CHAPTER ONE

INTRODUCTION

Sewage sludge is the solid material that is generated as the byproduct of municipal wastewater treatment. Biosolids are the treated sewage sludge that meets the criteria set for beneficial use. The term biosolids is used to emphasize the beneficial nature of this product. The United States Environmental Protection Agency (USEPA) defines biosolids as "the nutrient-rich organic materials resulting from the treatment of domestic sewage in a treatment facility. When treated and processed, these residuals can be recycled and applied as fertilizers to improve and maintain productive soils and stimulate plant growth" (USEPA, 1993).

Land application of biosolids is becoming the most common method of handling the waste residuals. Land application of human wastes to improve crop production is an age-old practice. Records of this practice date back to the Roman Empire. It is reported that Chinese returned sewage, referred to as "night soil", to land to improve soil productivity. Little sewage treatment occurred until the mid 20th century. The application of untreated waste raised concerns due to the emergence of health problems. In the U.S., restrictions on the disposal of pollutants to waterways were put forward by the Federal Water Pollution Control Act Amendments of 1972 (PL 92-500, 1972) which encourages the beneficial use of sewage. Subsequent legislation increased the regulations on land application of biosolids (USEPA, 1983). The United States Environmental protection Agency estimated that approximately 6.9 million Mg of biosolids were generated in 1998 (USEPA, 1999). Sixty percent of the biosolids generated were beneficially used either through land application, composting or as a landfill cover. This number is expected to increase to 7.6 million Mg by 2005 and to 8.2 million Mg in 2010.

The utilization of biosolids is expected to increase in the near future as production continues to grow. The ban on ocean dumping and the high cost associated with landfilling are some factors that are contributing to the recycling of biosolids to agricultural land (USEPA, 1999; Harrison et al., 1997). In many cases, land application of biosolids is less expensive than other disposal methods (Federal Register, Vol. 54, No.

23, pp. 5476-5902, February 6, 1989). Meanwhile, the quality of biosolids has continued to improve as industrial pretreatment of wastewater has advanced (Walker, 1998; Harrison et al., 1997; Stehouwer, et al., 1998). The USEPA expects that the use of biosolids will increase to 66% in 2005 and to 70% in 2010 (USEPA, 1999). Currently, the states of Arizona, Colorado, Delaware, Indiana, Iowa, Maine, Oregon, and Wyoming use more than 90% of the biosolids they generate, with Indiana using up to 100% (Biocycle, 2000).

Biosolids can be used for its soil conditioning and nutrient supplying capabilities. Macronutrients (e.g., N, P), micronutrients (e.g., Cu, Zn, Mo, Bo, Mn), and organic carbon can be beneficially recycled for crop production by applying biosolids to agricultural land. On the average, biosolids contain 3.2 % N, 2.3 % P, and 0.3 % K (Metcalf and Eddy, 1991). Although biosolids have generally low nutrient contents, their use reduce or eliminate the need for commercial fertilizers.

When treated and processed properly, biosolids can be land applied. Biosolids land application is regulated by USEPA 40 CFR Part 503, the Standards for the Use and Disposal of Sewage Sludge (USEPA, 1993). This rule demands that trace element contents of biosolids applied to land be below a specified concentration to ensure the safety of humans, animals, and plants. These standards were based on a risk assessment (USEPA, 1992) that was developed to evaluate the potential negative effects of pollutants in biosolids. The rule considers the exposure of human, animals, and plants to trace elements in biosolids through 14 possible pathways (Table 1). Each element has a reference pollutant load calculated for each pathway to avoid detrimental effects on exposed individuals (USEPA 1995). The limiting value for a certain metal is its smallest reference pollutant load (Table 2).

Table 1.1. Exposure pathways used in USEPA Part 503 (Harrison et al., 1997).

Pathway	Description of highly exposed individual.
Sludge →Soil→ Plant→ Human	Human (except home gardener) lifetime ingestion of plants grown in sludge-amended soil.
Sludge →Soil →Plant →Human	Home gardener lifetime ingestion of Plants grown in sludge-amended soil.
Sludge →Human	Human ingesting sludge.
Sludge →Soil→ Plant →Animal →Human	Human lifetime ingestion of animal products (animals raised on forage grown on sludge-amended soil).
Sludge →Soil→Animal →Human	Human lifetime ingestion of animal products (animal ingest sludge directly).
Sludge →Soil→ Plant →Animal	Animal lifetime ingestion of plants grown on sludge amended soil.
Sludge →Soil→ Animal	Animal lifetime ingestion of sludge.
Sludge →Soil→ Plant	Plant toxicity due to taking up pollutants when grown in sludge-amended soils.
Sludge →Soil→ Organism	Soil organism ingesting sludge/soil mixture.
Sludge →Soil→ Plant →Predator	Predator of soil organisms that have been exposed to sludge-amended soils.
Sludge →Soil→ Airborne dust→ Human	Adult human lifetime inhalation of particles (dust).
Sludge →Soil→ Surface water→ Human	Human lifetime drinking surface water and ingesting fish containing pollutants in sludge.
Sludge →Soil→ Air→ Human	Human lifetime inhalation of pollutants in sludge that volatilize to air.
Sludge →Soil→ Groundwater→ Human	Human lifetime drinking well water containing pollutants from sludge.

This, in turn, permitted the calculation of allowable pollutant concentration in biosolids for each metal (Table 3). Land application is also subjected to state regulations. Each state has the choice to opt for regulations that are more stringent than the federal standards (Harrison et al., 1997). Indeed, 37 states have promulgated regulations that are the same or more stringent than Part 503 (BioCycle*,* 1997).

Table 1.2. The limiting pathways according to the USEPA Part 503 risk assessment. (USEPA, 1992)

Element	Limiting pathway
As	Child ingesting sludge
Cd	Child ingesting sludge
Cu	Phytotoxicity
Pb	Child ingesting sludge
Hg	Child ingesting sludge
Mo	Animal eating feed
Ni	Phytotoxicity
Se	Child ingesting sludge
Zn	Phytotoxicity

Strict regulations and quality standards must be met before biosolids can be land applied. The Part 503 rule that regulate the use and disposal of biosolids, includes concentration limits for nine trace elements in biosolids, as well as standards for pathogen reduction, vector attraction, and record keeping and monitoring.

With respect to trace elements concentrations, Part 503 provides the following four sets of trace elements limits: the ceiling concentration limits, pollutant concentration limits, cumulative pollutant loadings and annual pollutant loadings rate. Pollutant concentration limits (mg kg^{-1}, dry weight basis) are one of the criteria necessary for biosolids to be considered as “exceptional quality” (EQ) status. Tracking is not required for land application of “EQ” biosolids, and such biosolids can be applied with no restriction in duration or amount.

Table 1.3. Pollutant limits in USEPA Part 503 Regulations (Harrison et al., 1997)

Pollutant	Max. Pollutant Conc. mg kg^{-1}	Max. Ceiling Conc. mg kg^{-1}	CPLR kg ha^{-1}	APLR kg ha^{-1} yr^{-1}
As	41	75	41	2
Cd	39	85	39	1.9
Cu	1500	4300	1500	75
Pb	300	840	300	15
Hg	17	57	17	0.85
Mo		75		
Ni	420	420	420	21
Se	100	100	100	5
Zn	2800	7500	2800	140
Applies to:	Bulk and bagged sludges.	All land-applied Sludges.	Bulk Non-EQ sludges.	Bagged sludges not meeting EQ.

CPLR. Cumulative Pollutant Loading Rate.
APLR. Annual Pollutant Loading Rate.

Ceiling concentrations (mg kg^{-1}, dry weight basis), are the maximum concentration of a pollutant that can be present in land-applied biosolids. Biosolids that contain higher concentrations of any of the specified pollutant cannot be land-applied.

Cumulative Pollutant Loadings (CPLR, kg ha^{-1}) apply to biosolids that meet Ceiling Concentration Limits but not Pollutants Concentration Limits for any of the nine pollutants. The CPLR is the maximum amount of each regulated pollutant that can be land applied to a site before application must stop. This requires that the applier track the amount of each pollutant applied. Annual Pollutant Loading Rates (APLR) applies to packaged biosolids. The APLR is the maximum amount of pollutants in biosolids that can be applied to a site during a 365-day period.

The Part 503 rule permits long-term application of biosolids to agricultural land on the assumption that the soil accumulation of trace elements, from biosolids meeting ceiling concentration limits, will not cause environmental or health hazards before the application must cease. An independent report by the National Research Council concluded that “established numerical limits (in the Part 503 rule) on concentrations of pollutants added to cropland by biosolids are adequate to ensure the safety of crops produced for human consumption”(National Research Council, 1996).

The land application of biosolids can significantly increase trace metal concentrations in soils. There is a concern by some scientists over the long-term fate of these trace metals in the environment. Harrison et al. (1997) asserted that "Current US federal regulations governing the land application of sewage sludges do not appear adequately protective of human health, agricultural productivity or ecological health." They argued that the EPA analysis did not adequately consider the variability in plant accumulation and soil sorption capacity of trace metals.

Most of the data used to develop Part 503 rule were from studies in which trace element uptake by plants was measured during the years of biosolids application. Research on the behavior of trace metals in soils, which have stabilized after biosolids application, is needed to understand the long-term effect of biosolids disposal on agricultural land.

OBJECTIVES

The purpose of this research was to investigate the effects of biosolids application on trace metals mobility in soils and their availability to plants 17-19 years after application. We will address this objective by examining the distribution of trace metals with soil depth, mass balance of applied trace metals, availability of biosolids-applied trace metals, uptake coefficients of Cd, Ni, Cu, and Zn in lettuce, radish, and barley, and the linearity of uptake curves.

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CHAPTER TWO

LITERATURE REVIEW

Introduction

The USEPA Part 503 rule permits long-term application of biosolids to agricultural land based on the assumption that the soil accumulation of trace metals from biosolids meeting ceiling concentration limits will not cause environmental or health hazard before the application must cease. The protectiveness of the Part 503 rule has been called into question based on assumptions regarding trace metal immobilization made in the underlying risk assessment (McBride, 1995; Harrison et al., 1997; Schmidt, 1997). Most studies used in the risk analysis were short term. With time, alternate metal biochemical equilibrium may be reached after the cessation of biosolids application as the organic matter decomposes. Thus, it is important to investigate the long-term fate of biosolids-added trace metals after cessation of biosolids application. Particularly important aspects that need to be investigated include long-term metal mobility and availability, plant trace metals uptake and toxicity, and leaching and colloidal mediated transport.

Soil factors affecting metal availability

Soil factors that may influence metal solubility and movement in biosolids amended soil include pH, total metal concentration, texture, and organic matter (Dowdy and Volk, 1983; Welch and Lund, 1987). Sauerbeck (1991) reported that metal concentration in soils and pH are more important than organic matter and texture in regulating the availability of Cd, Cu, Ni, and Zn to several plant species. Properties of the metal, soil binding sites, pH, concentration of complexing (organic/inorganic) agents, and competing cations are also factors that have been indicated to affect metal mobility and, therefore, uptake and leaching (Tyler and McBride, 1982).

Hooda (1997) noticed that total metal concentration, pH, and clay fraction of the soil affects trace metals concentration in plants. Plants that were grown at neutral soil pH and fine-textured soil tend to accumulate fewer trace metals. The solubility and , hence, the mobility of most metals increased with lower soil pH. Liming to agronomically acceptable pH levels will prevent metal toxicity and reduce uptake (Brallier et al., 1996; Hooda et al., 1996). Chang (1984) noted that soil texture did not appear to influence metal sorption since the Domino loam and Greenfield sandy loam soils exhibited the same metal accumulation characteristics. It is also reported that organic matter may form metal complexes that reduce metal movement (Tyler and McBride, 1982) or may increase metal movement at high pH (Kuo and Baker, 1980)

Trace metal binding capacity of biosolids

Iron, Al, and Mn oxyhydroxides and organic matter added to soil as biosolids increased the soil's capacity to adsorb and bind trace metals (Corey et al., 1987). Considerable amounts of organic matter are applied to soil with long-term biosolids application since organic matter comprises 20-60% of the dry matter of biosolids (Metcalf and Eddy, 1991). The biosolids constituents responsible for reduced metal availability in soil amended with biosolids have been a topic of debate for some years. Some researchers proposed that the organic matter component of biosolids is the primary factor controlling metal availability (Beckett et al., 1979). Other scientists (Corey et al., 1981; Corey et al., 1987; Essington and Mattigod, 1991) emphasized the role of inorganic components (Fe, Mn, and Al oxyhydroxides minerals) in the formation of heterogeneous precipitates in soils as well as the precipitate's ability to adsorb trace metals. Biosolids contain high levels of Mn, Fe, Al and other elements, which may play an important role in controlling metal availability in amended soil. The freshly precipitated hydrous oxides provide high surface areas with highly reactive sites for sorbing metal ions. Trace metals in biosolids exist as soluble, organically complexed, adsorbed, and co-precipitated with Al, Fe, P, and Ca solid phases (Lake et al., 1984; Jing and Logan, 1992). Fractionation of composted biosolids according to size, density, and magnetic properties revealed that trace metals were associated with fine and dense inorganic fractions (Essington and Mattigod, 1990; Hyun et al., 1998).

Researchers who believe that organic matter controls biosolids metal availability expect that metal binding capacity of biosolids to be reduced as the organic matter decomposes, “time-bomb” hypothesis. As a result, trace metals released are expected to behave as inorganic salts (McGrath et al., 1988; McBride, 1995). In a long-term study, Brown et al. (1998) found that the loss of organic matter did not increase the phytoavailability of Cd and suggested that organic carbon loss was not the primary factor limiting Cd phytoavailability to lettuce for this study. Similar findings were reported by other researchers (Mahler et al., 1987; Chang et al., 1997). They concluded that inorganic components play a more significant and persistent role in limiting phytoavailability than organic matter (Brown et al., 1998). Inorganic mineral forms (Fe and Al oxides, silicates, phosphates, and carbonates) may compose 30 to 60% of the digested primary biosolids (Metcalf and Eddy, 1991). Selective adsorption and precipitation of trace metals on oxide surfaces provide the basis of adsorption in biosolids amended soils. The potential for trace metals sorption in biosolids amended soils is further increased as newly formed oxides precipitate (Corey et al., 1987).

Li et al. (2001) reported an increase in Cd sorption in biosolids amended soil relative to the control soil. The removal of organic carbon reduced sorption of Cd but did not account for the observed increase in sorption, indicating that organic carbon is not the only factor responsible for increased sorption. Hettiarachchi et al. (2003) conducted a series of adsorption-desorption experiments on different fractions of soil taken from long-term experimental sites. A difference in adsorption capacity between the control and biosolids-amended soil could not be explained by the removal of only biosolids organic carbon; however, the removal of organic and Fe/Mn oxides eliminated the difference in adsorption between the control and biosolids amended soil. In desorption experiments, a higher hysteresis was noticed for biosolids amended soil. This difference in desorption hysteresis with control samples was again minimized when both oxides and organic carbon were removed. They concluded that the increased sorption capacity of soil is due to oxides and organic carbon fractions added with the biosolids

Trace element immobilization

The concept of long term metal immobilization in soil was challenged when mass balance calculations in a number of land application studies were unable to account for up to half of the biosolids applied trace metals (Williams et al., 1987; Alloway and Jackson, 1991; Dowdy et al., 1991; Brown et al., 1997; McBride, 1997; Richards et al., 1998; Berti and Jacobs, 1998). Researchers reporting such discrepancies have attempted to explain the apparent metal loss as due to leaching (McBride, 1995), metal losses via preferential flow (Dowdy et al., 1991), tillage losses (Williams et al., 1987), dispersion of trace metals due to tillage (McGrath and Lane, 1989), incomplete chemical extraction from soil (Dowdy et al., 1991), and bulk density estimations (Chang et al., 1984).

Uptake of trace metals in crops represents a small proportion (0.5-1%) of the metal removed and is usually neglected (McGrath, 1987). Leaching studies have reported contradictory results. Studies of metal leaching in soil columns (Emmerich et al., 1982; Miller et al., 1983) or field investigations (Chang et al., 1982, 1984; Williams et al., 1984, 1987; McGrath and Lane, 1989) generally concluded that trace metals were strongly bound to topsoil components. Other field investigations (Lamy et al., 1993) and soil column studies (Prakash et al., 1997; Frenkel et al., 1997) have documented rapid leaching of significant concentration of several trace metals, including Zn, Cu, Cr, and Cd. In an attempt to explain the absence of trace metals from shallow profiles, some researchers suggested that preferential flow combined with transport of trace metals as relatively non-adsorptive complexes can leach without accumulation in shallow subsoil (Camobreco et al., 1996; Richards et al., 1998).

Brown et al. (1997) reported substantial apparent losses of biosolids–applied trace metals from the zone of application nearly 20 years after biosolids application where little or no tillage had occurred. McBride (1999) argued that tillage cannot readily explain why some trace metals are lost at relatively higher rates than others.

Potential for colloids to facilitate metal leaching

Studies that examined trace metal concentration with depth in biosolids treated soil generally report increased metal concentration to a depth of 0.3 m but no measurable increase below 0.4 m (Chang et al., 1984; McGrath, 1989; Sloan et al., 1997). Barbarick et al. (1998) reported no leaching of trace metals, except Zn, below the plow layer from five or six biosolids applications during an 11-year period. However, Brown et al. (1997) detected movement of Pb, Zn, and Cu below 0.6 m in a Galestown sand (sandy siliceous, mesic Psammentic Hapludult) and below 0.8 m in a Christiana fine sandy loam (clayey, kaolinitic, mesic Typic Paleudult) from high application rates of 0 to 448 Mg/ha of a variety of limed and unlimed biosolids. Streck and Richter (1997) observed that 5% of applied Cd and Zn moved below 0.7-0.9 m.

McBride (1999) found that some trace metals including Cu, Zn, Ni, and Cd continued a gradual process of leaching from contaminated subsoil and through the shallow subsoil more than 15 years after biosolids application. McBride (1999) observed that 70% of the Zn, 82% of the Cd, and 90% of the Cu in percolates collected at 60 cm depth from a long-term study were not in the form of free cations. He indicated that the mobile trace metals were largely in complexed form, probably with dissolved organic matter. Kalbitz and Wennerich (1998) conducted percolation experiment using a lysimeter on undisturbed soil cores from a highly contaminated wetland soil. The results showed considerable mobilization of Zn, Cd, Cu, Hg, and Cr. Concentration of Cr, Hg, and Cu in the percolates were positively correlated with dissolved organic matter.

Richards et al. (1998) contended that the lack of significant increases in soil metal movement below the incorporation zone (Chang et al., 1984; Williams et al., 1987; McGrath and Lane, 1989; Unwin et al., 1989; Bell et al., 1991; Dowdy et al., 1991; Streck and Richter, 1997) cannot be used as evidence for metal immobility. Incomplete metal recovery in the calculation of mass balances between applied trace metals and trace metals present in the soil profile provide doubts as to the fate of the applied trace metals. Complexed trace metals may pass through the subsoil without leaving detectable 'tracks' since preferential flow paths typically occupy a small fraction of the subsoil volume

(McBride et al., 1997; Richards et al., 1998). Due to their physico-chemical properties, mobile soil colloids can potentially have greater metal sorption affinity than bulk soil (Karathanasis, 1999); therefore, trace metals could be preferentially bound to mobile colloid surfaces, which would enhance their transport to deeper depths rather than soil pore walls.

The velocity of solute movement to groundwater has been shown to be greatly enhanced via preferential flow (Steenhuis et al., 1995). Rapid flow of water can occur in these preferential networks after bypassing most of the soil matrix (Li and Shuman, 1996). This could increase soluble and ligand-complexed metal movement.

Antoniadis and Alloway (2002) employed this mechanism to explain the loss of trace metals from packed columns. In their study, 50 Mg ha^{-1} of biosolids was applied to the topsoil without incorporation. There was significant increase in Cd and Zn concentrations down to 8 cm and Ni concentration down to 10 cm after applying an equivalent of 900 mm water year^{-1}. Dissolved organic carbon, Ni, and Zn were found in the leachates of the 40-cm length columns at elevated concentrations, which led the researchers to suggest a possible role of dissolved organic carbon in facilitating the movement of Zn and Ni through the soil profile. This hypothesis was supported by Sawhney et al. (1994) and Guisquiani et al. (1992).

Dissolved organic carbon can play an important role in metal mobility. Dissolved organic matter bound trace metals may be more mobile than dissolved uncomplexed trace metals. Low molecular weight hydrophilic dissolved organic matter components are mobile and might influence metal transport (Bolton et al., 1991; Jardine et al., 1992; Zhu and Alva, 1993; McGrath et al., 1993; Christensen et al., 1996). Several studies reported the mobility of trace metals with soluble organics (Lund et al., 1976; Gerritse et al., 1982; Christensen, 1985; Dudley et al., 1986; Van Erp and Van Lune, 1991; DelCastilho et al., 1993; Persicani, 1995; Driscoll et al., 1995; Camobreco et al., 1996; Breault et al., 1996). Water dispersible colloidal particles migrating through preferential flow channels have significantly enhanced metal mobility (Mills et al., 1991; Liang and McCarthy, 1995; Ouyang et al., 1996). Sandhu and Mills (1987) reported that up to 90% of the eluted Cr and As from leaching columns were associated with trace metals oxides. Roy and Dzombak (1997) found that mobility and transport of Ni was enhanced by colloids in sand-packed columns. Karathanasis (1999) observed up to 50 times enhancement in metal transport due to colloids depending on metal, colloid, and soil properties in a laboratory column experiment, in which suspensions of colloids with a range of surface properties were applied at a constant flux to undisturbed soil media columns.

Correlations have been found between levels of dissolved organic matter and the concentrations of Hg, As, Cu, Cd, Zn, and Cr in leachates (Campbell and Beckett, 1988; McBride et al., 1997; Kalbitz and Wennrich., 1998). Temminghohh et al. (1997) observed that Cu mobility was enhanced in sandy soil in the presence of dissolved humic acid.

Soluble complexing ligands in biosolids cause certain trace metals, particularly Cu, to be more mobile than they would be in the absence of organics (Camobreco et al., 1996; Frenkel et al., 1997). Recent evidences suggest that a substantial fraction of dissolved Cd in biosolids treated soil can be complexed (Hamon et al., 1995). Dunnivant et al. (1992) found that Cd migration increased in the presence of stream derived dissolved organic matter. Michopolus (1999) demonstrated that soluble organic matter of low molecular weight derived from organic layers would not cause significant leaching from soil A horizon. Udin et al. (1993) reported a decrease in Cu mobility in the presence of dissolved organic matter. In a column transport experiment with sesquioxide coated sandy aquifer material, Han and Thompson (2003), found that the mobility of DOM followed the order of hydrophilic DOM > hydrophobic acid DOM > hydrophobic neutral DOM for the same molecular weight fraction. They also reported an increase in Cu transport associated with DOM in the early stages of transport (75 pore volumes). Dissolved organic matter components inhibited Cu transport in the later stages. In the long term, Cu mobility may be retarded by the presence of dissolved organic matter due to formation of ternary complexes and pH buffering by dissolved organic matter (Han and Thompson, 2003). Christensen et al. (1996) found that migration velocity of trace metals increased in the presence of dissolved organic matter from landfill leachate but did not exceed 1.2% of water migration velocity and concluded that effect of dissolved organic matter on mobility of Cu, Ni, and Zn may cause minor environmental problems or effects. Gerritse et al. (1982) reported that metal movement in homogenized soil columns has velocities of 0.01 to 0.1% of the velocity of water.

Long-term availability of biosolids-added trace metals

Most of the data used to develop Part 503 rule were from studies in which trace element uptake by plants were measured during the years of biosolids application with the assumption that trace element availability does not change after biosolids applications cease. It is imperative to understand what happens to trace metals bioavailability with time after cessation of biosolids application.

Some recent studies have demonstrated that the bioavailability of trace metals in biosolids amended soils does not increase years after land application of biosolids have stopped (Bidwell and Dowdy, 1987; Hyun et al., 1998). In fact, it has been reported that the highest bioavailability of biosolids-applied trace metals is during the period immediately following biosolids application (Bidwell and Dowdy, 1987; Chang et al., 1987, 1997; Sommers et al., 1991). Metal availability has been reported to decrease with time as organic decomposition rate decreases (Bidwell and Dowdy, 1987; Walter et al., 2002). The greatest rate of biosolids organic carbon mineralization occurs within days after application (Wiseman and Zilbilske, 1988). The rate of organic matter decomposition decreases greatly with time when no further application is made (McGrath et al., 2000; Bidwell and Dowdy, 1987).

Biosolids-applied trace metals are converted to more stable chemical forms with time. McGrath (1984) observed a decrease in DTPA extractable trace metals after biosolids application had ceased. DTPA extractable metal increased with total metal concentration but declined with time. In a laboratory incubation experiment, Parkpain et al. (1999) reported that most biosolids-added trace metals existed in easily mobile forms, soluble and exchangeable, but these trace metals reverted into less mobile pools after 12 weeks of incubation.

Biosolids organic matter often decomposes slowly. Organic matter decreased by up to 40% 24 years after termination of biosolids application with no change in Cd availability (McGrath et al., 2000). They indicated rapid biosolids organic matter decomposition during the initial years, but the recalcitrant fraction may remain in the soil for hundreds of years before soil organic matter revert to its background level (McGrath et al., 2000). Hyun et al. (1998) found no evidence that soluble Cd concentration or phytoavailability of Cd increased because of this decrease in organic carbon content. Frost and Ketchum (2002) reported that wheat plants grown on soils with high loads of aged, high trace metals biosolids had metal concentrations similar to plants grown on soils receiving much lower application of metal from commercial fertilizers. Mineralization of organic carbon during the 20 years incubation of high-metals biosolids did not result in increased concentration of trace metals in wheat plants. In long-term studies of trends of metal availability after termination of biosolids application, McGrath et al. (1987) and McGrath (2000) measured no change in availability. Sauerbeck and Styperek (1986) reported a decrease in availability at some sites, but the trend was inconsistent.

Plant uptake

Trace metals in crop tissues are often elevated as a result of biosolids application to land (Logan and Chaney, 1983; Corey et al., 1987; Berti and Jacobs, 1996; Hooda et al., 1996). Plant uptake is a critical pathway by which biosolids-borne, potentially toxic trace metals can enter the food chain (Chaney, 1990). Plant availability of trace metals differs widely among plant species and organs. Hooda et al. (1997) measured variability in the accumulation of Cd, Cu, Ni, Pb, and Zn in wheat, carrots, and spinach grown on biosolids -amended soils. Cadmium, Ni, and Zn increased in plants to a greater extent than Cu and Pb compared to their background levels suggesting that Cd, Ni, and Zn might pose the greatest hazard among the trace metals studied. Similar findings were reported by Keefer et al. (1986) and Smith (1994). Sauerbeck and Hein (1991) found that Ni uptake in 13 crops was dependant on plant species and organs. Nickel concentration was higher in grain and storage organs than in vegetative plant parts. Barley accumulates low amounts of Ni, lettuce is a medium accumulator, and radish absorbs high amounts of Ni.

Crop uptake of Cd increases with increasing Cd concentration in biosolids (Jing and Logan, 1992). The forms of Cd in biosolids are more bioavailable than other pollutant trace metals (Shuman et al., 2002). Chaudri et al. (2001) found seasonal and cultivar effects on grain Cd content in wheat grown on a sandy loam soil at pH 6.5 Many researchers have reported that accumulation of trace metals in biosolids-amended soils had no adverse effects on plant growth (Heckman et al., 1987; Payne et al., 1988; Xiu et al., 1991). Dowdy et al. (1978) reported that the Zn concentration in bean (*Phaseolus vulgaris* var tendergreen) never exceed 60 mg kg^{-1} in the edible bean tissue although 1520 kg ha^{-1} Zn was applied. In the same study, Cu concentrations in the bean increased from 2 mg kg^{-1} in the control to 8 mg kg^{-1} in the treatment that received 266 kg ha^{-1} Cu. Sloan et al. (1997) reported elevated Cd and Zn concentration in romaine lettuce because of 16 years of biosolids applications but plant tissue metal concentrations were largely within the normal ranges for the crop studied. Relative availability of biosolids-applied metals followed the order of Cd>Zn≥Ni>Cu>Cr>Pb (McGrath, 1996).

Few cases of toxicities have been reported however. Berti and Jacobs (1996) found that soybean grown on a sandy loam soil failed to grow on one of the treatments receiving biosolids in which the Zn and Ni content were well above the CPLR (USEPA, 1993). They attributed the growth depression to phytotoxic Zn and Ni concentrations in the soil of these plots. The researchers also reported phytotoxic concentration of Zn in corn for five years following 10 years of biosolids application. Nickel in corn samples was also greater than the tolerance level of 3 mg kg^{-1} suggested by Risser and Baker (1990).

Plateau vs. linear uptake response

Biosolids application will cause buildup in the concentration of trace metals in amended soil. Biosolids-applied trace metals are usually highly persistent in soils (Alloway and Jackson, 1991; McGrath and Lane, 1989). Concerns have been raised that the availability of added trace metals may increase after biosolids applications cease since the binding capacity of the biosolids is reduced as organic matter is mineralized (Beckett et al., 1979; McBride, 1995). The USEPA (1993) based the allowable biosolids trace metals additions on the assumption that plant uptake is a linear function of biosolids addition. Chaney and Ryan (1992) determined that actual plant uptake should be lower than that predicted by the linear regression model used to formulate the Part 503 Rule. Plant metal concentrations reach a maximum as the biosolids rate increase, due to a lower efficiency in trace metals uptake by plants at higher loadings (McBride, 1995).

For low-metal biosolids, the phytoavailability of the metal is controlled by the biosolids chemistry and plant uptake should follow a plateau response despite increased metal loading. Biosolids application increases the metal contents of soil and the soil sorption capacity; therefore, plant tissue metal concentration should attain a plateau at higher application rates of biosolids (Corey at al., 1987; Chaney and Ryan, 1993). This is made possible by the presence of organic and inorganic components in biosolids that have a high capacity for specific sorption of trace metals (Huyn et al., 1998; Jing and Logan, 1992; Luo and Christie, 1998). Chaney and Ryan (1992) stated, "All evidence available indicate that the specific metal adsorption capacity added with sludge will persist as long as trace metals of concern persist in the soil."

Soils have a fixed capacity to attenuate added trace metals. Slow decomposition of biosolids added organic matter could increase the solubility of trace metals (McBride, 1995). As metal concentrations in biosolids increase, the specific adsorptive capacity may become saturated and metal availability may approach that of metal salts (Corey, 1981; Corey et al., 1987). It is important to determine the source of biosolids protective effects (McBride, 1995).

The literature is replete with studies that support either the linear- or the plateau-type response. Several studies (Chang et al., 1987; Barbarick et al., 1995; Brown et al., 1998) provided evidence that biosolids trace metals uptake by wheat, corn (*Zea mays*), and several vegetables reached a maximum with biosolids application rate. Street et al. (1978) reported a plateau type relationship for Cd uptake in corn grown on biosolids - amended soil. The content of Zn and Cu in bean leaves reached a maximum that did not respond to further biosolids application (Dowdy et al., 1978). Logan et al. (1997) observed plateau response for Zn, Cd, and Cu by corn but linear response for lettuce (*Lactuca sativa*). Thirty-five years after application, Zn and Cd concentrations in radish displayed a plateau response; however, concentrations of total and free trace metals in soils did not display any plateau response (Hamon et al., 1999). Chaudri et al. (2001) found no plateau response between soil Cd and grain Cd up to a soil Cd concentration of

2.7 mg.kg^{-1} although a slight curvilinear relationship was apparent. McGrath et al. (2000) showed that total soil and crop metal concentrations increased linearly as a function of biosolids addition. In a 10-yr biosolids land application experiment, Chang et al. (1997) reported that actual plateau or time bomb effect were not evident up to a soil Cd concentration of 40 mg kg^{-1}. They suggested that hypotheses used in the risk assessment are only valid as long as concentrations of trace metals in biosolids do not exceed the upper limits for high quality biosolids and mass loadings do not exceed 1000 Mg ha^{-1}.

Plateau responses to metal uptake have been attributed to the attenuation of bioavailability by biosolids -added materials (Chaney and Ryan, 1993; Barbarick et al., 1995; Chang et al., 1996). McBride (1995) and Hamon et al. (1999) proposed that plateau-type response can also be explained by limited plant uptake and root to shoot translocation at higher soil solution metal concentration as evidenced by the difference in plant species response to metal concentrations in the same soil and the absence of plateau-type response in soil solution.

Hamon et al. (1999) argued that the plateau response observed was due to plant physiological factors and not the result of metal attenuation by increased sorption sites provided by biosolids. Plant response shifts from concentration-dependant at low soil metal concentration to concentration-independent at higher concentration when soil solution metal attains a specific threshold level (Baker, 1981). The saturation of the transport channel (Guerinot and Eide, 1999; Van der Zall et al., 1999) at high concentrations results in no increase in plant metal concentration.

Use of chemical extractants to assess metal availability

It is desirable to be able to monitor the potential for metal availability and toxicity using chemical extractants. Several methods have been proposed to assess metal availability in biosolids treated soil. Chemical extractants such as chelating agents, dilute acids and unbuffered salt solution are typically used to assess plant availability of trace metals. These extractants can be used to assess metal status in polluted soils with some modifications such as higher extraction ratio for DTPA (O'Connor, 1988; Esnaola et al., 2000) and higher extractant concentration for $CaCl_2$ (Esnaola et al., 2000). A mobile fraction assessed by salt solution is used to simulate trace metals uptake by plants. Complexing agents are suitable to predict potentially active metal fraction in soil (Gupta et al., 1996).

The use of weak unbuffered salt solutions or water as extractants would best represent plant available trace metals in soils (Hooda et al., 1997). Non-buffered salt solutions are now considered to give best prediction of the availability of trace metals in soils (Lebourg et al., 1996). Among salt solutions, $CaCl_2$ at 0.01 to 0.1M is one of the most often used and successful extractant for plant available trace metals (Sauerbeck and Stypereck, 1984; Ericksson, 1990; Novozamsky et al., 1993). It is a rapid and simple way to evaluate trace metals bioavailability (Beckett, 1989). Nonbuffered salt solutions extract soluble and easily exchangeable trace metals. Soil solution contains the most mobile fractions of trace metals and nonbuffered salt solution can thus simulate metal uptake rate and mobility of contaminants (Ure, 1996). Non-buffered salt solutions are used to study trace metals (Cd, Zn, and Ni) accumulated in soils as a result of human activity (Houba et al., 1990; Novozamsky et al., 1993) and can be used as an index of Cd uptake by crops (Whitten and Ritchie, 1991). Calcium chloride extractable Zn was found to be a good indicator of phytotoxicity (Houba et al., 1990). Results achieved with salt solution are more closely correlated with the appearance of toxicity symptoms than results obtained by extraction with dilute acid or complexing agent solution (Houba et al., 1996).

Sauerbeck and Styperek (1983) observed that $CaCl_2$ is a useful extractant to predict the availability of trace metals in soil. The use of 0.01 M $CaCl_2$ is reported to be a good predictor of Zn in green beans (Houba et al., 1990), Cd in subterranean clover (Whitten and Richie, 1991) and in lettuce and endive (Novozamsky et al., 1992). Plant trace metals content are best correlated to trace metals concentrations extracted with unbuffered salt solution (Novozamsky et al., 1992; Smilde et al., 1992). High correlations between $CaCl_2$ extractable Zn and Cd and their concentrations in maize plants (Smith et al., 1992) and Cd and Pb in vegetables (Ure, 1996) have been observed.

Calcium chloride is an attractive extractant due to its low cost, simplicity and reproducibility. Moreover, concentrations of the solution employed closely approximate the ionic strength in soil solution (Houba et al., 1990). Limitation of 0.01 M $CaCl_2$ is the low concentration of extracted trace metals, which are often below the detection limit of the usual analytical techniques (Houba et al., 1996; Ure, 1990). As a result, stronger extractants such as chelating agents or dilute acids are often used. Ethylenediaminetetraacetic acid (EDTA) and diethylenetriaminepentaacetic acid (DTPA) are the most commonly used chelating agents as a soil test for assessing plant availability of trace metals because of their greater extracting strength (Hooda et al., 1997). DTPA is the most extensively used and successful in assessing availability (Singh et al., 1984; Gatti et al., 1991; Akram et al., 1995). Mehlich-1 and Mehlich-3 have also been reported to be good predictor of micronutrient uptake by plants (Xiu et al., 1991; Cahoon, 1974; Wear and Evans, 1968; Evans et al., 1974; Fiskell et al., 1984).

Synthetic chelating agents are widely used extractants. They extract trace metals from the soluble, exchangeable, carbonate, and organically bound fractions (Walter et al., 2002). Chelating agents have been reported to dissolve Fe and Al oxides, however, which may result in overestimation of the available metal fraction (Sauerbeck and Hein, 1991). DTPA is the most popular chelating agent used because the concentration of trace metals extracted tends to correlate fairly well with metal uptake by plants (Bidwell and Dowdy, 1987; Sommers et al., 1991; Hooda and Alloway, 1994)

Ure (1996) found high correlation between DTPA extractable Cu, Zn, Ni, and Cd and their concentrations in lettuce and wheat. Rappaport et al. (1986) found good correlation between DTPA extractable Zn and its concentrations in corn on 14 different Virginia soils. Mitchell et al. (1978) reported significant correlations between DTPA extractable concentration and trace metals uptake by different plants in biosolids treated soils. Lake et al. (1984) showed that Cd and Zn uptake by vegetables is proportional to DTPA extractable concentrations in soil. DTPA was also well correlated with Ni uptake by carrots roots and leaves (Sauerbeck and Hein, 1991), with Cu, Zn, Cd and Ni uptake by winter wheat, carrots, and spinach (Hooda et al., 1997), and with Cd, Cu, and Zn uptake by Swisschard, lettuce, tobacco, and peanut (Miner et al., 1997).

Sequential extraction

Soil trace metals may be present as free or complexed ions in solution; adsorbed at the surface of clays, Fe and Mn oxyhydroxides and OM; inorganic precipitates of phosphates, sulfides and carbonates; occluded in amorphous materials such as Fe and Mn oxy-hydroxides, Fe sulfide or present in the crystal lattice of primary mineral (Lake et al., 1984; Tessier and Campbell, 1988; Moral, 2002). Biosolids addition to soil increases the concentrations of trace metals in all fractions but the distribution of trace metals is controlled by soil mineralogy and chemistry.

Chemical fractionation is a common operational approach to study metal bioavailability in soils (Smith, 1994; Mench et al., 1994; Sauerbeck and Hein, 1991; Bingham et al., 1984). Sequential extractions have been developed to extract different metal fractions. The main drawbacks of sequential extraction is its inability to distinguish among discrete geochemical fractions in soil, the nonselectivity of extractants, trace elements redistribution among phases and the effects of sequence of extractions (Qian et al., 1996; Miller et al., 1986). Sequential extraction is often considered as a qualitative approach to study temporal change in chemical forms and metal distribution among different phases. However, such extractions of trace metals in soils can give useful information for predicting bioavailability, leaching rates and transformation between chemical forms in soils (Miller at al., 1986; Sims and Kline, 1991; Tsalidas et al., 1995; McGrath, 1996; Ure, 1996).

Amending soil with biosolids alters the distribution of trace metals (Walter and Cuevas, 1999). Application of biosolids increased Cu and Zn in all fractions and increased Zn, Cu, Cd, Ni, Pb, and Cr were usually found in the more resistant fractions. Adding biosolids increased percent Cd in the exchangeable and specifically sorbed forms (Sloan et al., 1996; Basta and Sloan, 1999), in the organic and carbonate fractions (Taylor et al., 1996), and in the Fe/Mn oxide fraction (Illera et al., 2000). McGrath (1996) found that most Cd was present in forms easily available more than 15 years after application. The exchangeable and specifically sorbed fractions comprised 75% of the total Cd in amended soils.

Berti and Jacobs (1996) reported an increase in Zn concentration in all soil fractions except residual. The greatest concentration of Zn was in the water soluble, exchangeable, and acid soluble fractions. Nickel was found in all soil fractions but predominantly prevalent in the acid soluble and Fe oxide fraction, regardless of the total concentration in soils. The researchers also found that Cd measured in soil from biosolids treated plots resided primarily in the acid soluble and exchangeable fractions, while the highest concentration of Cu was found in the acid soluble fraction.

Sloan et al. (1997) reported that biosolids application increased the percent of Cd, Ni, and Zn in the soluble, easily exchangeable and specifically adsorbed fractions. More than 75% of Cu, Ni, and Zn were found in the relatively stable organic, oxides and inorganic residual fractions of control and biosolids amended soils. At high Cd and Zn loads, McGrath (1996) observed that the amounts of Cd and Zn extracted increased linearly with biosolids application in the exchangeable and specifically sorbed fractions. The greatest percent increase was reported in oxide fractions. Similar findings were reported by other researchers (Hickey and Kittrick, 1984; Sims and Kline, 1991; McGrath, 1996). Copper was found to be largely associated with the specifically sorbed and oxides fractions of biosolids amended soil (Hickey and Kittrick, 1984). Sims and Kline (1991) extracted equal amounts of Cu in organic and oxide fractions. McGrath and Cegarra (1992) found most Cu in the organic fraction when it was extracted before the oxide fraction. Miller et al. (1986) however, reported equal amounts of Cu in organic and oxides fractions when the later was extracted first.

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CHAPTER THREE

Recovery and Distribution of Biosolids-Derived Trace Metals in a Davidson Clay Loam Soil.

ABSTRACT

The long-term mobility of trace metals has been cited as a potential hazard by critics of EPA 503 rule governing the land application of biosolids. The objectives of this study were to assess the accumulation of Cu, Ni, Cd, and Zn within the soil profile, the distribution of exchangeable, specifically adsorbed, organic and oxide fractions of each metal and mass balance of Cu and Zn 17 years after biosolids application. Biosolids was applied to 1.5 x 2.3 m confined plots of a Davidson clay loam (clayey, kaolinitic, thermic, Rhodic Paleudult) in 1984 at 0, 42, 84, 126, 168, and 210 Mg ha^{-1}. The highest biosolids application supplied 4.5, 750, 43, and 600 kg ha^{-1} of Cd, Cu, Ni, and Zn, respectively. Soils were sampled to a depth of 0.9 m and sectioned into 5-cm increments after separating the Ap horizon. Total (EPA 3050B), bioavailable (Mehlich-I), sequential extraction, and dispersible clay analysis were performed on samples from the control, 126 Mg ha^{-1} and 210 Mg ha^{-1} treatments. Trace metals are still concentrated in the top 0.2 m with slight enrichment down to 0.3 m of soil. More than 80% of applied Cu and Zn are still found in the topsoil where biosolids was incorporated. Mehlich-I results showed a slight increase in metal concentration down to 0.35 m. Biosolids application increased the concentration of trace metals in all the extracted fractions, with a large proportion of Zn and Cd present in readily extractable forms. The major portion of Cu, Zn, and Ni is associated with the metal-oxides fraction. Dispersible clay content and water-soluble metal contents were low and were not affected by biosolids application. Results from this study showed that 17 years after biosolids application there was negligible movement of trace metals through the soil profile and consequently, there is a little risk of contamination of groundwater at this site.

INTRODUCTION

Land application of biosolids increases metal concentrations in soils (Sloan et al., 1998). The United States Environmental Protection Agency (USEPA) developed a risk assessment method (USEPA, 1992) to evaluate the potential negative effects of pollutants in biosolids. The USEPA 40 CFR Part 503 was promulgated as the Standards for the Use and Disposal of Sewage Sludge (USEPA, 1993). The Part 503 rule permits long-term application of biosolids to agricultural land with the assumption that the soil accumulation of trace metals from biosolids meeting ceiling concentration limits will not cause environmental or health hazard during or after the application period.

The protectiveness of the Part 503 rule has been called into question based on assumptions about trace metal immobilization made in the underlying risk assessment (McBride, 1995; Schmidt, 1997; Harrison et. al., 1997). The risk assessment assumes that Fe, Al, and Mn oxy-hydroxides and organic matter added to soil along with biosolids increase the soil capacity to adsorb and bind trace metals (Corey et al., 1987). Selective adsorption and precipitation of trace metals on oxide surfaces provide the basis for immobilization in biosolids-amended soils. Inorganic minerals (Fe and Al oxides, silicates, phosphates, and carbonates) may comprise 30 to 60% of digested primary biosolids (Metcalf and Eddy, 1991). The potential for trace metals sorption in biosolids amended soils is further increased by newly formed oxide precipitates (Corey et al., 1987).

The concept of long term metal immobilization in soil has been challenged when mass balance calculations at a number of land application studies were unable to account for up to half of biosolids-applied trace metals (Williams et al., 1987; Alloway and Jackson, 1991; Dowdy et al., 1991; Brown et al., 1997; McBride et al., 1997; Richards et al., 1998.). Researchers reporting such discrepancies have attempted to explain the apparent metal loss as due to leaching (McBride, 1995), dispersion of trace metals due to tillage (McGrath and Lane, 1989), and incomplete chemical extraction from soil or overestimation of trace metals loadings (Dowdy et al., 1991).

Most studies of metal leaching in soil columns (Emmerich et al., 1982; Miller and McFee, 1983) or field investigations (Chang et al., 1982, 1984; Williams et al., 1984, 1987; McGrath and Lane, 1989) conclude that trace metals are strongly bound to topsoil. Other field investigations (Lamy et al., 1993) as well as soil column studies (Prakash et al., 1997; Frenkel et al., 1997) have documented rapid leaching of significant concentrations of Zn, Cu, Cr, and Cd.

Suspended clay sized particles may facilitate the transfer of strongly sorbing contaminants, generally regarded as relatively immobile in soil. McBride et al. (1999) observed that Zn, Cd and Cu in percolates collected at 60-cm depth in a long-term study are largely in complexed form probably with dissolved organic matter. Correlations have also been found between levels of dissolved organic matter and the concentration of Hg, As, Cu, Cd, Zn, and Cr in leachates (Kalbitz and Wennrich, 1998; McBride et al., 1997;

Campbell and Beckett, 1988). Recent research evidence showed enhanced metal mobility associated with water-dispersed colloidal particles moving through soil macropores and fractures (Mills et al., 1991; Liang and McCarthy, 1995; Ouyang et al., 1996). Soluble complexing ligands in biosolids cause certain trace metals to be more mobile than they would be in the absence of organics (Frenkel et al., 1997; Camobreco et al., 1996).

Total metal concentration does not furnish sufficient information regarding the potential availability of elements (Srikanth and Reddy, 1991). Trace metals can be associated with different soil fractions, which affect their availability and mobility in soil. The distribution of various forms of trace metals in soil may also change with time. They may accumulate in soil in different proportions as water soluble, exchangeable, and organically bound or be associated with oxides (Salomons and Forstner, 1980). Sequential extraction can be used to characterize the distribution of the different chemical fractions of the trace metals in soil. (Berti and Jacobs, 1996; Lake et al., 1984).

Long-term application of biosolids at annual rates limited by N and P fertilizer application will cause a gradual increase in the concentration of trace metals in amended soil. Most of the data used to develop Part 503 rule were obtained from studies in which trace element availability was measured during the years of biosolids application. Research on the behavior of trace metals in soils, that have approached equilibrium years after biosolids application, is needed to understand the long-term effect of biosolids application on agricultural lands. A field test was initiated in 1984 on a highly weathered, deep, well-drained Davidson clay loam soil with controlled lateral flow field experimental plots (Rappaport et al., 1988). These plots received a single application of several rates of biosolids that contained considerably higher concentrations of Cu and Zn than currently found in land-applied biosolids. The rates of the trace metals applied and the experimental plot design provided an opportunity to investigate the fate of trace metals in soil amended with biosolids nearly 17 years earlier.

This research was initiated to characterize the long-term impacts of biosolids application on trace metals distribution and mobility in a weathered, well-drained piedmont soil. The objectives were: to investigate (i) the accumulation of biosolids-applied trace metals by depth, (ii) the distribution of Cu, Cd, Ni, and Zn into Soluble +

exchangeable, chemisorbed, organically bound, Fe oxide-bound fractions, and(iii) the percent of applied Cu and Zn remaining in soil 17 years after biosolids application.

MATERIALS AND METHODS

Background

A field test was established in the spring of 1984 to evaluate the use on cropland of aerobically digested biosolids from a wastewater treatment plant with considerable industrial input (Rappaport et al., 1987, 1988). The experiment was conducted at the current site of the Northern Piedmont Agricultural Research and Educational Center (NPAREC) in Orange, Virginia on a Davidson clay loam (clayey, kaolinitic, thermic Rhodic Paleudult). The chemical and physical properties of this piedmont soil when the original biosolids applications treatments were made are presented in Table 1.

Table 3.1. Chemical and physical properties of the Ap horizon of the Davidson clay loam used for the biosolids study in 1984 at the NPAREC. (Rappaport et al., 1988)

			Amorphous and Crystalline			Particle size distribution		
pH	OM	CEC	Al	Mn	Fe	Sand	Silt	Clay
	g kg^{-1}	cmol$_c$ kg^{-1}			mg kg^{-1}			
5.7	18	12.5	4740	1280	42500	153	471	376

Field experimental plots were constructed to prevent the lateral movement of the biosolids. The plots consisted of an isolated volume of soil that measured 2.3-m long x 1.5 m wide x 0.9 m deep. Isolation of this soil was accomplished by excavating a ditch 20 cm wide and 0.9 m deep around each plot and wrapping the sides of these soil blocks with 254-micrometer polyethylene film. Aluminum flashing extended 15cm above the soil surface to prevent lateral movement and runoff. Eventually, the Al-flashing was replaced by wooden boards 5-cm wide x 20-cm deep. An aerobically digested biosolids was applied by hand at rates of 0, 42, 84, 126, 168, and 210 dry Mg ha^{-1} in the spring of 1984. The treatments were arranged in randomized complete block design with four replicates.

The biosolids contained considerably higher concentrations of trace metals than are currently found in land-applied biosolids. The current ceiling limits and actual concentration of trace metals found in the biosolids are also shown in Table 2. Copper, Zn, and Pb concentrations were above the Annual Pollutant Limit (APL) limits for pollutant concentration biosolids and Cd and Ni were lower than exceptional quality limit; thus, Part 503 would require lifetime loading rates of this biosolids to be tracked. The 210 Mg ha^{-1} biosolids rate supplied 4.5 kg Cd, 750 kg Cu, 43 kg Ni and 600 Kg Zn per ha (Table 3). These amounts are below the maximum cumulative pollutant loading rate application of trace metals that can be applied to soils in biosolids that meets ceiling concentration limits. This rate provided 12% of the Cd, 50% of the Cu, 10% of the Ni, and 21% of the Zn permitted loadings. None of the trace metals is near the limits for ceasing application of pollutant concentration biosolids.

Table 3.2. Properties of the aerobically digested sewage sludge (ADSS) and Part 503 Ceiling Concentration Limit (CCL) and Pollutant Concentration Limit (PCL) standards for land applied biosolids. (Rappaport et al., 1988).

Trace metals	ADSS	CCL	PCL
		mg kg^{-1}	
Cd	21.5	85	39
Cu	3650	4300	1500
Ni	210	420	420
Pb	640	840	300
Zn	2980	7500	2800

Table 3.3. Quantity of biosolids, trace metals and total N and P applied at the NPAREC study site (Rappaport et al., 1988)

Biosolids	Macronutrients		Trace metals			
Application	N	P	Cd	Cu	Ni	Zn
Mg ha^{-1}			Kg ha^{-1}			
42	670	1380	0.9	153	8.6	125
84	1340	2760	1.8	304	17.2	248
126	2010	4140	2.7	456	25.8	372
168	2680	5520	3.6	608	34.4	496
210	3350	6900	4.5	760	43.0	620

Plot Management

The experimental plots were annually planted to Pioneer 3193 field corn (*Zea mays L.*). All plots received the same rates of N fertilizer according to Virginia Cooperative Extension (VCE) guidelines (Donohue and Heckendorn, 1994). Phosphorus and K fertilizers were applied according to VCE soil testing recommendations. Agronomic practices, seedbed preparation, pest control, and weeding were performed according to VCE recommendations. The aboveground portion of the crop was totally removed at physiological maturity. Lime applications in 1989 and 1997 were made to raise the pH to 6.

Soil Sampling and Processing

Soil cores were sampled from the 0, 126, and 210 Mg ha^{-1} treatment plots on June 19, 2001. Four 2.5-cm diameter cores were collected in plastic sleeves to a depth of 0.9 m using a Giddings hydraulic soil probe. After removing the cores, sodium bentonite was added to the holes to fill the lower 75 cm. The top 15 cm was filled with topsoil from the same plot.

The cores were kept intact in plastic tubes until they were sectioned by depth at 5-cm intervals after the Ap horizon had been separated in the laboratory. Samples were labeled, air-dried in clean plastic bags, and ground with a glass mortar and pestle to pass a 2-mm sieve.

Metal Analysis

Several digestion and extraction methods were employed for metal analysis. Trace metals were extracted by the Mehlich-I, EPA-3050B, and EPA-3052 methods and fractionation was accomplished with sequential extraction. The Mehlich-I method (Mehlich, 1953) uses a solution of 0.05M HCl and 0.0125 H_2SO_4 to extract a fraction that is an indicator of plant-available soil trace metals. The EPA-3050B (USEPA, 1996) method is a strong acid digestion procedure that extracts all elements that could become "environmentally available." The method extracts 75% to 90% of the total metal. It is not designed to extract elements bound within the silicate structure. The procedure utilizes a mixture of HCl, HNO_3 and H_2O_2 with supplemental heating to extract trace metals. Metal concentrations were determined using Thermo (Fitchburg, MA) Jarrell Ash ICAP (Inductively Coupled Argon Plasma-Atomic Emission Simultaneous spectrometer; ICP-AES). The EPA 3052 (USEPA, 1996) microwave digestion procedure was employed to determine total metal concentrations for mass balance estimation. Microwave methods are known to be rapid and give reproducible recovery with better precision than conventional hot plate digestion procedures (Millward and Kluckner, 1989). Briefly, 0.5-g aliquot of sieved air-dried soil was mixed with 9 ml HNO_3, 2 ml HF, and 3 ml HCl. Temperature of the mixture was raised to 140 ^{o}C in 6 min and maintained at 140 ^{o}C for an additional 9 min. Metal concentration was determined using Perkin Elmer (Norwalk, CT) model 3300 atomic Absorption spectrometer (AAS).

Fractionation

The distribution of Cu, Ni, Cd, and Zn within soluble + exchangeable (Exch.), specifically sorbed (SA), organically bound (Organic), and metal oxide (Oxide) fractions was measured in soil samples taken from the 0-15 cm depth as described by Miller et al. (1986). The procedure consisted of sequential extraction with 0.5 M $Ca(NO_3)_2$ (Exch), $Pb(NO_3)_2$ (SA), 0.1 $K_4P_2O_7$ (Organic), and oxalate reagent (0.175M $(NH_4)_2$ C_2O_4 +0.1 $H_2C_2O_4$) under UV irradiation (Oxide). Calibration blanks were routinely included in the analysis. Metal concentrations were determined using (ICP-AES).

Dispersible clay

Samples from the control, medium (126 kg ha^{-1}) and high (210 kg ha^{-1}) sludge application rates were analyzed for dissolved organic carbon, dispersible clay, and water-soluble trace metals in the manner described in Southern Cooperative Series Bulletin (1998). Five-gram samples were weighed into a centrifuge tube to which 50 ml of deionized water was added. After shaking for 14 hrs on a reciprocal shaker, each suspension was allowed to settle undisturbed for 2 hrs. The dispersed colloidal fraction was sampled from the top 2.5 cm. The aliquot was then oven dried at 110 ^{o}C in pre-weighed Al-boats and the dispersible clay quantified. The remaining suspension in each tube was then filtered (0.2 μm pore size polycarbonate membrane filter). The filtrate was analyzed for water-soluble trace metals using (ICP-AES).

Mass Balance

Trace metal recovery was calculated from metal crop recovery for the duration of the study and trace metals content in the upper 25 cm of the soil profile. Available data on crop yield and metal uptake compiled for the period 1985 to 2003 were used to estimate metal removal. Whenever data for either yield or uptake were missing, averages for the duration of the study were used. Average crop removal was estimated by multiplying the metal concentration (mg kg^{-1}) by total yield (kg plot^{-1}). The plant metal contents of the control plots were subtracted from each treatment to give the average net uptake per treatment. Averages over the years were added and the net total crop removal was estimated.

Soil bulk density was determined for each soil section. Each section (2.5-cm diameter x 5-cm high cylinder) was weighed, and its density calculated in order to estimate total soil mass. The average bulk density of the Ap horizon was 1.52 g cm^{-3}. For the second and third sections, a bulk density estimate of 1.58 g cm^{-3} was used. An estimated 30 kg of the Ap section of each plot was lost to annual sampling and a greenhouse pot experiment was added to total soil mass. Approximately 0.5 kg of soil sampled from each plot every year for routine lab analysis and 20 kg of soil was removed from each plot in 1995 for a greenhouse study.

Total soil mass of each section was multiplied by its respective metal concentration. The control for each depth was subtracted and the concentrations of each depth increments comprising the 0-25 cm soil depth were added. This total content was divided by metal loading in the biosolids to calculate recovery of the biosolids applied trace metals. Total metal concentrations for mass balance estimation were determined using EPA 3052 microwave digestion procedure. The solutions were then filtered and metal concentrations were determined by AAS.

Statistical Analysis

Two cores per replicate were used for analysis; therefore, all data are averages of 8 samples (4 replicates per treatment x 2 samples per replicate). The statistical analysis was performed with the SAS package version 8.2 for Windows (SAS Inst, 2002). Data was evaluated by analysis of variance (ANOVA) and by the least significant difference (LSD) mean separation procedures at the 0.05 level of significance (Steele and Torrie, 1980).

RESULTS AND DISCUSSION

Vertical translocation of trace metals

Mehlich-I

Soil profile Mehlich-I extractable Cu and Zn concentrations in the topsoil (0-15 cm) increased with biosolids rate. At the highest biosolids input rate (210 Mg ha^{-1}) the Cu and Zn concentration in the 0-15 cm depth increased from 3.4 and 3.7 mg kg^{-1} to 63 and 71 mg kg^{-1}, respectively. Metal movement occurred beyond the top horizon sampled. Slight enrichment in metal concentration was observed to a depth of 35 cm. No significant movement was observed below this depth (Fig.1a, 1b). At the 15-35 cm soil depth the concentration of Cu and Zn in the treated soil were significantly ($p<0.05$) higher than the control, but there were no significant differences in metal concentrations between the medium and high treatments plots.

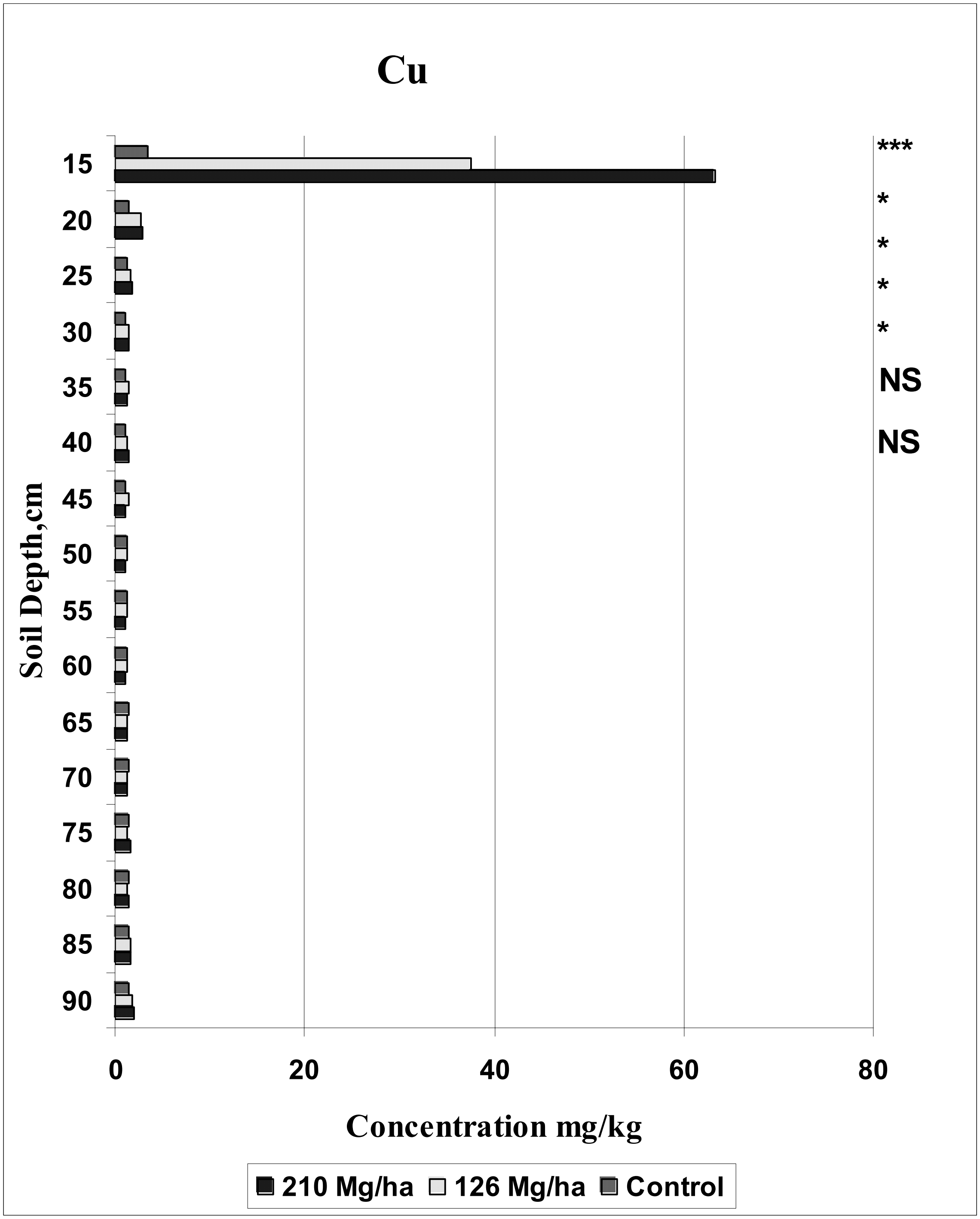

Fig.3.1a. Distribution of Mehlich-1 extractable Cu with soil depth 17 years after biosolids application. *, **, *** significant effect at 0.05, 0.01, 0.001 level of probability, respectively.

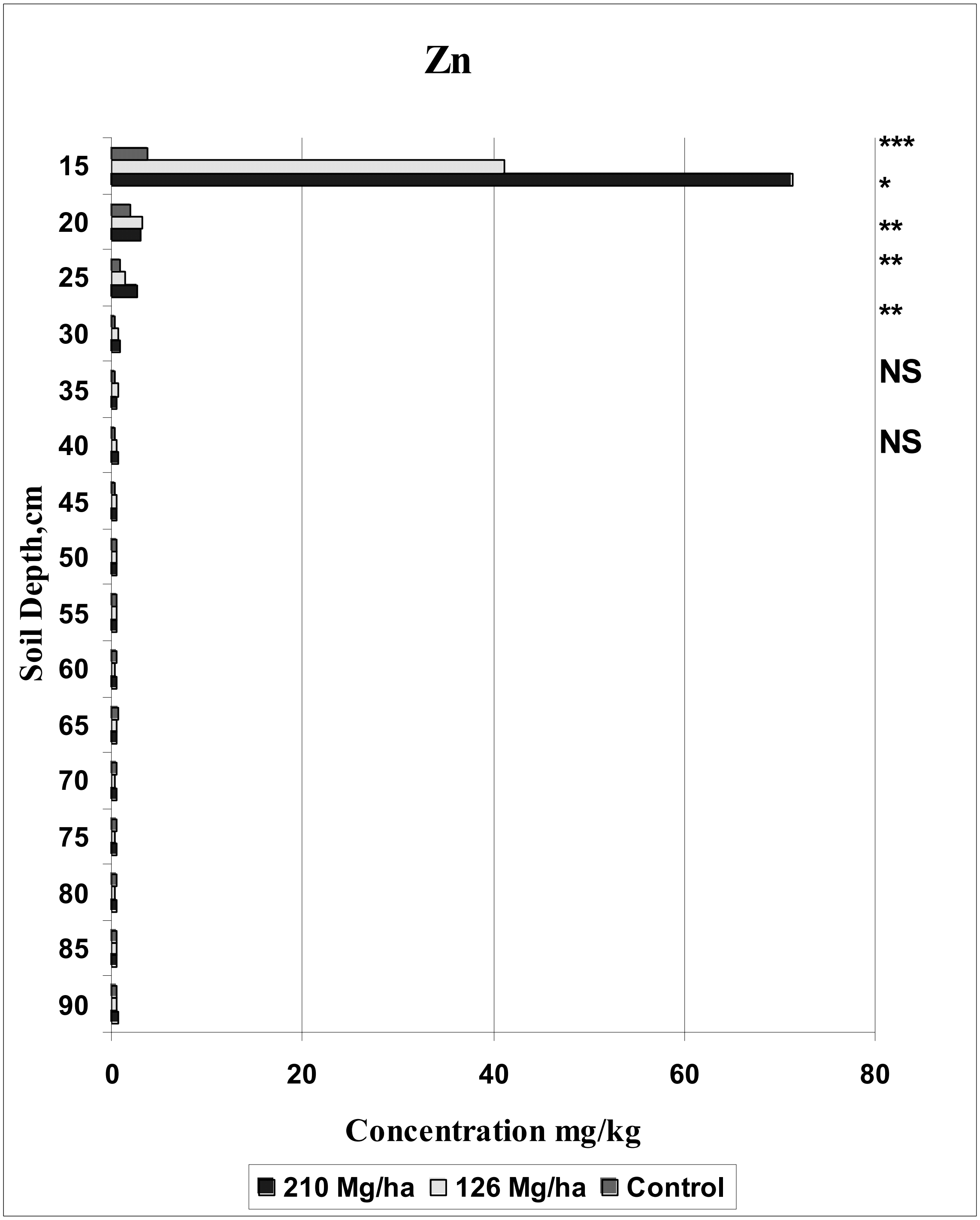

Fig.3.1b. Distribution of Mehlich-1 extractable Zn with soil depth 17 years after biosolids application. *, **, *** significant effect at 0.05, 0.01, 0.001 level of probability, respectively.

EPA-3050B.

Soil profile total metal (EPA-3050B), concentration data from the same treatments showed an increase in Cu, Zn, and Ni in the topsoil with increasing biosolids rate (Fig. 2a, 2b, 2c). Copper, Ni, and Zn concentrations increased from 60, 18, and 83 mg kg^{-1}, in the control, to 287, 28, and 264 mg kg^{-1} respectively in the plots receiving the highest biosolids application rate. This increase reflected the composition of the biosolids applied to the plots. The trace metals were still concentrated in the top 20 cm of the soil profile with minor enrichment down to 25 cm seventeen years after the application of biosolids. At the 15-25 cm soil depth there were no significant ($P<0.05$) differences between the medium and high biosolids treatments. Both were significantly higher than the control. There was no apparent movement of total Cu, Ni, and Zn below this depth.

The apparent lack of trace metals leaching is consistent with the results of other studies. Barbarick et al. (1998) reported no leaching below the plow layer of trace metals, except Zn from five or six biosolids application during an 11-year period. Chang et al. (1983) determined that more than 90% of Cd and Zn were concentrated in the top soil (0-15 cm) after six consecutive biosolids applications at an annual rate of 90 Mg ha^{-1}. Most studies reported the accumulation of trace metals in the zone of incorporation with minimal movement below the root zone, (Berti and Jacobs, 1998; Chang et al., 1982, 1983; Williams et al., 1984; McGrath and Lane, 1989; Brown et al., 1997; Sloan et al., 1997). Generally, they conclude that trace metals studied were still largely concentrated in the topsoil. An exception was noted by Robertson et al. (1982) who measured the movement of some trace metals to a depth of 0.4-0.6 m when liquid biosolids was applied at high rates to three Ultisols.

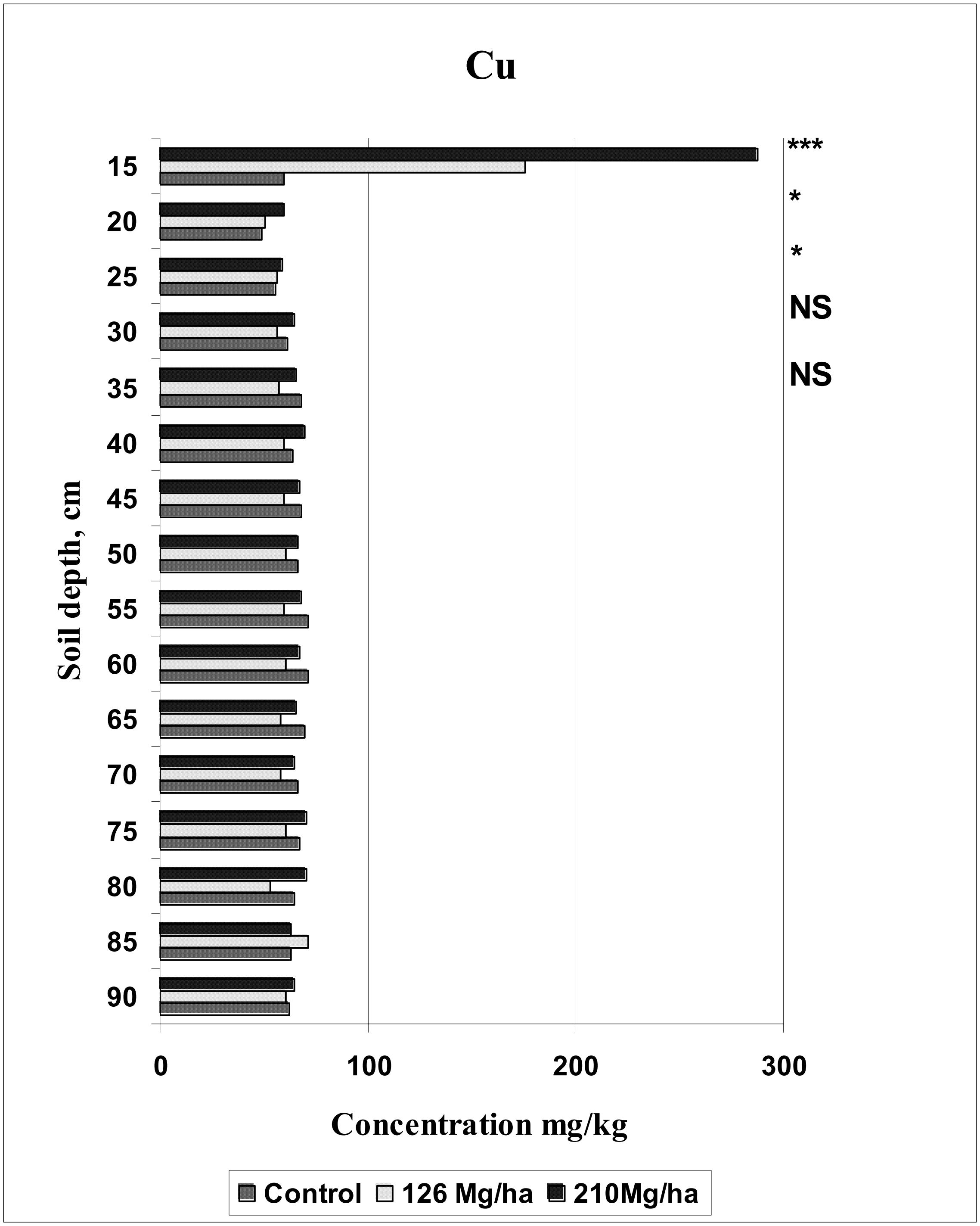

Fig 3.2a. Distribution of EPA-3050 extractable Cu with soil depth 17 years after biosolids application. *, **, *** significant effect at 0.05, 0.01, 0.001 level of probability, respectively. NS = non significant.

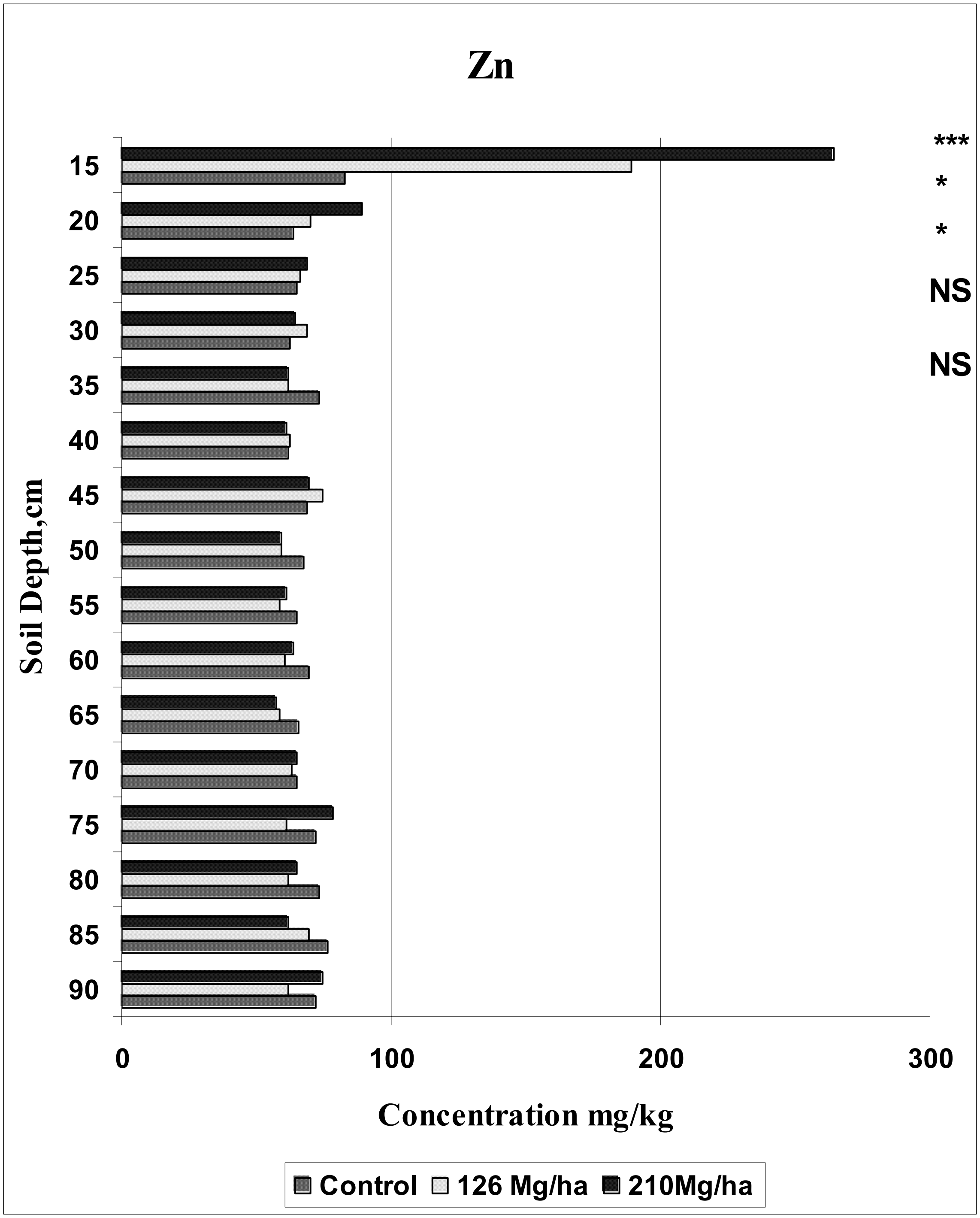

Fig 3.2b. Distribution of EPA-3050 extractable Zn with soil depth 17 years after biosolids application. *, **, *** significant effect at 0.05, 0.01, 0.001 level of probability, respectively. NS = non significant.

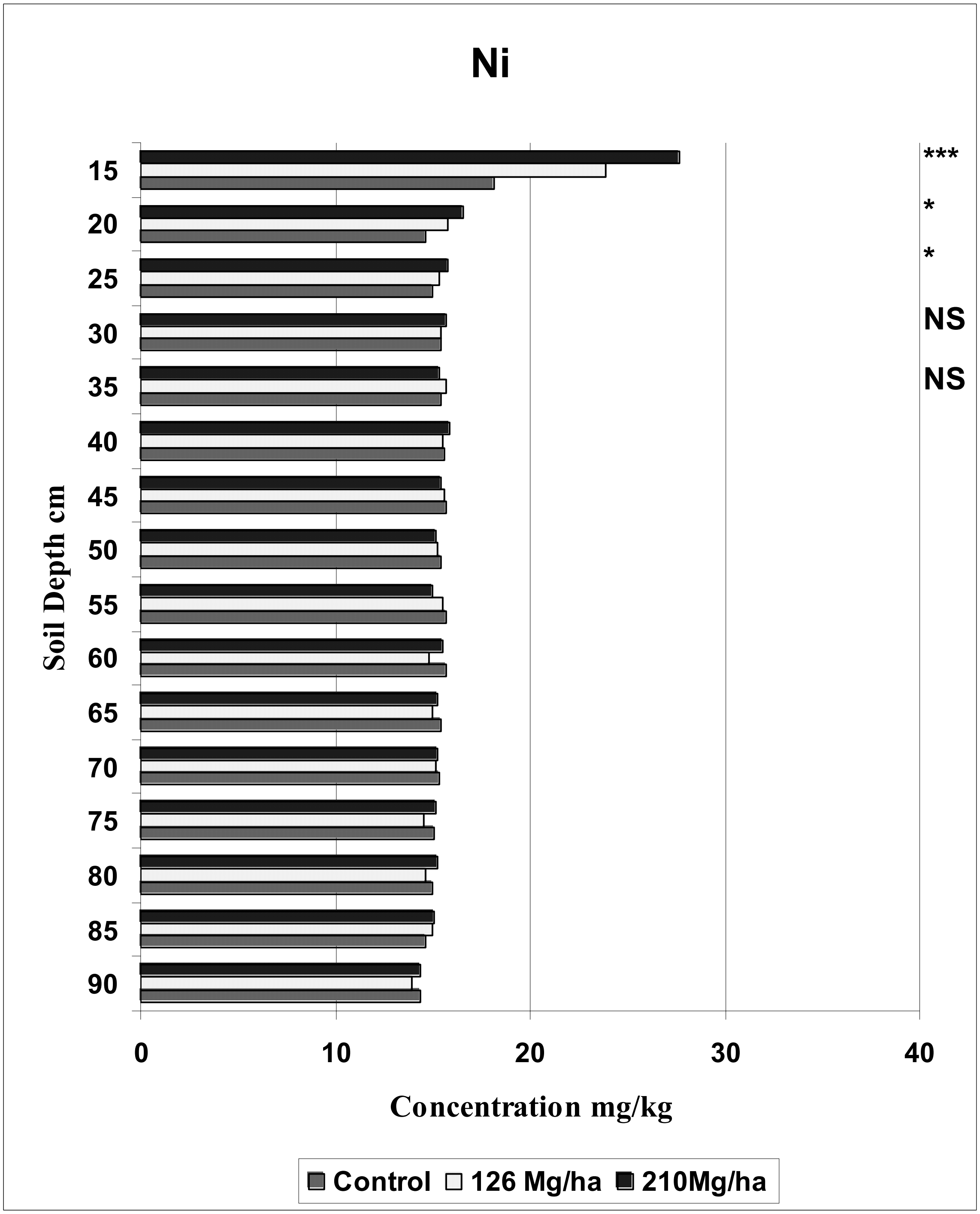

Fig 3.2c. Distribution of EPA-3050 extractable Ni with soil depth 17 years after biosolids application. *, **, *** significant effect at 0.05, 0.01, 0.001 level of probability, respectively. NS = non significant.

Dispersible clay

Suspended clay sized particles may facilitate the transfer of contaminants through soil. Recent evidence showed enhanced metal mobility associated with water-dispersed colloidal particles moving through soil macro-pores and fractures (Mills et al., 1991; Liang and McCarthy, 1995; Ouyang et al., 1996). There were no significant effects of biosolids application on dispersible clay content in the top 30 cm (Table 4). The amount of dispersible clays was low. It was highest in the 0-15 and 15-20 sections but dropped sharply at lower depth.

Table 3.4. Distribution of dispersible clay content by depth. (g kg^{-1})

Depth	Control	126 kg ha^{-1}	210 kg ha^{-1}
0-15cm	13.5	12.8	12.6
15-20cm	12.1	12.0	12.3
20-25cm	6.20	5.70	6.02
25-30cm	0.50	0.55	0.50

Karathanasis (1999) found that soil with low negative surface charge, large particle size, and high Fe- and Al-hydroxyoxides contents had the lowest metal colloid-mediated transport potential as compared to soils with high negative surface charge and organic carbon concentration. The Davidson soil is high in Al- and Fe- oxides and has a moderate charge, which might explain the low dispersible clay content (especially in the deeper horizons). The total metal concentration of Cu, Cd, and Zn associated with dispersible clay increased significantly as biosolids rates increased (Table 5).

Table 3.5. Total metal concentration of dispersible clay fraction in (mg kg^{-1}) as determined by EPA-3050B .

Treatment	Cu**	Zn**	Cd*	Ni
Control	87.6c	144c	2.29b	30.9
126 kg ha^{-1}	196b	223b	2.61b	34.3
210 kg ha^{-1}	252a	293a	3.20a	39.2

*, ** represent significant effect at 0.05, 0.01 level of probability, respectively. Column means for each treatment followed by different letters are significantly (p=0.05) different.

The water-soluble Zn concentration increased in the first (0-15cm) and the second (15-20 cm) layers only as biosolids loading increased. No significant differences were observed below this depth (Table 6). Data on Cu was inconclusive because the concentrations of many samples were below the detection limit.

Table 3.6. Water-soluble concentrations (mg kg^{-1}) of Cu and Zn in the top 30 cm of the soil profile as influenced by biosolids application.

	Control		126 kg ha^{-1}		210 kg ha^{-1}	
Depth	Cu	Zn	Cu	Zn	Cu	Zn
Ap	0.05	1.50	0.02	2.20	0.05	2.12
15-20cm	0.02§	1.25	0.05†	1.32	0.10†	2.68
20-25cm	0.02§	1.74	0.02†	1.83	0.02†	1.95
25-30cm	0.09	1.86	0.05	1.71	0.04	1.79

† Values are the averages of two samples. § Concentration in one sample only was detected.

These results strengthen our finding of limited metal movement through the soil profile. The potential for metal migration at this site at the current conditions appears to be very low.

Fractionation

Sequential extraction procedures have been used to determine the amounts and proportions of trace metals present in different soil fractions (Shuman, 1985, 1991). Fractionation can provide useful information regarding the distribution of the trace metals in question, the affinity of trace metals for different soil components and the potential mobility of trace metals under prevailing conditions. However, interpretation of sequential extraction results should be made with caution due to several problems encountered with this procedure, such as the sequence of extraction and the non-specificity of the extractants (Miller and McFee, 1983; Sheppard and Stevenson, 1995; Miller et al., 1986).

Biosolids application significantly ($P<0.001$) increased the Zn concentration in each of the four fractions examined in the surface 0-15 cm soil (Table 7a). Zinc concentrations increased from 1.0, 3.1, 26, and 1.4 mg kg^{-1} to 35, 45, 86, and 18.6 mg kg^{-1} in the exchangeable, specifically adsorbed, metal-oxide, and organic fractions, respectively, with the highest biosolids application rate. Almost 40% of the recovered Zn was still present in the exchangeable and specifically adsorbed fractions (Fig 3). The greatest percentage increase in Zn was observed in these two fractions; however, the metal-oxide fraction had the highest Zn concentration in the control, medium and high biosolids application rates plots. Other researchers reported similar patterns of Zn fractionation (McGrath and Cegarra, 1992; Sims and Kline, 1991; Sloan et al., 1997; Berti and Jacobs, 1996).

Table 3.7a. Distribution of Cu and Zn among exchangeable, specifically adsorbed, metal oxide, and organic fractions as determined by sequential extraction.

Fraction	Cu			Zn		
	Control	126 kg ha^{-1}	210 kg ha^{-1}	Control	126 kg ha^{-1}	210 kg ha^{-1}
Exch.***	0.24c	1.99b	7.40a	1.04c	14.3b	34.9a
SA***	2.90c	33.6b	62.4a	3.07c	26.3b	45.2a
Oxide***	27.7c	80.3b	126a	25.9c	57.6b	86.1a
Organic***	4.65c	29.8b	47.9a	1.35c	11.4b	18.6a

Rows means for each treatment followed by different letters are significantly ($p<0.05$) different.

The Cu concentrations increased in all four fractions (Table 7a). The highest application rate increased Cu concentrations significantly ($P<0.001$) from 0.2, 2.9, 27.7, and 4.6 mg kg^{-1} to 7.4, 62.4, 125.7, and 48 mg kg^{-1} in the exchangeable, specifically adsorbed, oxalate and organic fractions, respectively (Table 7a).

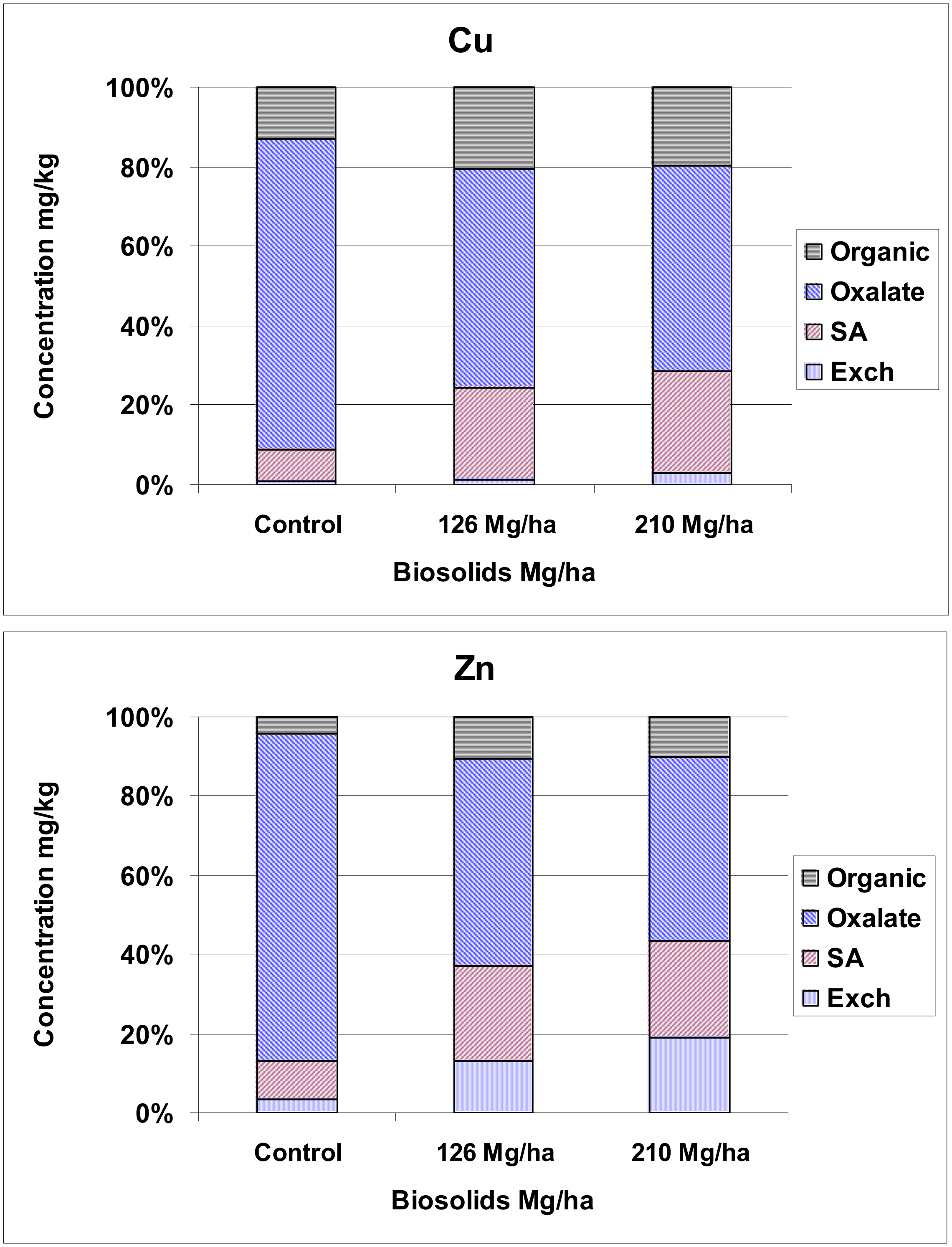

Fig 3.3. Percent Distribution of Cu and Zn among exchangeable, specifically adsorbed, metal oxides and organic fractions as determined by sequential extraction. Percentages are based only on the sum of the four fractions extracted.

The greatest percent increase was found in the specifically adsorbed and organic fractions (Fig 3). The metal-oxide fraction still contained the highest concentration of Cu among all determined fractions. Although strong organic complexes are known to form with Cu, our results indicated that the metal-oxide fraction appears more important in controlling Cu concentration. These results confirm the finding of other researchers (Sloan et al., 1997).

The concentrations of Cd and Ni applied to these plots were initially low. Cadmium was only detected in the exchangeable and specifically adsorbed fractions, forms that are readily bioavailable (Table 7b). Biosolids application increased the concentration of Cd in exchangeable and specifically adsorbed fractions. Soil Cd has been found predominantly in the soluble and exchangeable fractions (Assami et al., 1995; Ramos et al., 1994; Wasay et al., 1998). Soil Ni concentrations increased only in the oxide fraction at the 126 and 210 Mg ha^{-1} treatment and in the exchangeable and specifically adsorbed fractions at the high application rate (Table 7b). Nickel concentrations in the other fractions were below the detection limits of the ICP. The concentration of Ni in the metal oxide fraction increased from 5 mg kg^{-1} to 12 mg kg^{-1} at the highest application rate. Sloan et al. (1997) also reported the greatest increase in Ni concentration in the metal oxide fraction. Berti and Jacobs (1996) found Ni in all soil fractions but predominantly prevalent in the acid soluble and Fe oxide fraction regardless of the total concentration in soils.

Table 3.7b. Distribution of Cd and Ni among exchangeable, specifically adsorbed, metal-oxide, and organic fractions as determined by sequential extraction.

Fraction	Cd			Ni		
	Control	126 Mg ha^{-1}	210 Mg ha^{-1}	Control	126 Mg ha^{-1}	210 Mg ha^{-1}
Exch.	ND	0.98	1.32	ND	ND	0.53
SA	0.43b	0.60ab	0.66a	ND	ND	1.75
Oxide	ND	ND	ND	5.25b	10.5a	12.3a
organic	ND	ND	ND	ND	ND	ND

Rows means for each treatment followed by different letters are significantly ($p<0.05$) different. ND=not detectable; concentrations were below analytical detection limits.

Metal Recovery

Incomplete metal recovery has been reported in many biosolids land application studies (Williams et al., 1987; Alloway and Jackson, 1991; Dowdy et al. 1991; Brown et al., 1997; McBride, 1997; Richards et al., 1998). The apparent metal loss was attributed to leaching (McBride, 1995), dispersion of trace metals due to tillage (McGrath and Lane, 1989; Williams et al., 1987), incomplete chemical extraction from soil (Dowdy et al., 1991) and overestimation of trace metals loadings and bulk density estimation. This illustrates the difficulty of accounting completely for the elements applied. Improved recovery may be possible if the plots are well protected against soil loss and lateral dispersion and when strong acid digestion methods are used to extract most of the total trace metals from soil as was done in this study.

Uptake of trace metals by crops represents a small proportion (0.5-1%) of the metal removed (McGrath, 1987). Elements removed through plant harvest are not considered a major factor affecting recovery calculation. Our analysis of data on plant yield and metal uptake on these plots supported McGrath conclusion for Zn. Crop removal accounted for no more than 1.1% and 0.52% for the 126 Mg ha^{-1} and 210 Mg ha^{-1} application rates respectively (Table 8a). Cu uptake accounted for a much lower percentage (0.07%) of the total metal.

Table 3.8a. Net plant metal removal for the 1985-2003 time period.

Biosolids rate Mg ha^{-1}	Cu		Zn	
	mg kg^{-1}	%	mg kg^{-1}	%
126	0.17	0.08	1.83	1.10
210	0.21	0.07	1.43	0.52

Significant enrichment of trace metals occurred in the top 25 cm and, therefore, Cu and Zn extracted from this soil depth were used in the mass balance calculation. In general, approximately 85% of the applied Cu and more than 75% of the applied Zn are still present in the zone of incorporation (0-15 cm). When metal concentration in the 15-25 cm was included in the analysis, approximately 90% of the applied Cu and more than

85% of Zn were recovered (Table 8b). Twice as much Zn as Cu was recovered in the 15-25 cm depth. This might be explained by the higher mobility of Zn as shown by the fractionation results and water-soluble concentrations. Seventeen years after biosolids was applied to these plots, around 90% of Cu and Zn were still concentrated in the top 25 cm. This indicates that Cu and Zn moved slowly down the soil profile and, as a result, one may conclude that the risk of metal leaching and groundwater contamination at this site is low. Similar findings were reported by other researchers (Chang et al., 1983; Sloan et al., 1997).

Table 3.8b. Mass balance of Cu and Zn detectable at selected soil depth 17 years after biosolids application.

	126 kg ha^{-1}				210kg ha^{-1}			
	Cu		Zn		Cu		Zn	
	Kg ha^{-1}	%	Kg ha^{-1}	%	Kg ha^{-1}	%	Kg ha^{-1}	%
0-15cm***	395	86.6	304	81.7	679	89.3	493	79.5
15-20cm*	10.9	2.4	17.8	4.8	14.4	1.90	35.3	5.70
20-25cm*	9.2	2	10.4	2.8	11.2	1.50	20.5	3.30
0-25cm	415	91	332	89.3	705	92.7	549	88.5
Sampling removal	18.7	4.1	13.6	3.7	32.7	4.30	22.6	3.70
Total	425	95.1	346	93	737	97	572	92.2

*, *** Significant effect at 0.05 and 0.001 level of probability, respectively.

CONCLUSIONS

Soil was sampled with depth 17 years after a one-time biosolids application. The biosolids-applied trace metals were primarily concentrated in the topsoil (0-15 cm). Slight enrichment of metals was observed in the 15-35 cm layers with no apparent movement below that depth. Close to 90% of the applied trace metals Cu and Zn were recovered in the top 25 cm. Crop removal and accounted for <1% of the total trace metals applied. The lack of any indication of trace metal movement through the soil profile and the low dispersible clay content lead us to conclude that deviation of recoveries from

100% is more likely due to inherent variance in sampling, extraction, and analytical techniques as well as the possibility of overestimation of trace metals loadings. Leaching of trace metals down the soil profile as well as their removal by crops do not appear to be significant routes of metal loss from this site. Availability of trace metals was significantly greater in soils that received biosolids application. Mehlich-I extractable trace metals were much higher in biosolids treated soils than the control. Sequential extraction indicated that a considerable proportion of Zn and Cd are still present in the exchangeable forms. The major portion of Cu, Zn, and Ni is associated with metal-oxides fraction.

The Davidson soil has high clay content. It also has a high oxides content resulting in a well-structured and well-drained soil. Most of the applied Cu, Ni, and Zn are still present in the top 25 cm indicating that movement of Cu, Ni, and Zn at this site is extremely slow. Consequently, this indicates to us that there is a little risk of downward movement and contamination of groundwater at this site and for soil with similar conditions and management history.

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CHAPTER FOUR

Trace Metals Availability in a Biosolids-Amended Piedmont Soil 19 Years after Application

ABSTRACT

Concerns over the long-term availability of biosolids-applied trace metals to plants have been raised by many scientists. The objectives of this study were to assess the availability and uptake of Cd, Cu, Ni, and Zn by plants on plots established by single application of biosolids in 1984. Biosolids were applied to 1.5 x 2.3 m confined plots of a Davidson clay loam (clayey, kaolinitic, thermic, Rhodic Paleudult) in 1984 at 0, 42, 84, 126, 168, and 210 Mg ha^{-1}. The highest biosolids application supplied 4.5, 750, 43, and 600 kg ha^{-1} of Cd, Cu, Ni, and Zn, respectively. Radish (*Raphanus sativus* L.), lettuce (*Lactuca sativa* Var longifolia) and barley (*Hordeum vulgare*) were planted at the site. Extractable (0.005 DTPA, 0.01 M $CaCl_2$ and Mehlich-1) Cd, Cu, Ni, and Zn were determined on 15-cm depth samples from each plot. Simple linear regression between plant metal concentration and biosolids-added trace metals were computed to determine uptake coefficients (UC) of crops for each metal as outlined by USEPA Part 503 Rule. Biosolids treatments had no significant effect on the yield of the crops. Plant uptake of trace metals differed among crops. Plant tissue metal concentrations increased with biosolids rate but were within the normal range of these crops. Trace metal concentrations in plants generally correlated well with the concentrations extracted from soil with 0.005 M DTPA, 0.01 M $CaCl_2$ and Mehlich-1. Mehlich-1 gave the best correlation coefficients for Cu and Zn and, therefore, was the most reliable in predicting their availability and uptake by the crops grown. Availability of trace metals as measured by Mehlich-I, DTPA, and $CaCl_2$ extraction were higher in amended plots as compared to control and increased linearly in response to biosolids addition. Metal concentration in the plants exhibited a plateau response in most cases. Several linear increases were observed in some cases in 2003 when the soil pH decreased below 5.5. The UC values generated for the different crops were in agreement with the values set by the Part 503 Rule.

INTRODUCTION

Plant nutrients (e.g., N and P) and organic matter can be recycled by applying biosolids to agricultural land (Hyun et al., 1998; Berti and Jacobs, 1998). Repeated land application of biosolids, however, has resulted in increasing metal concentration in soils (Sloan et al., 1998). The persistence of biosolids borne metal in soils after application has ceased, continues to be an area of concern (McGrath, 1987). Trace metals in crop tissues are elevated as a result of biosolids application to land (Logan and Chaney, 1983;

Corey et al., 1987; Berti and Jacobs, 1996). Plant uptake is one of the major pathways by which biosolids-borne potentially toxic trace metals enter the food chain (Chaney, 1990).

The United States Environmental Protection Agency (USEPA) developed a risk assessment method (USEPA, 1992) to evaluate the potential negative effect of pollutants in biosolids. USEPA, 40 CFR Part 503 was promulgated as the Standards for the Use and Disposal of Sewage Sludge (USEPA, 1993). This rule is risk-based and considers the exposure of human, animals, and plants to biosolids trace metals through 14 possible different pathways. Each element has a reference pollutant load calculated for each pathway to avoid detrimental effects on highly exposed individuals (USEPA, 1995). The limiting value for a particular metal is its smallest reference pollutant load. This, in turn, permitted the calculation of allowable pollutants concentration in biosolids for each trace element (USEPA, 1993). The Part 503 rule permits long-term application of biosolids to agricultural land with the assumption that soil accumulation of trace metals from biosolids meeting ceiling concentration limits will not cause environmental or health hazard before the application must cease.

The protectiveness of the Part 503 rule has been questioned over some assumptions made in the underlying risk assessment (McBride, 1995; Schmidt, 1997; Harrison et al., 1997). Change in pollutant bioavailability after termination of biosolids application and relationship between plant metal uptake and metal loading are still contentious.

Harrison et al. (1997) argued that the EPA analysis did not adequately consider the variability in plant accumulation and soil sorption capacity of trace metals. Uptake coefficients (UC), which express the amount of a metal taken up by a plant compared to the amount applied to the soil, are critical to a number of the pathways in the risk assessment. The use of geometric means to generate the UC for different crops has been criticized as oversimplified (Harrison et al., 1997). These means included data from soils with a wide range of characteristics such as cation exchange capacity, pH, and texture. This has led many researchers to voice their concerns that the UC values used in the risk assessment are low, especially in regard to acidic soils (Harrison et al., 1997; Stern, 1993; Chaney and Ryan, 1994).

Long-term availability of biosolids-applied trace metals to soils has been another issue of concern. Debates on whether organic or inorganic components in biosolids are responsible for controlling metal availability continue. Some researchers proposed that the organic matter component of biosolids is the primary factor controlling availability (Beckett et al., 1979), while others (Corey et al., 1987; Essington and Mattigod, 1991; Hettiarachchi et al., 2003) stressed the role of inorganic components (Fe, Mn, and Al oxy-hydroxide minerals) in controlling metal bioavailability. Supporters of the first hypothesis assumed that metal would be released to soil system after cessation of biosolids application because of decomposition of organic matter (McBride, 1995). In this respect, the concentrations of trace metals are predicted to increase in plants as a linear function of their concentrations in soils (linear response curve). The risk assessment conservatively assumes that the relationship between metal added in biosolids and plant uptake will be linear despite data that suggest that uptake will attain a plateau response. This is a result of the adsorptive materials added to soils with the biosolids. According to the "plateau effect", the rate of trace metals uptake decreases as the biosolids-applied trace metals concentration in soils increase (USEPA, 1993).

Most of the data used to develop Part 503 rule were from studies in which trace metal uptake by plants were measured during the years of biosolids application. Concerns have been raised that the availability of added trace metals may increase after termination of biosolids applications (Beckett et al., 1979; McBride, 1995). It is crucial to understand the long-term effects biosolids application have on metal availability.

Different chemical reagents have been used to estimate the fraction of trace metals that are potentially available to plants. These results can be used as indicators of metal bioavailability in biosolids-amended soil. The most frequently employed reagents are chelating agents such as diethylenetriaminepentaacetic acid (DTPA) (Lindsay and Norvell, 1978). Others utilized non-buffered salt solutions such as $CaCl_2$, $Ca(NO_3)_2$, and NH_4NO_3, and Mehlich-1 to estimate metal availability. Trace metals extracted by DTPA are shown to correlate with plant metal uptake (Bidwell and Dowdy, 1987; Sommers et al., 1991; Hooda and Alloway, 1994). DTPA has often proven effective for assessing metal availability to plants. It is one of the most extensively used organic extractants (Akram et al., 1995). DTPA extracts trace metals from the soluble, exchangeable, and some of the organic fractions. A $CaCl_2$-extractable trace metals fraction has been also found to correlate closely with plant uptake (Sanders et al., 1986, 1987). Calcium chloride removes trace metals that are soluble and easily exchangeable. Non-buffered salt solutions such as $CaCl_2$ are considered among the best extractants to predict plant-available trace metals in soil (Hooda et al., 1997) and are widely used (Eriksson, 1990; Novosamski et al., 1993).

The objectives of our research were (i) to measure the long-term availability of biosolids-applied trace metals to a weathered piedmont soil, (ii) to calculate uptake coefficients of Cd, Ni, Cu, and Zn for these crops, and (iii) to determine whether uptake of biosolids derived trace metals continues linearly or reaches a plateau with increasing concentrations of trace metals added.

MATERIALS AND METHODS

Field Plots and Treatments

A field test was established in the spring of 1984 to evaluate the use of an aerobically digested biosolids from a wastewater treatment plant with a major industrial input on cropland (Rappaport et al., 1987, 1988). The experiment was conducted at the current site of the Northern Piedmont Agricultural Research and educational center (NPAREC) in Orange, Virginia on a Davidson clay loam (clayey, kaolinitic, thermic Rhodic Paleudult). The chemical and physical properties of this piedmont soil are presented in Table 1.

Table4.1. Chemical and physical properties of the Ap horizon of the Davidson clay loam used for the biosolids study at the NPAREC. (Rappaport et al., 1986)

			Amorphous and Crystalline			Particle size distribution		
pH	OM	CEC	Al	Mn	Fe	Sand	Silt	Clay
	$g\ kg^{-1}$	$cmol_c\ kg^{-1}$		$mg\ kg^{-1}$			$mg\ kg^{-1}$	
5.7	18	12.5	4740	1280	42500	153	471	376

Field experimental plots were constructed to prevent the lateral movement of biosolids constituents. The plots consisted of an isolated volume of soil, 2.3 x 1.5 m and 0.9 m deep. Isolation of this soil volume was accomplished by excavating a ditch 20 cm wide and 0.9 m deep and wrapping these soil blocks with 254-micrometer polyethylene film. Aluminum flashing was placed around the plots to prevent lateral movement and runoff. Connection of the aluminum flashing to the plastic ensured total lateral isolation. Eventually, the Al flashing was replaced by wooden 5 cm wide by 20 cm deep boards that encompassed the plots. In the spring of 1984, an aerobically digested biosolids was applied at rates of 0, 42, 84, 126, 168, and 210 dry Mg/ha. The treatments were arranged in a randomized complete block design with four replicates.

The biosolids contained considerably higher concentrations of trace metals than currently found in typical land-applied biosolids. The current ceiling limits and actual concentration of trace metals found in the biosolids are shown in table 2. Copper, Zn, and Pb concentrations were above the exceptional quality (EQ) limits for pollutant concentration biosolids and Cd and Ni were lower than EQ limit; thus, Part 503 would require lifetime loading rate of this biosolids to be tracked. The 210 Mg ha^{-1} biosolids rate supplied 4.5 kg Cd, 750 kg Cu, 43 kg Ni, and 600 kg Zn per hectare (Table 3). These amounts are below the maximum cumulative pollutant loading rate application of trace metals that can be applied to soils in biosolids that meets ceiling concentration limits. This rate provided 12% of the Cd limit, 50% of the Cu limit, 10% of the Ni limit, and 21% of the Zn limit. None of the trace metals is near the limits for ceasing application of pollutant concentration biosolids, but the amounts added are higher than would be applied from the continuous addition of agronomic rates of biosolids since 1984, based on the average quality of currently generated biosolids. Thus, the application rates of the trace metals provide a scenario that can test the original assumptions of the 503 risk assessment.

Table 4.2. Properties of the aerobically digested sewage sludge (ADSS) and Part 503 Ceiling Concentration Limit (CCL) and Pollutant Concentration Limit (PCL) standards for land applied biosolids. (Rappaport et al., 1986)

Trace metals	ADSS	CCL	PCL
Cd (mg kg^{-1})	21.5	85	39
Cu (mg kg^{-1})	3650	4300	1500
Ni (mg kg^{-1})	210	420	420
Pb (mg kg^{-1})	640	840	300
Zn (mg kg^{-1})	2980	7500	2800

Table 4.3. Quantity of biosolids, trace metals and total N and P applied at the NPAREC study site (Rappaport et al., 1986)

Sludge	Macronutrient		Trace metals			
Application	N	P	Cd	Cu	Ni	Zn
Mg ha^{-1}			Kg ha^{-1}			
42	670	1380	0.9	150	8.6	120
84	1340	2760	1.8	300	17.2	240
126	2010	4140	2.7	450	25.8	360
168	2680	5520	3.6	600	34.4	480
210	3350	6900	4.5	750	43	600

Cropping System

A three-year rotation was initiated in the spring of 2001 to assess the phytotoxicity of Cu, Ni, and Zn and the phyto-accumulation of Cd, Cu, Ni, and Zn by barley (*Hordeum vulgare L.*), radish (*Raphanus sativus L.*), and lettuce (*Lactuca sativa* Var *longifolia*) cultivar Paris Island Cos. Barley was planted in late October, 2001 only, and the radish and lettuce were planted in early April, 2001, 2002, and 2003. The plots had been limed to a pH of approximately 6.0 in 1998-1999 to minimize acid pH-induced Al, Cu, and Zn phytotoxicity and trace metal uptake. The change in soil pH during the duration of the study is presented in Table 4. Commercial fertilizer nitrogen (as ammonium nitrate) at 120 kg N ha^{-1}, P (as triple superphosphate) at 22 kg P ha^{-1}, and K (as muriate of potash) at 83 kg K ha^{-1} were applied to each plot (rep x treatment) every spring as required according to the Virginia Cooperative Extension soil testing recommendations (Donohue and Heckendorn, 1994).

Table 4.4. Soil pH (average of 4 replicates) in the biosolids-amended soil experiment from 2001 to 2003.

Biosolids	pH		
Application Mg ha^{-1}	2001	2002	2003
0	5.83	5.65	5.38
42	5.95	5.65	5.41
84	6.03	5.58	5.32
126	6.03	5.75	5.34
168	5.88	5.58	5.33
210	5.95	5.63	5.47

Sampling and Sample Preparation

Barley samples were collected in March 2002 by cutting the whole above ground portion. Drought conditions from fall 2001 to spring 2002 reduced seedling germination and stand vigor; thus, samples were collected for metal concentration but not biomass production. Samples were washed under tap water, rinsed with de-ionized water to ensure cleanliness and dried in a forced air oven at 70°C for 48 hrs.

Each plot contained two rows of lettuce with minimum of 14 plants per row and two rows of radishes with a minimum of 40 plants per row. The plants were harvested when physiologically mature. Radish tops were separated from globes, and radish and lettuce samples were prepared following standard washing procedures. The plant tissues were dried in a forced air oven at 70°C for 72 hrs. Dry weight was calculated by weighing fresh and dry sub-samples of four lettuce and fourteen radish plants randomly chosen. Crops were visually monitored throughout the growing season for any sign of deficiency or toxicity.

Air-dried samples were ground in a stainless steel Wiley mill to pass 0.5mm sieve in preparation for chemical analysis. Ground samples were stored in paper bags and placed in the oven at 65°C to remove any moisture added during grinding and handling of the samples.

Plant Analysis

Plant tissue was digested using a nitric acid microwave method. A 0.5 g aliquot of each ground sample was weighed and placed in a digestion vessel to which 10 ml of trace metal grade HNO_3 acid was added. Vessels were tightly closed and transferred to the Ethos Plus 800 Microwave Labstation (Milestone Microwave Lab Systems, Germany). Digestion followed a three-step program. The first stage program brought the sample to 140^oC in 5 min, the second stage permitted a slow rise of temperature to 190^oC in 10 min, and the third stage allowed the digest to remain at 190^oC for an additional 10 min. Following the 25 minute program, the samples were allowed to cool before they were transferred to 20 ml plastic vials. Reagent blanks and NIST standard samples (1573a) were routinely included in the analysis. Samples were analyzed for Cd, Cu, Ni, and Zn using a Thermo (Fitchburg, MA) Jarrell Ash ICAP (Inductively Coupled Argon Plasma-Atomic Emission Simultaneous spectrometer; ICP-AES).

Soil Analysis

Mehlich-I (0.05M HCl and 0.0125 H_2SO_4) (Mehlich, 1953), neutral salts (0.01 M $CaCl_2$) (Novozamski et al., 1993) and diethylenetriamine pentaacetic acid extracting solution (0.005 M DTPA, 0.1 M TEA, and 0.01 M $CaCl_2$, adjusted to pH 7.3) (Lindsay and Novell, 1984) solutions were employed to extract various soil trace metals fractions as potential indicators of plant-available trace metals. The soil extracts were analyzed for Cd, Cu, Ni, and Zn with an inductively coupled plasma atomic emission spectrophotometer (ICP-AES).

Statistical Analysis

Plant weight and trace metal concentration data were evaluated by analysis of variance and by the least significant difference (LSD) mean separation procedures at the 0.05 level of significance (Steele and Torrie, 1980). Extractable soil trace metals data were also evaluated by LSD mean separation procedures at the 0.05 level of significance. The UC (plant uptake slopes) values were calculated with the linear regression statistical method. The slope of the linear regression line of the metal taken up by a plant compared to the amount applied to the soil was taken as the UC for those trace metals. Relationships between plant metal concentrations and DTPA-, Mehlich 1-, and $CaCl_2$-extractable trace metals were determined by Pearson correlation coefficients.

RESULTS AND DISCUSSION

Rappaport et al. (1988) reported a linear increase of DTPA extractable Cu and Zn because of biosolids application. The levels of extractable Cu and Zn in 1984 reached 129 and 78 mg kg^{-1} respectively at the highest application rate (Table 5). Anderson et al. (1997) also reported a linear increase in DTPA extractable Cu and Zn concentrations with biosolids application at the site; however, the concentrations of both metals were lower than those extracted in 1984 despite the lower soil pH. This decreasing trend continued in 2001. Our results have shown a linear increase of Cu and Zn extracted with DTPA. However, the amounts extracted were much lower than the concentrations reported in 1984 (Table 5) which indicates a significant decrease of metal availability with time.

Bioavailability of trace metals in biosolids amended soil typically does not increase years after land application has ceased (Bidwell and Dowdy, 1987; Hyun et al., 1998). Chang et al. (1987, 1997) and Sommers et al. (1991) reported that the highest availability of trace metals is during the period immediately following biosolids application. In other studies, the availability of trace metals has been reported to decrease with time as organic decomposition rates decreased (Bidwell and Dowdy, 1987; Walter et al., 2002; McGrath, 1984). With time, metals may convert into less available forms (Parkpain et al., 1999).

Table 4.5. Long-term effect of biosolids application on DTPA extractable Cu and Zn from soil.

Biosolids application Mg ha^{-1}	Zn mg kg^{-1}		Cu mg kg^{-1}	
	1984	2001	1984	2001
0	1.64	2.7	1.4	3.2
42	19.2	9.1	24.9	12.6
84	38.9	19.8	53	25.4
126	52.4	27.9	73.4	33.7
168	73.2	35.5	120	43.3
210	78.2	46.7	129	53.6

Soil organic matter increased with biosolids application rates (Fig 1). Following biosolids application, the levels of organic matter were 7 to 31 g kg^{-1} higher in plots that received biosolids as compared to the control. By 1992, the levels of organic matter for the 42 and 84 Mg ha^{-1} treatments had not changed appreciably. The 126 and 168 Mg ha^{-1}, levels had decreased by about 4 g kg^{-1}. The highest decrease was reported in the 210 Mg ha^{-1} treatment, where organic matter content decreased by as much as 10 g kg^{-1}. There was no change in the level of organic matter with time after 1992. Analyses performed in 1995 and 2001 showed that the organic matter contents are similar to those of 1992.

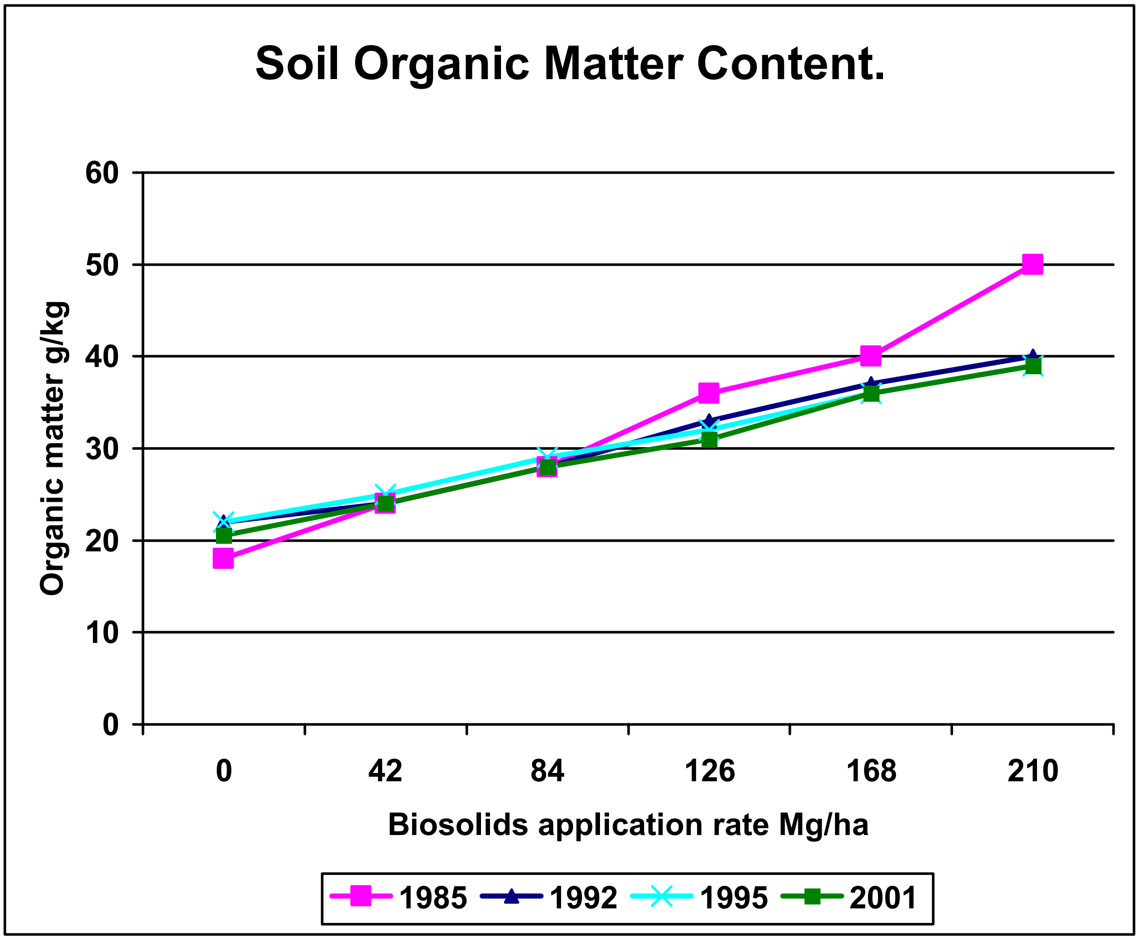


Fig. 4.1. Long-term effect of biosolids application on soil organic matter.

Biosolids applied organic matter often decomposes slowly. Studies reported that the greatest rate of biosolids organic carbon mineralization occurs within days of biosolids application (Wiseman and Zilbilske, 1988). Organic matter decomposition rate decreases greatly with time, and biosolids applied organic matter can remain in soils for centuries before soil organic matter revert to its background levels (McGrath et al., 2000; Bidwell and Dowdy, 1988)

Crop Yield

Biosolids application rate did not affect the yield of lettuce, radish globes, and shoots as indicated by ANOVA (Table 6). There were no visual signs of toxicity or deficiency observed in any of the crops. Lettuce yield significantly increased with biosolids rate in the first season, but this trend was not repeated in the following two seasons. This suggests that plant growth was little affected by the biosolids addition.

Table.4.6. Effect of biosolids treatment on dry weight of romaine lettuce and radish.

		Biosolids application rates Mg ha^{-1}					
		0	42	84	126	168	210
Crop	Year	Weight kg ha^{-1}.					
Lettuce	2001	710b	1056a	919ab	1212a	1139a	1131a
	2002	681	731	847	887	803	855
	2003	664	902	792	858	911	916
Radish globes	2001	641	760	676	708	757	684
	2002	806	751	664	815	832	667
	2003	548	647	708	678	719	623
Radish tops	2001	780	1029	800	1018	971	887
	2002	615	609	568	589	681	647
	2003	496	632	592	577	623	629

Row means for each treatment followed by different letters are significantly ($p<0.05$) different

Metal uptake.

The trace metals concentrations in the dry matter of barley, radish globes and tops, and lettuce are summarized in tables 7, 8, and 9. The concentrations of the different trace metals analyzed in all crops of all biosolids-amended treatments were higher than the control but remained well within the values observed for uncontaminated soils (Table 10, Kabata-Pendias and Pendias, 1991). Trace metal concentrations increased with biosolids metal rate, but none attained toxic concentrations and all were within the range considered sufficient for agronomic crops. Similar results were reported by others (Hooda et al., 1997; Keefer et al., 1986; Smith, 1994).

The concentrations of Cd, Ni, and Zn in lettuce leaves were greater in biosolids - amended soils than their concentrations in plants grown in the control plots (Table 7). Three times as much Cd and 2.3 times as much Zn accumulated in lettuce grown in the biosolids plot as compared to background concentrations. Tissue Zn concentration was greater than 100 mg kg^{-1} at the highest biosolids application rate. Lettuce is indicative of vegetables with high potential for Cd uptake and transport to edible crop tissues. Lettuce is the most responsive of the garden vegetables to changes in soil Cd concentration (Brown et al., 1996). Sloan et al. (1997) reported elevated Cd and Zn concentration in romaine lettuce because of 16 years of biosolids applications.

Table 4.7. Trace metals concentrations in the dry matters of lettuce.

		Biosolids Application Rate Mg ha^{-1}					
		0	42	84	126	168	210
		Concentration mg kg^{-1}					
Cu	2001	8.47	11.2	9.19	9.65	8.47	10.1
	2002	8.43	9.44	9.86	10.7	10.1	10.0
	2003	9.21	10.6	10.1	9.92	10.8	9.44
Zn	2001	45.3d	63.8bc	75.6b	92.3a	92.5a	108a
	2002	42.4d	71.8c	100b	108ab	122a	118ab
	2003	46.8d	68.8c	85.4bc	97.8ab	111a	101ab
Cd	2001	0.32d	0.54c	0.73b	0.88ab	098a	0.98a
	2002	0.51c	0.92b	1.16a	1.20a	1.21a	1.27a
	2003	0.26b	0.60a	0.75a	0.81a	0.79a	0.75a
Ni	2001	0.80	1.39	1.19	1.05	1.04	1.11
	2002	1.05b	1.54b	2.11a	2.24a	2.46a	2.22a
	2003	0.34c	0.47bc	0.64b	0.62b	1.16a	1.13a

Row means followed by different letters are significantly different at the 0.05 probability level.

The concentrations of Cu, Zn, and Ni in radish globes and tops from biosolids -amended soils are shown in tables 8 and 9. Trace metals concentrations were higher than in the plants from the control plots.

Table 4.8. Trace metals concentrations in the dry matters of radish globes.

		Biosolids Application Rate Mg ha^{-1}					
		0	42	84	126	168	210
		Concentration mg kg^{-1}					
Cu	2001	2.69d	4.32c	4.71c	5.42bc	6.31ab	6.70a
	2002	3.24d	5.07c	6.24b	6.36b	7.47a	7.87a
	2003	2.67d	3.99cd	5.36c	7.23b	8.10b	10.5a
Zn	2001	37.8c	50.8b	56.5b	68.1a	71.9a	73.3a
	2002	31.8c	40.1c	52.1b	53.0b	62.5a	62.8a
	2003	28.7e	43.2d	52.6c	66.5b	76.0a	81.6a
Cd	2001	0.19	0.16	0.14	0.15	0.20	0.22
	2002	0.24	0.26	0.20	0.25	0.30	0.26
	2003	0.13	0.15	0.16	0.16	0.20	0.15
Ni	2001	0.30	1.06cd	0.91de	1.41bc	1.87a	1.57ab
	2002	0.46e	0.67de	1.02cd	1.16bc	1.53ab	1.65a
	2003	0.30	0.46c	0.46c	0.63c	0.95b	1.29a

Row means followed by different letters are significantly different at the 0.05 probability level.

Cd concentrations were not significantly affected by the biosolids additions. Barley grown in biosolids-amended plots accumulated significantly higher Zn and Cu (Table 9). Nickel and Cd were below the detection limit of the instrument. Concentrations of the trace metals in the different crops clearly showed that metal uptake differs widely between crops. The accumulation was dependent on the species grown as well as the specific metal examined.

Table 4.9. Trace metals concentrations in the dry matters of radish tops.

		Biosolids Application Rate Mg ha^{-1}					
		0	42	84	126	168	210
		Concentration mg kg^{-1}					
Cu	2001	5.43d	7.12c	6.66c	8.30ab	7.67bc	8.83a
	2002	6.85c	9.47b	10.3ab	10.6ab	11.6a	11.3ab
	2003	4.56c	5.71bc	5.98b	7.58a	6.85b	8.01a
Zn	2001	37.3d	42.0cd	46.9bc	52.7b	54.6b	63.3a
	2002	34.6d	48.9c	68.7ab	61.1bc	74.3a	70.3ab
	2003	32.7e	48.5d	63.8c	67.5bc	77.1b	95.4a
Cd	2001	0.54	0.66	0.47	0.57	0.43	0.42
	2002	0.80	0.76	0.73	0.70	0.73	0.75
	2003	0.35	0.35	0.31	0.31	0.33	0.32
Ni	2001	2.20	2.52	2.15	2.40	1.80	1.99
	2002	1.17c	1.70bc	1.94ab	1.95ab	2.40a	2.17ab
	2003	0.47	0.69c	0.68bc	0.77b	1.09a	1.24a
Barley							
Cu	2001	11.1d	15.6c	17.1ab	16.6abc	17.6a	16.3bc
Zn	2001	44.7d	78.4c	85.2bc	90.6b	104a	100a

Row means followed by different letters are significantly different at the 0.05 probability level.

Table 4.10. Critical concentrations of trace metals in plant tissues (Kabata-Pendias and Pendias, 1991).

Metal	Considered toxic	Tolerable in Agronomic Crop	For 10% yield loss
Zn	100-400	300	100-500
Cu	20-100	50	10-30
Cd	5-30	3	10-20
Ni	10-100	50	110-30

Several studies have indicated that trace metals in crop tissues are elevated as a result of biosolids application to land (Logan and Chaney, 1983; Corey et al., 1987; Berti and Jacobs, 1996; Hooda et al., 1997; Chaudri et al., 2001). Jing and Logan (1992) reported that crop uptake of Cd increased with increasing biosolids Cd concentration. Dowdy et al. (1978) reported that the Zn concentration in bean (*Phaseolus vulgaris* var tendergreen) never exceeded 60 mg kg^{-1} in the edible bean tissue although 1520 kg ha^{-1} Zn was applied. Copper concentration increased from 2 mg kg^{-1} in the control to 8 mg kg^{-1} in the treatment that received 266 kg Cu ha^{-1}.

In contrast, Berti and Jacobs (1996) observed that soybean grown on a sandy loam soil failed to grow on one of the plots receiving biosolids in which the Zn and Ni content are well above the CPLR (USEPA, 1993). They attributed the response to phytotoxic Zn and Ni concentrations in the soil of these plots. The authors also reported phytotoxic concentration of Zn in corn for five years following 10 years of biosolids application. They also found that Ni in the corn samples was greater than the tolerance level of 3 mg kg^{-1} suggested by (Risser and Baker, 1990).

Some recent studies have demonstrated that the bioavailability of trace metals in biosolids amended soils does not increase years after land application of biosolids has stopped (Bidwell and Dowdy, 1987; Brown et al., 1998; Hyun et al., 1998). In fact, several long- term biosolids studies have shown that plant availability of biosolids trace metals is greatest during the first year after application (Bidwell and Dowdy, 1987; Chang et al., 1987, 1997; Sommers et al., 1991).

Soil Test

The biosolids-amended soils have significantly higher trace metals concentration than the control. The concentration of Cu and Zn extracted with 0.05 M DTPA, 0.01 M $CaCl_2$, and Mehlich-1 and Ni extracted with $CaCl_2$ and DTPA increased linearly in response to biosolids additions (fig. 2a, b, and c). The increase in DTPA- and $CaCl_2$-extractable Cd with metal addition decreased with biosolids rate but no clear plateau was reached (Fig 2d). Available metal concentration was dependant on the total metal content of soil. Metal concentration in soils and pH are found to be important factors in regulating the availability of Cd, Cu, Ni, and Zn to several plant species (Dowdy and Volk, 1983; Welch and Lund, 1989; Sauerbeck, 1991).

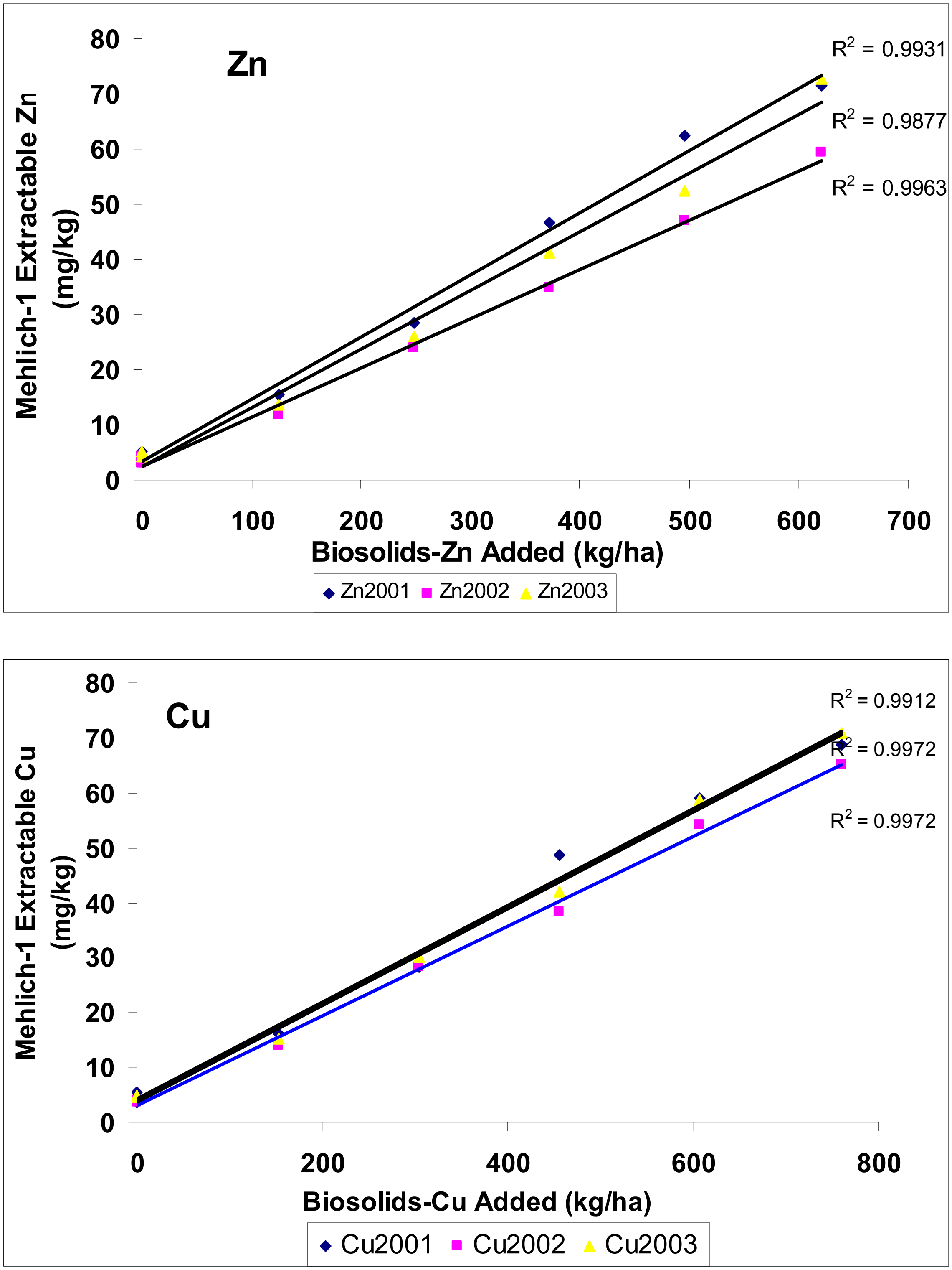
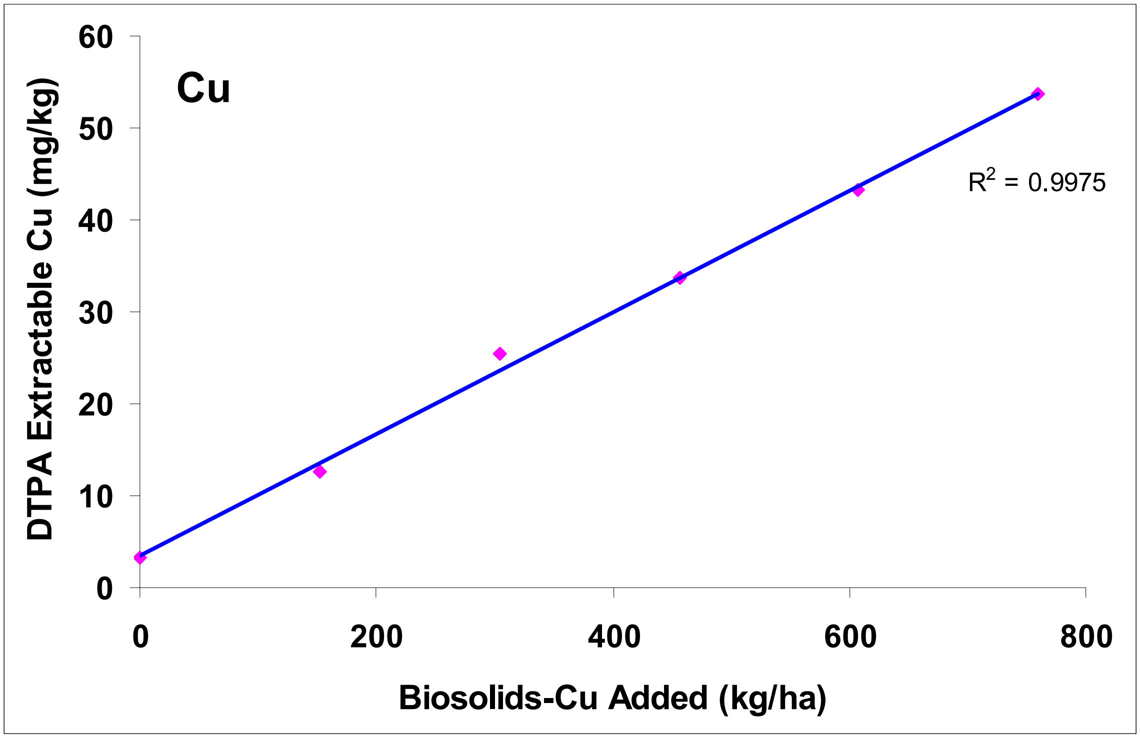


Fig 4.2a. Effects of biosolids application on the levels of Mehlich1-extractable Cu and Zn.



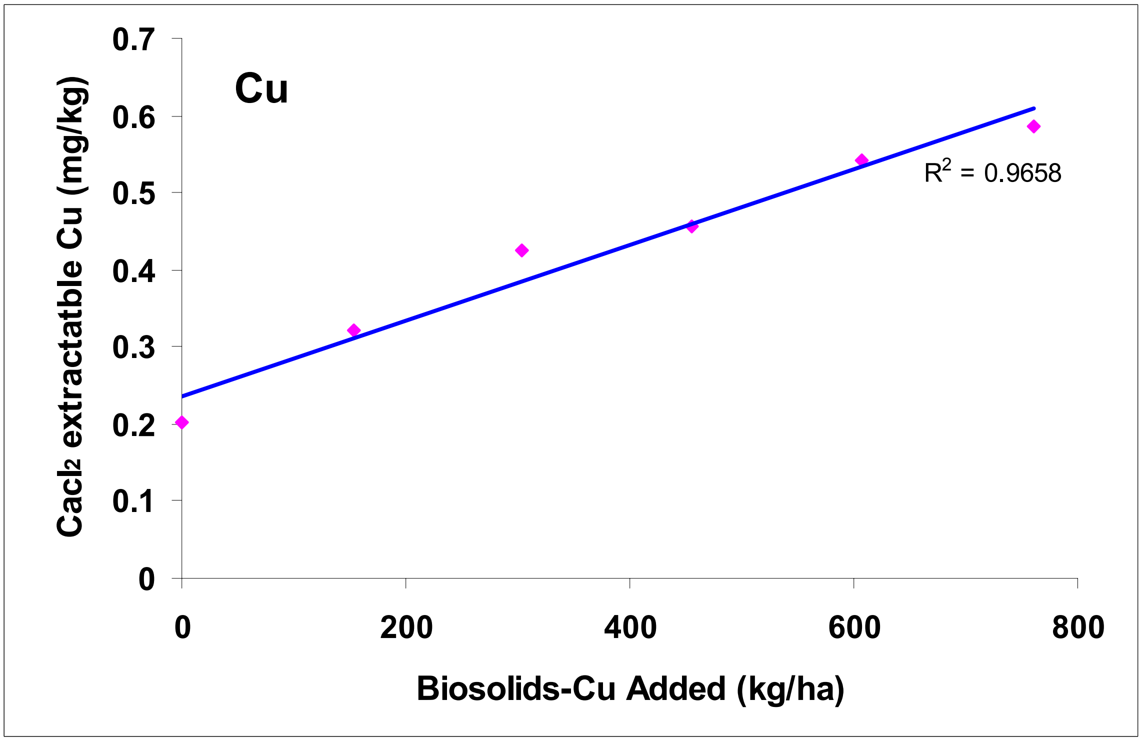


Fig 4.2b. Effects of biosolids application on the levels of DTPA and $CaCl_2$ extractable Cu 17 years after application.

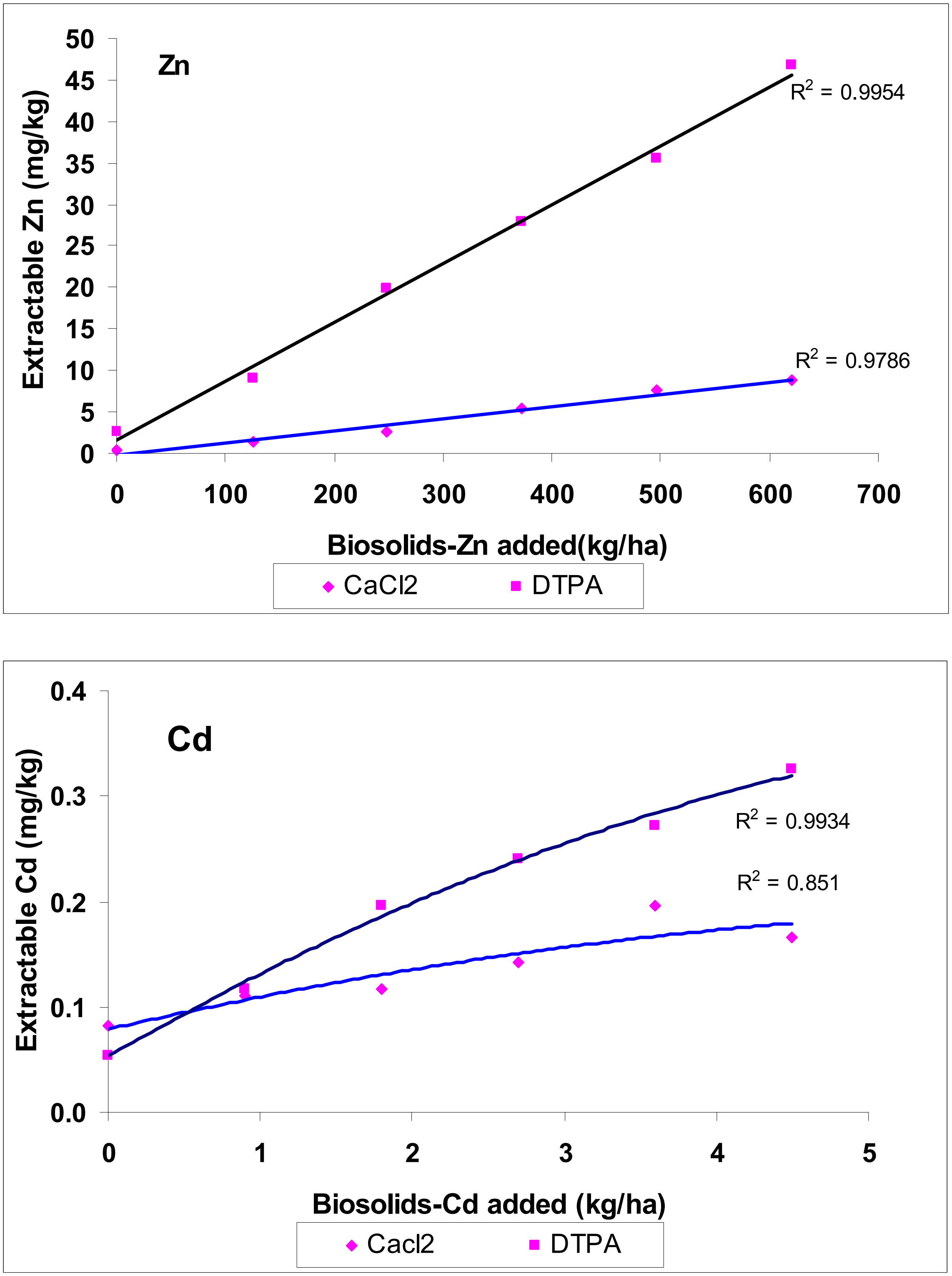


Fig 4.2c. Effects of biosolids application on the levels of DTPA and $CaCl_2$ extractable Cd and Zn 17 years after application.

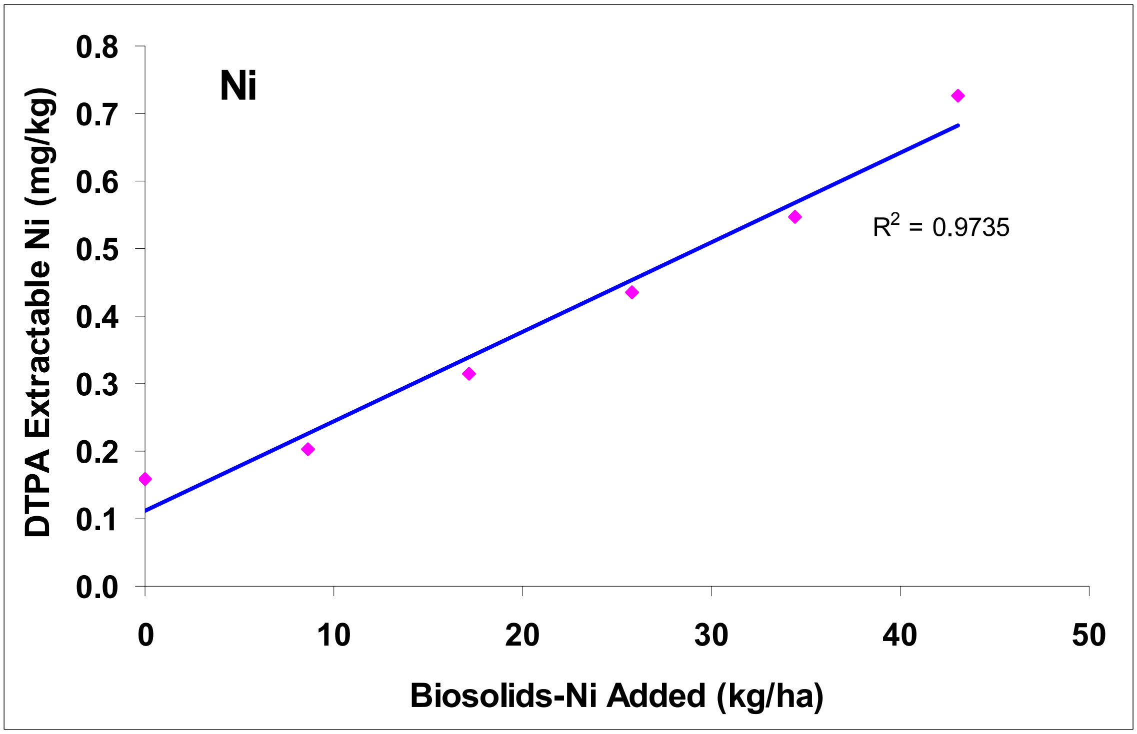


Fig 4.2d. Effects of biosolids application on the levels of DTPA extractable Ni 17 years after application.

The rankings among the extractants were Mehlich-1>DTPA>> 0.01 $CaCl_2$ across all rates. The concentration of available Cu and Zn differed between growing seasons, but there were no increasing or decreasing trends.

The concentrations of Cd, Cu, Ni, and Zn in radish globes and tops, lettuce and barley crops were correlated with their bioavailable fractions in soil extracted with three different reagents (0.05 M DTPA, 0.01 M $CaCl_2$, and Mehlich-1) using Pearson correlation coefficient (Table 11). Extractable fractions increased markedly in the biosolids-amended soil as compared to the control. Zn extracted with all three reagents correlated significantly with its content in the crops. Zn availability was best predicted by the Mehlich-1 test. Calcium chloride and DTPA gave lower, though significant, correlation coefficients. Similarly, Cu concentrations in radish globes and tops and in barley were significantly correlated to its available concentration in soils. Mehlich-1 gave the best relationships as evidenced by the higher R^2 among the three extractants. DTPA better predicted availability of Cd in lettuce (R^2= 0.83) and Ni in radish globes (R^2=0.77) than $CaCl_2$. For Ni and Cd in the other plants, the relationship was not significant because

Ni and Cd contents of plants did not show any significant increase. In general, prediction of plant available trace metals depended on the plant species and specific metal. Hooda et al. (1997) reported similar findings. Our results showed that the Mehlich-1 test gave the best correlation for Cu and Zn. Except for Cu in lettuce, Mehlich-1 consistently correlated with Cu and Zn in each of the crops and, therefore, appears to be a reliable test to measure available metal fraction to the different crops grown at this site.

Table 4.11. Pearson correlation coefficients for Cu and Zn uptake by all crops examined and 0.01 M $CaCl_2$, DTPA and Mehlich-1 extractable soil trace metals.

Extractant	Year	Cu Pearson Correlation Coefficient.			
		Barley	Radish tops	Radish globes	Lettuce
$CaCl_2$	2001	0.510	0.438	0.638	0.361
DTPA	2001	0.660	0.766	0.872	0.008
Mehlich-1	2001		0.794	0.848	-0.011
	2002	0.688	0.784	0.899	-0.045
	2003		0.677	0.868	0.082
Extractant	Year	Zn Pearson Correlation Coefficient			
		Barley	Radish tops	Radish globes	Lettuce
$CaCl_2$	2001	0.747	0.824	0.848	0.766
DTPA	2001	0.817	0.848	0.881	0.799
Mehlich-1	2001		0.877	0.905	0.908
	2002	0.8434	0.854362	0.8639	0.8594
	2003		0.8675	0.9161	0.7121

The concentrations of trace metals extracted with $CaCl_2$ and DTPA were, in general, low which might affect their ability to reflect trace metals availability to plants. The concentration and soil: solution ratio we used may have been low for metal enriched soils. Novell (1984) suggested that no more than half the complexing capacity should be used when extracting trace metals with a complexant; otherwise, the concentration will be influenced by the concentration of the other available trace metals (Esnaola et al., 2000), and incomplete extraction could result. A higher soil: solution ratio should then be used as a better test of plant availability (Esnaola et al., 2000). The concentrations of trace metals extracted with $CaCl_2$ were the lowest. Metal exchange with Ca and metal dissolution might have been incomplete due to the low concentration used (0.01M). At a higher concentration, the complexing capability of chloride with Cd, Cu, and Zn would favor dissolution and desorption of those trace metals (Esnaola et al., 2000). A higher concentration may have better reflected the amounts of plant available trace metals.

Plant uptake coefficients

The USEPA was criticized for using the geometric means to generate the uptake coefficient (UC) values for different crops in their risk assessment. This approach was deemed non-conservative by some as it masks the effect of variation in soil properties. Some researchers voiced concerns that these UC values are low for acid soils common in the eastern United States (Harrison et al., 1997; Stern, 1993).

Table 12 depicts the range of UC of the different trace elements generated in this study and the UC values used in the USEPA risk assessment. The slope of the linear regression curve represents the UC or the efficiency of metal transfer from soil to plants. The UC values establish the relationship between rate of metal uptake by crops and the amount of contaminant added to soil. Higher uptake coefficients imply that greater metal fractions will accumulate in the plant.

Table 4.12. Uptake coefficients of the different trace metals in lettuce and radish tops and globes.

Lettuce	Study UC	Regression coefficient	EPA UC†
Cd	0.126-0.167	0.684-0.922	0.182
Cu	0.0002-0.001	0.002-0.213	0.001-0.197
Ni	0.0005-0.025	0.002-0.908	0.001-0.075
Zn	0.096-0.129	0.878-0.969	0.020-2.240
Radish globes			
Cd	0.007-0.017	0.229-0.431	0.029-0.123
Cu	0.005-0.010	0.914-0.990	0.001-0.015
Ni	0.024-0.029	0.783-0.989	
Zn	0.058-0.089	0.915-0.987	0.006-0.206
Radish tops			
Cd	0.001-0.017	0.087-0.419	0.032
Cu	0.004-0.007	0.781-0.896	
Ni	0.001-0.022	0.414-0.918	
Zn	0.041-0.096	0.859-0.985	

† Range/average UC used in the EPA risk assessment analysis.

The metal uptake data clearly shows that the UC of trace metals varied widely between crops. Lettuce had a higher concentration of Zn and Cd and the slope of the regression line is steeper relative to their concentration in soils. Lettuce had a Cd UC 10 times higher than radish globes and tops. The Zn UC were between 1.1 and 2.3 times higher in lettuce and varied among years This indicates that lettuce leaves can accumulate greater amount of Zn and Cd than the other crops for a given soil metal concentration. Metal uptake data confirms these observations (Tables7, 8, and 9).

In contrast, Cu had the smallest UC values, which were consistent in all three crops. The Ni UC values were similar among the crops. The highest Ni UC was observed in radish globes. The UC values for the different trace metals in this study were similar to those used in the USEPA risk analysis (Table 12).

Uptake curve

The relationships of metal uptake by plants to their added concentration in soils are shown in Fig. 3a-3k. Metal concentration in plants displayed a plateau response in most cases. Some linear responses were also observed, however. Most of the linear responses were observed for the 2003 growing season, when the pH was below 5.5.

Zinc uptake by lettuce increased with biosolids rate and plateaued between 372 and 496 kg Zn applied per ha (Fig. 3a). Lettuce Cd concentration followed the same pattern (Fig. 3b). Cd accumulation by other plants was below detection limits of our instrumentation. Radish Zn increased with increasing biosolids, while the Zn concentration in globes (Fig. 3c) showed a plateau-type response. The Zn concentration response in radish tops (Fig. 3d) differed among growing seasons. Zinc in radish tops increased linearly in 2001 and 2003 seasons while it leveled off at higher concentrations in 2002. Barley also showed a plateau type response (Fig. 3e).

Copper uptake by radish tops (Fig. 3f) and Barley (Fig. 3g) increased with biosolids rate and plateaued at 608 kg Cu applied per ha. Lettuce Cu concentration response to biosolids Cu was not significant. The concentrations of Cu in the radish globes (Fig. 3h) increased linearly in 2003 but showed a plateau-type response in the other growing seasons.

The response to higher Ni concentrations in soils differed among crops and among growing seasons for the same crop (Fig. 3i, j, and k). Both linear as well as plateau-type response curves were observed. The increase in Ni concentration was linear for the 2003 growing season. The increase in Cd and Zn uptake peaked before tissue levels reached toxic concentrations. Despite the increase in Cd and Zn in lettuce, yield was not affected. Cadmium and Ni accumulation by the barley leaves were not calculated because the concentrations of both trace metals were below the detection limits of the atomic absorption spectroscopic method. Both Zn and Cu significantly increased but plateaued at high biosolids application rate.

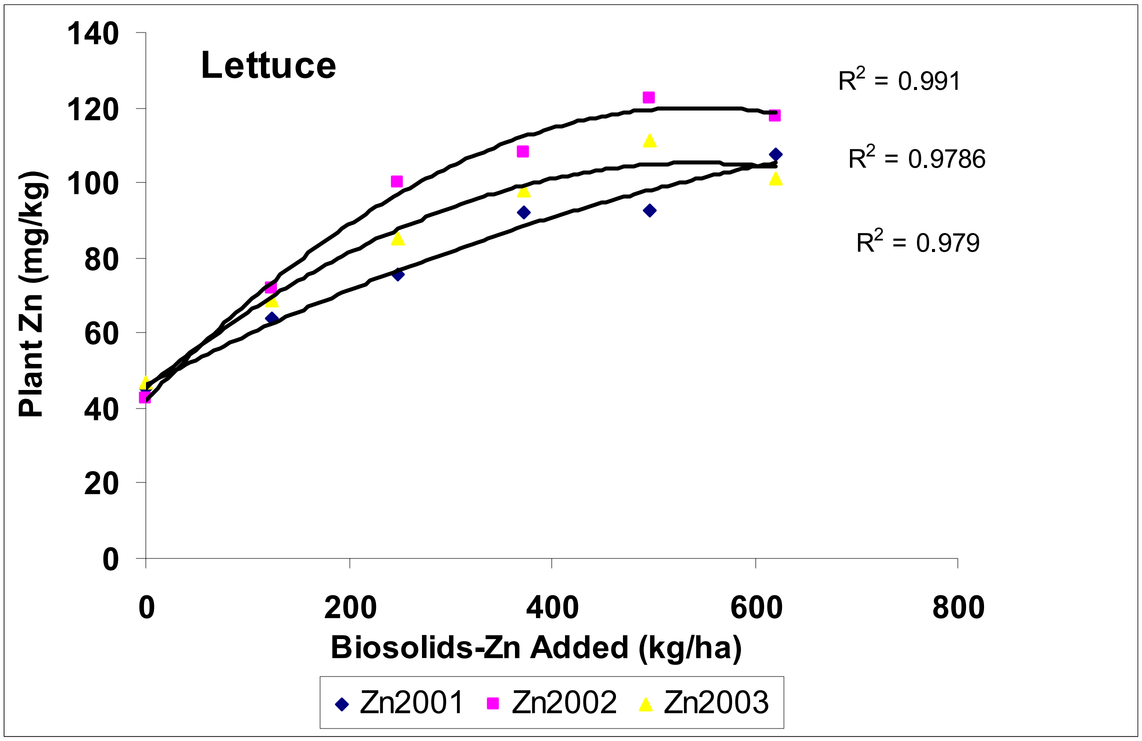


Fig 4.3a. Zinc accumulation in lettuce as a function of biosolids applied Zn

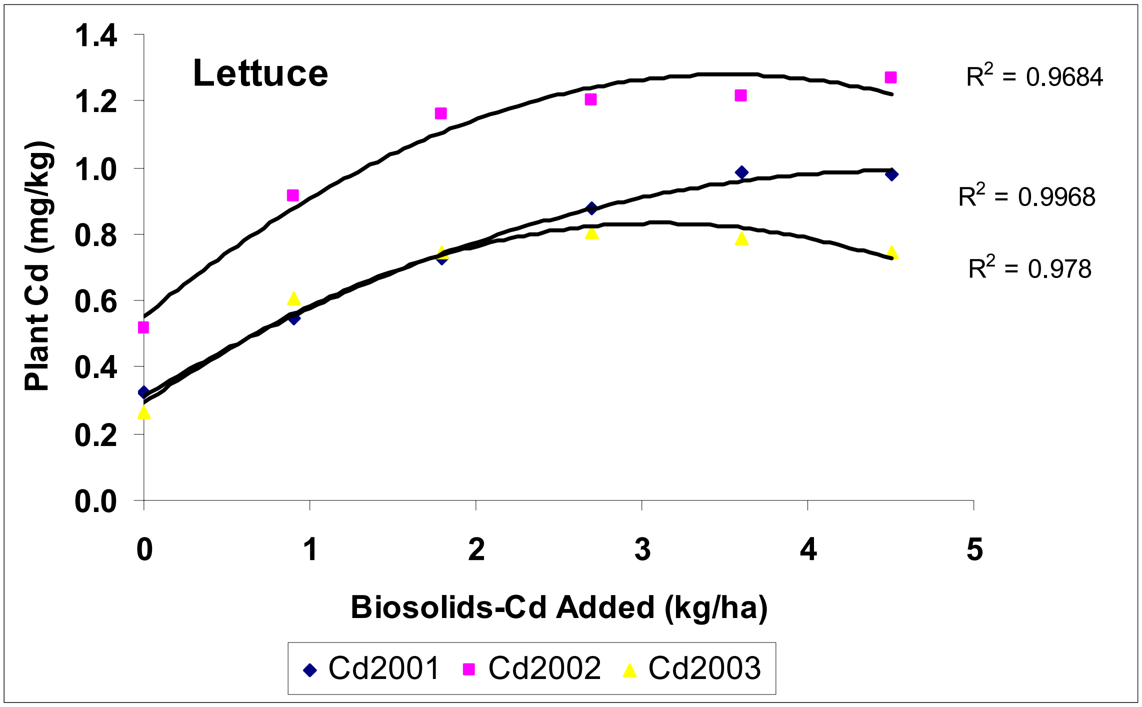


Fig 4.3b. Cadmium accumulation in lettuce as a function of biosolids applied Cd

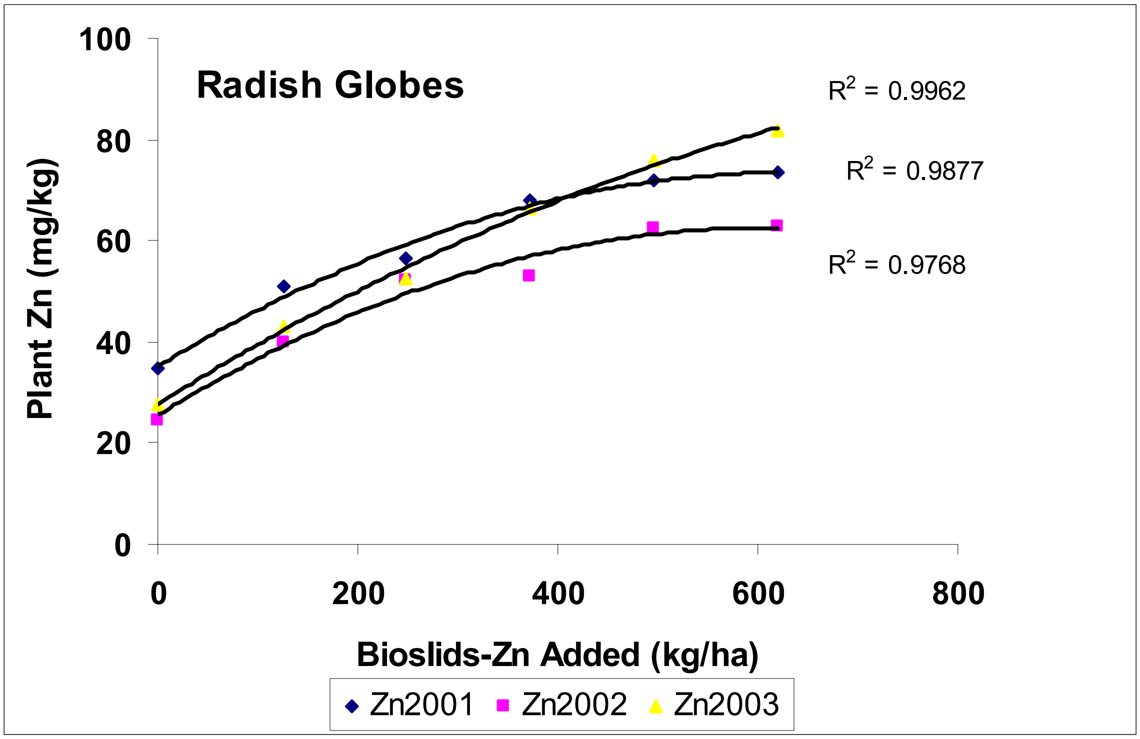


Fig 4.3c. Zinc accumulation in radish globes as a function of biosolids applied Zn

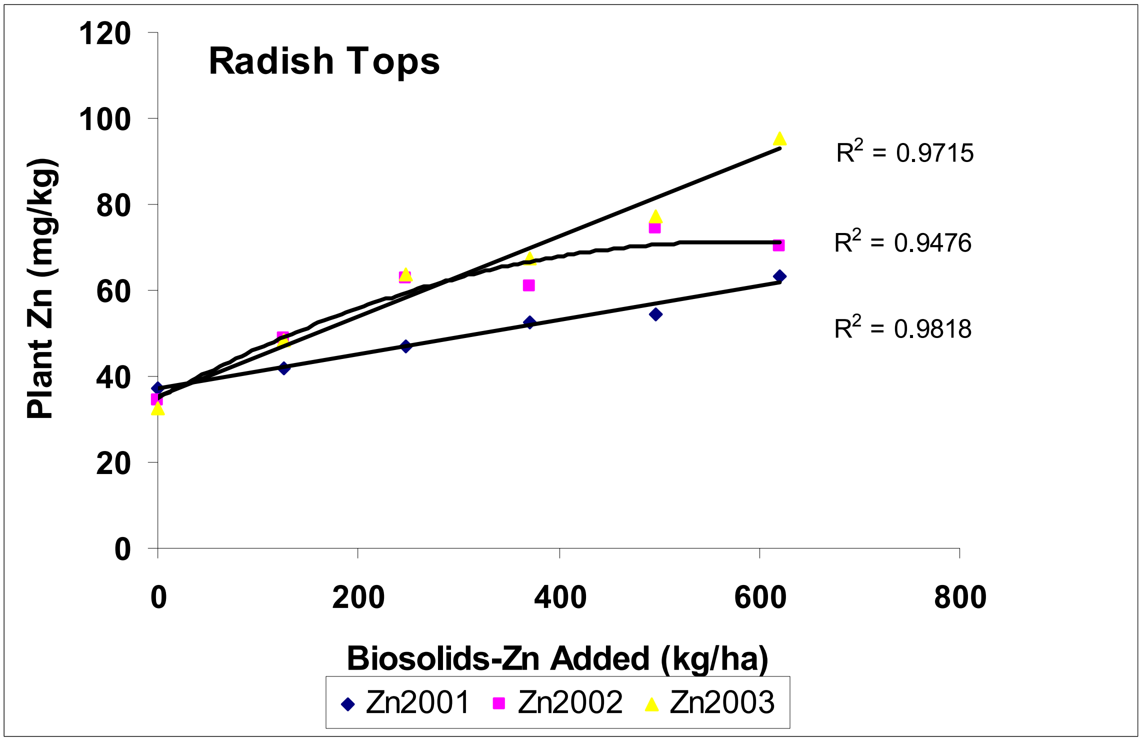


Fig 4.3d. Zinc accumulation in radish tops as a function of biosolids applied Zn.

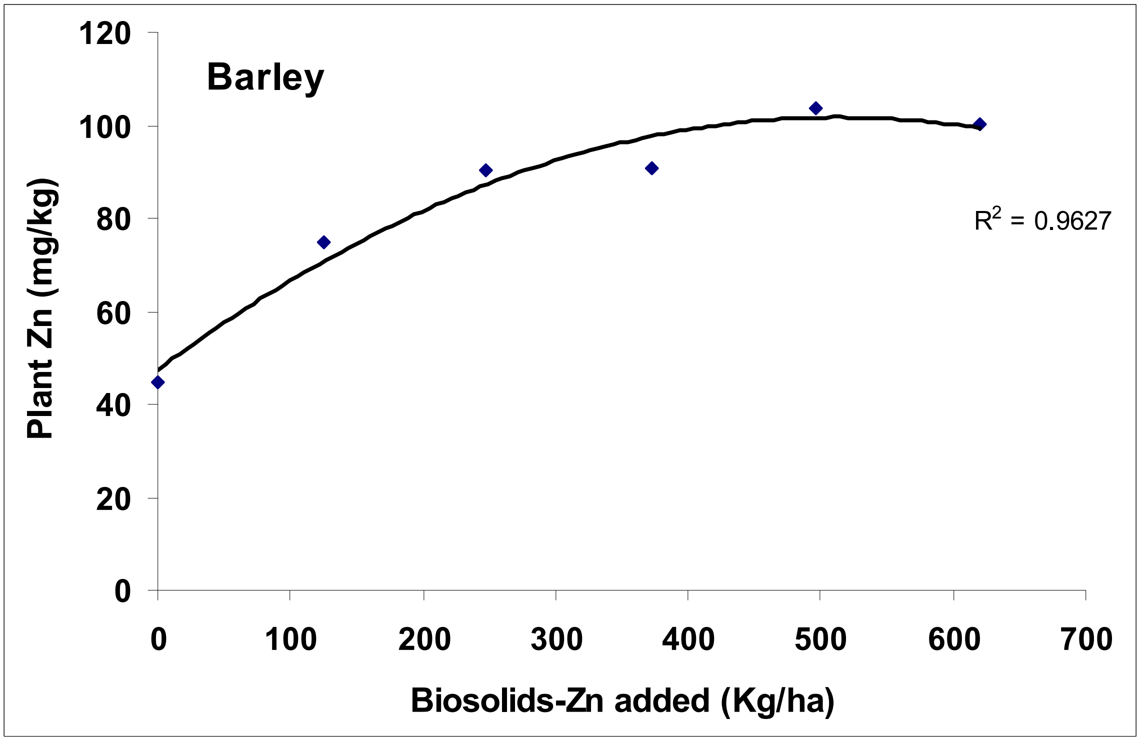


Fig 4.3e. Zinc accumulation in barley as a function of biosolids applied Zn

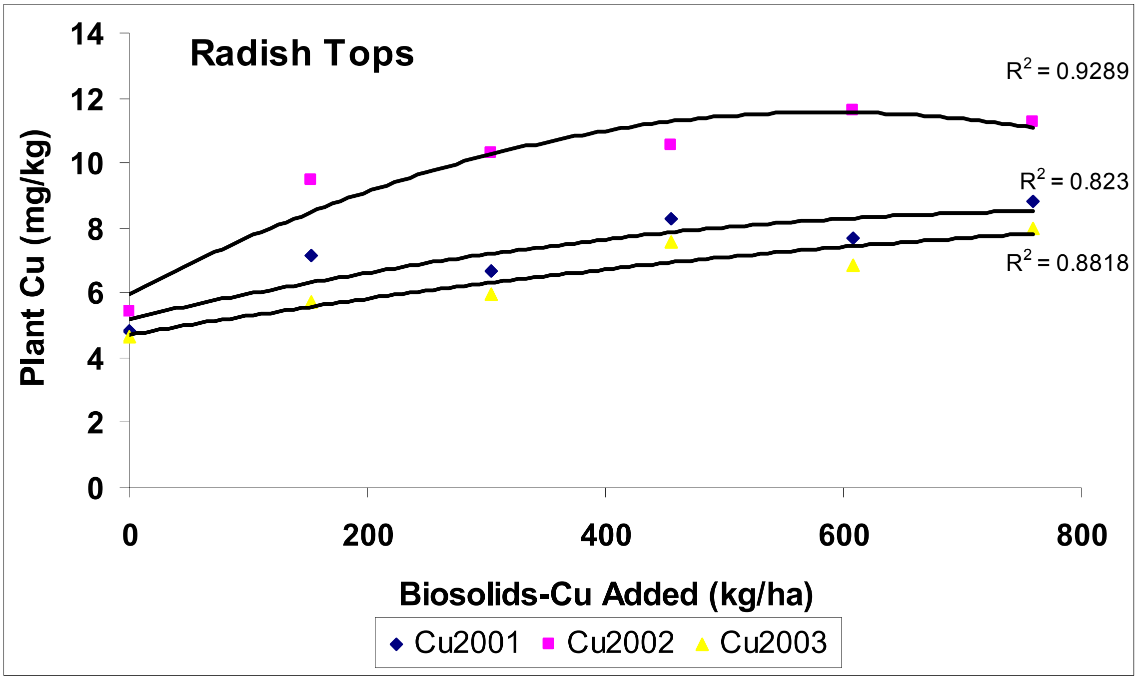


Fig 4.3f. Copper accumulation in radish tops as a function of biosolids applied Cu

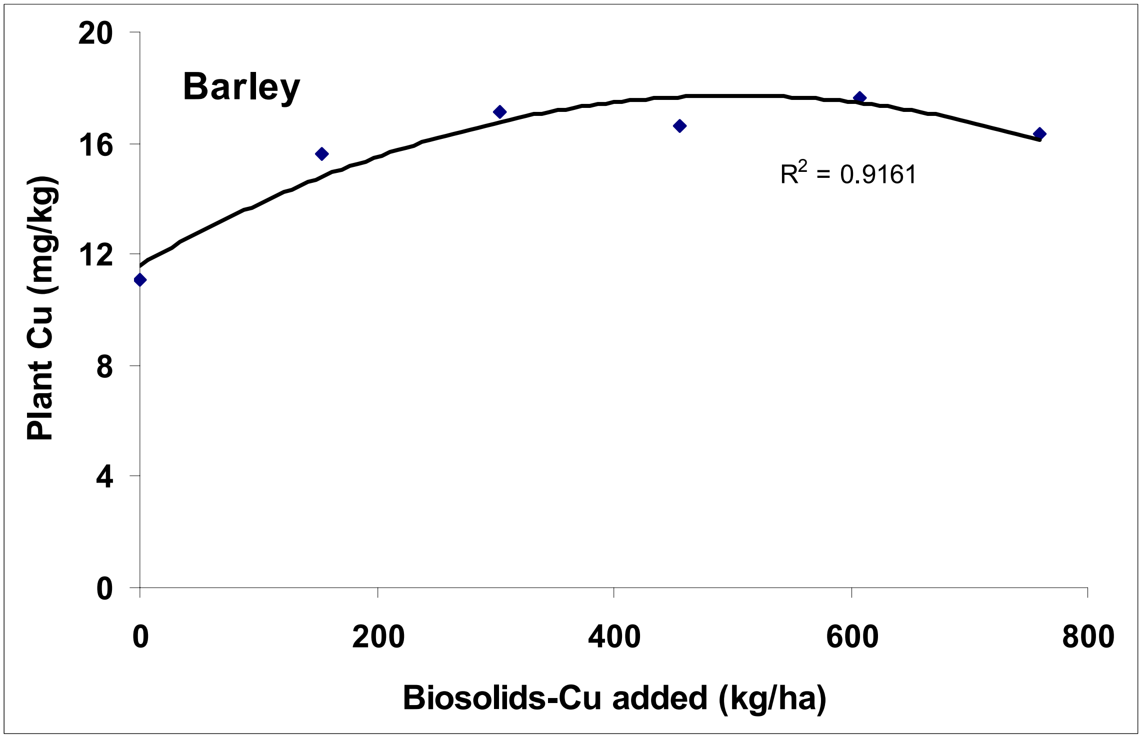


Fig 4.3g. Copper accumulation in barley as a function of biosolids applied Cu

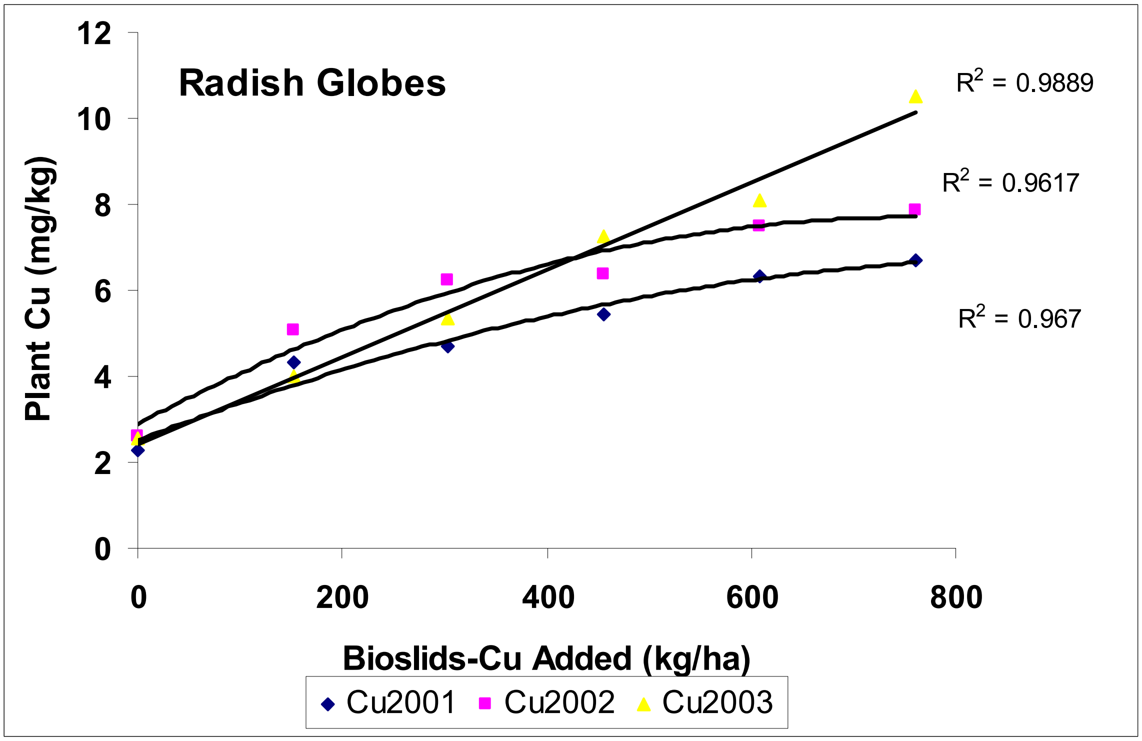


Fig 4.3h. Copper accumulation in radish globes as a function of biosolids applied Cu.

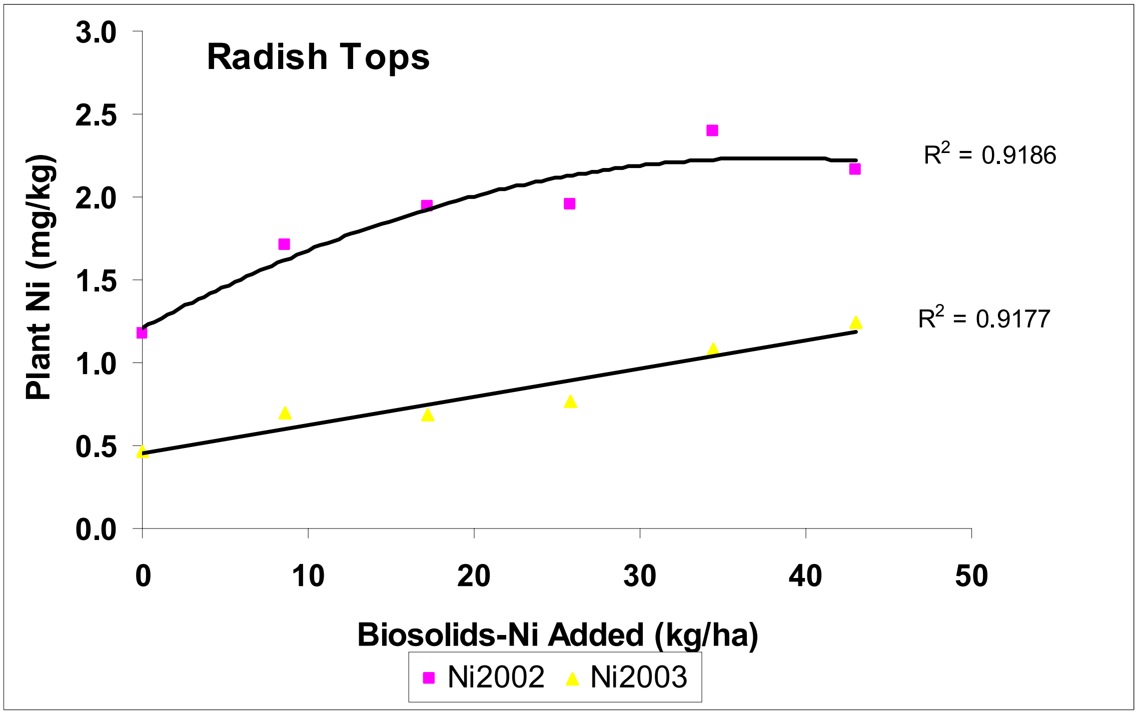


Fig 4.3i. Nickel accumulation in radish tops as a function of biosolids applied Ni.

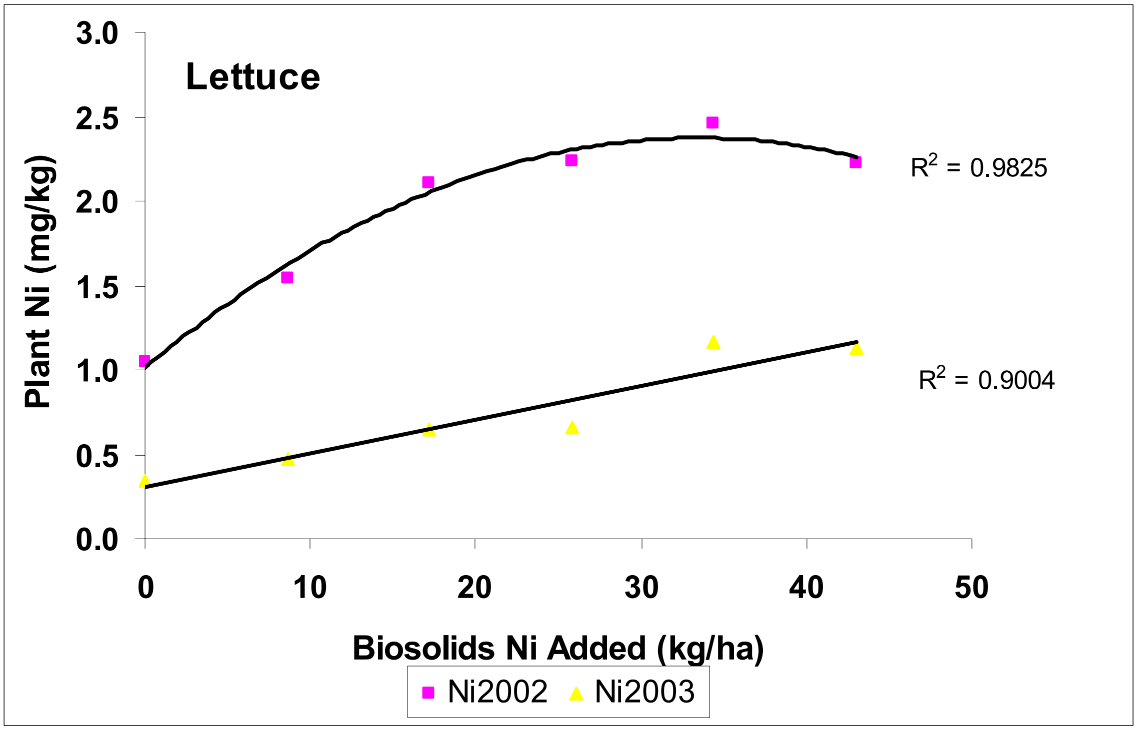


Fig 4.3j. Nickel accumulation in lettuce as a function of biosolids applied Ni.

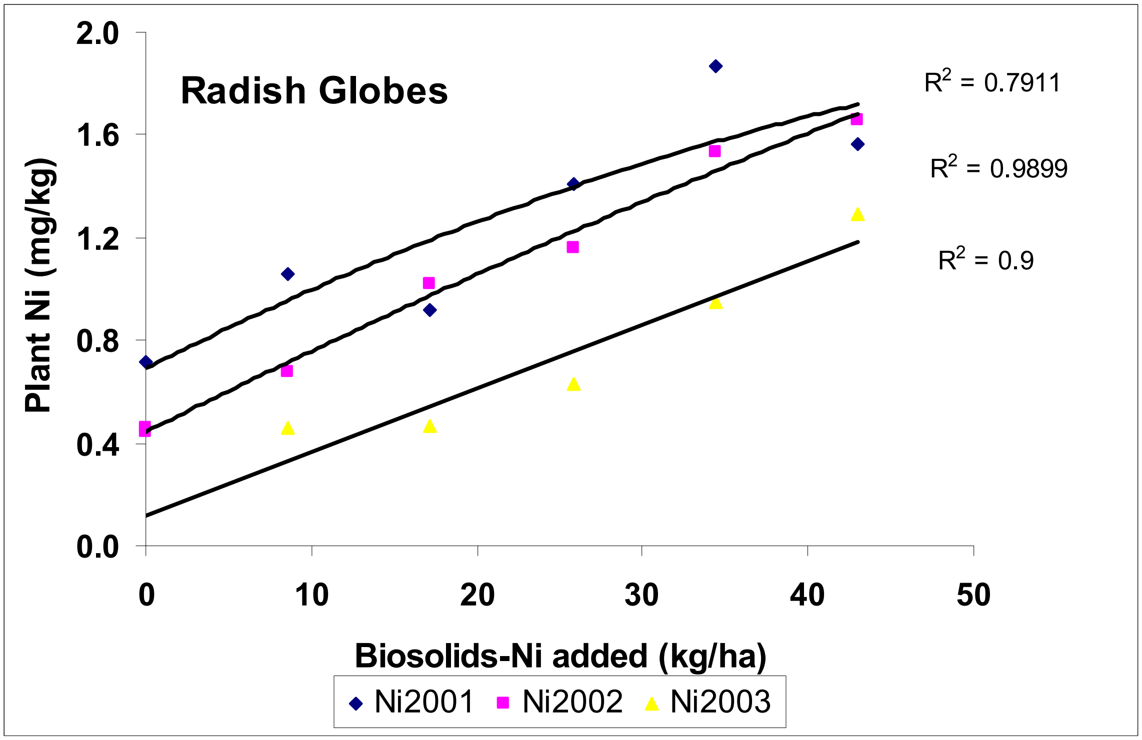


Fig 4.3k. Nickel accumulation in radish globes as a function of biosolids applied Ni

The USEPA (1993) based the allowable biosolids trace metals additions on the assumption that plant uptake is a linear function of biosolids addition. Chaney and Ryan (1992) demonstrated that actual plant uptake should be lower than that predicted by the linear regression model used to formulate the part 503 rule as long as plant concentration reached a maximum as the biosolids rate increased (plateau theory). Brown et al. (1998) confirmed that Cd uptake of lettuce follow this trend. Several studies (Chang et al., 1987; Barbarick et al., 1995; Logan et al., 1997; Brown et al., 1998) have provided evidence that biosolids trace metals uptake by wheat, corn and several vegetables reach a maximum with biosolids application rate.

Linear uptake curves were also reported in several studies. Logan et al., (1997) demonstrated that lettuce exhibited linear (decreasing with time) uptake curves. Chaudri et al. (2001) found a slight curvilinear relationship between wheat grain Cd concentration and soil Cd as well as soil pore water free Cd.

Many explanations have been proposed to explain these types of responses. Corey et al. (1987) stressed the importance of biosolids components in controlling metal availability. Corey et al. (1987) suggested that metal uptake would approach a maximum (plateau) at high biosolids metal loadings. Chaney and Ryan (1993) proposed that higher biosolids application increased not only the metal concentration, but also the metal adsorption capacity of soil. Thus, metal availability at high biosolids application will level off as a result. Metal attenuation by precipitation reactions could also limit solubility of the different trace metals (Christensen and Tjell, 1984).

McBride (1995) suggested that plant mechanisms would account for the observed decrease in uptake at high metal loadings. These mechanisms include exclusion of trace metals, limited transport from root to shoot, saturation of the carrier system responsible for metal transport into globes (Hamon et al., 1995) and root avoidance. Metal concentration in plants grown at this site displayed a plateau response to trace metals from biosolids-applied in most cases. Response was plant species dependant and varied among growing seasons. These observations show that in addition to soil reactions, plant-uptake control mechanisms might also play an important role in regulating metal uptake.

Hamon et al. (1999) proposed that if attenuation of metal concentration is due to biosolids chemistry, bioavailability will plateau at some biosolids loading rate, and metal uptake by plants will follow the same trend. Available Cd extracted with $CaCl_2$ and DTPA started to plateau at the highest biosolids rate. Concentration of the other trace metals in soils did not display this kind of response. The total amounts of biosolids-applied metals may not have been high enough to demonstrate the plateau response.

CONCLUSIONS

Despite the high concentrations of Cu and Zn applied to the site from a biosolids meeting the Ceiling Concentration Limits for the two trace metals, no adverse effect on plant growth or excessive amounts of metal uptake were noted. None of the trace metals reached toxic concentrations. Concentrations in all crops at all biosolids-amended treatments were well within the sufficiency range observed for agronomic crops (Kabata-Pendias and Pendias, 1991). Our results showed that the uptake of trace metals differed widely among plant species. Lettuce accumulated the highest concentrations of Cd and Zn, whereas radish showed the highest increase in Cu content as compared to their background levels.

Available metal concentrations were dependant on the total metal content of soil. The biosolids-amended soils have significantly higher trace metals concentration than the control. The quantity of Cu and Zn recovered by the three extractants from the biosolids amended plots were in the order of Mehlich-1>DTPA>>$CaCl_2$. DTPA extracted more Cd and Ni than $CaCl_2$. The amounts of trace metals extracted with these two reagents were low. Higher reagents concentration or soil-to-solution ratio may have provided different results.

Cu and Zn extracted with the three reagents correlated significantly with its content in the different crops. Pearson correlation coefficients were used to relate trace metals concentrations in crops to their extractable soil concentrations. Cu and Zn availability was best predicted with the Mehlich-1 test. In general, DTPA and $CaCl_2$ extractable Ni and Cd did not correlate well with plant metal uptake. The concentrations of trace element accumulation in plants were correlated with their level in biosolids. Availability of the trace metals to plants appeared to be associated with total metal added.

Concentration of available Cd extracted with $CaCl_2$ and DTPA started to plateau at the highest biosolids rate. Concentrations of $CaCl_2$, and DTPA and Mehlich1 extractable Cu, Zn, and Ni increased linearly with biosolids application.

Metal uptake by plants displayed both plateau- and linear-type responses. Most of the linear responses were observed for the 2003 growing season, where the pH dropped to below 5.5. Available soil trace metals showed a linear increase as a result of higher biosolids additions. Therefore, it is hard to conclude whether plateau response by plants is the result of metal attenuation in soils or it is a combination of soil and plant physiological factors.

The UC of trace metals varied widely between crops and with year for a given crop but agreed with the values used in the USEPA risk assessment. Cu had the lowest UC. Cd had the highest UC in lettuce; Zn UC was highest in radish globes and tops.

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CHAPTER FIVE

CONCLUSIONS

The research site was established in 1984 (Rappaport et al., 1987). One-time biosolids application was made to 1.5 x 2.3 m confined plots of a Davidson clay loam (clayey, kaolinitic, thermic, Rhodic Paleudult) at rates of 0, 42, 84, 126, 168, and 210 Mg ha^{-1}. The biosolids contained considerably higher concentrations of several trace metals including Cu and Zn than biosolids generated currently. Copper, Zn and Pb concentrations were above the pollutant concentration limits for exceptional quality (EQ) biosolids; thus, Part 503 would require lifetime loading rates of this biosolids to be tracked (USEPA, 1993). The 210 Mg ha^{-1} biosolids rate supplied 4.5 kg Cd, 750 kg Cu, 43 kg Ni and 600 Kg Zn per hectare. These amounts are below the maximum cumulative pollutant loading rate. None of the trace metals is near the limits for ceasing application of pollutant concentration biosolids. Field and laboratory studies were conducted in 2000-2003 to investigate the long-term effects of biosolids on trace metals mobility and availability in a well-weathered Davidson clay loam Piedmont soil. Our results were in agreement with the assumptions put forward in the risk assessment analysis. Seventeen years after application, trace metals were still concentrated in the zone of soil incorporation, and the yield of barley, lettuce, and radish were not affected by biosolids addition. None of the concerns put forward by critics of the USEPA risk assessment was substantiated.

1-No adverse effects on plant growth or excessive amounts of metal uptake were noted. The trace metals concentrations in all crops at all biosolids-amended treatments were well within the sufficiency range observed for agronomic crops (Kabata-Pendias and Pendias, 1991).

2-The uptake of trace metals differed widely among plant species. Lettuce accumulated the highest concentrations of Cd and Zn, whereas radish showed the highest increase in Cu content as compared to their background levels.

3-The biosolids amended soils have significantly higher trace metals concentration than the control. Available soil trace metals showed a linear increase because of higher biosolids additions.

4-The quantity of Cu and Zn recovered by the three extractants from the biosolids amended plots were in the order of Mehlich-1>DTPA>>$CaCl_2$. DTPA extracted more Cd and Ni than $CaCl_2$.

5-Copper and Zn availability was best predicted with the Mehlich-1 test. In general, DTPA and $CaCl_2$ extractable Ni and Cd did not correlate well with plant metal uptake.

6-Metal uptake by plants displayed both plateau- and linear-type responses. Most of the linear responses were observed for the 2003 growing season, where the pH dropped to below 5.5.

7-The UC of trace metals varied widely between crops but agreed with the values used in the USEPA risk assessment. Copper had the lowest UC. Cadmium had the highest UC in lettuce; Zn UC was highest in radish globes and tops.

8-The biosolids-applied trace metals were primarily concentrated in the topsoil (0-15 cm). Small fractions were observed in the 15-35 cm layers with no apparent movement below that depth.

9-Close to 90% of the applied trace metals Cu and Zn were recovered in the top 25 cm. Crops removal accounted for <1% of the total trace metals applied.

10-The leaching of trace metals down the soil profile as well as their removal by crop do not appear to be significant routes of metal losses from this site.

11-Sequential extraction indicated that a considerable proportion of Zn and Cd are still present in forms considered bioavailable (exchangeable and specifically adsorbed). The major portion of Cu, Zn and Ni is associated with the metal-oxides fraction.

12-Based on these results one can assume that there is little risk of downward movement and contamination of groundwater at this site and for soil with similar conditions and management history.

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