concentration of sulfur and phosphorus in control soil (C1) and soil-sludge (S1) (n = 12 for each). Error bars show standard deviation.



The sludge soil contains unusually high concentrations of sulfur and phosphorus compared to most agricultural soils. Sulfur in the sludge soil is approximately two times higher than in the control soil, and phosphorus is approximately three times higher than in control soil. By comparison with an agricultural field site, soil from Musgrave Research Farm of Cornell University, Aurora, NY, contains around 900 ppm phosphorus and 250 ppm sulfur. The soil from this Aurora farm has been fertilized every year for many decades. To summarize, these results

show that soil amended with a sewage sludge nearly half a century ago still contains a high level of organic matter which is degrading very slowly, and quite elevated concentrations of phosphorus and sulfur that are likely associated in part with the residual organic matter.

One potential reason for the slow degradation of organic matter in the sludge soil is its high concentration of metals. (Hattori, 1996) studied the decomposition of two sludges: one with Cd and one without Cd as a control. At 30°C, the control sludge decomposed in 4 weeks. The sludge with Cd did not decompose. The heavy metals adsorbed onto organic matter in soil decrease enzymatic activity, which may inhibit organic matter decomposition (Tyler 1974; Ebregt et al., 1977). Another study in the East Midlands of the UK, where biosolids were applied for around 100 years had an elevated level of organic matter of 10% with quite high concentrations of heavy metals Ni, Cu, Zn, Cd, and Pb were 114, 198, 565, 10, and 246 mg/kg, respectively (Mossa et al., 2020).

The measured concentrations of selected trace elements and toxic metals in sludge soil (S1) and control soil (C1) based on analysis of the acid soil digests using ICP-OES are shown in Table 2.2.

**Table 2.2.** Total concentration of metals, sulfur and phosphorus in sludge soil (S1) & control soil (C1). Standard deviations are given in parentheses (n = 12 for each measurement).

	<b>Sludge Soil (2015)</b>	<b>Control Soil (2015)</b>
	<b>mg/kg</b>	<b>mg/kg</b>
<b>Cadmium</b>	12.7(0.4)	0.2(0.01)
<b>Chromium</b>	187 (6.8)	22 (0.5)
<b>Copper</b>	128(4.2)	16(0.4)
<b>Manganese</b>	661(12.4)	684(7.9)
<b>Nickel</b>	49(1.12)	20(0.4)
<b>Lead</b>	155(3.9)	113(4.6)
<b>Zinc</b>	678(3.2)	90 (1.5)
<b>Silver</b>	3.3(0.1)	-
<b>Phosphorus</b>	2150(53.9)	815(18.8)
<b>Sulfur</b>	599 (23.7)	350(7.8)
<b>Iron</b>	27071(86.4)	22081(38)

The concentrations of all metals except Mn, and Pb are much higher in the sludge soil than control soil. Manganese and lead have almost the same concentrations in the sludge-amended and control soils. Since the concentration of Mn in uncontaminated mineral soils of NY State is typical around 600-700 mg/kg, it appears that the sludge soil contains a normal level of Mn, suggesting that the applied sewage sludge did not contain an excessive concentration of this trace metal. In contrast, the Pb concentrations in both the sludge soil and control soil are higher than expected for uncontaminated mineral soils of New York State; this high soil Pb level is almost certainly the residue of Pb arsenate spray that was used in the apple orchard to control codling moth in the first part of the 20<sup>th</sup> century. Normal background levels of Pb in New York rural soils are typically in the range of 10 to 20 mg/kg.

The trace metals with much higher concentrations in S1 than in C1 (Cd, Cr, Cu, Ni, Zn, and Ag) are present in the sludge soil as a result of the heavy sludge amendment, as the particular sludge applied in the orchard contained very high concentrations of these particular metals (McBride et al., 1997). These trace metals are not typically found in such high concentrations unless soils have been severely contaminated by anthropogenic activities. For



example, the concentration of Cd is approximately 65 times higher in the sludge soil than the control soil. The concentrations of Cu and Zn are 8 times higher in the sludge soil than in the control soil. The level of Ni in the sludge soil is approximately 2.5 times higher than in the control soil. These excessively high levels of metals in the soil, if they have a significant degree of bioavailability, can negatively impact soil microbial activity and plant growth. In addition, plant uptake of Cd in particular could produce crops that are toxic to animals or humans (Wuana et al., 2011; Heffron et al., 1980). These considerations will be the subject of the following chapters of this thesis.

## **Discussion and Conclusion**

The results presented in this chapter showed that the sludge soil and control soil have very different chemical and physical properties despite the fact that 40 years has passed since the sludge application. The soil treated with sewage sludge (S1) in 1977 has today a higher pH, higher level of organic matter, phosphorus and sulfur, and higher concentrations of toxic metals including Cd, Zn, Cu, and Ni than a nearby untreated soil. While Zn and Cu are essential micronutrients for plant and animal life, excessive uptake of these elements can negatively affect plant growth (Wuana et al., 2011). Although most soil Ni is immobilized by adsorption processes, Ni can become mobile at low pH or when any organic matter it combines with is degraded, and as a result can leach into groundwater (Wuana et al., 2011).

Cd is highly soluble in water and has been shown to interfere with the uptake, transport, and use of several essential elements (Ca, Mg, P, and K) and water by plants. Cd reduces the absorption of nitrate and its transport from roots to shoots by inhibiting nitrate reductase activity in the shoots (Saha, 2015). Shahid et al., (2016) noted that the bioavailability of Cd depends on the pH of the soil, with a pH around 4-4.5 increasing the bioavailability of Cd compared with a pH of 7.5. But Heffron et al., (1980) studied corn grown in sludge fertilized soil with near-neutral pH sludge (which contained a high concentration of Cd) and soil without sludge. Corn grown in

sludge contained a high level of Cd despite the neutral pH. (One reason for this may be that plant root exudates could solubilize Cd in the rhizosphere, promoting uptake into the roots even at higher pH.) After sheep were fed this corn, their research found that the kidneys and livers of these livestock contained high concentration of Cd.

In 1997, McBride and Richards analyzed the same sludged soil at the Cornell Orchards site that is under study here, but only 20

years after the sludge application. In 1997, the level of soil organic matter was measured as 12%, whereas the level of organic matter in the sludge soil was measured as 7.4% in 2015 (this study). Since the organic matter levels in 1997 and 2015 were analyzed using the same method, loss on ignition (LOI), it therefore seems likely that the level of organic matter in the sludge soil has decreased since 1997. Furthermore, between 1997 and 2015, the measured soil levels of toxic metals appeared to decrease. Cd, Cu, and Zn decreased by approximately 2.5 times and Ni by approximately 2 times (see Table 2.3). Consistent with the loss of organic matter, it seems likely that the concentrations of metals decreased as organic matter-bound metals were released into soil solution and leached.

However, the validity of this comparison between the soil measurements made in 1997 and 2015 must be viewed with caution. For instance, the plot where sludge was applied is around 0.4 acres in area, and the amount of sludge applied was not uniform across the plot. Thus, the soil samples selected for analysis for the two studies could have come from locations with different initial sludge metal loadings. Additionally, the analytical instruments in use today have much higher sensitivity than those used in 1997.

**Table 2.3.** Concentration of trace elements and organic matter in sludge soil measured in 2015 and 1997.

	<b>Sludge Soil (2015)</b>	<b>Sludge Soil (1997)</b>
	<b>mg/kg</b>	<b>mg/kg</b>
<b>Cadmium</b>	13	34
<b>Chromium</b>	188	490
<b>Copper</b>	129	321
<b>Nickel</b>	49	93
<b>Zinc</b>	678	1660
<b>Silver</b>	3.3	11
<b>Phosphorus</b>	2150	5630
<b>Sulfur</b>	599	1260
	%	%
<b>Organic matter</b>	7.4	12

Knowledge of the total concentration of toxic metals in contaminated soils is insufficient to evaluate the environmental impact of metal contamination. The evaluation of metal behavior, including solubility, mobility, bioavailability, and toxicity requires the identification of an appropriate method for quantification and qualification of metal bonding to specific phases in soil. While high levels of mature organic matter in sewage sludges, which resist decomposition, can retain metals and protect plants and microbes from metal toxicity for years or decades, inorganic residues such as phosphates, silicates, and iron, aluminum and manganese oxides also exist in sewage sludges which in the long term may either adsorb or form insoluble precipitates with metals. To better understand how these metals behave in the environment, the following chapters analyze their behavior in sewage sludge-amended soil using the sequential extraction method to determine metal bonding and form and using a leaching test to measure mobility and the rate of toxic metal loss from the surface soil. Plant growth is also assessed to evaluate any phytotoxicity.

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# Chapter 3: Characterization of Toxic Metal Availability in a Long-Term Sewage Sludge-Amended Soil using Sequential Extraction

## Introduction

Knowledge of the total concentration of toxic metals in contaminated soils is insufficient to evaluate the environmental impact of metal contamination. In order to more fully assess metal behavior in soil, including solubility, mobility, bioavailability, and toxicity, selective chemical extraction tests are frequently employed. Sequential extraction, a method involving repeated dissolution of the metals in a soil using increasingly aggressive reagents, has been considered to be a way to evaluate the lability of metals and the likely solid phases with which the metals are associated. A number of sequential extraction methods for metals in soils have been developed. One of the most commonly used is that of Tessier et al (1979), which uses five chemical extraction steps to determine the amounts of metals associated with the different phases of the metals retained in soil and sediments. This method presumes to extract in sequential steps metals adsorbed on exchange sites, carbonates, iron and manganese oxides, and organic matter. Metal not extractable by any of these steps is considered to be in a residual state that is essentially unavailable. However, in this procedure, steps 3 and 4 both use strongly acidic solutions which are not highly selective, with step 3 almost certainly releasing metals from sites in addition to the intended Fe and Mn oxide phase. Therefore, this selectivity error in step 3 carries over into step 4, with the result that the fraction of metals bound to organic matter may be severely underestimated by the Tessier and similar sequential extraction schemes. Because of this poor selectivity as well as the problem of metal reabsorption during the extraction steps (Zimmerman et al., 2010), the accuracy and validity of this method are questionable when applied to determine metal bonding properties in contaminated soils such as sewage sludge-amended soils.

The European Community Bureau of Reference (BCR) proposed a somewhat simplified three-step sequential extraction method, which is similar to the Tessier method but combines the first 2 steps into a single step that presumes to extract soluble, exchangeable and carbonate-bound metals. It therefore suffers from the same weaknesses as the Tessier method. For example, the low pH (1.5-2.0) in the second extraction step of the BCR sequential extraction method is poorly selective in spite of its intended purpose of dissolving only metals bound to Fe and Mn oxides, and is very likely to release metals from various mineral-bound and organic-bound sites (Kim et al., 2006). Thus, the BCR method, like the Tessier method, fails to distinguish metal bonding to soil organic matter from other mechanisms of metal retention in soils such as chemisorption on minerals and precipitation with phosphates or silicates.

Most published studies of sequential extraction have used the Tessier or BCR methods in an attempt to speciate metals in the solid phase of soils. Awokunmi et al. (2015) analyzed soil from cocoa wasteland farms with a low level of organic matter using the Tessier procedure as a basis. Li et al (1995) also used the Tessier procedure but changed extractants in step 4, using 0.02 M HNO<sub>3</sub> and 30% hydrogen peroxide to extract metals bound to organic matter and sulfides. The extraction procedures of the Tessier and the BCR method are particularly unsuitable for organic soils, in which the fixation of heavy metals may be controlled by the chemistry and mineralogy of sulfur compounds. Although the selectivity of the standard extractants in the Tessier procedure may be unsuitable for soils rich in organic matter, it may be more appropriate and useful for sandy soils (Vodyanitskii, 2006).

Here, I describe a new method for sequential extraction for quantification and qualification of metal bonding to specific phases in soil. I use this method to compare the availability of toxic metals in soil contaminated by metals from a heavy application of municipal sewage sludge some 40 years ago to that of an untreated (control) soil from the same field. These two soils have very different levels of organic matter, pH, and total concentrations of metals as is shown in Tables 2.1 and 2.2 (shown below; originally shown in Chapter 2).

**Table 2.1 (reprinted from Chapter 2).** pH, organic matter content, and texture analysis of sludge soil and control soil.

<b>Sample</b>	<b>pH</b>	<b>Organic matter (%)</b>	<b>Sand (%)</b>	<b>Silt (%)</b>	<b>Clay (%)</b>
<b>C1</b>	5.1	2.8	3.0	75.2	21.8
<b>S1</b>	6.7	7.4	12.0	62.6	25.3

**Table 2.2 (reprinted from Chapter 2).** Total concentration of metals, sulfur and phosphorus in sludge soil (S1) & control soil (C1). Standard deviations are given in parentheses (n = 12 for each measurement).

	<b>Sludge Soil (2015)</b>	<b>Control Soil (2015)</b>
	<b>mg/kg</b>	<b>mg/kg</b>
<b>Cadmium</b>	12.7(0.4)	0.2(0.01)
<b>Chromium</b>	187 (6.8)	22 (0.5)
<b>Copper</b>	128(4.2)	16(0.4)
<b>Manganese</b>	661(12.4)	684(7.9)
<b>Nickel</b>	49(1.12)	20(0.4)
<b>Lead</b>	155(3.9)	113(4.6)
<b>Zinc</b>	678(3.2)	90 (1.5)
<b>Silver</b>	3.3(0.1)	-
<b>Phosphorus</b>	2150(53.9)	815(18.8)
<b>Sulfur</b>	599 (23.7)	350(7.8)
<b>Iron</b>	27071(86.4)	22081(38)

The sequential extraction method employed here reduces the pH level progressively, employing reagents commonly used in agricultural soil testing. This extraction method allows the present study to analyze available nutrients, including sulfur and phosphorus, in addition to the toxic metals of concern. The results of this sequential extraction help characterize and explain the behavior of potentially toxic metals including cadmium (Cd), copper (Cu), nickel (Ni), zinc (Zn), and lead (Pb) in a field-aged sewage sludge-amended soil. Extraction of metals using



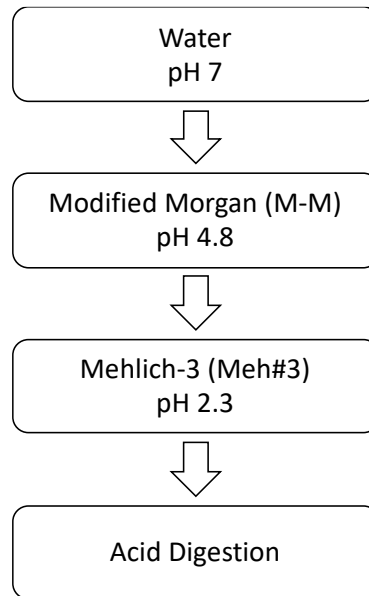
a progressively decreasing pH level provides a measure of the strength of adsorption and the possible mineral or organic phases responsible for adsorption.

## Materials and Methods

### The Sequential Extraction Method

A schematic presentation of the sequential extraction method used in the present study is shown in shown Fig. 3.1.

**Figure 3.1.** Flow diagram of sequential extraction.



The sequential extraction method of soils consists of the following four steps with all extracts filtered and analyzed using ICP – OES and EPA method 6010B (see Appendix for analytical details).

1. The first step extracts with Deionized Water (DI) at pH 7 to determine the solubility of metals in water.
2. The second step extracts with Modified Morgan solution ( $\text{NH}_4$  acetate buffer, pH 4.8) to measure the weakly-bound fraction of metals, including metals bonded to carbonate, sulfate and weak organic complexes.

3. The third step uses Mehlich-3 solution with a strongly acidic pH (2.3) to solubilize metal cations bound to phosphate compounds, silicate clays, and organic matter, and Mn and Fe oxides.
4. The final step involves digestion of the soil with nitric acid perchloric acid (adapted from EPA method 3050-3051) to solubilize metals retained in the immobile (residual) fraction.

### **Collection and analysis of soil samples**

In 2015, soil samples were collected from the surface soil (0-30 cm deep) at the Cornell Orchards (the “Orchard”) in Ithaca, NY. Two types of samples were collected: soil that had been amended with municipal sludge (“sludge soil”, S1) where a high-Ca sludge (pH 6.7) was applied in 1978 (Heffron et al., 1980), and soil had not received sludge treatment (“control soil”, C1). From 1979 to 1983, the sludge soil was cropped with the addition of some commercial fertilizer. After the soil was limed in 1985 and deep plowed, it remained fallow for much of the subsequent period and was planted to sod grass in 1986 (McBride et al., 1997). The Orchard soil is a silty-clay loam (pH 5.1), Hudson series, classified as fine, illitic, mesic Glossaquic Hapludalf (McBride et al., 1997). All soil samples were thoroughly homogenized and passed through a 2mm sieve.

### **Results**

The results from the sequential extraction method applied to soil from the long-term sewage sludge application site in the Cornell Orchard (S1) and to a control soil from the same Orchard (C1) are shown in Table 3.1 for the metals of concern, including Cd, Zn, Cu, Ni and Pb.

**Table 3.1.** Results of the Sequential Extraction Method applied to sludge soil (S1) and control soil (C1) from the Cornell Orchard Site. Values are mean concentration (mg/kg) of elements in soil (n=6). Standard deviations in parentheses.

	<b>Sample</b>	<b>Cd</b>	<b>Zn</b>	<b>Cu</b>	<b>Ni</b>	<b>Pb</b>	<b>Mn</b>	<b>Fe</b>
<b>Water</b>	C1	0.001 (0.001)	0.06 (0.003)	0.07 (0.03)	0.01 (0.007)	0.04 (0.003)	0.65 (0.05)	8.84 (1.2)
	S1	0.01 (0.002)	0.3 (0.06)	0.30 (0.04)	0.06 (0.005)	0.03 (0.11)	0.21 (0.07)	9.27 (0.12)
<b>Modified Morgan</b>	C1	0.19 (0.005)	1.99 (0.007)	0.65 (0.024)	0.36 (0.0007)	11.07 (0.25)	144.7 (1.09)	42.4 (1.33)
	S1	4.99 (0.005)	46.50 (0.005)	3.57 (0.023)	1.16 (0.003)	4.97 (0.012)	79.2 (0.21)	5.7 (0.05)
<b>Mehlich-3</b>	C1	0.03 (0.001)	1.52 (0.07)	0.20 (0.01)	0.21 (0.03)	1.02 (0.04)	144.4 (0.37)	121.5 (2.6)
	S1	1.23 (0.03)	57.02 (2.7)	8.69 (0.18)	1.12 (0.02)	4.24 (0.3)	101.3 (0.16)	69.2 (2.5)
<b>Digestion after extraction</b>	C1	0.00	74.97 (0.004)	16.03 (0.003)	19.10 (0.001)	95.06 (0.04)	430 (0.4)	21812 (0.8)
	S1	7.2 (0.004)	578 (0.18)	125.4 (0.01)	48.0 (0.01)	143.3 (0.05)	501 (0.4)	27943 (8.1)
<b>Sum of concentration of metals from sequential extraction</b>	C1	0.23	78.5	17	19.7	95.06	720	21985
	S1	13.4	682	138	50.3	143.3	682	28026
<b>Total concentration of metals from trace element analysis (see Ch. 1)</b>	C1	0.24	90	17	20	113	684	22081
	S1	12.7	678	129	49	155	661	27071

The sequential extraction test revealed that only a very small fraction of the total metals (Cd, Zn, Cu, Ni, Pb) in the soils were extractable in water. The relatively mild extractant, Modified Morgan solution (pH 4.8) extracted a larger fraction than water, with Cd being the most effectively extracted by this reagent. This also suggests the ease with which Cd may accumulate in crops. For soil S1, the fraction of total metals extracted by Modified Morgan followed the order Cd >> Zn >Pb> Cu> Ni. In the control soil (C1), a large fraction of the total Cd was solubilized by the Modified Morgan extraction step, and Pb was also more readily extracted by this step than was the case for the S1 soil. The efficiency of metal extraction from soil C1 by Modified Morgan was Cd >>Pb>Cu >Zn > Ni. In the sludge soil (S1), where the amount of organic matter and total sulfur are still very high, chalcophilic metals such as Zn and Cd may be chelated by ligands containing thiol functional groups. These functional groups ultimately are degraded by oxidation over time, releasing sulfate and the metal ions to soluble forms (Martinez et al., 1999; Martinez et al., 2006). The concentration of sulfur in the sludge-soil is still about 600 mg/kg, about twice that of the control soil (350 mg/kg). It is possible that thiol ligands in the residual organic matter of soil S1 may still account for the lower relative Modified Morgan-extractable fraction of total Pb and Cd from soil S1 (0.035 and 0.37, respectively) than from soil C1 (0.116 and 0.82, respectively). Nevertheless, the high extractability of Cd by this mild extracting reagent indicates a high lability and bioavailability of Cd in both the control and sludge soils.

The third step in sequential extraction was the Mehlich-3 solution. This extractant has a strongly acidic pH (2.3) owing to the reagent 0.1 M HNO<sub>3</sub> and was initially designed to extract important soil nutrients including phosphate, potassium and calcium, but trace metals bound to silicate clay minerals and also some inorganic complexes of Mn and Fe oxides are readily extractable with Mehlich-3 (Beck, 1999). Table 3.2 displays the extraction of Mn, Fe, P and S in Modified Morgan and Mehlich-3 in the sequential test. Analysis of the data shows that Mehlich-3 is the more aggressive extractant. Sulfur in the Mehlich-3 extract is 10 times higher than in

Modified Morgan extract, despite the fact that extraction in Modified Morgan was done first. Sulfur compounds may control the fixation of heavy metals in organic matter (Vodyanitskii, 2006). The higher amount of sulfur in Mehlich-3 extract suggests that Mehlich-3 destroys or oxidizes part of the organic matter in the soil. Although Fe, Mn and P were extracted in Modified Morgan, they were even more extractable in Mehlich-3. This suggests that Mehlich-3 can extract some inorganic or organic complexes of Mn and Fe oxides (Beck, 1999).

**Table 3.2.** Extractable soil concentrations of Fe, Mn, P, and S in the sequential extraction test.

Samples	Fe	Mn	P	S
	mg/Kg	mg/Kg	mg/Kg	mg/Kg
<b>Modified Morgan</b>				
Control	42.4	144.7	270	4.3
Sludge Soil	5.7	79.2	722	6.5
<b>Mehlich-3</b>				
Control	121.5	144.4	16.5	60.7
Sludge Soil	69.2	101.3	164	62.7

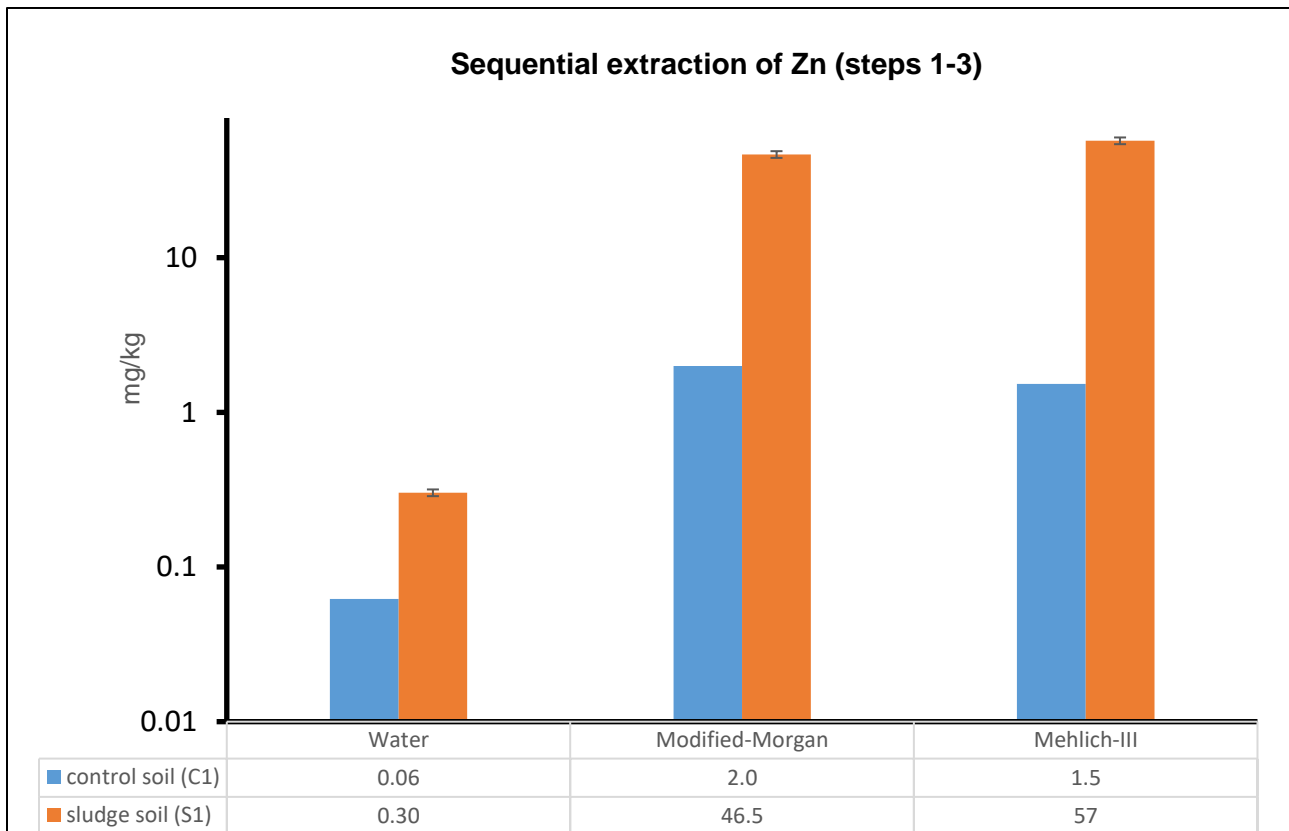
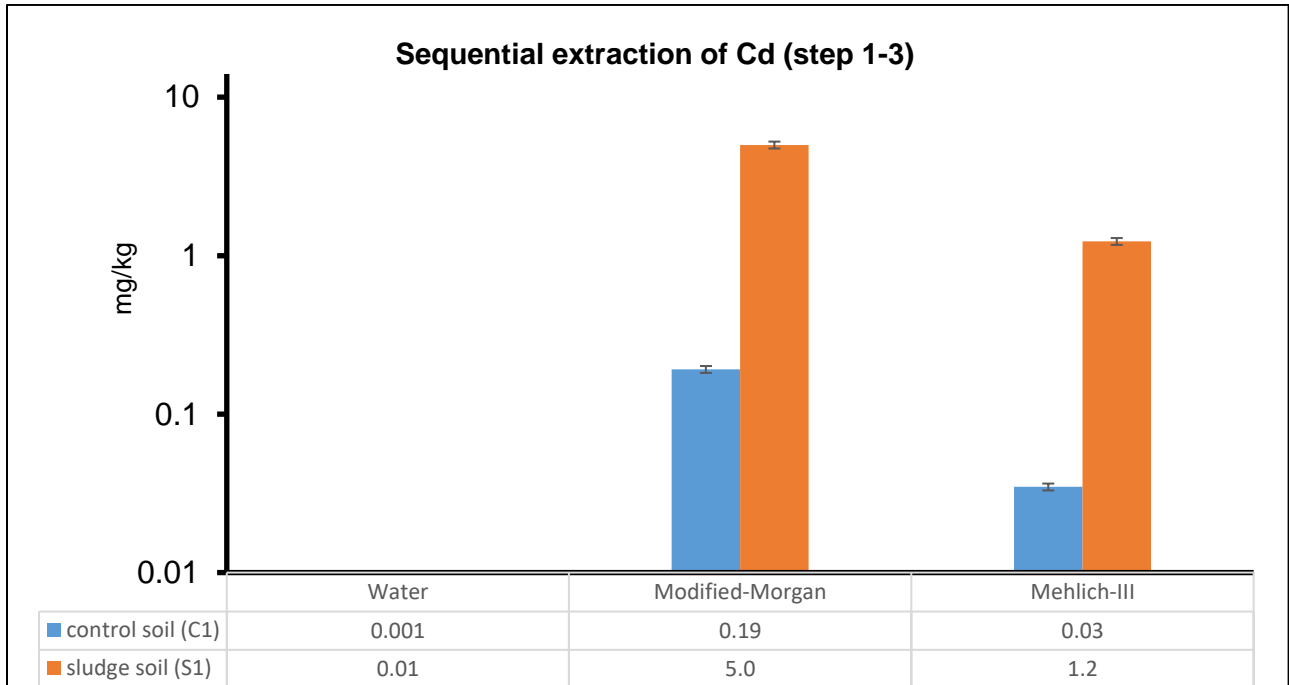
Previous studies have shown that Mehlich-3 extracts on average about 30% of trace metals such as Cu, Zn, Cd and Ni, but closer to 50 % of Pb (Sims et al., 1991; Minca et al., 2013). This extractant solubilized a larger fraction of Cu, and Zn from the sludge soil than Modified Morgan, presumably because of its very strongly acidic nature; however, similar fractions of Pb and Ni were extracted by Mehlich-3 compared to the previous Modified Morgan step, suggesting very strong bonding of these two metals in sludge soil S1. A much lower fraction of Cd extracted by Mehlich-3 from S1 is probably the consequence of the large fraction solubilized by Modified Morgan in the previous step, leaving a very strong bonded “residual” form of Cd comprising roughly half the total Cd in soil S1. In the control soil (C1), Mehlich-3 extracted only between 1 and 2% of the total soil Zn, Cu, Ni and Pb. Cadmium was the exception to this, with 13 % of total Cd extracted. Mehlich-3 extracted a larger fraction of Zn, Cu, Ni and Pb from soil S1 than from C1, but a smaller fraction of Cd. This may indicate that Mehlich-3 can more readily extract most metals bound to the organic matter of the sludge soil.

The sum of the total concentrations of the extractable metals Cd, Cu, Ni, Zn and Pb in the sequential extraction, added to the residual concentration of these metals obtained from acid digestion, should equal the total concentration of these metal in the soils S1 and C1 as calculated from a separate analysis of the soils for total metals after acid digestion. There is very good mass balance agreement between the sums of the extractable fractions and the separately determined totals as shown in Table 3.1, providing evidence that the sequential extraction method is precise and has a low cumulative error.

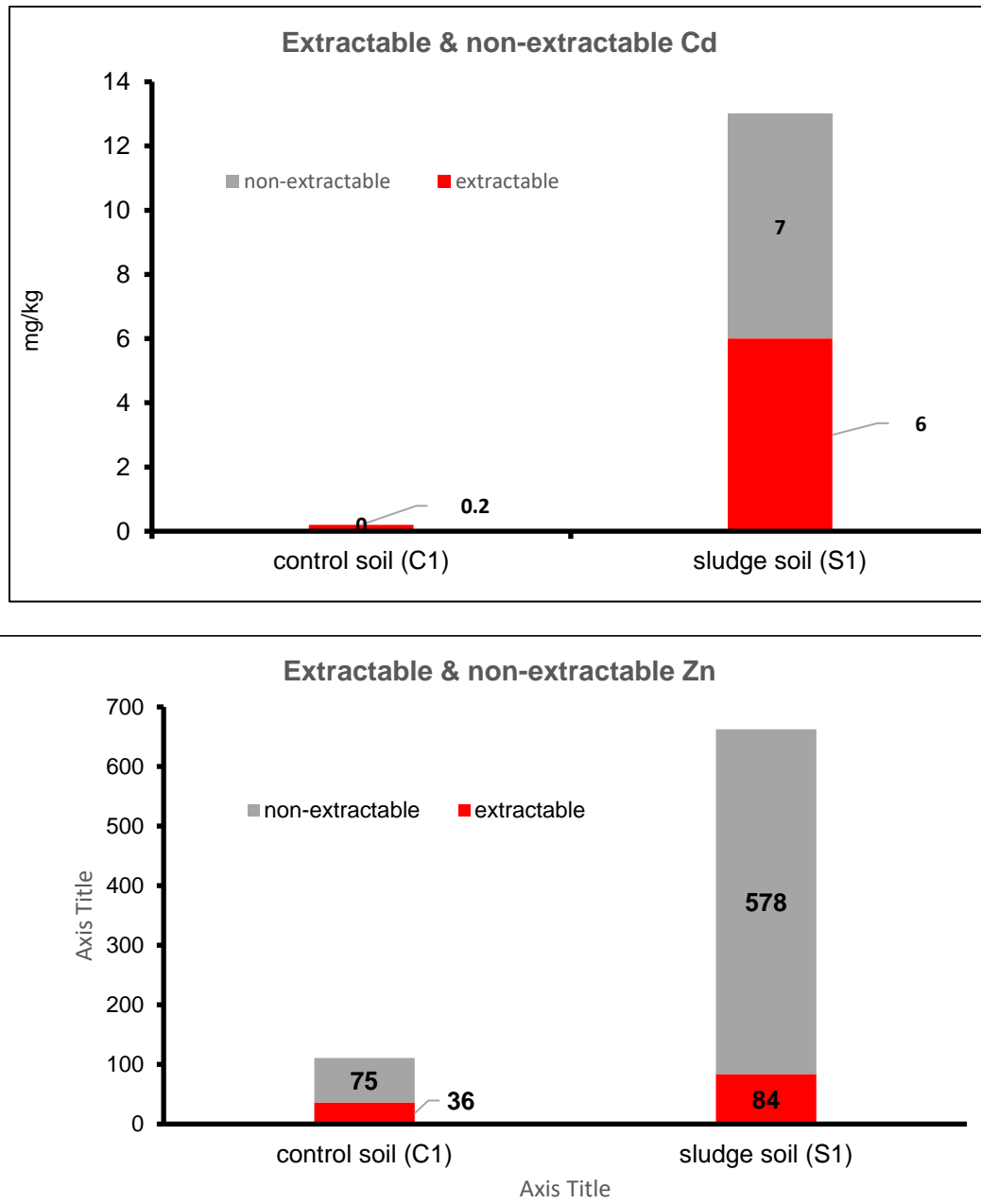
### **Extractable and non-extractable Cd and Zn fractions**

Figure 3.2 displays graphically the sequential extraction results for Cd and Zn. In Figure 3.3, the total extractable (recovered in steps 1–3 of the sequential extraction method) and non-extractable (recovered only in step 4 of the sequential extraction method by digestion of the soil) fractions of Cd and Zn are shown. Approximately half of the total Cd in sludge soil is extractable (6 mg/kg of Cd is extractable and 7 mg/kg is non-extractable or residual). Nevertheless, despite the neutral pH and high level of organic matter, Cd is very mobile in the sludge soil. For Zn, only one-sixth of the total concentration was extractable from the sludge soil. Although the sludge soil contains a higher total concentration of Zn than the control soil, a smaller fraction of the total Zn is extractable from the sludge soil than from the control soil (~13% for sludge soil versus ~32% for control soil). It is possible that much of the Zn in the sludge soil is strongly complexed with organic matter, but it must also be considered that the sludge soil pH is near neutral (compared to the control soil which has a strongly acidic pH and low organic matter content). In the control soil, essentially all of the Cd is extractable, and one-third of the Zn is extractable, as expected at the lower pH of this soil.

**Figure 3.2.** Results from the first three steps of sequential extraction of Cd (top chart) and Zn (bottom chart).



**Figure 3.3.** Extractable and non-extractable fractions of Cd and Zn (after digestion of the soil) from sludge-soil (S1) and control soil (C1) samples.



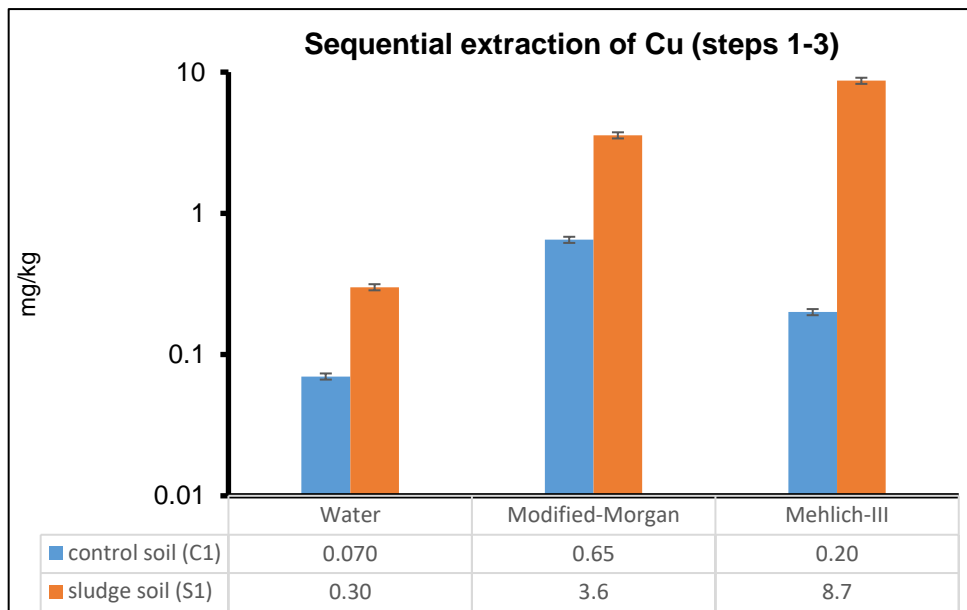
**Extractable and non-extractable Cu fractions**

Copper generally is more strongly associated with soil organic matter than most other metals (McBride, 1994). The sludge soil S1 with higher organic matter content than the control soil could be expected to bond Cu very strongly. As the pH decreases with each step of the

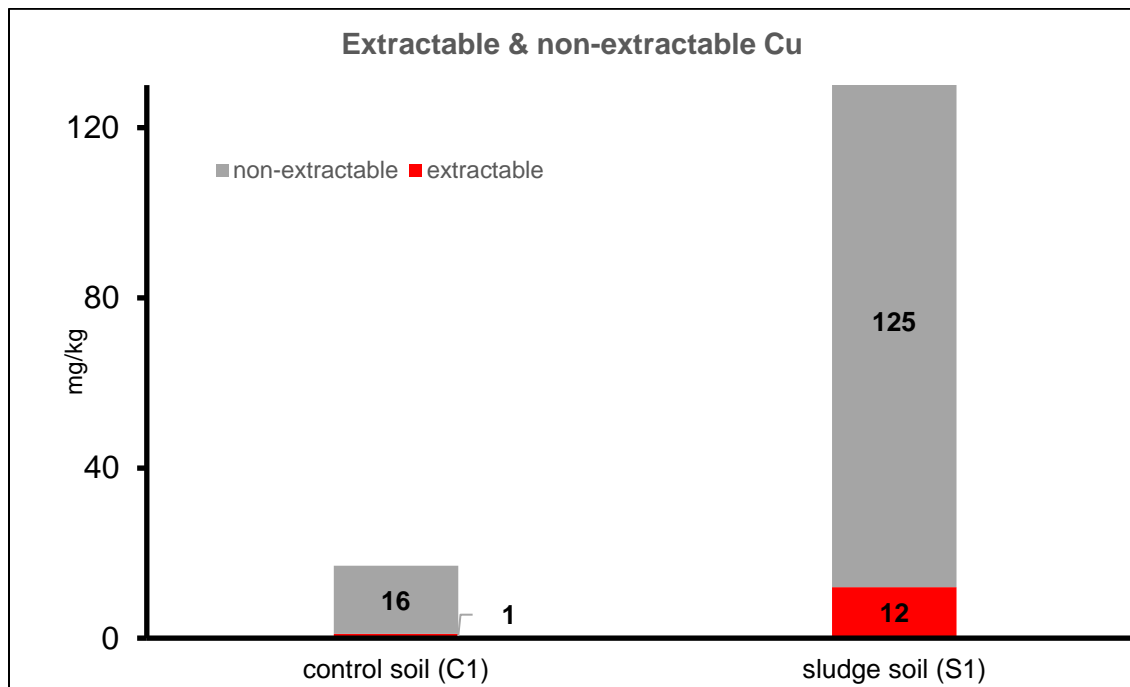


sequential extraction method, functional groups on the organic matter are increasingly protonated, releasing  $\text{Cu}^{2+}$  and other metal ions. As shown in Table 3.1 and Figure 3.4, Cu was only weakly extracted from soil S1 by water, but was extracted to some degree by Modified–Morgan solution, and to an even greater extent by Mehlich-3 solution at pH 2.3. Thus, the extractable concentration of Cu depended strongly on the pH of the extract. However, as shown in Figure 3.5, only about 10% of Cu was extractable from the sludge soil by the first 3 sequential extraction steps, a likely indication of the very strong affinity of Cu for residual organic matter in the soil.

**Figure 3.4.** Results from the first three steps of sequential extractions Cu for control soil and sludge soil.



**Figure3.5.** Extractable and non-extractable fractions of Cu (after digestion of the soil) from sludge-soil (S1) and control soil (C1) samples.



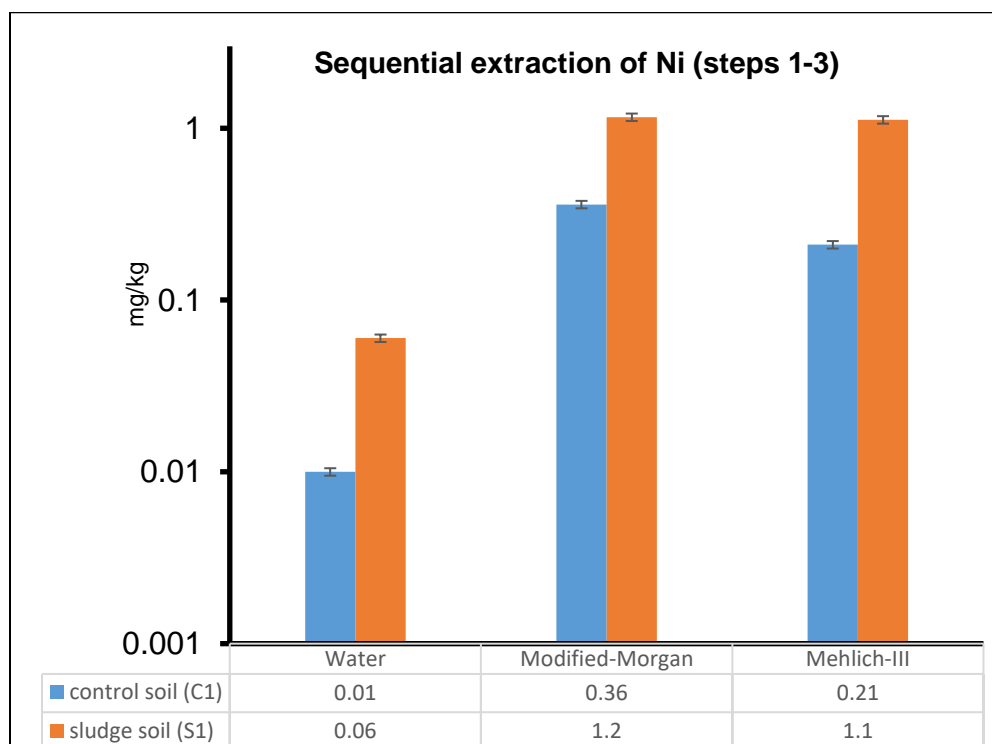
Copper behaves differently in the control soil (C1), where organic matter content is low compared to the sludge soil (S1). The total concentration of Cu in the control sample (C1) is 8 times lower than in S1, and pH in the control sample (C1) is also lower (around 5.6). Mehlich-3 solution is acidic enough to dissociate some Cu from bonds with organic matter, although only about 6% of the total Cu in soil S1. The control soil (C1) has not been fertilized for more than 30 years and therefore the organic matter is at a very low level. Nevertheless, the amount of Cu extractable from soil C1 is only about 5% of the total. The much higher extractable concentration of Cu in the sludge soil S1, in comparison with the control soil (C1), shows that application of high-Cu sewage sludge to soil can continue to affect the available quantity of Cu in this soil after almost half a century.

## Extractable and non-extractable Ni fractions

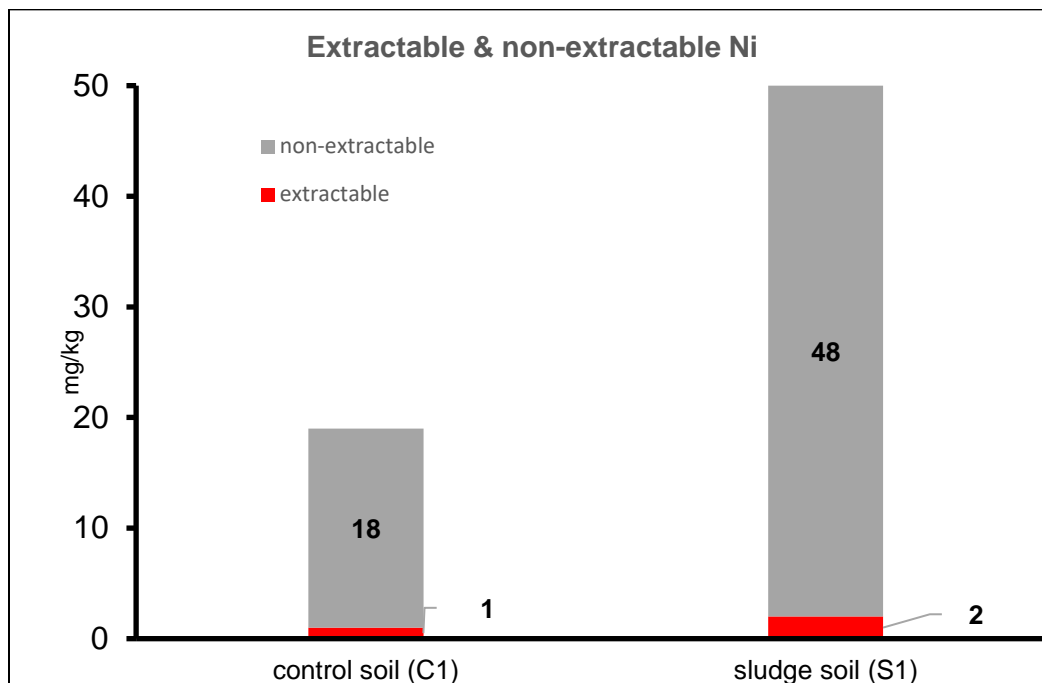
Figure 3.6 displays graphically the sequential extraction results for Ni. In Figure 3.7, the total extractable (recovered in steps 1–3 of the sequential extraction method) and non-extractable Ni (recovered only in step 4 of the sequential extraction method by digestion of the soil) are shown.

The extractable Ni is a very small fraction of total Ni in both the sludge soil (4%) and control soil (5%) samples (see Figure 3.7). The Ni may have complexed with insoluble organic matter, or chemisorbed on surfaces of silicate minerals, Fe oxides or Mn oxides.

**Figure 3.6.** Results from the first three steps of sequential extractions of Ni.



**Figure 3.7.** Extractable and non-extractable fractions of Ni (after digestion of the soil) from sludge-soil (S1) and control soil (C1) samples.



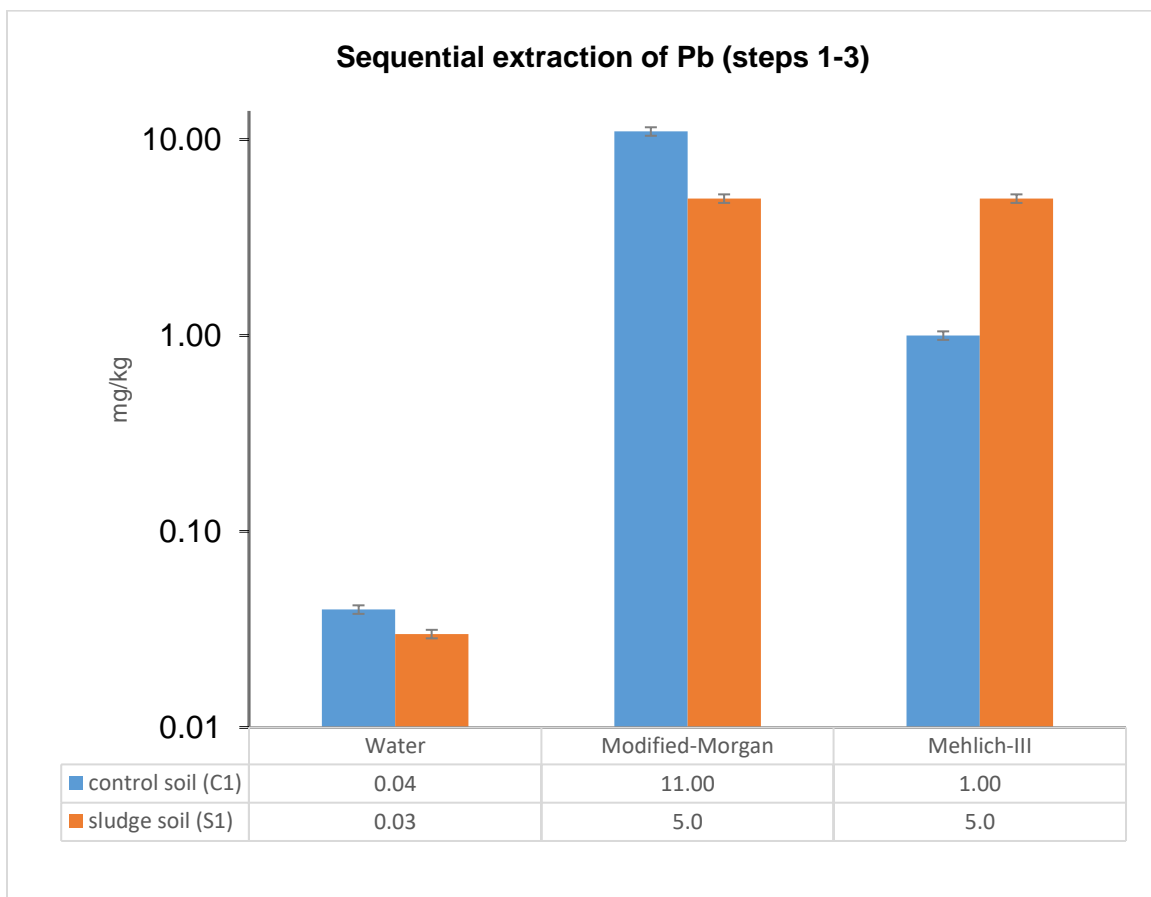
### Extractable and non-extractable Pb fractions

Figure 3.8 displays graphically the sequential extraction results for Pb. In Figure 3.9, the total extractable (recovered in steps 1–3 of the sequential extraction method) and non-extractable Pb (recovered only in step 4 of the sequential extraction method by digestion of the soil) are shown.

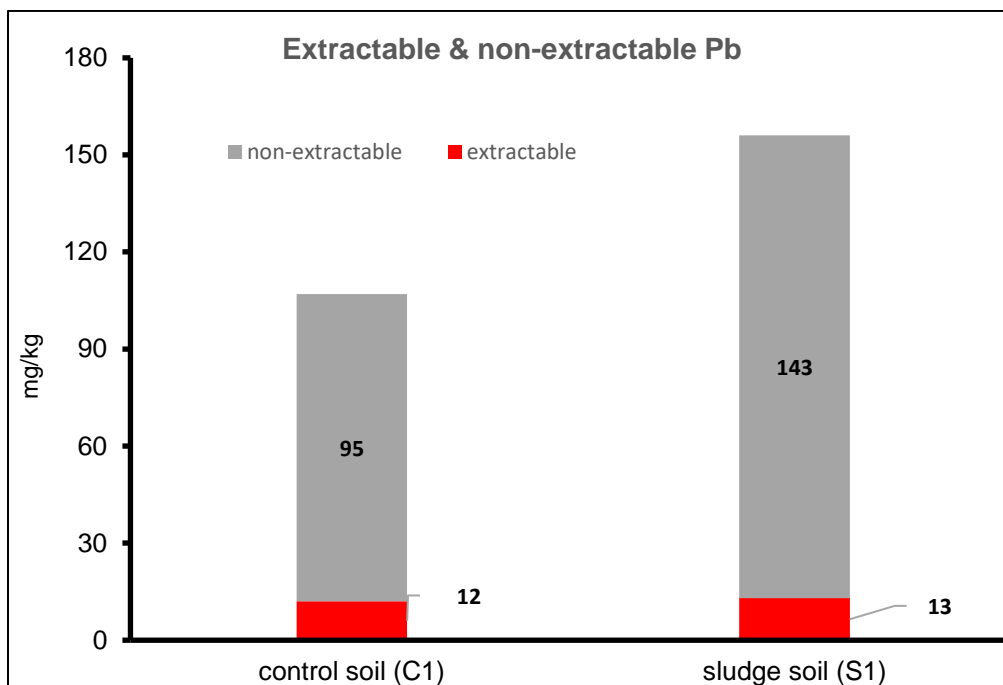
The total concentrations of Pb in soils S1 and C1 are relatively similar, 155 ppm and 113 mg/kg, respectively (see Table 2.2). However, Pb behaves differently in the sequential extraction procedure for these two soils. Factors known to impact the extractability of Pb in soils include soil pH levels, soil organic matter, and concentrations of phosphates, sulfates, and carbonates in soils. The amount of Pb extractable in water is similar for both soil C1 and S1, around 0.03-0.04 mg/kg. However, Modified Morgan solution extracts more Pb from the control

soil (C1) than from sludge soil (S1), a result consistent with the lower pH and organic matter content of soil C1. Very strong retention of Pb is a well-described characteristic of soil organic matter (McBride, 1989). There is also a very high concentration of phosphorus in the sludge soil, around 2%, raising the possibility that Pb retention in this soil could be in part due to the formation of the relatively insoluble Pb phosphate (Kim et al., 2006, Mossa et al., 2020). While Modified–Morgan solution with pH of 4.8 may partially solubilize Pb phosphates, Mehlich-3 solution with a much lower pH of 2.3 would largely dissolve this mineral as well as dissociating Pb<sup>2+</sup> ions from functional groups of organic matter.

**Figure 3.8.** Results from the first three steps of sequential extractions of Pb.



**Figure 3.9.** Extractable and non-extractable fractions of Pb (after digestion of the soil) from sludge-soil (S1) and control soil (C1) samples.



## Discussion and Conclusion

Based on the sequential extraction results, Ni appears to be the least mobile metal in the sludge soil S1 in comparison with other metals. Conversely, there are substantial labile fractions of Cd and Zn in this soil, rendering the sewage sludge-amended soil potentially toxic to plants and soil biota despite the long term stabilization process of field aging for 40 years. However, about half of the Cd in the sludge soil is not extractable after 40 years of aging, and is in the residual fraction. This result is consistent with other observations on the long-term fate of soil Cd which suggest that slow fixation into a non-bioavailable “pool” (10-20% or more) occurs (Hamon et al., 1998). Recently, Mossa et al. (2020) have reported that between 20 and 37% of the Cd in long-term sewage sludge-amended field soils was in a labile (bioavailable) form as determined by stable isotope exchange methods. Median metal lability followed the order: Cu (37.0) > Cd (28.3) > Zn (22.1) > Ni (18.8) > Pb (4.8) in soils from these long-term application sites, with the values in parentheses being lability as a percent of total metals in the soil. Although they stated

that their results supported the sludge “protection hypothesis in which biosolids constituents help immobilize potential toxic metals”, their observations on metal lability only supported this hypothesis for Pb and Cd. In fact, the labile fraction of Cd only decreased at very high sewage sludge loadings, while appearing to increase at lower cumulative applications. Increased sewage sludge cumulative applications actually increased the % lability of Zn, Cu and Ni, apparently as a result of weaker metal binding as soil metal levels increased. This means that potential bioavailability of these three metals increases in a disproportionate (greater than linear) manner with increasing metal loading to the soil, magnifying the potential for toxic effects on plants and soil biota at higher metal loadings.

In comparison to that study, the present results based on extractability of the metals revealed the following order of metal lability: Cd (46.5) >> Zn (15.2) > Cu (9.1) > Pb (6.5) > Ni (4.7), with the values in parentheses being lability as a percent of total metals in the soil. These results again suggest that Pb and Ni are likely to have the lowest bioavailability. However, the sequential extraction method gives a substantially higher estimate of lability than the isotope exchange method for Cd, but a lower estimate for Zn and Cu. These predictions of lability by different methods need to be tested and validated using biological assays such as plant uptake studies of these metals.

A substantial fraction of organic matter in sewage sludge has persisted in the field site studied here, and appears to be resistant to decomposition over a time frame of several decades at least. This organic matter may have a critical role in retaining metals and protecting plants and microbes from metal toxicity for decades. On the other hand, the unusually high concentration of phosphorus (2150 mg/Kg) in the sludge soil may be particularly effective in limiting the extractability of Pb by forming insoluble Pb phosphate minerals. Many sewage sludges also contain high concentrations of metal carbonates and sulfides when first land-applied, but these minerals are generally unstable, solubilizing in the soil environment and potentially increasing metal solubility over several years (McBride 1995).

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# Chapter 4: Long Term Leaching of Toxic Metals in a Sewage Sludge-Amended Soil

## Introduction

Leaching is a natural soil process whereby soluble chemicals in the form of ions and molecules are moved downward in the soil profile by water from rain or snow melt. Through the leaching process, a topsoil could theoretically recover to some degree from the harmful effects of contamination by toxic metals. At the same time, however, long-term leaching of toxic metals such as Cd, Cu, Ni, Zn, and Pb could contaminate groundwater. Although long-term studies of toxic metal leaching from soils under field conditions are rare, a few such studies have reported that after sewage sludge is applied to soil and a large fraction of the organic matter has decayed, changes in the chemical properties of the soil can alter metal solubility and mobility (McBride, 1995). For example, the Woburn Market Garden experiment in the UK reported that the concentration of free cationic and soluble forms of Cd, Cu, Ni, Zn, and Pb changed several decades after sewage sludge application to soil (McGrath, 1994). Cu and Ni had the highest total solubility, ranging from 0.06 to 0.27 mg/L for Cu and from 0.04 to 0.12 mg/L for Ni. Less than 2% of the dissolved concentration of these two metals was present in the soil in free cation form. Although there was a high concentration of Zn in the sewage sludge-amended soil, it was less soluble than Cu and Ni, ranging from 0.008-0.079 mg/L. These observations suggest the importance of metal-organic complexation in controlling the long-term solubility and leachability of metals, as Cu and Ni complex more strongly with natural organic matter than Zn (McBride 1989). This fact, and the chemical similarities between Cd and Zn, most likely makes Zn the most important trace metal competitor for Cd adsorption sites in most soils (Christensen et al., 1999). Christensen (1987) showed that the presence of Zn in the concentration range  $10^{-6}$  to  $10^{-5}$  M reduced Cd adsorption by 25-50%. The organic matter of soils contains functional groups

which act as Lewis bases, some of which are relatively soft Lewis bases (e.g., sulfhydryl groups) that have a strong bonding affinity for chalcophilic metals such as Cd. Much of the humified organic matter of mineral soils is bound to minerals. Therefore, Cd may be preferentially bound to mineral-organic complexes (Christensen et al., 1999).

Grunewald (1994) described solubility of metals in farm soils in Germany that had been fertilized with sewage sludge for over a century but discontinued a long time ago. As such, long-term accumulation of heavy metals created conditions favorable to increased metal mobility in spite of the high levels of added organic matter. Soluble Zn was around 1% of the total soil Zn and water-soluble Cu and Pb in this surface soil was more than 1 mg/L. Cd was detected at 30 cm depth below the soil surface, and well water of farms with long-term use of sewage sludge was contaminated with Cd at concentrations exceeding health standards.

De Haan (1980) showed that the greatest leaching of metals such as Cu, Ni, Cd, and Zn occurs immediately after sewage sludge application to soil since biological activity and dissolved organic matter are particularly high in the early most active stages of organic matter decomposition. Most sewage sludges have pH near neutral or in the alkaline range, with some alkaline-stabilized sludges having pH values above 10. Thus, amendment of soils with sewage sludges can raise soil pH as well as dissolved organic matter (DOM) since organic matter is more soluble at nearly neutral or alkaline pH than at acid pH (Roy et al., 1998; Japenga et al., 1992; Kalbitz et al., 1998; McBride et al., 1997). Thus, the greatest risk of metal leaching to groundwater may occur in the first few months or years after sludge application (Lamy et al., 1993; de Haan, 1980). The importance of soluble metal-organic complexes in the speciation of dissolved metals was demonstrated by Aguirre-Gomez (1995), who showed that Cu and Pb dissolved in sludge-treated soil from the Cornell Orchard were in the form of organic complexes, while only a small fraction of dissolved Cd and Zn were complexed with DOM.

The current study further investigates the behavior of toxic metals in sewage sludge-amended soil using a leaching test to measure mobility and the rate of toxic metal loss from the

surface soil. Specifically, this research examines how the chemical properties of the soil (pH, level organic matter, concentration of metals) impact the solubility and mobility of metals.

## **Materials and Methods**

In 2015, soil samples were collected from the surface soil (0-30 cm deep) at the Cornell Orchards (the "Orchard") in Ithaca, NY. Two types of samples were collected. The soil that had been amended with municipal sludge ("sludge soil", S1) where a high-Ca sludge (pH 6.7) was applied in 1978 (Heffron et al., 1980). From 1979 to 1983, it was cropped with the addition of some commercial fertilizer. After the soil was limed in 1985 and deep plowed, it remained fallow for much of the subsequent period and was planted to sod grass in 1986 (McBride et al., 1997). The control soil (C1) was taken from an area of the same field that had not received sludge treatment. The Orchard soil of this field site is a silty-clay loam with pH 5.1, Hudson series, classified as fine, illitic, mesic Glossaquic Hapludalf (McBride et al., 1997). All collected samples were thoroughly homogenized and passed through a 2mm sieve prior to conducting chemical tests and the leaching experiment.

The metal leaching experiment was conducted in the laboratory in an effort to simulate natural rainfall-driven leaching conditions for soils in the field. Buchner funnels with a volume of about 200 ml were fitted with filter papers and filled with samples of the control soil (C1) and sewage sludge soil site (S1), then saturated with deionized water (Fig.4.1). The funnels were covered to minimize the evaporation of water from the soils. After seven days equilibration, leachate water was extracted from the soils using a vacuum pump, filtered and analyzed by ICP-OES for Cd, Cu, Ni, Pb, Zn, and S by the method described in the Appendix (Soil water and plant tissue analysis methods).

**Figure 4.1.** Buchner funnels and pump used in the leaching experiment.



## Results and Discussion

### Soil leaching test

As shown in Table 4.1, the concentrations of metals and sulfur in leachate from the sludge-amended soil (S1) are higher than in the control soil (C1). The results of the leaching test suggest that the metals Cd, Cu, Ni, Zn, and Pb, are gradually leaching from the sludge-amended surface soils presently, and have almost certainly been leaching since the time of the sludge application approximately 40 years earlier. However, the low rate of metal leaching losses predicted from this experiment indicates that total soil metal concentrations in the field would be only slightly reduced by long-term leaching. Although the low leaching rate after 40 years is likely to be at a near-steady condition, it is likely that much higher leaching losses of metals would have occurred immediately following the application of sewage sludge at the site

(de Haan, 1980). The continued loss of sulfur in leachate (Table 4.1) suggests that organic sulfur is still being released by oxidation to sulfate as organic matter is mineralized.

**Table 4.1.** The measured mean concentrations (mg/L) of metals and sulfur in the water from the leaching test for each soil sample (n=12). Standard deviations are given in parentheses.

Soil	Concentration in leachate water (mg/L)					
	Cd	Cu	Ni	Pb	Zn	S
<b>C1</b>	0.00 (0)	0.03 (0.001)	0.01 (0)	0.04 (0.01)	0.14 (0.02)	13.3 (0.9)
<b>S1</b>	0.12 (0.2)	0.19 (0.16)	0.03 (0.01)	0.09 (0.1)	1.30 (0.5)	25.2 (1.3)

The results of this test indicate that the sludge soil releases a higher concentration of toxic metals in spite of a higher level of organic matter and a much higher pH (6.7) than the control soil (pH 5). This is largely because of the much higher concentration of total toxic metals in the sludge soil. Compared to the concentrations of metals leached from control soil, the leached Cu from the sludge soil is 6 times greater, Ni is 3 times greater, Pb is 2.3 times greater, and Zn is 9 times greater. Cd was not detectable in leachate water from the control soil. In addition to the metals, sulfur is also more mobile in the sludge soil than in the control soil. Sulfur-containing thiol functional groups in soil organic matter are effective ligands for strongly binding chalcophilic metals. Over time, sulfur in sulfides oxidizes to sulfate, potentially releasing metals into a soluble form in soil (Martinez et al., 2006), and the near-neutral pH of the sludge soil promotes the movement of soluble organic matter (DOM) as a carrier for toxic metals via preferential flow paths from the surface to the subsoil (McBride et al., 1997).

This potential mechanism of metal leaching provides an explanation for the result seen with Pb. Although the total and available concentrations of Pb are almost the same in the sludge and control soils, the leachate Pb concentration is 2.3 times higher from the sludge soil than in the control soil. Pb is known to strongly complex with DOM at near-neutral soil pH, with organo-

Pb complexes accounting for more than 90% of dissolved Pb at soil pH above 6 (Sauvé et al., 1998).

### **The distribution coefficient (K<sub>d</sub>) calculation**

To determine the relative mobility of metals based on their water solubility in the sludge and control soil leaching experiments, distribution coefficients (K<sub>d</sub>) were calculated. The distribution coefficient (K<sub>d</sub>) of a metal is the ratio of that metal's total concentration (mg/kg) in soil to soluble metal concentration in the aqueous phase, which in this case is the leachate (mg/L). The K<sub>d</sub> value is an inverse indicator of relative mobility of metals in soils.

The top row of Table 4.2 shows the K<sub>d</sub> of the metals in 2017, the year in which the leaching experiment described here was conducted, whereas the bottom row shows K<sub>d</sub> values calculated for soils collected 20 years after the initial sludge application. At that earlier time, the most mobile metals were Ni and Cu, but this changed by 2017. Cu and Ni became less mobile, whereas Cd and Zn became more mobile with time. The most recent (2017) results show that Cd is the most mobile metal with the lowest K<sub>d</sub>, a result consistent with many studies showing Cd to be one of the least strongly retained toxic metals in soils (McLaughlin et al., 1999). For example, Hamon et al. (1998) studied the bioavailability of Cd in soil which was fertilized with superphosphate (which contains Cd) from 1948 to 1975. Soil and wheat samples were collected in 1995 from that area. The cadmium concentration in wheat was 0.19-1.4 mg/kg. The study showed that only 1-1.5% of total Cd was being immobilized in this soil per year. In another case, in the East Midlands of the UK, the application of sewage sludge in farms was continued for over 100 years. The concentration of Cd in these soils reached a maximum of 48.6 mg/kg and the lability of Cd at the long-term site is a maximum of 11.1mg/kg, or 37.3% of the total concentration of Cd. Lability was determined using stable isotope methods (Mossa et al., 2020).

**Table 4.2.**  $K_d$  values (L/kg) of toxic metals in sludge soil collected in 2017 and 1997 (McBride et al., 1998) at the Orchard site where sludge had been applied in 1978.

	<b>Cd</b>	<b>Zn</b>	<b>Cu</b>	<b>Pb</b>	<b>Ni</b>	<b>Cr</b>
<b>2017</b>	105	520	665	1757	1943	5256
<b>1997</b>	2500	4800	360	13000	240	7500

The results of the  $K_d$  calculations taken from the same field site nearly 20 years apart suggest that the mobility of metals change over time. These changes may depend on the components of organic matter that decompose or oxidize. Mobility can be enhanced in the high-organic matter soil by the formation of soluble organic complexes (Martinez et al., 1999). Cd and Zn are likely to be complexed by organic matter through thiol sulfur as functional groups (Martinez et al., 2006). The results of the leaching test show that mobility of sulfur is two times higher in the sludge soil than in the control soil despite the 40 years of soil leaching in the field. Oxidation of the sulfur in organic matter of sewage sludge likely resulted in a delayed higher mobility of Zn and Cd (Martinez et al, 1999). Cu and Pb form complexes more strongly with solid organic matter and therefore are less mobile than Zn or Cd (Stevenson, 1976). Nevertheless, a shift of the  $K_d$  for Pb to a lower value over time could indicate that the loss of some Pb bonding sites in organic matter, possibly thiol groups, during organic matter decomposition caused Pb retention to be somewhat weakened. Conversely, it is possible that Ni, which became less mobile over the long term, had strongly bonded to Mn, Al, or Fe oxide surfaces, as Ni is known to have a high affinity for these secondary minerals (Kim et al., 2006). McBride (1989) demonstrated affinity sequences for amorphous Fe hydroxides, Al hydroxides, and the silanol groups for the metals Pb, Cu, Zn, Ni, and Cd. Each type of mineral surface had its own affinity order, but Ni consistently had a higher affinity for oxides compared to the other metals (except Cd).



Ashworth et al. (2004) and Gove et al. (2001) described a high level of Ni leaching in freshly amended sludge soil. Based on the  $K_d$  for Ni measured in 2017 and in 1997 at the Orchard site, we observed a similar trend (Ni in 1997 was more mobile than 20 years later). Cr in the form of  $Cr^{3+}$  is very strongly adsorbed on soil surfaces, especially by iron and aluminum oxides when the pH is above 5, so that its extremely low mobility as indicated by a high  $K_d$  in Table 4.2 is expected (Wuana et al., 2011).

Cd and Zn are revealed to be presently the most mobile metals in the sludge soil, and therefore may pose a hazard for human health, soil health and agriculture. Despite the fact that Zn is one of the essential elements for plants, microorganisms, animals, and humans, a high quantity of Zn in soil may cause both direct and indirect threats (e.g., the direct threats occur through negative effect of metals on crop growth and yield; and indirect threats occur through transfer through the food chain with a potentially negative impact on human health). (Wuana et al. 2011).

### **Velocity of metal leaching in sludge soil**

Leaching is one of the factors that could over the long-term lead to the “natural” recovery of badly contaminated soils from toxicity. To estimate the velocity of metal leaching each year (from the surface into the subsoil), I used the convection-dispersive equation (McBride, 1994):

$$\frac{v_M}{v_W} = \frac{1}{[1 + (\rho_B/\theta)Kd]}$$

Where:

$v_M$  = downward velocity of metal in the soil column (cm/year)

$v_W$  = downward velocity of water in the soil column (cm/year)

$\rho_B$  = bulk density of soil ( $g/cm^3$ )

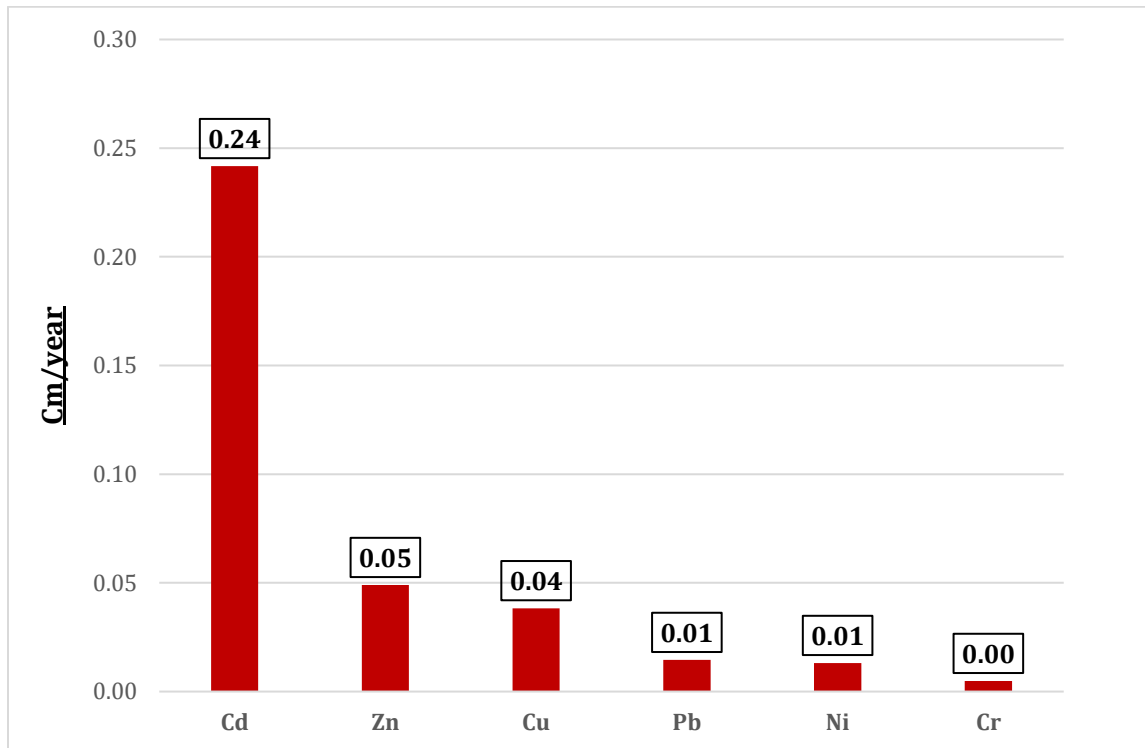
$\theta$  = fractional porosity of soil

$Kd$  = distribution coefficient

Here, the downward velocity of water is estimated from annual rainfall (36 inches) minus evapotranspiration (15 inches) in Ithaca, NY, resulting in about 51 cm/year. Soil bulk density is estimated to be 1.0 g/cm<sup>3</sup>, and porosity is approximately 0.5 for this mineral soil.

In this equation,  $1 + (\rho_B/\theta)K_d$  is the retardation factor, estimating the degree to which the metal's movement is reduced. Large retardation factors can mean that the leaching of metal cations from the surface into the subsoil is slow. Because the  $1 + (\rho_B/\theta)K_d$  factor is constant for all metals, this means that the retardation factor for sludge soil depends only on the metal's  $K_d$ . The higher the  $K_d$ , the more slowly a metal leaches into the subsoil.

**Figure 4.2.** Calculated downward velocity of metals (cm/year) in the sludge soil.



The results indicate that all metals are still slowly leaching into the subsoil, with the most mobile metal by far being Cd, which is 4.8 times more mobile than Zn. However, the velocity of Cd moving from the surface into subsoil is still only 0.24 cm per year. If the extractable amount of Cd in the sludge soil is about 6 mg/kg based on data from the sequential extraction chapter

(total Cd is 13 mg/kg), this means it would take more than a century to remove this Cd from the soil surface. Zn, Cu, Pb, and Ni migrate even more slowly in the soil. But at the same time, these metals are predicted to have discernable migration compared with the extremely immobile Cr. As mentioned above, Cr<sup>3+</sup> strongly adsorbs on mineral and organic surfaces, especially Fe and Al oxides, under the near-neutral pH conditions of the sludge soil. Mandal et al. (2001) studied Cr bound in sludge surrounding a tannery waste dumping area by sequential extraction. The concentration of Cr in exchangeable form decreased from the top to the bottom layer of the soil. Solubility of Cr<sup>3+</sup> decreased as the solution pH was increased above 5. The order of sequential extraction of metals in this study was Cd > Pb > Zn = Cu > Mn > Ni > Fe = Cr. The highest percentage of Cr was found in the Fe-Mn bound phase. This study indicated that iron in soil can hold Cr. Mandal et al. (2001) also cites other sequential extraction studies that found the same order of extraction of these metals, although the fractions and chemicals used were different among the studies (Tack et al., 1995; Ratuszny et al., 2009; Sarkar et al., 2004; Kierczak et al., 2008).

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# Chapter 5: Plant Uptake of Toxic Metals from a Contaminated Soil 40 Years after Sewage Sludge Application

## Introduction

Plants grown in contaminated soils can take up toxic metals which are released into soluble complexed forms with dissolved organic matter (DOM) during microbial degradation of organic-rich materials such as sewage sludges (Antoniadis et al., 2002). Nevertheless, there has not been a strong correlation established between total metals in soils and the concentration of metals in the tissues of plants. For example, Payne et al. (1988) studied the application of up to 415 kg Cu and 897 kg Zn per hectare (as  $\text{CuSO}_4$  and  $\text{ZnSO}_4$ ) to the Davidson silky clay from 1967 through 1986 and determined that this application did not reduce corn grain and silage yield compared to control plots. The study also found that the concentrations of Cu and Zn in corn tissues did not increase above the normal range as a result of the metal additions. Actual uptake via roots into shoots depends on many factors including translocation efficiency of metals from roots to shoots of plants, plant species and cultivar, environmental conditions, and competing ions McBride (1995). Although the total concentrations of toxic metals measured in soils are typically used to assess potential damage to soil health, soil organic matter plays a key role in mitigating phytotoxicity. For example, Cu accumulates over decades in vineyards from the use of Bordeaux mixture fungicide, but total soil Cu in the range of several hundred milligrams per kilogram does not cause phytotoxicity in all plant species (Payne et al., 1988) because of strong bonding of Cu with organic matter. For the same reason, organic soils can contain even higher total concentrations of Cu without causing phytotoxicity (Belanger et al., 1986; McBride, 2001; Hattori, 1996) The stable organic matter in soils with a high concentration of heavy metals is expected to degrade very slowly, allowing a

small fraction of the organic-bound metals to transition into a soluble form that could be taken up by plants.

The present study investigates the phytoavailability of toxic metals in soil sampled from an orchard field site which was polluted with sewage sludge in 1978, and where a substantial fraction of the organic matter added with this amendment has decomposed over more than 40 years. Metal phytoavailability in a nearby unpolluted soil was assessed in this study as a control, although this soil did contain Pb and As at concentrations greater than normal background because of historical application of Pb arsenate as an insecticide. While a thin fescue grass (*Festuca*) grows on the control area, the sewage sludge site has been colonized by goldenrod (*Solidago*) species and bull thistle (*Cirsium vulgare*) (see Figure 5.1).

**Figure 5.1.** Images of plant species adapted to the soil in the field on the control and sludge soil sites.

### **Control soil site**



**Fescue Grass**





**Bull thistle**



**Goldenrod**

## **Materials and Methods**

Soils collected from the orchard field sludge site (S1) and control site (C1) have been described in Chapter 2. Some of their key properties are shown in Table 2.1, and their heavy metal concentrations are listed in Table 2.2.

**Table 2.1 (reprinted from Chapter 2).** pH, organic matter content, and texture analysis of sludge soil (S1) and control soil (C1).

<b>Sample</b>	<b>pH</b>	<b>Organic matter (%)</b>	<b>Sand (%)</b>	<b>Silt (%)</b>	<b>Clay (%)</b>
<b>C1</b>	5.1	2.8	3.0	75.2	21.8
<b>S1</b>	6.7	7.4	12.0	62.6	25.3

**Table 2.2 (reprinted from Chapter 2).** Total concentration of metals, sulfur and phosphorus in sludge soil (S1) & control soil (C1). Standard deviations are given in parentheses (n = 12 for each measurement).

	<b>Sludge Soil (2015)</b>	<b>Control Soil (2015)</b>
	<b>mg/kg</b>	<b>mg/kg</b>
<b>Cadmium</b>	12.7(0.4)	0.2(0.01)
<b>Chromium</b>	187 (6.8)	22 (0.5)
<b>Copper</b>	128(4.2)	16(0.4)
<b>Manganese</b>	661(12.4)	684(7.9)
<b>Nickel</b>	49(1.12)	20(0.4)
<b>Lead</b>	155(3.9)	113(4.6)
<b>Zinc</b>	678(3.2)	90 (1.5)
<b>Silver</b>	3.3(0.1)	-
<b>Phosphorus</b>	2150(53.9)	815(18.8)
<b>Sulfur</b>	599 (23.7)	350(7.8)
<b>Iron</b>	27071(86.4)	22081(38)

In August 2015, stem and leaf tissue samples were collected from the three plant species growing in the field sludge site (S1) and control site (C1). The samples were cleaned of soil particles, spread in a thin layer, and dried overnight at 70°C in a forced draft oven. The dry

samples were ground in a small Wiley Mill with a 40 mesh screen. The ground tissue samples were digested using a temperature-controlled hotblock (Questron Technologies Corp.) in fluorocarbon (TFM) digestion vessels (100 mL capacity). Each plant sample had 12 replicates, plus a control sample, a certified sample and a blank sample. The method for digestion of samples is described in detail in the Appendix. The acid-digested tissue samples were analyzed for Cd, Cu, Ni, Pb, Zn, Mn, and P using ICP-OES (Spectro Analytical Instrumentations, Inc.). The method used was adapted and modified from EPA method 6010-B and is described along with quality control procedures in the Appendix.

To determine whether the sludge soil itself affects fescue grass growth, a laboratory plant assay was conducted with sludge soil and control soil. Fescue grass seeds (“Agway Shady Green Grass Seed”) were planted in 6 pots with 700 ml volume, 30 g of seeds per pot. Three pots contained control soil (C1) and three contained sludge soil (S1). During germination and growth, the pots were watered as needed with DI water. I collected and analyzed the grass tissue from the six pots by the same method used for analysis of plant tissues from the field site. Above-ground tissue samples were collected, dried and digested as described above, and analyzed for metals by ICP.

I also conducted a plant assay using soybeans in sludge soil (S1) to assess impacts of this contaminated soil on an important agricultural crop plant. I planted untreated soybean seeds in 4 pots containing sludge soil (S1) and in 4 pots an uncontaminated agricultural soil from the Musgrave Research Farm in Aurora, NY (A1). Each pot contained 300g soil. The pots were watered with DI water. Pots were situated in a growing chamber with conditions: temperature 25°C day / 23°C night, humidity 50%, light 16 hours day (Soybean protocol, 2016) Six weeks after emergence, the above ground tissues were cut and washed with DI water. The tissues were dried in an oven at 70°C and then ground to a size of 2 mm. 0.5 gram ground samples were digested with 5 ml nitric acid and 3 ml perchloric acid and then analyzed with ICP-OES. (For method details, see Appendix.)

## Results and Discussion

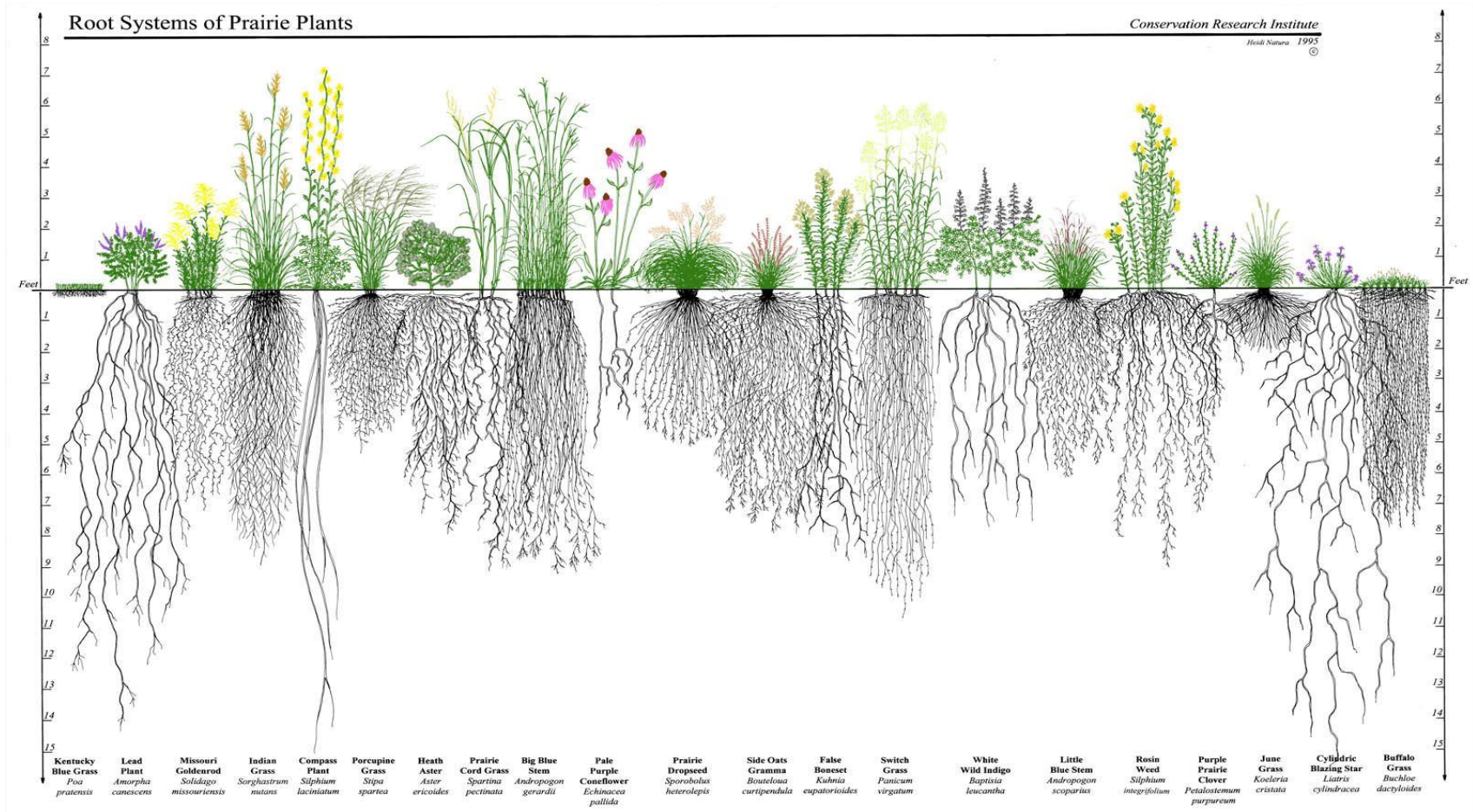
### Analysis of plants colonizing the S1 and C1 field sites

The species of plants growing at the sewage sludge site (*Solidago sp.* and *Cirsium vulgare*) have very different root systems from grasses which may have allowed them to adapt to their different environments by being more or less efficient in element uptake. The morphology of root systems and the depth of their penetration into the soil varies among different species and with different soil factors. Each species of plant has a certain characteristic form of root system and a preferred depth of growth (Figure 5.2). Root systems (root length and root size) are plastic, able to respond to localized stress from toxic metals in soils and reduce their exposure to these metals by avoidance (Khare et al., 2017; Remans et al., 2012; Potters et al., 2007; Moradi et al., 2009). As a result, some plants that are metal “excluders” may be achieving this by root avoidance of the most contaminated regions in the soil. This inherent physiological response in plant roots impacts phytoextraction of toxic metals in the field and concentrations of these metals measured in the above-ground stems and leaves.

Fescue grass and goldenrod have diffuse (fibrous), branching and very slender root systems (see Figure 5.2). As a plant with a shallow root system, grass is best suited to grow in regions of scanty rainfall, which usually reaches only the upper few inches of soil. For fescue roots, the minimum soil depth needed is 10 cm; however, for deeper root penetration and the benefits that it brings, the accepted standard for turf grass is 15 cm (Emerald View Turf Farms, 2011; Fuller et al., 1954). Plants with deep root systems are more firmly anchored in soil than those with shallow roots. Goldenrod has an extensive root system which is very deep (up to 3 m) and fibrous with 5- to 12-cm long rhizomes (horizontal underground stems) which absorb water and nutrients deeply underground. Figure 5.2 compares root length of fescue grass and goldenrod and shows how deep root systems extend underground. Bull thistle has a taproot system (Figure 5.3), in which the primary root grows most rapidly and remains the

largest root. Horizontal roots of the plant may extend 4 m or more and vertical roots may grow 2 to 5 meters deep. These observations suggest that the root systems of bull thistle and goldenrod would be better suited to limiting toxic metal exposure and uptake than fescue grass because of their deeper root systems that could readily avoid the high concentrations of Cu, Zn, Pb, Cd and other metals localized in the soil surface (10-20 cm).

**Figure 5.2.** Root systems of various plants



**Figure 5.3.** Taproot system of bull thistle.



The above-ground plant tissue results for the ICP analysis of fescue grass growing on the control site (C1), and goldenrod and bull thistle growing on the sewage sludge soil site (S1) are shown in Table 5.1.

**Table 5.1.** Heavy metal content in fescue from the control site (C1) and in Goldenrod and Bull Thistle from the sewage sludge (S1) sites. Mean concentration (mg/kg) of elements in plants (n=12). Standard deviations are given in parentheses.

Trace element	Unit	Fescue Grass (C1)	Goldenrod (S1)	Bull Thistle (S1)
Cd	mg/kg	0.13(0.01)	0.10(0.01)	0.44(0.02)
Cu	mg/kg	2.55(0.38)	6.87(0.19)	13.7(0.05)
Ni	mg/kg	0.77 (0.07)	0.51(0.03)	0.70(0.04)
Pb	mg/kg	0.45 (0.02)	0.12(0.03)	0.42(0.02)
Zn	mg/kg	17.3 (1.05)	21.8(0.82)	29.7(0.78)

The results of the tissue analyses show that all of the plants contain relatively normal levels of Cd, Cu, Ni, Pb and Zn despite the fact that the Goldenrod and Bull Thistle were grown in the highly metal-contaminated sludge soil (Table 5.1). The relatively low Zn and Cd levels in above-ground tissues of Goldenrod and Bull Thistle grown on the sludge site suggest avoidance of the sludge contamination due to these plants' deeper roots. Previous experiments with more shallow-rooted crop plants grown on these same soils have shown much higher concentrations (McBride et al., 2004; Tai et al., 2016).

On the control site (C1), grass is growing in soil with low pH (around 5) and a low level of organic matter. Concentrations of Cu and Zn are in the lower range of levels expected for turf-grasses, which are typically in the range of 40-65 mg/kg Zn and 5-8 mg/kg Cu (Bryson et al., 2014).

Goldenrod's ability to grow in the sludge soil without taking up high levels of bioavailable metals can be explained by the fact that the roots of goldenrod are very deep and have widespread rhizomes which may take up nutrients and trace metals from zones in the soil that are not severely contaminated by sewage sludge metals. Bull thistle has stout taproot roots



which run deep underground where subsoil is virtually free of toxic metals (Fuller et al., 1954). The root physiologies of goldenrod and bull thistle probably explain why these perennial species can thrive and outcompete fescue grass in the contaminated area. Besides this competitive pressure, the phytotoxic effects of metals such as Zn, Cu, Cd and Ni may contribute to reduced growth of fescue at the sewage-sludge contaminated site.

#### **Laboratory assay of fescue grown on sludge soil (S1) and control soil (C1).**

During the first five days after germination, grass grew well in all pots with control soil C1 and sludge soil S1 (Figure 5.4). However, by ten days after seedling emergence, grass in the pots with sludge soil became yellow (Figure 5.5 and 5.6), a visual symptom of a nutrient deficiency or toxicity. Results of the ICP analysis of heavy metals in the above-ground tissues are shown in Table 5.2.

**Figure 5.4.** Grass growing in the two soils C1 (right) and S1 (left), 5 days after emergence.



**Figure 5.5.** Grass growing in the two soils C1 (right) and S1 (left), 10 days after seedling emergence.



**Table 5.2** Element content in the tissue above ground of fescue grass grown in pots. Standard deviations are given in parentheses.

Element	Grass grown in sludge soil (S1) (mg/kg)	Grass grown in control soil (C1) (mg/kg)
Al	242(0.6)	306 (3.8)
As	1.7(0.03)	0.3(0.03)
Ca	16167(21)	6302(26)
Cd	2.2(0.01)	0.1(0.01)
Cr	2.5(0.1)	0.9(0.2)
Cu	31.5(0.02)	2.5(0.03)
Fe	370(0.9)	613(5.1)
K	19334(39)	9006(38)
Mg	6687(5.2)	1777(5.4)
Mn	37.3(0.03)	248(0.34)
Mo	3(0.01)	0.4(0.04)
Ni	6.4(0.08)	0.8(0.004)
P	11218(4.44)	1640(7.02)
Pb	1.8(0.05)	0.4(0.012)
S	7631(2.2)	1371(1.8)
Zn	100.8(0.14)	17.3(0.22)

These tissue analyses (Table 5.2) show that concentrations of the trace metals (Cd, Cu, Ni, Pb, Zn) are 5 to 20 times higher in fescue grass grown in sludge soil (S1) than in grass grown in the control soil (C1). For example, Cd is about 20 times higher, while Zn and Ni are 6 times higher in the grass grown in sludge soil. Toxic metals such as Cd and Ni may impair the absorption and uptake of essential micronutrients, resulting in reduced growth and nutrient deficiency (Jalil et al., 1994). Although total Pb concentration was similar in the two soils (114 mg/kg in the control samples (C1), and 155 mg/kg in the sludge soil samples (S1)), Pb concentration was 5 times higher in the tissue of the grass grown in sludge soil pots despite the higher pH of the sludge soil. Some likely reasons for this are that metals such as Pb can complex with soluble organic matter near neutral pH (sludge soil pH 6.7; control soil pH 5.1) (Sauve et al., 1998), or that root exudates may decrease soil pH and solubilize metals. The presence of several potentially plant-toxic metals simultaneously in the soil at high

concentrations, particularly Zn, Cu and Ni, can create an additive or even synergistic effect which increases phytotoxicity (McBride, 1995). Although Cu and Zn are essential micronutrients, high concentrations of these trace metals may induce deficiency of other micronutrients such as Fe and Mn, a situation that has been observed to cause crop plants to be chlorotic and sickly at long-term sludge application sites (Leeper, 1978; McBride 1995). High concentrations of Cu could be a result of high levels of Cd in the soil, which increases Cu uptake (Gayomba et al., 2013).

The sludge soil contained a much higher level of total phosphorus than the control soil (Table 2.2, see above). Therefore, as expected, the level of P was much greater in the fescue grown in the sludge soil than in the control soil (Table 5.2). Although the level of Mn in the two soils was almost equal, the level of Mn was much greater in grass grown in the control soil than in grass grown in sludge soil (Table 5.2). In fact, Mn in fescue grown in soil S1 is 7 times less than in grass grown in soil C1. Fescue grass normally contains quite high levels of Mn, in the 200-2000 mg/kg range (Hull, 2001). Thus, it appears that the grass grown in soil S1 has a Mn deficiency. There are several possible explanations for this. One possible explanation is that high concentrations of Cu and Zn could suppress the ability of the plant to take up and translocate Mn. There are only a few cases in which sludge soils have been studied over long periods of time (McBride, 1995). However, three cases of long-term application of sewage sludge on farms were described by Leeper (1978), who suggested that experience with two of the farms (near Paris, France and Melbourne, Australia) provided evidence in support of the application of sewage sludge to land. However, crops grown at the Paris farm had Mn deficiency, possibly as a result of redox processes that led to Mn depletion in the soil due to sewage sludge application, or as a result of high levels of heavy metals such as Zn and Cu which impeded plant uptake and translocation of Mn. At a third sewage farm (near Berlin, Germany), the severity of chlorosis in crops was linked to higher soil levels of Cu and Zn. All three of these farms had high levels of organic matter in the soil due to continued sludge

application, making it likely that an increase in dissolved organic matter (DOM) associated with organic matter decomposition caused an increase in the solubility of metals (McBride, 1995). While the concentrations of free metal cations in soil solution (rather than total dissolved metal concentration) are the best indicators of metal toxicity, these have not been studied in sludged soils over long periods of time (McBride, 1995).

One final possible explanation for the low levels of Mn in the fescue grown in sludge soil is that a high level of P in the sludge soil reduces root hair development and results in less Mn uptake (Pedas et al., 2011; Gahoonia et al., 1997; Zhu et al., 2010; Miura et al., 2011). Phosphorus interacts with micronutrients other than Mn, e.g., Zn, Fe, and Cu (Murphy et al., 1981; Broadley et al., 2010; Petez-Novo et al., 2011). Phosphate fertilization of plants with low Zn status may lead to visible Zn deficiency symptoms, a phenomenon referred to as P-induced Zn deficiency (Cakmak et al., 1987). However, Zn and Cu deficiency were not observed in this study despite the very high soil available P because the sludge soil contains very high levels Zn and Cu.

The results of this fescue assay have shown that the high concentration of P added to the soil from sewage sludge is possibly responsible for reduced Mn uptake by fescue roots, which can result in Mn deficiency in the shoot. This interaction between P and Mn has been previously observed in barley plants (Pedas et al., 2011). The fescue grass grown in the soil control pots (C1) contains 248 mg/kg of Mn and 1604 mg/kg of P, whereas the grass grown in sludge soil pots (S1) contains 37 mg/kg of Mn and 11218 mg/kg of P (Table 5.2). The practical implication is that increased soil P levels that result from repeated long-term sewage sludge application to farm fields may be expected to lead to increased prevalence of Mn deficiency in crops.

**Laboratory assay of soybean grown in sludge soil (S1) and agricultural soil from Musgrave Research Farm in Aurora, NY (A1)**

Figure 5.6 shows the leaves of soybeans growing in sludge soil (S1) pots. Yellow spots became visible on the soybean leaves 5 weeks after seeding emergence in sludge soil (S1).

Figure 5.7 shows the leaves of soybeans growing in agricultural soil (A1) 6 weeks after seeding emergence. The leaves appeared relatively healthy, and no yellow spots were observed.

**Figure 5.6.** Soybeans growing in sludge-soil (S1) 6 weeks after seedling emergence.



**Figure 5.7.** Soybeans growing in agricultural soil (A1) 6 weeks after seedling emergence.



**Table 5.3** Elemental contents in soybean shoots grown in pots. Standard deviations are given in parentheses.

<b>Element</b>	<b>Sample (A1)</b>	<b>Sample (S1)</b>
	<b>mg/Kg</b>	<b>mg/Kg</b>
As	0.2 (0.1)	0.99 (0.04)
Cd	-	6.59 (0.3)
Cr	1.1 (0.4)	4.0 (0.5)
Cu	5.9 (1.1)	13.56 (1.1)
Fe	150 (36)	76.6 (9.7)
Mg	4623 (429)	5970 (232)
Mn	84.3 (2.5)	31.4 (4)
Mo	1.9 (1)	9.3 (1.8)
Ni	0.8 (0.7)	17.5 (0.7)
P	1391 (181)	4219 (168)
Pb	-	6.5 (0.4)
S	1618 (120)	2260 (238)
Zn	17.0 (1.6)	240.5 (11)

Analysis of the soybean shoots and leaves sampled 6 weeks after seedling emergence showed that stems and leaves of soybeans grown in sludge soil contain somewhat higher than normal levels of toxic metals such as Cd (6.6 mg/kg), Ni (17.5 mg/kg), and Pb (6.5 mg/kg). The micronutrients, Cu and Zn, were also found at higher level in the soybeans, with Cu at 13.6 and Zn at 240.5 mg/kg. The average level of Cu in field-grown soybeans is 3-6 mg/kg and Zn is around 20 mg/kg (Bryson et al., 2014). The results of the soybean growth experiment are consistent with the results of the fescue grass growth experiment. The concentrations of Cd, Ni, Pb, Cu, Zn, phosphorus, and sulfur are higher in soybeans grown in sludge soil S1 than in agricultural soil A1. Conversely, Mn and Fe in soybeans grown in sludge soil S1 are several times lower than in soybeans grown in agricultural soil A1. The leaves of soybeans grown in sludge soil S1 had signs of chlorosis six weeks after seedling emergence (Figure 5.6). It is likely that a deficiency of Mn and high concentrations of Cu, Zn and P promoted chlorosis and phytotoxicity, as seen in other studies with soil from the same sludge site (Tai et al., 2016; McBride et al., 2004). Although those studies used different species of plants, including lettuce, amaranth, and red clover, the high concentration of toxic metals in the sludge soil resulted in severe phytotoxicity across different species.

## **Conclusion**

Toxic metals in a soil that had received a heavy application of contaminated sewage sludge are still available to plants some 40 years later. The toxic metals of concern include Cd, Ni, Zn, Cu and Pb, and were taken up into above-ground tissues of fescue grass and soybeans in laboratory assays. In spite of high concentrations of organic matter that effectively bind these metals, slow release of these metals into soluble form during degradation of the organic matter renders them available for absorption and uptake by plant roots. The very high soil P levels in the sludge-amended soil appeared to have a role in causing reduced Mn uptake and Mn deficiency in the plants. The results here suggest that agriculturalists should be taking a



cautionary approach to the repeated application of heavy-metal contaminated sewage sludge to farmlands.

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## Chapter 6: Methods for measuring trace silver in sludge, plant tissue and soil samples

### Introduction

Silver is a rare element, which occurs naturally in its pure form as a white, ductile metal, and in ores. It has an average abundance of about 0.1 ppm in the earth's crust and about 0.3 ppm in soil. The major source of elevated silver levels in cultivated soils is the application of sewage sludge and sludge effluents as agricultural amendments. Silver in the ionic form ( $\text{Ag}^+$ ) is toxic to soil microorganisms and inhibits bacterial enzymes (Domsch 1984). For example, Colman et al. (2013) studied how plants and microorganisms responded to the presence of silver nanoparticles in biosolids in a field experiment. They studied two samples: biosolids with a concentration of silver of 0.02 mg/kg and biosolids with added engineered silver nanoparticles (AgNPs) with a concentration of silver of 0.14 mg/kg. The results of the test showed that the amount of microbial biomass was significantly lower in the soil treated with the biosolids containing AgNPs than in soil treated with biosolids alone. Changes in microbial biomass and activity were accompanied by changes in microbial community composition. The most notable changes in microbial activity in the soil treated with the biosolids containing AgNPs were lower extracellular enzyme activity and an increase in the flux of nitrous oxide ( $\text{N}_2\text{O}$ ), contributes to ozone depletion in the stratosphere (Ravishankara et al., 2009). These extracellular enzymes are often used as indicators of the microbial potential to decompose organic matter (Burns, 1982). This study and another study by Westerhoff et al. (2015) found valuable elements including silver (10mg/kg) in municipal sludge applied agricultural land.

The sludge applied at the Cornell Orchard site contains toxic metals, including silver (Table 2.2).

**Table 2.2 (reprinted from Chapter 2).** Total concentration of metals, sulfur and phosphorus in sludge soil (S1) & control soil (C1). Standard deviations are given in parentheses (n = 12 for each measurement).

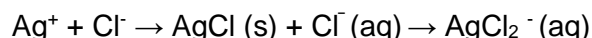
	<b>Sludge Soil (2015)</b>	<b>Control Soil (2015)</b>
	<b>mg/kg</b>	<b>mg/kg</b>
<b>Cadmium</b>	12.7(0.4)	0.2(0.01)
<b>Chromium</b>	187 (6.8)	22 (0.5)
<b>Copper</b>	128(4.2)	16(0.4)
<b>Manganese</b>	661(12.4)	684(7.9)
<b>Nickel</b>	49(1.12)	20(0.4)
<b>Lead</b>	155(3.9)	113(4.6)
<b>Zinc</b>	678(3.2)	90 (1.5)
<b>Silver</b>	3.3(0.1)	-
<b>Phosphorus</b>	2150(53.9)	815(18.8)
<b>Sulfur</b>	599 (23.7)	350(7.8)
<b>Iron</b>	27071(86.4)	22081(38)

To determine the concentrations of silver in sludge, soil, and plant samples requires a robust and sensitive analytical method such as ICP-OES. The key factor in detecting low concentrations of silver in plant samples containing a high level of organic carbon and in soil (which commonly has a low level of organic carbon) is that solid samples must be digested and prepared in the correct way for ICP-OES. The main methods used in the acid digestion of sediments, sludge, and soil are US EPA methods 3050B (Kimbrough et al., 1989) or 3051A (Link et al., 1998). However, both of these methods are designed for samples with high concentrations of silver (around 2000 mg/kg in a 2 g sample). A low concentration of silver in biosolids, soil, and plant samples requires special methods of digestion of the solid material prior to analysis by ICP-OES. The method described in this chapter uses chloride ions (Cl<sup>-</sup>) to

precipitate the silver cations ( $\text{Ag}^+$ ), which allows high recovery of silver from soil, sludge or plant tissue samples containing a low silver concentration.

### **Chemical Properties of Silver**

Most environmental samples contain trace quantities of Cl, which causes Ag to precipitate out of solution as solid AgCl. However, the solubility of AgCl increases with increasing Cl concentration due to the formation of complex anions with the general formula  $\text{AgCl}_n^{1-n}$ . By adding HCl, the  $\text{Cl}^-$  concentration is increased, and the  $\text{AgCl}_n^{1-n}$  complex anions that are formed are stabilized in solution:



This method is applicable to the hotblock assisted acid digestion of plant tissue and sludge for the determination of silver as the complex  $\text{AgCl}_n$  (McMurry et al., 1995).

Samples of sludge, plant tissue or soil are digested using a hotblock and analyzed using inductively coupled plasma-optic emission spectroscopy (ICP-OES) at the wavelength 328.068 nm for Ag (Rutzke, 1999; Rutzke, 2003).

### **Materials and Methods**

The method below describes the digestion of sludge, plant tissue, and other samples containing a high concentration of organic carbon, as well as soil samples which typically have a low level of organic carbon.

#### **Materials**

All preparation of samples and solutions used  $18 \text{ M}\Omega \text{ cm}^{-1}$  deionized water (DI). Sample digestion was performed with trace metal grade concentrated nitric acid (Fisher), trace metal grade concentrated hydrochloric acid (Fisher), 30% hydrogen peroxide (J.T. Baker), and stock silver standards (ICP grade) from Inorganic Ventures. Reference materials used were: Metals in Sewage Sludge ERA Water Company Cat #160 (concentration of silver is 78 mg/kg), trace

metals in soil ERA Water Company Cat #540 (concentration of silver is 45 mg/kg), and domestic sludge from NIST (National Institute of Standard and Technology) Cat # 2781 (concentration of silver is 98 mg/kg).

### **Instrumentation**

Sample digestion was performed with a temperature controlled graphite hot block (Questron Technologies Corp.) in 40 ml fluorocarbon (TFM) digestion vessels. Analysis of prepared samples was performed on an ICP-OES (Spectro Analytical Instrumentations, Inc.). All sample preparation and analysis steps were performed in low-light or yellow-light environments to minimize redox activity or precipitation of silver contained in samples.

### **Sludge and plant sample preparation (for samples with high organic carbon)**

Sludge samples of 0.50 g were weighed out in triplicate. An additional three samples were spiked with an internal Ag standard of concentration 2 mg/L. The internal standard was prepared from a standard concentration of 1000 ppm (Inorganic Ventures). Six matrix blank samples, the certified samples, and the spiked samples were digested in preparation for ICP-OES analysis according to the following method: 10 ml of concentrated hydrochloric acid was added to each 50 mL digestion vessel containing the sample. After 5 min, 3 ml of concentrated nitric acid was added. 10 min later 0.5 ml 30% hydrogen peroxide was added.

The digestion vessels were then placed in the graphite hot block for digestion. The temperature was ramped to 110 °C over 40 min and held at 110 °C for the next 60 min. Then, the temperature of the graphite hot block was ramped to 160 °C over 20 min and held at 160 °C for another 10 min. Afterward, vessels were cooled to room temperature. The resulting digested samples were each diluted to a volume of 20 ml with DI water.

### **Soil sample preparation (for samples with low organic carbon)**

Certified reference samples of ERA water company Cat #540 and spiked soil samples with a concentration of silver of 1 mg/L from Musgrave Research Farm in Aurora, NY (Aur) were



prepared in triplicate. Samples of 0.50 g were weighed into digestion vessels and 10 ml of concentrated hydrochloric acid was added. After 5 min, 3 ml of concentrated nitric acid was added. Matrix blank samples were prepared by digestion of six replicates of control soil samples. All samples were placed in the graphite hot block for digestion. The temperature was immediately ramped to 160°C over 10 min and held for 3 min. Afterward, vessels were cooled to room temperature and diluted to a volume of 20 ml with DI water.

### **ICP-OES analysis of samples**

Digested samples were analyzed for total silver content against calibration standards prepared from stock standard (1000 mg/L) over the concentration range of 0 – 5 mg/L. Calibration standards were prepared daily prior to analysis to account for potential photodegradation. The silver signal was measured at wavelength 328.068 nm. Quality control samples consisted of acid matrix-matched calibration verification standards that were analyzed immediately following calibration and again after a maximum of every 10 samples. Carryover was assessed by analysis of calibration blank samples after measurement of the calibration verification samples. All systems were washed with 30% hydrochloric acid to avoid precipitation of silver. This high concentration of hydrochloric acid was used to keep the silver in solution (McMurry et al., 1995).Results

### **Method detection limits and reproducibility**

Method limits of detection and quantification were determined for all matrices by analyzing the silver signal of six replicates of matrix blanks, taking the standard deviation of the detected silver concentration, and multiplying by the student's t-value at 99% confidence interval for the appropriate degrees of freedom. The method limit of detection for sludge and soil was 1.59 ppb. The lowest acceptable calibration standard was 2 ppb.

## Analytical recoveries: accuracy and precision

Analytical recovery for all samples was calculated using the following equation:

$$\%Recovery = \frac{[Ag]_{measured}}{[Ag]_{nominal}} 100\%$$

The results of the analysis of silver in sludge are shown in Table 6.1.

Recovery of Ag from spiked sludge samples was 98.3-98.4%. The standard deviation for triplicate measurements for silver digests of spiked samples was 0.074 ppm. Recovery for metals in sewage sludge, Cat #160 (MSL#160) was 98-97.4% (standard deviation 0.005 ppm). Recovery of metals in domestic sludge from National Institutes of Standard and Technology, Cat # 2781(DSNIST 2781) was 96.7-98.9% (standard deviation 0.026 ppm).

The results of the analysis of silver in soil are shown in Table 6.2. The observed recovery from soil samples was 97-99%. The recovery from spiked Aurora soil samples using a commercially purchased silver (Ag) solution in nitric acid DI water also assessed, with recovery of 99% (standard deviation 0.49 ppm). Commercial soil samples (metal in soil cat # 540) provided an average recovery of 98% (standard deviation 0.04 ppm).

## Discussion

A robust digestion method is required in order to adequately determine the low concentration of silver in soil, sludge, and plants in chemical laboratories where equipment may contain trace concentrations of chloride ion ( $Cl^-$ ). A high degree of robustness is also necessary to provide accurate analytical results in samples with variable chemical distribution such as sludge and soil. The precision and accuracy in the results of the spiked samples and reference materials demonstrates the high recovery of the method developed and used here.

The method described in this chapter was applied to determine the concentration of silver in aged sludge that was originally applied at Cornell Orchards in 1977. Using this method, the concentration of silver was measured to be 3.3 mg/kg in samples which were collected from the

sludge site in 2015. In the 1990s, sludge samples taken from the same site were measured to contain 11 mg/kg silver (McBride et al., 1997). However, it is difficult to make a direct comparison between these measurements. One reason for this is that the previous measurement used a different technique, neutron activation analysis. Also, although the measured samples were both taken from Cornell Orchards, an uneven distribution of sludge in the soil may have resulted in the samples containing different amounts of sludge.

Another consideration is that over 25 years, bioturbation by bacteria and other organisms may have reduced the concentration of silver in the surface soil, such non-sulfur bacteria reduce silver in soil and biosolids, which can be useful in the recovery of soil by biotechnological methods (He et al., 2017). Colman et al. (2013) showed that significant reductions in microbial biomass and changes in microbial activity after the application of biosolids to soil were a result of Ag released from  $\text{Ag}_2\text{S}$  (Colman et al., 2013). Another study demonstrated that bioturbation by earthworms also plays a role in the vertical transport of  $\text{Ag}_2\text{S}$  in soil (Baccaro et al., 2019). This study examined the transport of silver in soil columns, with and without earthworms, with  $\text{Ag}_2\text{S}$ -NPs (silver sulfide nanoparticles) added to the top of the columns. The initial Ag concentration was approximately 6 mg/kg at the top of the columns. After 28 days, the concentration of Ag in the bottom layers of the columns with worms was significantly above background levels (approximately 1 mg/kg), representing movement of about 9% of the total silver from the top. The columns without worms did not show significant movement of silver to the bottom. The earthworms themselves accumulated up to 1–2 mg/kg dry body weight Ag.

Silver is a growing concern in the environment due to its increased use in consumer products. Therefore, it is important to develop reliable methods for the measurement of silver in environmental samples, including samples with high concentrations of organic carbon (such as sludge and plant samples) and samples with low concentrations of organic carbon (such as soil samples). These types of samples are often critical for horticultural and agricultural studies. Further method development and studies will also be necessary to understand the speciation of

silver in digested samples and its relationship to the determined concentration in biological and soil samples.

**Table 6.1.** Results of the analysis of silver in sludge.

<b>sample</b>	<b>Ag 328.068 mg/l</b>	<b>Ag 328.068 mg/kg</b>	<b>recovery %</b>	<b>Concentration of reference material</b>
	ICP - OES			
SL Spike	1.96		98.4	1 mg/l
SL Spike	2.00		98.4	
SL Spike	2.10		98.3	
STDEVA	<i>0.074</i>			
MSL#160	1.99	79.7	97.8	78 mg/kg
MSL#160	1.99	79.6	98.0	
MSL#160	2.00	80.0	97.4	
STDEVA	<i>0.005</i>			
DSNIST 2781	2.48	99.1	98.9	98 mg/kg
DSNIST 2781	2.48	99.3	98.7	
DSNIST 2781	2.53	101.0	96.9	
STDEVA	<i>0.026</i>			

**Table 6.2.** Results of the analysis of silver in soil.

<b>sample</b>	<b>Ag 328.068 mg/L</b>	<b>Ag 328.068 mg/Kg</b>	<b>recovery %</b>	<b>Concentration of reference material</b>
	ICP - OES			
AUR-1	0.99		99	1 mg/l
AUR-1	0.90		99	
AUR-2	1.03		99	
STDEVA	<i>0.49</i>			
Metal in soil #540	1.10	44.04	98	
Metal in soil #540	1.06	42.35	98	
Metal in soil #540	1.14	45.55	97	45 mg/kg
STDEVA	<i>0.04</i>			

## References for Chapter 6

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## Chapter 7: Conclusion

The results presented in this dissertation show that 40 years after a single application of sewage sludge, the soil contains high concentrations of toxic metals. These metals are still gradually migrating out of the surface soil into the subsoil, as leaching the results of test 2015 and 1997 year the show that the concentration of toxic metals and organic matter are decreasing. At the same time a large fraction of heavy metals are immobilized in the surface soil, and a much smaller fraction of these metals are still soluble. In the course of my research I have observed that physical, chemical, and biological processes in soils significantly impact and modify toxic metal solubility and bioavailability. This was confirmed by the  $K_d$  values which show that the mobility of metals appears to have changed over time as organic matter from the sewage sludge amendment stabilized and decomposed. The tests conducted in my research confirmed that the concentrations of bioavailable trace metals in soil are governed by a variety of reactions, including complexation with organic and inorganic ligands, ion exchange and adsorption, precipitation and dissolution of solids, and acid-base equilibria, as shown in the sequential extraction test. Organic matter plays a key role in modifying metal availability to plant roots by the formation of soluble metal-organic complexes and by reducing free metal cation concentrations in soil solution. At the same time, high concentration of metals in organic matter released in soluble form during the decomposition of organic matter make the soil solution rich with toxic metals that can harm plants and other biological life in the soil and leach into underground water. The metals in organic matter decelerate the decomposition of organic matter. Thus, the soil where sewage sludge was applied retains high levels of toxic metal, making it less suitable for agriculture for as long as a century. Soil pH is also a critical factor in controlling the solubility of the free metal cations; more acidic soils have a higher solubility of toxic metals and a larger fraction of dissolved metals in the toxic free ionic form as this research has shown.



This study found toxic metals such as Cd, Ni, Zn, Cu and Pb in tissues of fescue grass and soybeans which were grown in sludge soil in the lab. Plants do not grow as well in the sludge soil compared with control soil or soil from Musgrave Research Farm in Aurora, NY. It is possible that multiple heavy metals present in the soil at lower concentrations can create a synergistic effect that increases toxicity to roots and results in yield reductions greater than if a single metal was present at a higher concentration. Increased soil P levels may also be expected to lead to increased prevalence of Mn deficiency in crops. Most of the agriculturally productive soils in this country are presently in use; food production cannot simply be relocated if existing farmland is degraded. Our best agricultural soils need to have their productivity and crop quality protected, not for 10, 20, or even 100 years, but in perpetuity. To this end, a cautionary approach to the application of sludge to soil would be wise.

# Appendix

## Methods

### A. Digestion of Plant Tissue Samples

I have used a hotblock with controlled temperature (Questron Technologies Corp.) for digestion for plant tissue samples from the field, fescue grass from pots in control soil and sludge soil, and soybeans in sludge soil.

The acid digestion method for plant tissue using a hotblock is applicable for the following elements: copper, cadmium, chromium, lead, manganese, nickel, phosphorus, sulfur, zinc.

#### Summary of Method

I weighted 0.5 g of the plant tissue sample and digested in 4 ml of mixed acids (60% of concentrated nitric acid and 40% of perchloric acid) with an extra 1ml perchloric acid, while ensuring that the sample does not completely dry out.

The tissue sample and mixed acids were placed into a fluorocarbon (TFM) vessel. The open vessel was heated on the hotblock unit. After cooling, the vessel contents were allowed to settle and then diluted to a 20 ml volume and analyzed using the appropriate SW-846 method (Ref.1 of Appendix).

#### Apparatus & Materials

- Hotblock “Vulcan-84” with a temperature control (Questron Technologies Corp.)
- Fluorocarbon (TFM) digestion vessels (50 mL capacity)
- Volumetric graduated cylinder, 50 mL capacity.
- Bottle-top Dispensers
- Analytical balance, 300 g capacity, and minimum  $\pm 0.01$  g.

## Procedure

0.5g of dried tissue samples were weighed in the fluorocarbon vessel. The vessels were set up in the robot digestion system “Vulcan-84” (Questron Technologies Corp.). In each vessel with a tissue sample,  $4 \pm 0.1$  mL of acid mix (60% of concentrated nitric acid and 40% of perchloric acid) was added, and an extra 1 ml of perchloric acid was added to avoid completely drying out the sample. The temperature of the hotblock was ramped to 120 °C over 30 min and held at 120 °C for the next 60 min. Then, the temperature was ramped to 180 °C over 20 min and held at 180 °C for another 10 min. At the end of the program, the solution’s color was checked to confirm that it was clear. The vessel was allowed to cool off for a minimum of one hour before removing it from the hotblock. When the vessel cooled off to room temperature, the samples were transferred into a volumetric graduated cylinder and diluted to 20 ml with DDI water. The samples were allowed to settle until the supernatant was clear. The digestion samples were prepared for elemental analysis using ICP-OES (manufactured by Spectro, Inc.).

## Quality control

Quality control was reference material 1570a “Trace Elements in Spinach Leaves” of the National Institute of Standards & Technology (NIST) (Table A4). Before digestion, 1ppm yttrium was added to each vessel with tissue samples as an internal standard.

**A-A.** Analysis of samples using ICP-OES: the method “Plant-Water” for plant tissue samples, water extraction of sequential extraction test step 1 and leaching test.

An ICP-OES instrument was calibrated with a multi-element solution. Four standards were made. A blank was used for standard I. The highest concentration was used for standard IV. The final concentrations of the standards in 2% HNO<sub>3</sub> solutions are shown in Table A1.

**Table A1.** Standard solution solutions “Plant-Water” Method

	<b>Standard</b>	<b>Standard</b>	<b>Standard</b>	<b>BLK</b>
<b>Element</b>	<b>IV</b>	<b>III</b>	<b>II</b>	<b>I</b>
	<b>mg/l</b>	<b>mg/l</b>	<b>mg/l</b>	<b>mg/l</b>
Cd	3	1	0.2	0
Cr	3	1	0.2	0
Cu	3	1	0.2	0
Mn	3	1	0.2	0
Ni	3	1	0.2	0
P	300	100	20	0
Pb	3	1	0.2	0
S	120	40	8	0
Zn	3	1	0.2	0

The calibration blank (Standard I) was used in establishing the analytical curve. The calibration blank was prepared by acidifying with DDI water to the same concentrations of acids found in the standards and samples.

#### Conditions for ICP-OES

The instrument was set up with the following operating parameters: Forward power - 1.4 kW; Coolant gas - 13.5 LPM; Plasma gas - 1.2 LPM; Sample gas - 0.8 LPM; Sample uptake rate - 0.9 ml/min using a peristaltic pump and a fixed cross flow nebulizer. The instrument was allowed to become thermally stable before beginning, at 60 minutes of operation prior to calibration.

#### Quality control

Data of samples did not exceed the line calibration range. As a method blank, a volume of reagent water was carried through the same preparation process as a sample to determine if contamination or any memory effects were occurring. The spike sample recovery was within  $\pm$  10% of the actual value (Table A2).

**Table A2.** Quality assurance objective -water spike samples by measurement.

<b>Element</b>	<b>Recovery (%)</b>	<b>RSD (%)</b>
Cd 214.438	99.89	4.82
Cr 205.552	99.67	2.80
Cu 324.754	99.53	4.89
Mn 257.610	99.79	4.11
Ni 231.604	100.25	6.13
P 213.618	98.20	3.37
Pb 220.351	105.93	9.78
S 182.034	88.21	4.60
Zn 213.856	100.06	3.87

**Table A3.** Wavelengths and Method Detection Limit.

<b>Detection Limit of Plant –Water Method</b>	
<b>Element Type</b>	<b>DLMW</b>
Cd 214.438	4.94E-05
Cr 267.716	0.000281
Cu 324.754	0.0003
Mn 294.921	0.000237
Ni 231.604	0.000254
P 213.618	0.001077
Pb 220.353	0.00101
S 182.034	0.001519
Zn 206.200	6.20E-05

**Table A4.** Results of analysis of reference material of 1570a “Trace Elements in Spinach Leaves” of NIST

<b>Element</b>	<b>1570A reference material (mg/kg)</b>	<b>NIST data of spinach leaves 1570a (mg/kg)</b>
Cd 214.438	2.83	2.89
Cr 267.716	0.61	-
Cu 324.754	11.00	12.2
Mn 257.611	74.63	75.9
Ni 231.604	2.12	2.14
P 213.618	5001	5180
Pb 220.353	0.18	0.2
S 182.034	4487	4600
Zn 206.200	81.70	82.0

## **B. Digestion of soil samples for trace metals chapter 1 and sequential extraction of fourth step**

For analysis soil for trace metals from chapter #1 and residue of metals in sequential extraction in control soil and sludge soil, I digested samples using a hotblock with controlled temperature (Questron Technologies Corp.) and analyzed the samples using ICP-OES.

The acid digestion method for soil and sludge soil using a hotblock is applicable for the following elements: copper, cadmium, chromium, lead, manganese, nickel, phosphorus, sulfur, zinc.

### Summary of Method

A representative sample of up to 0.5 g is digested in 4 ml of acid mix (60% of concentrated nitric acid and 40% of perchoric acid) and 1ml hydrofluoric acid.

The sample and acid are placed in a fluorocarbon (TFM) vessel. The open vessel is heated on the hotblock unit. After cooling, allow the vessel contents to settle, and then dilute to 50ml volume and analyze the appropriate SW-846 method (Ref. 1).

### Apparatus & Materials

- Hotblock “Vulcan-84” with a temperature control (Questron Technologies Corp.)
- Fluorocarbon (TFM) digestion vessels (50 mL capacity)
- Volumetric graduated cylinder, 50 mL capacity.
- Bottle-top Dispensers
- Analytical balance, 300 g capacity, and minimum  $\pm 0.01$  g



## Reagents

- Concentrated nitric acid, HNO<sub>3</sub>. "BEAKER INSTRA -ANALYZED" CAS#: 769-37-2. If the method is blank, use the acid.
- Per-choric acid, HClO<sub>4</sub>, "BEAKER INSTRA -ANALYZED" CAS#: 10450-60-9.
- Hydrofluoric acid, HF, "BEAKER INSTRA -ANALYZED" CAS#: 7664-39-3".
- Reagent Water (DDI).

## Procedure

0.5g of dried soil samples were weighed in the fluorocarbon vessel. The vessels were set up in the robot digestion system "Vulcan-84" (Questron Technologies Corp.). In each vessel with a control soil or soil sludge sample, 4 mL of acid mix (60% of concentrated nitric acid and 40% of perchoric acid) and 1 ml of hydrofluoric acid were added. The temperature of the hotblock was ramped to 180 °C over 10 min and held for 20 min. At the end of the program, the solution's color was checked to confirm that it was clear. The vessel was allowed to cool off for a minimum of one hour before removing it from the hotblock. When the vessel cooled off to room temperature, the samples were transferred into a volumetric graduated cylinder and diluted to 50 ml with DDI water. The samples were allowed to settle until the supernatant was clear. The digested samples were prepared for elemental analysis using ICP-OES (Spectro, Inc.).

## Quality control

The quality controls were the reference materials "Metals in soil" cat # 540 and Metals in sewage sludge Cat # 160 of the ERA Water Company (Table B4). Before digestion, 1ppm scandium was added to each vessel with control soil and sludge soil samples as an internal standard.

**B-A.** Analysis of samples using ICP-OES by SPECTRO by the Method “Soil” for trace metals and residue metals of fourth step of sequential extraction test.

Instruments were calibrated with a multi-element solution, with 4 standards. The final concentrations of the standards in 2% HNO<sub>3</sub> solutions are shown in Table B1.

**Table B1.** Standard solution solutions “Soil” Method

	Standard	Standard	Standard	BLK
Element	IV	III	II	I
	mg/l	mg/l	mg/l	mg/l
Cd	2	0.4	0.2	0
Cr	20	4	2	0
Cu	20	4	2	0
Mn	20	4	2	0
Ni	5	1	0.5	0
P	100	20	10	0
Pb	10	2	1	0
S	50	10	5	0
Zn	50	10	5	0

The calibration blank (Standard I) was used in establishing the analytical curve. The calibration blank was prepared by acidifying DDI water to the same concentrations of acids found in the standards and samples.

#### Conditions for ICP-OES

The instrument was set up with the following operating parameters: Forward power - 1.4 kW; Coolant gas - 13.5 LPM; Plasma gas - 1.2 LPM; Sample gas - 0.8 LPM; Sample uptake rate - 0.9 ml/min using a peristaltic pump and a fixed cross flow nebulizer. The instrument was allowed to become thermally stable before beginning, at 60 minutes of operation prior to calibration.

## Quality control

Data from the samples did not exceed the line calibration range. As a method blank, a volume of reagent water was carried through the same preparation process as a sample to determine if contamination or any memory effects were occurring. Soil samples from Musgrave Research Farm in Aurora, NY were spiked and analyzed. The spike sample recovery was within  $\pm 10\%$  of the actual value (Table B2).

**Table B2.** Quality assurance objective – soil spike samples by measurement.

<b>Soil from Musgrave Research Farm in Aurora, NY</b>					
<b>Element</b>	<b>recovery%</b>	<b>RSD%</b>	<b>Acceptance range</b>		<b>MDL</b>
Cd	95	0	102	102	0.004
Cr	96	1	100	96	0.010
Cu	100	0	98	96	0.005
Mn	99	0	92	90	0.006
Ni	106	0	96	94	0.028
P	98	2	93	78	0.033
Pb	99	0	78	68	0.035
S	98	2	100	90	0.107
Zn	93	3	273	124	0.032

**Table B3.** Wavelengths and Method Detection Limit.

<b>Detection Limit of Soil Method</b>	
<b>Element</b>	<b>MDL (<math>\mu\text{g/g}</math>)</b>
Cd 214.438	0.0041
Cr 267.716	0.0098
Cu 324.754	0.0046
Mn 294.921	0.0065
Ni 231.604	0.0281
P 213.618	0.0327
Pb 220.353	0.0349
S 182.034	0.1071
Zn 206.200	0.0321

**Table B4.** Results of analysis of reference material of Metals in soil cat# 540, metals in Sewage sludge cat # 160 of ERA a water company.

<b>Sample</b>	<b>REF 540 metals in soil</b>	<b>ERA data cat #540</b>	<b>REF 160 metals in sewage sludge</b>	<b>ERA data cat #160</b>
	<b>mg/Kg</b>	<b>mg/Kg</b>	<b>mg/Kg</b>	<b>mg/Kg</b>
Cd 214.438	93	90.00	125	124
Cr 267.716	181	187.00	124	122
Cu 324.754	140	141.00	900	902
Mn 257.611	601	612.00	550	553
Ni 231.604	84	86.80	100	104
P 214.914	600	-	41411	-
Pb 220.353	90	92.40	128	129
S 182.034	275	-	20383	-
Zn 213.856	316	312.00	901	907

### **C. Method of extraction of control soil samples and sludge soil in Modified Morgan solution (second step of sequential extraction test).**

#### General Discussion

The Modified Morgan solution is a universal extraction solution used in many professional soil labs and universities for finding the nutrients that will dissolve and be available for plants.

- Modified Morgan Solution contains 0.62N  $\text{NH}_4\text{OH}$  and 1.25N  $\text{CH}_3\text{COOH}$ , buffer pH 4.8 with concentrated  $\text{NH}_4\text{OH}$  or acetic acid.
- The soil samples and the master soil of Cornell Nutrient Analysis Laboratory (CNAL) after water extraction were air dried, weighed, and loaded into a 125 ml Erlenmeyer flask.
- 30ml Modified Morgan solution was added to the flask with soil and to another empty flask (see schema of sequential extraction).
- Flasks were placed into racks in the box on the shaker, secured firmly by closing the lid of the box, and shaken for fifteen (15) minutes.
- Racks were removed from the shaker. The contents of the flasks were poured into the funnel filters and allowed to filter until all the liquid was in the test tubes.
- The funnel racks were removed, and the soil on the filter papers was air dried and weighed to prepare for the next step of sequential extraction.

Aliquots of the extraction were analyzed using ICP-OES by the “Modified Morgan” method.

### C-A. Conditions of method “Modified Morgan” for ICP-OES

The instrument was set up with the following operating parameters: Forward power - 1.4 kW; Coolant gas - 13.5 LPM; Plasma gas - 1.2 LPM; Sample gas - 0.8 LPM; Sample uptake rate - 0.9 ml/min using a peristaltic pump and a fixed cross flow nebulizer. The instrument was allowed to become thermally stable before beginning.

**Table C1.** Standard solution solutions “Modified Morgan” Method

	Standard	Standard	BLK
Element	M2	M1	I
	mg/l	mg/l	mg/l
Cd	1	0.5	0
Cu	1	0.5	0
Ni	1	0.5	0
Pb	1	0.5	0
Zn	1	0.5	0

#### Quality control

Data from the samples did not exceed the line calibration range. As a method blank, a volume of reagent water was carried through the same preparation process as a sample to determine if contamination or any memory effects were occurring. Soil samples from Musgrave Research Farm in Aurora, NY were used as a master soil for quality control. The master soil data were collected by CNAL.

**Table C2.** Detection of limit of method of “Modified Morgan”, ICP-OES

<b>Detection Limit of Modified Morgan Method</b>		
<b>Elements</b>	<b>DLM</b>	<b>ratio of DLM and Method BLK</b>
<b>Type</b>	<b>ppm</b>	<b>%</b>
Cd 214.438	0.00021	0.021
Cu 324.754	0.00016	0.016
Ni 231.604	0.00270	0.270
Pb 220.353	0.00338	0.338
Zn 213.856	0.00019	0.077



**D. Method of extraction of control soil samples and sludge soil in Mehlich-3 solution (third step of sequential extraction test).**

Mehlich-3 is an extraction solution that estimates plant availability of most macro- and micro-nutrients in soils of acidic to neutral pH using a dilute acid-fluoride-EDTA solution of pH 2.3.

The composition of solution is: 0.2N CH<sub>3</sub>COOH + 0.25N NH<sub>4</sub>NO<sub>3</sub> + 0.015N NH<sub>4</sub>F + 0.013N HNO<sub>3</sub> + 0.001M EDTA (ethylenediaminetetraacetic acid). This extract was developed by Dr. Adolf Mehlich in 1984. The solution used for the extraction was made by Texas Scientific Products LLC, CAS # 48-0101-4.

- The soil samples, and master soil of Cornell Nutrient Analysis Laboratory (CNAL) after the Modified Morgan extraction of step 2 of sequential extraction of test were air dried, weighed and loaded into a 125 ml Erlenmeyer flask.
- 20ml Mehlich-3 solution was added to the flask with soil and another empty flask (see schema of sequential extraction).
- Flasks were placed into racks in the box on the shaker, secured firmly by closing the lid of the box, and shaken for fifteen (15) minutes.
- Racks were removed from the shaker. The contents of the flasks were poured into the funnel filters and allowed to filter until all the liquid was in the test tubes.
- The funnel racks were removed, and the soil on the filter papers was air dried and weighed to prepare for the next step of sequential extraction.

Aliquots of the extraction were analyzed using ICP-OES with the “Mehlich-3” method.

#### D-A. Conditions of method “Mehlich-3” for ICP-OES

The instrument was set up with the following operating parameters: Forward power - 1.4 kW; Coolant gas - 13.5 LPM; Plasma gas - 1.2 LPM; Sample gas - 0.8 LPM; Sample uptake rate - 0.9 ml/min using a peristaltic pump and a fixed cross flow nebulizer. The instrument was allowed to become thermally stable before beginning, for at least 60 minutes of operation prior to calibration.

**Table D1.** Standard solution solutions “Mehlich-3” Method

	<b>Standard</b>	<b>Standard</b>	<b>BLK</b>
<b>Elements</b>	<b>STD 3</b>	<b>STD 2</b>	<b>STD1</b>
	<b>mg/l</b>	<b>mg/l</b>	<b>mg/l</b>
Cd	1	0.5	0
Cu	1	0.5	0
Ni	1	0.5	0
Pb	1	0.5	0
Zn	5	2.5	0

#### Quality control

Data from the samples did not exceed the line calibration range. As a method blank, a volume of reagent water was carried through the same preparation process as a sample to determine if contamination or any memory effects were occurring. Soil samples from Musgrave Research Farm in Aurora, NY were used as a master soil for quality control. The master soil data were collected by CNAL.

**Table D2.** Detection of limit of method of “Mehlich-3”, ICP-OES

<b>Detection Limit of Mehlich-3 Method</b>	
<b>Elements</b>	<b>DLM</b>
<b>Type</b>	<b>ppm</b>
Cd 214.438	0.0020
Cu 324.754	0.0009
Ni 231.604	0.0016
Pb 220.353	0.0005
Zn 213.856	0.0007

### Schema of Sequential Extraction

<b>SAMPLE ID</b>	<b>H2O Extraction Wt., g @ 15 min agit.</b>	<b>H2O Extraction Vol., ml @ 24.5C</b>	<b>Soil Wt. Recovered, g</b>	<b>Modified Morgan Extraction Wt., g @ 15 min agit.</b>	<b>Modified Morgan Extraction Vol., ml @ 26.0C</b>	<b>Soil Wt. Recovered, g</b>	<b>Mehlich-3 Extraction Wt., g @ 15 min agit.</b>	<b>Mehlich-3 Extraction Vol., ml @ 26.0C</b>	<b>Soil Wt. Recovered, g</b>
3C-1	15.049	30.0	14.484	14.368	30.0	13.223	13.105	20.0	11.790
3C-2	15.025	30.0	12.727	12.606	30.0	11.468	11.380	20.0	10.572
3C-3	15.004	30.0	14.123	14.058	30.0	13.134	13.098	20.0	12.055
3S-1	15.001	30.0	14.240	14.103	30.0	12.089	12.040	20.0	10.786
3S-2	15.009	30.0	13.890	13.819	30.0	12.666	12.621	20.0	11.499
3S-3	15.041	30.0	13.383	13.329	30.0	11.980	11.963	20.0	11.272
Aurora	15.028	30.0	13.879	13.822	30.0	12.560	12.538	20.0	11.335

C= "control soil "replicates

S= "soil sludge" replicates

## E. Determination of Soil pH.

### Background

Soil pH is a measure of hydronium ion ( $\text{H}_3\text{O}^+$ , or more commonly  $\text{H}^+$ ) activity in the soil solution. Soil pH influences many facets of crop production and soil chemistry, including availabilities of nutrients and toxic substances, activities and nature of microbial populations, and activities of certain pesticides.

Soils with pH values below 7 are referred to as "acid" and those with pH values above 7 as "alkaline". Soils at pH 7 are referred to as "neutral."

A suspension of two parts water to one part soil (2:1 ratio) is prepared and allowed to stand at room temperature for one (1) hour. The pH can then be determined with a manual pH meter.

### Apparatus

- pH Meter LIGNIN Robotic pH system
- Electrodes: Ross combination pH electrodes from Thermo Scientific (Orion 815600) for manual determination. Lignin brand electrodes and amplifiers.  
LIGNIN #505-765-5742
- 3 oz. plastic cups
- Pipetting machine: Oxford automatic variable speed dispenser, Catalog No. 470, Oxford Laboratories Inc., Foster City, CA 94404 capable of delivering 20 ml (optional).
- Soil Scoop, 20  $\text{cm}^3$  (cc).
- Stirring rod.

## Reagents

- Buffer, pH 7.00: Available from laboratory supply houses. Calibrate a pH meter with a certified pH 7.00 buffer. Read the pH of the prepared solution, and adjust the pH to 7.00.
- Buffer, pH 4.00: Available from laboratory supply houses. Calibrate a pH meter with a certified pH 4.00 buffer. Read the pH of the prepared solution; it should read pH 4.00 without adjustment.
- Deionized water.

## Procedure

1. Place cups in a rack. Label cups with sample IDs.
2. Using the 20 cm<sup>3</sup> scoop, scoop a portion of soil from each sample. Also, scoop 1 portion of soil for the master soil of CNAL.
3. The pipetting machine is set to deliver 20 ml, add 40 ml (2X) of distilled water to each cup with sample.
4. Stir each sample several times, until a homogeneous slurry is achieved. Rinse the stirring rod with distilled water.
5. Allow to stand for one (1) hour.

## **F. Soil Organic Matter by Loss on Ignition.**

### Background

A 1 cm<sup>3</sup> tared scoop is used to place a known weight of soil into a tared crucible. The sample is then ashed for two hours at 500°C. The percent weight loss on ignition of the soil is calculated. Soil samples are expressed as percent organic matter by use of a calibration equation developed from a comparison of the loss on ignition results to the results obtained by a modified Wakley-Black procedure.

### Apparatus

- Oven - capable of maintaining a temperature of 105°C.
- Muffle furnace - capable of maintaining a temperature of 500°C.
- Crucibles, 10-ml, porcelain.
- Balance - minimum capacity of 50gm, +/-0.001gm.
- Scoop - 1 cm<sup>3</sup> capacity.

### Procedure

1. Place a crucible on a tared balance, and record the weight.
2. Using the 1 cm<sup>3</sup> scoop, place 2 level scoops of the soil sample into the crucible.  
Record the weight of the crucible and soil sample. Place in 105°C oven for 1 hour, cool for 10 minutes and record weight. This first set of weights is used for the % moisture determination.
3. Place the crucible containing sample into a muffle furnace, which has equilibrated to 500°C, and ash the sample for two hours.
4. Remove the crucible containing the sample, cover with aluminum foil and allow to cool for 45mins-1 hour.
5. Tare the balance. Record the combined weight of the crucible and ashed sample.

## Calculations

$$1. \% \text{ Moisture} = \frac{B-C}{C-A} \times 100$$

$$2. \text{ Moisture correction factor} = \frac{100 + \% \text{ Moisture}}{100}$$

$$3. \% \text{ Loss-on-Ignition} = \frac{C-D}{C-A} \times 100$$

$$4. \text{ Organic matter: \% OM} = (0.7 \times \text{LOI}) - 0.23$$

Where:

A = weight of crucible

B = weight of crucible + fresh soil sample

C = weight of crucible + sample after drying @ 105-110°C for 1 hour

D = weight of crucible + sample after ashing @ 500°C for 2 hours

The moisture correction factor is used as a multiplication factor to convert parameters measured on fresh or air dried samples to a dry weight basis.



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