Abstract of a thesis submitted in partial fulfilment of the requirements for the Degree of Doctor of Philosophy.

Bioavailability of Cadmium, Copper, Nickel and Zinc in Soils Treated with Biosolids and Metal Salts

by

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It is widely accepted that bioavailability, rather than total soil concentration, is preferred when assessing the risk associated with metal contamination. Despite this, debate continues on what constitutes a bioavailable pool and how to best predict bioavailability, especially in relation to crop plants. The overall aim of this thesis was to assess and validate measures of cadmium (Cd), copper (Cu), nickel (Ni) and zinc (Zn) bioavailability in a range of soils amended with metal salts and biosolids. Six potential measures of bioavailability were investigated and compared: total metal; 0.04 M EDTA extraction; 0.05 M Ca(NO₃)₂ extraction; soil solution extracted using rhizon probes; effective solution concentration (Cₑ) determined using diffusive gradients in thin films (DGT); and modelled free ion activities (WHAM 6.0). These were compared to shoot metal concentrations obtained from plants grown in three soils with contrasting properties treated with biosolids and metal salts. The first study involved a wheat seedling (Triticum aestivum) assay carried out under controlled environmental conditions on incubated soils treated with metal salts and biosolids. Results showed that the presence of biosolids resulted in increases of DOC, salinity, Ca and Mg in soil solution as well as total concentrations of Cu and Zn, dry matter was also adversely affected by increased levels of salinity. The addition of biosolids did not significantly alter the extractability or solubility of Cd, Cu, Ni and Zn although concentrations of Cd in shoots were significantly lower in plants grown in biosolids amended soils compared with unamended soils. The second study involved a field experiment that used 20 cm diameter by 30 cm deep soil monoliths of the same three soils treated with metals and biosolids, and perennial ryegrass (Lolium perenne) was grown for 24 months. Results revealed the addition of biosolids significantly increased the amount of DOC, salinity, Ca and Mg in solution. The presence of biosolids also significantly altered the bioavailability of Cd, Cu, Ni and Zn, as measured by soil solution, Cₑ and free ion activity. However, this change had little effect on plant metal uptake. The length of time following treatment application had the greatest effect on soil chemistry and metal availability, resulting in pH decreases and increases in DOC, soil solution salinity, Ca and Mg. The free ion activities of each metal increased with time, as did soil solution Cd and Zn and Cₑ-Cu, with results for Zn indicative of migration through the soil profile with time. Plant uptake of Ni and Zn also changed with time. Nickel concentrations in shoots decreased, while concentrations of Zn in shoots increased. The findings from the two studies demonstrated that biosolids increased the amount of DOC, salinity, Ca and Mg present in soil solution. In the lysimeter study measures of metal availability were affected in soils amended with biosolids, but this did not affect shoot concentrations. The overall predictive strengths of the six potential measures of bioavailability was investigated using results from the previously described experiments and related studies carried out by ESR and Lincoln University using nine different soils amended with combinations of biosolids and metal salts. Of the four
metals Ni provided the strongest correlations between metal bioavailability and shoot concentrations, with 0.05 M Ca(NO₃)₂ extraction giving the strongest relationship for Ni concentrations in shoots ($r^2 = 0.73$). This suggests that the solubility of Ni is highly indicative of shoot concentrations and that Ca(NO₃)₂ is a robust measure of Ni bioavailability. In addition Ca(NO₃)₂ provided the best estimate of Zn bioavailability ($r^2 = 0.65$), and CE-Cd provided the best measure of Cd bioavailability, although it could only describe 47% of shoot Cd concentration. Results for Cu were typical of previously described studies as assays of Cu availability are almost always poorly correlated with shoot concentrations, with total Cu having the strongest relationship ($r^2 = 0.34$). Methods based on the extractability and solubility of Cu in soils were poor indicators of Cu concentration in shoots. Overall, the addition of biosolids did not alter the outcome of these bioavailability assays, and results indicated that total metal concentrations present in the soils and biosolids matrix, plus length of time since soil treatment, had a greater affect on metal bioavailability.

**Keywords**: Bioavailability, Cd, Cu, Ni, Zn, biosolids, wheat (*Triticum aestivum*), ryegrass (*Lolium perenne*), soil monoliths, pot trials
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Figure 6.3 Relationships determined between 20 day old wheat seedlings, pasture ryegrass concentrations and Cd concentrations obtained using various methods; (A) Total Cd, (B) EDTA-extractable Cd, (C) Ca(NO\(_3\))\(_2\) extractable Cd, (D) soil solution, (E) effective concentration, (F) free ion activity (\(n = 272\) for wheat and \(n = 188\) for ryegrass).

Figure 6.4 Relationships determined between 20 day old wheat seedlings, pasture ryegrass concentrations and Cu concentrations obtained using various methods; (A) Total Cu, (B) EDTA-extractable Cu, (C) Ca(NO\(_3\))\(_2\) extractable Cu, (D) soil solution, (E) effective concentration, (F) free ion activity for 20 day old wheat seedlings and ryegrass( \(n = 439\) for wheat and \(n = 277\) for ryegrass).

Figure 6.5 Relationships determined between 20 day old wheat seedlings, pasture ryegrass concentrations and Ni concentrations obtained using various methods; (A) Total Ni, (B) EDTA-extractable Ni (C) Ca(NO\(_3\))\(_2\) extractable Ni, (D) soil solution, (E) effective concentration, (F) free ion activity (\(n = 295\) for wheat and \(n = 208\) for ryegrass).

Figure 6.6 Relationships determined between 20 day old wheat seedlings, pasture ryegrass concentrations and Zn concentrations obtained using various methods; (A) Total Zn, (B) EDTA-extractable Zn (C) Ca(NO\(_3\))\(_2\) extractable Zn, (D) soil solution, (E) effective concentration, (F) free ion activity (\(n = 439\) for wheat and \(n = 220\) for ryegrass).

Figure 6.7 Relationships determined between shoot Cd concentration and soil Cd concentrations obtained using various methods; (A) Total Cd, (B) EDTA-extractable Cd, (C) Ca(NO\(_3\))\(_2\) extractable Cd, (D) soil solution, (E) effective concentration, (F) free ion activity. A linear regression line is fitted for each plot. \(n = 399\)

Figure 6.8 Relationships determined between shoot Cu concentration and soil Cu concentrations obtained using various methods; (A) Total Cu, (B) EDTA-extractable Cu, (C) Ca(NO\(_3\))\(_2\) extractable Cu, (D) soil solution, (E) effective concentration, (F) free ion activity.
concentration, (F) free ion activity. A linear regression line is fitted for each
plot. n = 592......................................................................................................... 139

Figure 6.9 Relationships determined between shoot Ni concentration and soil Ni
concentrations obtained using various methods; (A) Total Ni, (B) EDTA-
extractable Ni, (C) Ca(NO₃)₂ extractable Ni, (D) soil solution, (E) effective
concentration, (F) free ion activity. A linear regression line is fitted for each
plot. n = 425................................................................. 140

Figure 6.10 Relationships determined between shoot Zn concentration and soil Zn
concentrations obtained using various methods; (A) Total Zn, (B) EDTA-
extractable Zn, (C) Ca(NO₃)₂ extractable Zn, (D) soil solution, (E) effective
concentration, (F) free ion activity. A linear regression line is fitted for each
plot. n = 594................................................................. 141
Chapter 1
Literature Review

1.1 The concept of bioavailability in relation to metals in soils

The definition of bioavailability of elements and compounds varies according to the subject of the study. The concept of bioavailability is driven by the organisms or solution phase used for the measurement of the contaminant availability. For example, the bioavailable fraction is often defined as the fraction of contaminant extractable in a chemical reagent based on correlation with the total contaminant uptake by plants (Allen et al., 2001; Alloway et al., 1988; Chojnacka et al., 2005; Fairbrother et al., 2007; McBride et al., 1997a; McLaughlin et al., 2000). In these cases bioavailability is largely a measure of contaminant solubility (Chaney et al., 2000; Degryse et al., 2009a; Hooda, 2007).

Considerable debate exists in the literature on the precise definition of what constitutes a bioavailable metal pool (Hooda, 2007). Metal bioavailability has been linked to metal ion activity in soil solution, the exchangeable metal fraction and more recently to the concentration of metals that cause ecotoxicity (Antunes and Kreager, 2009; Antunes et al., 2007; Hooda, 2007; Loftis et al., 2007; Menzies et al., 2007; Rooney et al., 2006; Rooney et al., 2007b; Sauve, 2001; Sauve, 2003; Smolders et al., 2009; Speir et al., 2007). However, there is still no general consensus among the scientific community on the components involved in determining bioavailability. This may be due to the many inconsistencies between the bioavailable fraction measured and the uptake of metals by plants and the largely undescribed processes that may influence bioavailability occurring in the rhizosphere (Basar, 2009; Gregory, 2006; Jones et al., 2004; Menzies et al., 2007).

Even though it is widely acknowledged that metal bioavailability, rather than total concentration, is critical when accurately assessing the risk associated with soil contamination, debate continues on what constitutes a bioavailable pool and how to best predict bioavailability, especially in relation to crop plants (Alloway, 1995b; Alloway et
al., 1988; Gray and McLaren, 2006; McLaughlin, 2002; McLaughlin et al., 2000; Nolan et al., 2005; Oliver et al., 2004; Speir et al., 2003b).

1.1.1 Consequences of excess metals in the soil environment

Unlike organic contaminants which degrade over time (e.g. pesticides and hydrocarbon contaminants), metals can remain in the environment and amounts can only be reduced by transportation elsewhere such as uptake and removal by plants (phytoremediation), which has been observed in many New Zealand and international cases (Adams et al., 2001; Almas et al., 2004; Almas et al., 2007; Chaignon et al., 2003; Chapman, 2008; Georgieva et al., 2002; Gray et al., 1999c; Gray et al., 2002; Knights et al., 2001; McBride, 1995; McLaren et al., 2003; Nwachukwu and Pulford, 2008). The accumulation of metals in soil, particularly Cd, Cu, Ni and Zn, is of concern in agricultural production systems due to the potential threat of adversely affecting food quality (safety and marketability), crop growth (through phytotoxicity), or environmental health (soil flora and fauna). Agricultural exports are increasingly marketed internationally on the basis of an environmentally sound image so the issue of managing and regulating metal contamination of agricultural soils has become an important issue (McLaren et al., 2003; 2004; McLaughlin et al., 2000; McLaughlin et al., 2007; Speir et al., 2003b; Warne et al., 2008a; Warne et al., 2008b; Zarcinas et al., 2004).

Cadmium occurs naturally in soils, however for plants and animals Cd is a non-essential element that is bioaccumulating and is toxic at relatively low concentrations (Webb, 1975; Webb and Daniel, 1975). Biological effects resulting from excess Cd in soils has been extensively examined, particularly in relation to phosphate fertilizer applications (Bolan and Duraisamy, 2003; Gray et al., 1999c; Loganathan et al., 2003; Longhurst et al., 2004; McLaughlin, 2002; McLaughlin et al., 1996; McLaughlin et al., 1995; Nicholson et al., 2006; Roberts and Longhurst, 2002; Roberts et al., 1994). Increased concentrations of Cd in agricultural land from amendments such as mineral fertilizers and biosolids have led to enhanced Cd uptake by pasture plants. The consequence of this is potentially significant for Cd absorption by grazing livestock (Roberts et al., 1994). In
soils, Cd is a highly mobile metal in relation to leaching and availability to plants (McLaughlin, 2002; McLaughlin et al., 2000). Cadmium contamination of soil is also typically associated with Cu and especially Zn contamination, as most of the common Cd containing agricultural and industrial products added to soils also contains these elements (McLaughlin et al., 2000).

Copper, Ni and Zn, unlike Cd, are essential elements required in small amounts for the healthy functioning of animal and plant metabolic systems (Alloway et al., 1990; Bolan et al., 2003; Palmer and Guerinot, 2009; Reichman, 2002). However, excessive Cu is toxic to soil microorganisms, plants and grazing animals (Bolan et al., 2003; Giller et al., 2009; Speir et al., 2007; Vogeler et al., 2008) and in plants Cu toxicity causes foliar interveinal chlorosis, with the leaves becoming necrotic with increasing Cu exposure (Reichman et al., 2006). Moreover, elevated Cu soil concentrations have been shown to decrease the microbial activity in soils (Antunes et al., 2007; Sauve et al., 1997; Vogeler et al., 2008). This reduction of microbial activity as a result of Cu toxicity also negatively impacts upon the health of the plant as soil microbes and functioning plant systems are intricately linked (Cloutier-Hurteau et al., 2008; Dumestre et al., 1999; Giller et al., 2009; Khan et al., 2005).

The toxicity of Ni is almost always less than Cu, Zn and especially Cd (McLaughlin et al., 2000). Documented cases of Ni toxicity resulting from excess Ni present in the soil have resulted predominantly from practices such as smelting, ore refining, mining, combustion of fossil fuels and long term application of biosolids to agricultural land (Antoniadis and Alloway, 2002a; Antoniadis and Alloway, 2003b; Doig and Liber, 2007; Marschner and Marschner, 1995; McNear et al., 2007; Tye et al., 2004). Nickel plays an important role in lipid metabolism, hematopoiesis and several other biological functions in some organisms (Kozlova et al., 2009). Toxic responses to excess Ni in soils are less clear than those described for Cd, Cu or Zn (Kozlova et al., 2009; Marschner and Marschner, 1995). However, the most frequently documented effect of Ni toxicity is foliar chlorosis and in some instances decreased root growth (Himmelbauer et al., 2005; Marschner and Marschner, 1995; Molas and Baran, 2004). Research into the occurrence of Ni in soils and relationships between plant and soil microorganisms has focused
primarily on plants, especially hyperaccumulating species (i.e. *Thlaspi* species, Penny-crest) grown on serpentine soils and their role in promoting plant growth and Ni accumulation (Himmelbauer *et al.*, 2005; Idris *et al.*, 2006; Ma *et al.*, 2009; Puschenreiter *et al.*, 2005; Wenzel *et al.*, 2003).

Contamination of soil by excess Zn occurs primarily as a result of industrial activities such as mining and smelting (Almas *et al.*, 2006; Almas *et al.*, 2007; Chen *et al.*, 2009; Degryse and Smolders, 2006; Gutierrez-Ruiz *et al.*, 2007) or the long term application of biosolids to agricultural land (Antoniadis, 2008; Collins *et al.*, 2003a; Hirsch *et al.*, 1993; Horswell *et al.*, 2006; Kim *et al.*, 2007). However, occurrences of Zn toxicities in plants are far less widespread than reported cases of Zn deficiency (Alloway, 2009; Broadley *et al.*, 2007). Zinc is the most common crop micro deficiency, especially in high pH soils containing low concentrations of Zn (Alloway, 2009). In cases where Zn is present in soils in biologically toxic concentrations, symptoms of Zn toxicity typically include a general chlorosis of younger leaves (Reichman, 2002), as well as an overall decrease in leaf size compared with unaffected plants (Broadley *et al.*, 2007; Marschner and Marschner, 1995; Reichman, 2002).

### 1.2 Soil properties and processes that affect metal bioavailability

Bioavailability and mobility of metals in soils are controlled by a series of soil properties and processes such as, metal speciation, soil pH, soil texture, organic matter content, plant type and agronomic management (Chojnacka *et al.*, 2005; Lair *et al.*, 2007; Lair *et al.*, 2006; McBride *et al.*, 1997a; McLaughlin *et al.*, 2000). Metals present in soils can occur in several fractions such as; soil solution, exchangeable, sorbed and organically bound, bound and occluded onto oxides and clay minerals, residual, and within the primary lattice phase of minerals (Alloway, 1990; McBride, 1994). Plants and soil organisms are unable to access the total pool of metals present in the soil, of these metal fractions, the most immediately bioavailable pool of metals are metals present in soil.
solution, with other metal fractions being less available (Marschner and Marschner, 1995; Reichman, 2002).

### 1.2.1 Total metals in soils

Metals and metalloids occur in varying concentrations in all soils, most are essential at some levels for biological life such as Co, Cu, Mn, Ni, Se and Zn (low levels). Toxic metals, is a term that usually refers to those metals with non-essential biological roles, such as As, Cd, Pb, Hg, Tl and U (Alloway, 1995b; Alloway et al., 1990). The total concentration of metal in a soil includes all fractions of metals from those present in soil solution, to exchangeable amounts present on organic, clay and oxide surfaces, and to those occluded in the mineral matrices, from which rates of desorption are relatively slow (McBride, 1994). While total metal concentration in soils is still frequently used to estimate bioavailability, this assessment is considered insensitive to adequately reflect relevant metal availability (McBride et al., 1997b; McLaughlin et al., 2000; Sauve, 2003).

### 1.2.2 pH

Soil pH is regarded as the fundamental independent variable that controls ion exchange, dissolution/precipitation, redox reactions, adsorption, and complexation reactions occurring within the soil (McBride, 1994). Soil solution pH is intimately involved in the complex equilibria between metal speciation, solubility, adsorption on colloids and sites available for cation exchange (McBride, 1994; Reichman, 2002; Sauve, 2001). The binding of metal cations is strongly affected by pH, firstly due to the competition between protons and metal cations for humic binding sites, and secondly because of cation hydrolysis (Tipping, 1998; Tipping et al., 2003a).

Soil pH is recognised as the major factor controlling Cd and especially Zn availability, (Antonladis et al., 2008; Carrillo-Gonzalez et al., 2006; Gray and McLaren, 2006; McBride et al., 1997a; McBride et al., 1997b; Rooney et al., 2007b). Studies examining the factors that influence Cd availability revealed that pH was the dominant soil variable...
affecting solution Cd (Andrews et al., 1996; Gray and McLaren, 2006; Gray et al., 2003; Gray et al., 1999b; 1999d; Loganathan et al., 2003; Rachou et al., 2004). In addition, organic matter, CEC and total soil Cd were also found to be important factors determining Cd bioavailability (Gray and McLaren, 2006).

In many studies, Ni solubility and toxicity in soils was positively related to soil pH and total Ni content, while negatively related to total carbon content (Antoniadis and Alloway, 2002a; Antoniadis and Alloway, 2002b; McLaughlin et al., 2000; Rooney et al., 2007b). While pH has clearly been established as the key soil property controlling the solubility of Cd and Zn, the circumstances for Ni differs as organic matter has a more dominant role in influencing Ni solubility than for Cd or Zn (Sauve and Parker, 2005) and plant uptake of Ni (Hough et al., 2003; Hough et al., 2005).

The effect of pH on Cu speciation is less well defined and while some studies examining the influence of pH on the availability and toxicity of these metals found that soil solution pH does affect Cu solubility and speciation (Chaignon et al., 2003), other studies have found little direct relationship between pH and Cu availability (Sauve and Parker, 2005; Sauve et al., 1997). The reason is that Cu has a strong affinity for organic matter (Burton et al., 2005a; Burton et al., 2005b; McBride, 1994) and organic matter, especially dissolved organic matter is a more important determinant of Cu solubility and bioavailability than pH (Amery et al., 2007; Amery et al., 2008; Reichman, 2002; Sarathy and Allen, 2005).

### 1.2.3 Organic matter

Organic matter is a fundamental component of the soil and influences biological, chemical and physical soil properties (McBride, 1994; Stevenson, 1994). In particular, organic matter is involved the retention and release of nutrients (i.e. Ca, Cu, Fe, K, Mg, Mn) by cation exchange and adsorption of potentially toxic organic compounds, such as pesticides and industrial wastes (McBride, 1994).
Organic matter in soils is commonly and collectively referred to as humic substances, and this can be further divided into humic acid, fulvic acid, and humin based on solubility (McBride, 1994). Humic and fulvic acids contribute to the majority of cation binding properties in soils (Tipping, 2005) and dissolved organic carbon (DOC) is the main source of fulvic acids which are known to increase the carrying capacity of soil solutions for strongly organic complexing metals such as Cu (Amery et al., 2007; Ashworth and Alloway, 2007; Bolan and Duraisamy, 2003; Cattani et al., 2006; Chaignon et al., 2003; Sauve et al., 1997), and in some instances Ni (Antoniadis and Alloway, 2002b; Ashworth and Alloway, 2004; Doig and Liber, 2007). Metal ions can be complexed by the COO- and COOH- groups present in both DOC and solid organic matter to form stable complexes (McLaren and Crawford, 1973a; Stevenson, 1994). Consequently, the amount of organic matter, in particular DOC increases the opportunity for forming stable organo-metal complexes (McBride, 1994). In general these stable soluble organo-metal complexes are considered largely unavailable for plant uptake (Reichman, 2002), and whilst the amount of metal in solution may increase, metals, especially Cu may be less available as a result of complexing with DOC (Amery et al., 2008; Kalis et al., 2006).

Several studies have shown that the presence of DOC does effect the solubility and bioavailability of Cd (Antoniadis and Alloway, 2002b; Antonladis and Tsadilas, 2007; Gray and McLaren, 2006; Gray et al., 1999a; McLaughlin, 2002; McLaughlin et al., 2006). A study by Antoniadis and Alloway (2002b) found that increased amounts of DOC from biosolids application to land were correlated with an increase in Cd solubility as well as plant uptake of Cd. In contrast, the case of Cd applied to soils as Cd contained in biosolids, Cd was shown to be less available for plant uptake than the more water soluble forms, such as Cd salts (McLaughlin et al., 2006). Additionally, a study by Antonladis and Tsadilas (2007), observed that the complexation of Cd by DOC and retention in organic matter were less likely to occur in the presence of Cu, Ni or Zn as Cd does not compete for these sites as well as these metals. Thus while DOC plays an important role in determining the solubility and availability of Cd, it appears to be more influenced by the presence of other cations such as Ca (Antonladis and Tsadilas, 2007; Antonladis et al., 2008; Gray and McLaren, 2006).
Copper forms strong coordination complexes with organic matter, hence Cu present in soils is relatively immobile, with a large proportion associated with organic matter in the solid phase (Ashworth and Alloway, 2007; McBride et al., 1997b; McLaren and Crawford, 1973a; Reichman, 2002; Stevenson, 1994). In experiments carried out by Burton et al. (2005a and 2005b) it was revealed that Cu was sorbed predominantly to soil organic matter and Cu interactions with DOC were shown to influence the sorption-desorption process that primarily control Cu behaviour in soil (McBride, 1995; McLaren and Crawford, 1973b). Sauve et al. (1997) also confirmed the importance of organic matter in determining Cu solubility and bioavailability with a study on Cu contamination of a range of soils, showing that greater than 90% of Cu was bound organically irrespective of the soil pH.

Nickel ions also form strong coordination complexes with organic matter, because of the metal’s relatively high electronegativity which is second only to Cu in soil environments (McBride, 1994). Information regarding Ni availability in soils in relation to plant uptake or toxicity is substantially less than for Cd, Cu or Zn (McIlveen and Negusanti, 1994; Weng et al., 2003). Studies that have documented the effects organic matter, specifically DOC have had on Ni solubility and bioavailability revealed that complexation by DOC did increase Ni availability to plants and mobility through soil (Antoniadis and Alloway, 2002a; 2002b; Ashworth and Alloway, 2004). However, pH and competitive absorption are thought to be equally influencing of Ni solubility and bioavailability (Antonladis and Tsadilas, 2007; Ponizovsky et al., 2008; Puschenreiter et al., 2005).

Zinc solubility is also affected by organic matter and readily forms complexes with DOC, although it does not compete as well as Ca, Cu or Ni for these sites (McBride et al., 1997a). Zinc uptake in plants as well as Zn mobility down through the soil profile has also been correlated with the amount of DOC in soil solution (Antoniadis and Alloway, 2002a; Antoniadis and Alloway, 2002b; Ashworth and Alloway, 2004). However in these studies and several others the concentration and species of Zn in solution was found to be more strongly related to pH than on the amount of DOC (Antonladis et al., 2008; Broadley et al., 2007; Imtiaz et al., 2006; Lock and Janssen, 2001).
While organic matter, especially DOC clearly affects the behaviour of metals in soils, the amount of DOC in soil solution is most likely controlled by the amount of oxides and clays present in the soil. The presence of these other soil constituents, are known to form highly stable complexes with organic matter (McBride, 1994) and the influence of these on metal behaviour in the soil are described in the following sections.

1.2.4 Clays and oxides

Cadmium, Ni, Cu and Zn in soils are usually present in trace amounts, hence sorption processes (often referred to as specific absorption or chemi-adsorption) are generally accepted as the primary mechanisms responsible for controlling the solubility of these metals (McBride et al., 1997a; McBride, 1994). Specific adsorption usually takes place on the surfaces of clays and oxides (principally of Al, Fe, Mn and Si), with the clay fraction (commonly defined as the portion of fraction size < 2µm) usually the most influential for cation adsorption reactions, due to its high surface area, hydroxyl groups and large number of available binding sites (McBride, 1994).

Trace metals, such as Cd and Ni can also be co-precipitated or incorporated into the oxyhydroxide minerals, and in extreme cases (e.g. severe industrial pollution, or geothermal environments), precipitation of metals as distinct minerals can occur (Alloway, 1995a; McBride, 1994).

1.2.5 Redox conditions

The reduction/oxidation conditions in a soil can strongly affect the pH and speciation, and hence toxicity of metals in solution, and this is particularly well documented for As, Sb, Cr, Fe and Mn, which are several times more biologically toxic and mobile in their most reduced form (Allen et al., 2001; Alloway, 1995b; Horswell and Speir, 2006; Oorts et al., 2008). However Cd, Cu, Ni and Zn in soils are mostly present in the divalent form, as the monovalent forms are highly unstable and hence these metals are not significantly
reduced under low redox conditions (Reichman, 2002; Whitehead, 2000). Thus redox conditions are considered to play a much smaller role in the solubility and bioavailability of Cd, Cu, Ni and Zn.

1.2.6 Rhizosphere processes

The rhizosphere is defined as the volume of soil affected by the presence of roots of growing plants, which is generally accepted as the area 1-2 mm from the root surface (Gregory, 2006). Biological associations with plant roots that may potentially affect plant availability of metals are mycorrhiza fungi, rhizobia (N-fixing bacteria associated with legumes), and other soil bacteria and fungi, whose roles are largely undefined owing to the vast number of bacterial species present in the rhizosphere. The biogeochemistry of Cd, Cu, Ni and Zn in the rhizosphere environment remains largely undescribed (Puschenreiter et al., 2005; Wenzel et al., 2003). The reason for this is that the rhizosphere involves many physical, chemical and biological components, which are intricately and intimately linked. Moreover the majority of rhizosphere research to date has mostly focused on plant nutrition and major nutrient (e.g. P and N) and C cycling in soil ecosystems (Gregory, 2006; Jones et al., 2004).

1.2.6.1 pH changes

Rhizosphere influence on the pH of the surrounding soil, is probably the most distinguishable and measureable chemical change that would influence the bioavailability of metals. The pH of the rhizosphere can vary by up to 2.5 pH units from that of the bulk soil solution, depending on the species of plant and buffering capacity of the soil (Marschner, 1995; Reichman, 2002). This is mostly due to the excretion of $\text{H}^+/\text{HCO}_3^-$ and to a lesser degree organic acids to balance the internal charge difference as a result of cation and anion uptake by roots, and also the production of CO$_2$ from microbial activity (Jones et al., 2004; Marschner and Marschner, 1995). In addition, certain plants, referred
to as Strategy II plants (e.g. *Zea mays*), are known to acidify the surrounding media by production of phytosiderophores which are non-protein amino acids released from the roots to form soluble complexes with Fe$^{3+}$ when experiencing deficiencies of Fe and also Mn (Marschner and Marschner, 1995; Reichman, 2002; Reid *et al.*, 2003a).

### 1.2.6.2 Mycorrhiza and root exudates

Mycorrhizae are soil fungi symbiotically associated with the roots of most plant species. Mycorrhiza obtain photosynthates and in return increase the plant uptake of P and trace elements (Jones *et al.*, 2004; Reichman, 2002). This increase in plant uptake of P and trace elements by mycorrhiza association is achieved by increasing the surface area from which nutrients are sourced (Reichman, 2002). Root exudates have been hypothesized to be involved in the enhanced mobilization and acquisition of many nutrients from the soil as well as the detoxification of metals. However, with few exceptions there is little mechanistic evidence from soil systems to support this theory of metal detoxification (Jones *et al.*, 2004; Krpata *et al.*, 2009; Zhao *et al.*, 2001). There are many root exudates released into the rhizosphere, but for the most part their role is not clearly understood (Gregory, 2006). In P deficient soil, these exudates typically include low molecular weight organic anions such as citrate, oxalate, malate, lactate and fumarate (Jones *et al.*, 2004). How these exudates affect metal bioavailability has been the subject of several studies (Cattani *et al.*, 2006; Collins *et al.*, 2003a; Collins *et al.*, 2003b; Courchesne *et al.*, 2008; Degryse *et al.*, 2008; Lombnaes *et al.*, 2008; Lu *et al.*, 2007). These findings revealed changes in the speciation and solubility of Cd, Cu and Zn were significantly increased in the presence of organic anions such as citrate and oxalate. However, the extent to which these organo-metal complexes were being formed was affected by pH and the concentration of organic anions and of competing cations such as Ca (Collins *et al.*, 2003b; Lombnaes *et al.*, 2008; Lu *et al.*, 2007). At present there is a dearth of information regarding the role of organometallic complexes in plant enhanced nutrient acquisition (Courchesne *et al.*, 2008; Jones *et al.*, 2004).
1.2.6.3 Rhizobia and microbial activity

In legumes a symbiotic association with rhizobia bacteria exists, that results in the bacterial fixation of N\textsubscript{2} from the atmosphere to supply the plant with accessible nitrogen (N). As a result legumes take up more cations that anions and acidify their rhizosphere as a consequence (Gregory, 2006). This modification of solution pH through N assimilation by rhizobia has in some cases lead to a reduction in the populations of these simbionts in metal contaminated soils (Chaudri et al., 2008; Kopittke et al., 2007). In another study by Cloutier-Hurteau et al. (2008), rhizosphere relationships between pH, microbial biomass N and Cu\textsuperscript{2+} also indicated that bacteria influenced Cu availability by modifying the pH of the solution via N assimilation.

Microbial mineralization of organic matter has been suggested to partly supply metals such as Cu, Ni and Zn to the solution fraction of the rhizosphere through root decay (Cloutier-Hurteau et al., 2008). Links have been established between urease activity, biomass variables, organic matter and DOC, and water soluble Cu that indicate the effect that bacteria have on Cu speciation and bioavailability (Cattani et al., 2006; Chaperon and Sauve, 2007; Cloutier-Hurteau et al., 2008; van Hees et al., 2004; Vogeler et al., 2008). However, the precise mechanisms and the degree of influence that bacteria have on the speciation of Cu in the rhizosphere still remain largely unknown (Cloutier-Hurteau et al., 2008).

The influence of bacteria in the rhizosphere of Ni accumulating plants and plants grown on serpentine soils has been described in a number of studies (El-Aziz et al., 1991; Idris et al., 2004; Idris et al., 2006; Ma et al., 2009). It was found that certain bacteria enhanced the plant availability of Ni by directly enhancing Ni accumulation in plant tissues from the production phytosiderophores which induced the dissolution of Ni bearing mineral, and indirectly by promoting shoot and root biomass (Ma et al., 2009; Wenzel et al., 2003).
1.3 Metals in plants

The response of plants to elevated metals concentrations has generated a substantial amount of literature and is driven by two primary questions (Palmer and Guerinot, 2009):

- can crops be grown safely and productively at elevated concentrations?
- how and why do certain species function successfully at these concentrations?

Thus, the following section describes various uptake and tolerance/toxicity mechanisms.

1.3.1 Mechanisms of metal uptake and transport

Cations, including micronutrients (i.e. Fe, Cu, Mn, Zn and Ni) and contaminants (i.e. Cd and Pb) are passively taken up through ion channels. The reason for this is that micronutrients and contaminants such as Cd are all charged to varying degrees hence membrane voltages will have a significant impact on their movement into the cells. Hence electrochemical gradients applying to the transport of a particular nutrient largely define the possible mechanisms for uptake (Reid et al., 2003a; Reid, 2001). Most plants have a membrane potential between -120 mV and -180 mV, and based on thermodynamics, major cation nutrients, such as Ca and Mg, and metal uptake is classified as passive (Reichman, 2002; Reid et al., 2003a).

Metal uptake occurs in small fluxes, usually as a result of small external solution concentrations and also their low internal requirements (Reid et al., 2003a). Because of the small fluxes of micronutrients across the membrane, it is difficult to ascertain if ion channels are solely responsible for uptake. As a result, there are few reliable data on trace element uptake into intact plant cells, and evidence for particular transport mechanisms remains largely indirect (Reid et al., 2003a).

Calcium ions are transported across the root membrane via non-selective channels and it is hypothesised that uptake (low affinity) of metals from typical soil solution concentrations may also occur via this pathway (Reid et al., 2003b). There are many examples of competition between trace metals for uptake (at the root surface) and also
between trace metals and macronutrient cations such as Ca and K (Heenan and Campbell, 1981; Kalis et al., 2006; Reid et al., 2003a; Warne et al., 2008b).

Examples of cation competition at the root surface suggest that non-selective channels facilitate the entry of both essential and non-essential metals. Hence competition for transport sites would favour the uptake of those cations at higher concentrations, thereby exacerbating nutrient deficiencies (Palmer and Guerinot, 2009; Reid et al., 2003a; Welch, 1995). Conversely, when supply of micronutrients is too low to meet the nutritional requirements of the plant there appears to be some molecular and physiological evidence for the induction of high-affinity trace metal transport systems in response to deficiency (Reid et al., 2003a). For example, in the case of induced Fe deficiency in Arabidopsis, protein expression (IRT1) resulted in the root membrane that also strongly stimulated the uptake of Mn and Zn, but not Cu (Connolly and Guerinot, 2002; Vert et al., 2002). Hence, Fe deficient plants can also accumulate high concentrations of Mn and Zn.

1.3.2 Toxicity responses to elevated metal concentrations in soils

Copper, Ni and Zn are essential elements for plant nutrition and development, yet the same properties that make these transition metals essential can also make them toxic in excess (Alloway, 2009; Broadley et al., 2007; Chaney and Oliver, 1996; Longhurst et al., 2004; Marschner and Marschner, 1995; Palmer and Guerinot, 2009). Copper and Ni are most often used for their redox properties, with Cu and Ni essential for the structure and catalytic function of the enzymes (Marschner and Marschner, 1995; Palmer and Guerinot, 2009). Zinc is primarily used for its ability to act as a lewis acid (Palmer and Guerinot, 2009). Moreover, the amount of metal taken up by plants varies significantly with species as well as marked differences in metal tolerance among species, which has been documented in numerous studies (Broadley et al., 2007; Gray and McLaren, 2005; Macnicol and Beckett, 1985; Marschner and Marschner, 1995; McLaughlin et al., 1996; Naidu et al., 2003; Reichman et al., 2006).
1.3.2.1 Cadmium
Of all the heavy metals, Cd is recognised as posing the bigger threat to agricultural food quality (Chaney and Oliver, 1996; McLaughlin et al., 1996). Cadmium is recognised as the most mobile and bioavailable of the cationic trace metals and Cd has no known biological role in higher plants and therefore the presence of Cd is discussed in terms of tolerance and toxicity (Alloway, 1995a; Kiekens and Cottenie, 1985; Sloan et al., 1997). Most general symptoms of Cd toxicity in plants are stunted growth and chlorosis of younger leaves (An, 2004; Dheri et al., 2007) and a decrease in root elongation (Sun et al., 2005; Wang et al., 2009). However, concentrations of Cd in crop plants are likely to exceed food safety standards before phytotoxicity levels are attained (McLaughlin et al., 1996; McLaughlin et al., 2000).

1.3.2.2 Copper
Interveinal foliar chlorosis is a common initial symptom of Cu toxicity (Lock et al., 2007; Reichman, 2002). With increasing exposure to excess Cu, leaf tips and margins of shoots can become necrotic and, in acute toxicity, leaves may become wilted before eventually becoming necrotic (Marschner and Marschner, 1995; Reichman, 2002). For most crop species, the critical toxicity level of Cu in the leaves is above 30 µg g\(^{-1}\) dry weight (Macnicol and Beckett, 1985). Excess Cu supply in the soil can also lead to root growth inhibition and in plants more sensitive to Cu, inhibition of root elongation and damage of the plasma membrane of root cells (Marschner and Marschner, 1995), and can also adversely affect the nodulation process of legumes (Kopittke et al., 2007). Even though high Cu concentrations can adversely affect shoot growth, typically it is root growth and form that is affected before any signs of above ground growth abnormality has been detected (Reichman, 2002).

1.3.2.3 Nickel
Generally, the toxicity of Ni is almost always less than Cu, Zn and especially Cd (McLaughlin et al., 2000), and for crop plants critical toxicity levels are in the range of 10 µg g\(^{-1}\) to > 50 µg g\(^{-1}\) dry weight (Macnicol and Beckett, 1985; Marschner and
Marschner, 1995) for sensitive and moderately tolerant species. As with Cu, root growth and form is also affected in the presence of excess Ni in soil (Rooney et al., 2007b), and in sensitive species, root growth is severely inhibited at concentrations < 5 µM of Ni (Marschner and Marschner, 1995). The occurrence of Ni hyperaccumulators (e.g. genus Alyssum) and Ni tolerant plants growing on serpentine soils has been the focus of several studies as a result of their potential use in the phytoremediation of metal contaminated soils (Chaney et al., 2008; Chaney et al., 1997; Himmelbauer et al., 2005; Wenzel et al., 2003). In Ni hyperaccumulating species growing on serpentine soils, the leaves can reach 10–30 mg g⁻¹ dry weight, compared with 0.001 - 0.01 mg g⁻¹ dry weight in most crop species (Marschner and Marschner, 1995). Tolerance in Ni hyperaccumulators is mostly achieved by complexation of Ni with organic ions such as malate and citrate (Homer et al., 1991).

1.3.2.4 Zinc

Chlorosis of the younger leaves is typically the first symptom of Zn toxicity also with plants exhibiting Zn toxicity symptoms having smaller leaves than control plants (Reichman, 2002). With progressive exposure to toxic levels of Zn, this can develop into a reddening of the leaves and in severe cases lead to necrosis of the leaf tip (Marschner, 1995). In roots, the toxicity is manifested as a reduction in the growth of the main root, fewer and shorter lateral roots and a yellowing of the root (Broadley et al., 2007; Marschner and Marschner, 1995; Reichman, 2002). The critical toxicity levels in leaves of crop plants are from as low as 100 µg g⁻¹ to more than 300 µg g⁻¹, with the latter value being more typical (Marschner and Marschner, 1995). However, when the supply of plant available Zn is high, an induced deficiency of Fe and Mg can result because of the similar ion radius of Zn²⁺ and Fe²⁺ (Marschner and Marschner, 1995).
1.4 Methods for determining metal bioavailability in soils

Although bioavailability is recognized as the most relevant and critical aspect of metal fraction to measure, there is still remains no general consensus on how speciation and bioavailability can be best measured (Hooda, 2007; McLaughlin et al., 2000; Menzies et al., 2007; Zhang et al., 2001). Attempts to find suitable soil tests for estimating metal bioavailability have varied from measuring concentrations of the immediately bioavailable metal to also including measures of capacity of the soil to replenish the metal or buffer the metal concentration as it is removed from the soil solution (Reichman, 2002). This has resulted in the development of a multitude of soil tests of which, six methods currently used for estimating bioavailability will be discussed in this section.

1.4.1 Total extractable metals

The use of total metal concentrations in soil is still the most commonly used value to determine metal bioavailability and ecotoxicity (Menzies et al., 2007). Strong acids, such as HF, HClO₄, HNO₃ and aqua regia (HNO₃/HCl) are commonly used to extract the ‘total’ metal in a soil (Blakemore et al., 1987). However, as discussed in section 2.2 many factors influence the bioavailability of metals to plants and organisms, making total metal a typically poor measure of bioavailability (McBride et al., 2003). However, there have been some good relationships documented between total metal and plant responses (Reichman, 2002) although these were soil specific, thereby demonstrating the use of total metal as a robust measure of bioavailability as ineffective (McLaughlin et al., 2000).

1.4.2 Chelation methods

Chelation techniques involving aminopolycarboxylic acids such a diethylene-triamine-penta-acetic acid (DPTA) and ethylene-diamine-tetra-acetic acid (EDTA) have been used
extensively to determine the phytoavailability of metals in soils (Hooda and Alloway, 1994b; McLaughlin et al., 2000; Meers et al., 2008; Reichman, 2002). These tests have been commonly used to determine metal deficiencies and have been shown to be good predictors of deficiencies (Reichman, 2002). Moreover, environmentally more acceptable chelating agents such as EDTA have also been assessed as a potential soil amendment for the remediation of metals in contaminated soils as an alternative to phytoextraction (Meers et al., 2008).

Although chelating compounds have been successfully used to determine metal deficiencies in soils, the use of these methods to determine phytotoxic concentrations of metals has been less so, with plant responses typically poorly correlated with DPTA or EDTA extractable metals (McLaughlin et al., 2000; Menzies et al., 2007; Reichman, 2002). There are a number of reasons proposed for this lack of strong correlation between plant and soil concentrations of metals: these methods were developed for use in soils containing low amounts of organic matter; the concentration of metals in the soils may exceed the chelating ability of the compound; the use of EDTA requires the compound to be pH buffered, typically pH 6 (McLaren et al., 1984) that would have a significant effect on metal solubility, particularly Cd and Zn (Menzies et al., 2007; Reichman, 2002).

1.4.3 Weak and neutral salt extractants

Weak and neutral salt extractants such as CaCl₂, Ca(NO₃)₂ and NaNO₃ have been evaluated in a number of recent studies (Datta and Young, 2005; Ettler et al., 2007; McBride et al., 2009; Meers et al., 2009; Menzies et al., 2007; Nolan et al., 2005) with promising results. The use of neutral salts as a method to predict bioavailability is based on the hypothesis that plant available metals are mostly sourced on soil mineral surfaces and can be easily displaced by other cations such as Ca and Na (Andrews et al., 1996; Houba et al., 2000; McBride et al., 2003; McLaughlin et al., 1999). In a review of extractants used to estimate metal availability, Menzies et al. (2007) found that neutral salt solutions tended to provide the best relationship between soil extractable metal and
plant tissue concentration. The review compared the relationships of six of these extractants (1 M NH₄OAc and 1 M NH₄NO₃ for Cd, 1 M NaNO₃ for Zn and 0.01 M CaCl₂ for Cd, Ni and Zn), and apart from NH₄NO₃ for Cd, all had $r^2 > 0.5$. Although good relationships were observed the use of neutral and weak salt extraction are relatively new, and consequently datasets are limited.

Of these extractants CaCl₂ has been the most extensively reviewed, particularly in relation to assessment of plant available Cd (Ozkutlu et al., 2007; Weggler et al., 2004; Weggler-Beaton et al., 2000). The reason for this is that increased chloride concentrations has been shown to increase Cd concentration in soil solution and Cd uptake by plants (McLaughlin et al., 1994; Weggler et al., 2004). Studies comparing CaCl₂ extractable Cd and plant tissue concentrations have found reasonably successful correlations $r^2 > 0.5$ (Houba et al., 2000; Weggler et al., 2004) and these findings suggest that this type of extractant may also be suitable for metals such as Cu, Ni and Zn (Menzies et al., 2007). In addition, a study by Meers et al. (2009) to determine the effect of the counter ion (Cl⁻ vs NO₃⁻) found that at sufficiently high ionic strengths (e.g. 0.1 M) Ca(NO₃)₂ can be used to give an estimate of the total exchangeable pool, whereas weaker extractions similar to the ionic strength of the soil solution (0.01 M) allows for a quantitative soil response of the metal released as a function of increasing Ca²⁺ in the extractant under relevant soil solution conditions.

### 1.4.4 Metals in soil solution (water extractable)

Soil solution is the fraction of metal considered immediately available for plant uptake (Gray and McLaren, 2006). Soil solution is defined as the aqueous liquid phase of the soil and its solutes (McBride, 1994). However, there appears no standard approach to the extraction and measuring of soil solution and methods of extraction range from centrifuging, to leaching, to extraction using Rhizon probes, and all of these techniques require differing ratios of soil to water (Degryse et al., 2009a; Reichman, 2002).
The amount of metal in soil solution and the ability of the soil to buffer metal concentrations in the soils have been described respectively as the intensity \( I \) and quantity \( Q \) factors (Tiller et al., 1972), and relationships between these, as the buffer power \( C \) (McLaughlin et al. 2000). The factors that affect \( I \) and \( Q \) in soil solution include the total concentration of metals in the soil, soil pH, cation exchange capacity, the nature of the exchange sites and the mass balance of metals and good correlations between soil solution Cd, Cu and Zn have been found with these soil properties (McBride et al., 1997a). McLaughlin et al. (2000) also provided a comprehensive review of factors affecting plant and soil biota metal availability through the modification of \( I \) and \( Q \).

Several investigations (Gray et al., 1999b; McBride et al., 1997a; Sauve et al., 2000a; Tambasco et al., 2000) have proposed that soluble metal concentrations in soils can be predicted using a semi-empirical equation. The theoretical background to this competitive adsorption model has been presented by McBride et al. (1997a), in which metal solubility in soils can be derived from a semi-empirical approach that assumes that the free metal (i.e. Cd\(^{2+} \)) and H\(^+ \) compete for adsorption on soil exchange sites. This suggests that these models may be useful for deriving certain metals loadings in soils and may also be used for risk assessment of contaminated sites. Even though some good relationships have been found with metal concentrations in solution and shoot concentration in some studies, these appeared dependent on soil type, plant species and metal, hence total metal in soil solution may not be a robust method for determining bioavailability (Reichman, 2002). The reason that total metal in soil solution may not be a good predictor of bioavailability is that not all forms of metal in solution are thought to be available for plants and soil organisms and it had been demonstrated that free ion and certain labile complexes of metals are available for uptake, while a significant portion of metal species in solution are relatively unavailable (Arnold et al., 2007; Broadley et al., 2007; Degryse et al., 2006a; 2006b; McLaughlin et al., 1997; Sauve, 2003; Slaveykova et al., 2009; Stacey et al., 2008).
1.4.5 Diffuse gradient in thin films

Previous extraction methods discussed also allow an insight into the geochemical forms of metals in the soil. However, these methods do not account for the ability of the soil to sustain the solution concentration following the depletion by plant uptake (Zhang and Davison, 2001). The diffusive gradient in thin film technique usually abbreviated to DGT was initially developed by Davison and Zhang, (1994) for the purpose of measuring in situ concentration and fluxes of trace element species in aqueous solutions (Degryse et al., 2003). It has since been modified to quantitatively measure the supply of labile trace metals in soils (Degryse et al., 2009b; Hooda et al., 1999; Zhang et al., 1995; 1998; 2001).

The DGT device houses a resin-gel layer that is separated from the soil by a diffusion gel layer and a protective membrane. The technique relies on the accumulation of solutes onto the binding agent (such as the cation exchange resin) after passing through the uniform and well-defined diffusion gel layer. The binding agent acts as a localised sink for the solute thus causing a depletion of trace metals in the pore water and a re-supply from the labile metal pool on the solid phase, thereby introducing a diffusional flux to the DGT device from the surrounding solution; a process that simulates plant metal uptake (Degryse et al., 2003; Lehto et al., 2006a). The DGT-measured fluxes are affected by concentrations in the soil solution and the re-supply from the soil solid phase. This re-supply is dependent on the amount of labile metal sorbed onto the solid phase and the rate of exchange between the solid and the solution phase. This technique is unique in the respect that the resin-gel is selective only to labile ions.

In predicting metal uptake by plants, successful comparisons were made between kinetics of metal release in soils and prediction of the corresponding concentrations in plants using DGT (Zhang et al., 2004). Good correlations between metal concentrations in plants and metals have been measured by DGT in the same soil (Davison et al., 1999). Other studies demonstrated that under conditions where capacity and the kinetics of supply from the soil limit uptake, the controls on uptake to DGT and plant are similar (Degryse et al., 2009b; Nolan et al., 2005; Oporto et al., 2009; Zhang et al., 2004). The
use of DGT to predict metal uptake over a variety of plant species and soils types has been successful in some cases (Degryse et al., 2009b; Ernstberger et al., 2002; Nolan et al., 2005; Zhang et al., 2001). However, despite the growing use of this method to assess metal availability, particularly to plants, there is no standardised protocol for the preparation of soils prior to DGT deployment.

1.4.6 Speciation modeling and free ion activity

It is a widely held view that free metal ion activity is often a much better indicator of metal availability in soils than the total metal content (Cances et al., 2003; Degryse et al., 2009a; Lofts et al., 2004; Lofts et al., 2007; McBride et al., 1997a). Metals in soil solution may be present as free ions (e.g. Cd\(^{2+}\)) or as complexes with inorganic anions (e.g. Cl\(^{-}\), CO\(_3\)^{2-}, SO\(_4\)^{2-}), or organic anions (e.g. citrate and malate), or complexed with other forms of DOC. However, depending on the metal, the free ion may be the dominant form in solution and the free ion is generally the most reactive species in solution and thus can be a major determinant in bioavailability (Almas et al., 2006; Degryse et al., 2009a). Analytically quantifying the amount of free ion activity and labile species has only been attempted in a few studies (Sauve et al., 1995; Sauve et al., 2000b) and thus insufficient rigorous work has been done to confirm the hypothesis that free ion activity is the majority bioavailable species (Nolan et al., 2003; Reichman, 2002).

Assemblage models such as WHAM (Tipping et al., 2003b) consider the soil environment as a set of independent reactive surfaces and combines several models to describe sorption onto organic matter (soluble and solid), clays and oxides (Degryse et al., 2009a). These models require a considerable amount of information and several assumptions are made, about the percentage of active organic matter (typically 65 % fulvic acid in solution) and surface areas of the clays and oxides. Studies have shown that the complexation of Cu with DOC is less than predicted by WHAM (Buekers et al., 2008; Nolan et al., 2003; Sauve et al., 1997) and because few studies have described the complexation of Ni by DOC it is highly likely that the amount complexed may greatly differ than what would be predicted by WHAM (Degryse et al., 2009a; Nolan et al.,
Generally only a few studies have compared measured solid and aqueous distribution of metals in soils with predicted values obtained from assemblage models (Amery et al., 2008; Buekers et al., 2008; Tipping et al., 2003b), and findings reveals that predictions and measured distributions differed considerably and, hence adjustments to the model were necessary. Studies that have compared predicted free ion activity with plant metal uptake found good relationships with Cd and Zn in spinach (*Spinacia oleracea*) and ryegrass (*Lolium perenne*) (Almas et al., 2006; Hough et al., 2005), and Ni$^{2+}$ activity and toxic effects on barley roots (Rooney et al., 2007b) indicated that the free ion activities of Cd, Ni and Zn represent a significant proportion of the total bioavailable pool.

### 1.5 The use of biosolids as a soil amendment

Biosolids (sewage sludge) is the residual product of wastewater treatment and has been used widely in the agriculture industry for several years as it provides a valuable source of organic matter and macronutrients, as well as providing a means of disposing of an unwanted waste (Antoniadis and Alloway, 2001; McLaren et al., 2003; McLaughlin et al., 2007; Merrington and Smernik, 2004; Oliver et al., 2005; Smith, 2009; Speir et al., 2003b; Sullivan et al., 2006a). However, biosolids also contain organic and metal contaminants and there are some concerns regarding their environmental fate, and for this reason its agricultural use is regulated (McLaren et al., 2005; Speir et al., 2003a; 2003b; Sullivan et al., 2006b).

Two major environmental concerns arise from metal contaminants in biosolids following land application, namely the contamination of shallow groundwater from metal-rich leachate and the accumulation and uptake of metals in soils and plants (Ashworth and Alloway, 2004; Smith, 2009; Speir et al., 2003b). As a result of these environmental risks, rules and guidelines controlling both the quantity and quality of biosolids applied to land have been developed to assist in sustainable management and to provide advice on the safeguards necessary to protect public health (microbiological) and for metal contaminants. The focus on metal contamination is aimed primarily at preventing metal
accumulation in the food chain and toxic effects on plants and soil fauna (Alloway and Jackson, 1991; Antoniadis and Alloway, 2002a; 2003a; McLaren et al., 2007; McLaughlin et al., 2000; 2006; 2007; Speir et al., 2007).

Conversely, biosolids have been used extensively as soil amendments to reduce the bioavailability and phytotoxicity of metals in contaminated soils (Basta et al., 2001; Brown et al., 2005; Brown et al., 2007; Navas et al., 1999; Nwachukwu and Pulford, 2009; Stuczynski et al., 2007; van Herwijnen et al., 2007b). The hypothesis for the use of these soil amendments is that added organic matter may induce sorption or precipitation reactions for metals, resulting in increased metal retention on the solid phase and thus a reduction in the metal fraction available for plant uptake (Brown et al., 2005; Merrington et al., 2003; Nwachukwu and Pulford, 2009).

The efficacy of biosolids amendment at retaining metals has been examined by several studies (Antoniadis and Alloway, 2002b; 2003b; Antonladis and Tsadilas, 2007; Ashworth and Alloway, 2004; 2007; Brown et al., 2005; McBride et al., 1997b; McLaughlin et al., 2006; Merrington and Smernik, 2004; Merrington et al., 2003). The results from these studies have been varied with respect to the type of metal. Studies by Antoniadis and Alloway (2002b) and Ashworth and Alloway (2004) observed that biosolids possess relatively poor Ni retention capabilities. While Antoniadis and Alloway (2003a), van Herwijnen et al. (2007b) and Nwachukwu and Pulford (2009) observed an increase in the solubility of Ni and Zn overtime. Cadmium availability was not significantly affected, or availability decreased in the presence of biosolids (McLaughlin et al., 2006; Merrington and Smernik, 2004; Merrington et al., 1997). The efficacy of biosolids at reducing the bioavailability of metals is generally inconclusive but overall findings indicate potential risks in applying biosolids over longer time periods with respect to metal bioavailability and mobility.
1.5.1 Biosolids research and management in New Zealand

Metal pollution of agricultural land in New Zealand is concerning, although it is less severe than has been documented in many European countries (McLaughlin et al., 2000). However, New Zealand is economically dependent on agricultural products, which in-turn is dependent on maintaining adequate nutrient levels in soils. New Zealand environmental regulations and guidelines rely largely on data generated in northern hemisphere countries, which has led to unsuitable levels being chosen for several trace elements, especially Cd, Cu, Ni and Zn (Cavanagh and Coakley 2005; McLaughlin et al., 2000). As discussed in previous sections, the bioavailability of metals can be environmentally significant for several years after introduction (i.e. from the amendment of biosolids) and to predict metal bioavailability is the goal of ongoing research.

The majority of biosolids and soils research to date in New Zealand has been undertaken collaboratively by Environmental Science and Research Ltd. (ESR) and Lincoln University. The outcome of this research has helped to define guidelines for the safe and beneficial use of biosolids. Research to date has covered the trialing of five different New Zealand soils amended with biosolids and biosolids spiked with metal salts. These studies have assessed leachate quality, changes in soil chemistry plant metal concentrations and effects on soil organisms (Horswell et al., 2003; 2006; 2007; McLaren, 2005; McLaren et al., 2003; 2004; 2005; Yeates et al., 2006; Yuan, 2009) as well as impacts to groundwater quality (Speir et al., 2003b). Metal fractionation was examined in metal spiked biosolids and in leachate from high-rate application of biosolids to land (McLaren and Clucas, 2001).

Results from the metal spiked biosolids trials showed that after three years of continuous monitoring, soil pH had decreased and the concentrations of Cd, Ni and Zn in the drainage leachate had increased (McLaren et al., 2003; 2005). However, the lower pH had little effect on, or decreased the concentrations of Cr, Cu and Pb. Additionally, there were substantial differences observed between the soils, in the context of increased metal leachate, and Speir et al. (2003b) also showed that soil (coarse textured sand) could be strongly acidified by the sludge addition which resulted in considerable metal solubility,
mobility and bioavailability for plant uptake. A combination of plant cover, soil organic matter, clays and oxides contributed to the metal binding capacities of the soils and were responsible for the relatively low levels of leaching observed (McLaren et al., 2004). Speir et al. (2003b) confirmed that metals present in the biosolids used were largely unavailable as they were strongly complexed with organic matter contained in biosolids. However, it was observed under certain circumstances (low soil pH and clay content), metals contained in biosolids were more likely to move through the soil profile with the potential to contaminate groundwater (McLaren et al., 2004).

It is recognised that biosolids utilisation and management needs to be effectively managed in New Zealand and this has resulted in guidelines developed for the safe application of biosolids to land (NZWWA, 2003). These grade biosolids according to both stabilisation and contamination. Currently, the limits for metal contaminants in biosolids are given as total concentrations, which are set out in Table 1.1. As further research into determining the best measure of metal bioavailability continues, it is expected that guidelines will subsequently incorporate the available metal fraction with specified ecological receptors into land management practices (Cavanagh and Coakley 2005).

Table 1.1  **Biosolids classification by contaminant levels grades a + b (Cavanagh and Coakley, 2005).**

<table>
<thead>
<tr>
<th>Contaminant</th>
<th>Grade a (mg kg⁻¹)</th>
<th>Grade b (mg kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>As</td>
<td>20</td>
<td>30</td>
</tr>
<tr>
<td>Cd</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>Cr</td>
<td>600</td>
<td>1500</td>
</tr>
<tr>
<td>Cu</td>
<td>100</td>
<td>1250</td>
</tr>
<tr>
<td>Pb</td>
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<td>300</td>
</tr>
<tr>
<td>Hg</td>
<td>1</td>
<td>7.5</td>
</tr>
<tr>
<td>Ni</td>
<td>60</td>
<td>135</td>
</tr>
<tr>
<td>Zn</td>
<td>300</td>
<td>1500</td>
</tr>
</tbody>
</table>
Chapter 2
Research Aim and Objectives

2.1 Aim

The overall aim of this thesis was to assess and validate measures of bioavailable Cd, Cu, Ni and Zn across contrasting soil types, ranges of metal concentration and presence of biosolids. This was accomplished by assessing six potential measures of bioavailability across a range of soil types spiked with metal salts in the presence and absence of biosolids using wheat (*Triticum aestivum*) and ryegrass (*Lolium perenne*) as biological response models.

2.2 Objectives

The overall aim of the research programme was fulfilled by the following three major objectives which were completed between 2006 and 2009:

1. Examining the effects of biosolids amendment on the bioavailability of Cd, Cu, Ni and Zn in contrasting soil types placed in controlled environmental conditions. This was achieved using wheat as the model plant system, where wheat seedlings were germinated in three soils spiked with increasing levels of metal salts which had been incubated for two and 24 weeks;

2. Examining the effects of biosolids amendment and metal salt applications on the bioavailability of Cd, Cu, Ni and Zn in contrasting soil types managed under field conditions over a 24-month period. This was achieved by using ryegrass as the model plant system, where ryegrass was grown in three soils spiked with increasing levels of metal salts, to which biosolids had been added to the lowest metal salt concentration treatment;
3. To assess the overall strength of the relationships determined between metal concentrations in two plant species and potential measures of Cd, Cu, Ni and Zn availability from a range of New Zealand soils amended with a combination of biosolids and metal salts. This was achieved by combining results in objectives 2 and 3 and data obtained from closely related biosolids and metal salt plot field studies carried out by Environmental Science and Research Ltd. (ESR) in collaboration with Lincoln University.

2.3 Structure

The previous chapter consisted of an introductory literature review that outlines mainstream concepts regarding bioavailability and methodologies used to predict bioavailability. Chapter 1 also briefly described plant mechanisms responsible for metal uptake and responses to metal toxicity and the use of biosolids as a soil amendment. Chapter 3 contains a list of soil descriptions and localities for each of the soil assessed in this study and similar studies carried out by ESR in collaboration with Lincoln University. Description of sample preparation and analytical methods common to studies described in subsequent chapters are also included.

The three main objectives are individually addressed and were the focus of Chapters 4, 5 and 6 respectively. Chapter 4 evaluated the effects of biosolids amendment on the bioavailability of Cd, Cu, Ni and Zn in soils maintained under controlled environmental conditions, using wheat as the biological response model; Chapter 5 investigated the effects of biosolids amendment and metal salt applications on the bioavailability of Cd, Cu, Ni and Zn in soils managed under field conditions using ryegrass as the biological response model; and Chapter 6 assessed the overall strength of the relationships determined between metal concentrations in wheat and ryegrass shoots, and measures of Cd, Cu, Ni and Zn bioavailability from a range of New Zealand soils amended with biosolids and metal salts. The findings from Chapters 4 – 6 were individually discussed at
the end of each chapter, while Chapter 7 concluded this thesis with a general discussion concluding remarks and approaches for further study.
Chapter 3
Soil Classification and Methodology

3.1 Soil localities and classifications

Three soils from the Canterbury region, a Halkett sandy loam (HK), Summit/Rapaki silt loam (SM) and Wakanui silt loam (WK) (Table 3.1) were selected for this study based on their contrasting soil properties (e.g. organic matter content, mineralogy and texture). The HK and WK soils were located in the wider Christchurch area, Canterbury (Figure 3.1), where the mean annual rainfall of the area is about 620 mm in the north-east and about 690 mm in the south-west (Cox, 1978). The HK soil series were formed on sand dunes and are located mostly within the north-west of the district. Wakanui soils were developed in fine greywacke alluvium brought in by ancient overflow channels of the Waimakariri River (Cox, 1978).

Summit soil was taken from the Port Hills area (elevation 275 to 300m a.s.l.) adjacent to the Canterbury Plains (Figure 3.1). These soils were derived from loess and basaltic material (Griffiths, 1974). The mean annual rainfall of the Port Hills area is approximately 1100 mm. The mean annual daily temperature for Canterbury is 11.0° C, day length is approximately 15.5 hours in mid summer (January) and 9 hours in mid winter (July) (Cox, 1978). Soils used in the joint Environmental Science and Research Ltd (ESR) and Lincoln University biosolids and metal salt trials are also included in Table 3.1. Biosolids for use in experiments described in Chapters 4 and 5 were collected in September 2006 from the Bromley wastewater treatment plant in Christchurch, New Zealand.
Figure 3.1  Localities of soils used in experiments described in Chapters 4, 5 and 6.
Table 3.1 Characteristics and locations of experimental soils.

<table>
<thead>
<tr>
<th>Soil series</th>
<th>NZ soil classification</th>
<th>NZ Genetic Classification</th>
<th>Location within New Zealand</th>
</tr>
</thead>
<tbody>
<tr>
<td>Halkett sandy loam</td>
<td>Typic Sandy Recent Soil</td>
<td>Yellow-brown sands</td>
<td>West Melton</td>
</tr>
<tr>
<td>Summit silt loam</td>
<td>Orthic Brown Soil</td>
<td>Upland Yellow brown earth</td>
<td>Port Hills</td>
</tr>
<tr>
<td>Wakanui silt loam</td>
<td>Mottled Immature Pallic Soil</td>
<td>Gley soil – Yellow-grey earth</td>
<td>Prebbleton, Ladbrooks and Lincoln</td>
</tr>
</tbody>
</table>

Soils used in Lincoln University and ESR trials

<table>
<thead>
<tr>
<th>Soil series</th>
<th>NZ soil classification</th>
<th>NZ Genetic Classification</th>
<th>Location within New Zealand</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ashley Dene deep fine sandy loam&lt;sup&gt;1&lt;/sup&gt;</td>
<td>Typic Fragic Pallic Soil</td>
<td>Yellow-grey earth, weakly leached, moderately gleyed</td>
<td>Canterbury</td>
</tr>
<tr>
<td>Egmont black loam&lt;sup&gt;1&lt;/sup&gt;</td>
<td>Typic Orthic Allophanic Soil</td>
<td>Yellow-brown loam, moderately leached</td>
<td>Taranaki</td>
</tr>
<tr>
<td>Foxton black sand&lt;sup&gt;1&lt;/sup&gt;</td>
<td>Typic Sandy Brown Soil</td>
<td>Yellow-brown sand</td>
<td>Wellington</td>
</tr>
<tr>
<td>Horotiu sandy loam&lt;sup&gt;4&lt;/sup&gt;</td>
<td>Orthic Allophanic Soil</td>
<td>Yellow-brown loam</td>
<td>Hamilton</td>
</tr>
<tr>
<td>Lismore silt loam&lt;sup&gt;3&lt;/sup&gt;</td>
<td>Orthic Brown Soil</td>
<td>Udic Ustochrept (USDA)</td>
<td>Canterbury</td>
</tr>
<tr>
<td>Tahunanui loamy sand&lt;sup&gt;4&lt;/sup&gt;</td>
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<td>Brown soil</td>
<td>Nelson</td>
</tr>
<tr>
<td>Otaki&lt;sup&gt;1&lt;/sup&gt;</td>
<td>Weathered Fluvial Recent Soil</td>
<td>Recent soil</td>
<td>Wellington</td>
</tr>
<tr>
<td>Templeton fine sandy loam&lt;sup&gt;1,2,4&lt;/sup&gt;</td>
<td>Immature Pallic Soil</td>
<td>Intergrade between yellow-grey earth and recent soil</td>
<td>Canterbury</td>
</tr>
<tr>
<td>Waikou silt loam&lt;sup&gt;1&lt;/sup&gt;</td>
<td>Typic Orthic Pumice Soil</td>
<td>Yellow-brown loam</td>
<td>Hamilton</td>
</tr>
</tbody>
</table>

<sup>1</sup> Lysimeter trials involving biosolids only, managed under field conditions
<sup>2</sup> Pasture field trial involving biosolids spiked with metal salts
<sup>3</sup> A forest soil field trial involving the application of biosolids spiked with metal salts
<sup>4</sup> Metal salt plot trials managed under field conditions
3.2 Characterisation of soils and biosolids

All soils were air-dried at 20°C before being ground and sieved through a 2 mm stainless steel sieve. Soils were then stored in polyethylene bags at room temperature prior to analyses.

**Amorphous Al and Fe oxides:** Air-dried soil (2.0 g) was mixed with 50 ml of 0.2 M oxalic acid and placed on an end-over-end shaker for 4 hours (in darkness). Samples were centrifuged at 2000 rpm for 10 minutes before being filtered through Whatman No. 42 filter paper (McKeague and Day, 1966). Iron and Al were analysed using an Inductively Coupled Plasma Optical Emissions Spectrophotometer (ICP-OES) Varian 720-ES fitted with a SPS-3 auto-sampler and ultrasonic nebuliser. Amorphous Al and Fe results are shown in Table 3.2.

**Cation exchange capacity and exchangeable bases:** The cation exchange capacity (CEC), base saturation and exchangeable bases of biosolids and soils was determined by ammonium acetate leaching at pH 7.0 (Blakemore et al., 1987). Ca, Mg, Na, K and total NH$_4$-N were analysed in the leachate using atomic absorption spectrophotometry (AAS) Shimadzu AA-670 (Shimadzu Oceania, Australia) and Flow Injection Analyser (FOSS FIAstar 5000 triple channel analyser, Foss Tecator, Hoganas, Sweden) (Table 3.2 and Table 3.3). Cation exchange capacity was calculated from NH$_4$-N results. Total exchangeable bases (TEB) were the sum of Ca, Mg, Na and K. The percentage of base saturation is derived from the TEB/CEC.

**Soil particle size distribution:** Representative soil samples were sent to the University of Waikato New Zealand for texture analyses. Soil particle size distribution was determined using the Mastersizer 2000 (Table 3.4).
**Soil pH:** Approximately 10 g ± 0.2 of prepared soil was mixed with 25 ml of deionised water and left for six hours to equilibrate (Blakemore *et al.*, 1987). Soil pH was recorded using a Mettler Toledo pH meter (Mettler Toledo, U.S.A.), (Table 3.2).

**Total carbon:** Total carbon was determined from 0.5 g of prepared soil (Gray *et al.*, 1999a) placed in pre-weighed silica crucibles heated to 1300˚C by a LECO 2000 CNS Analyser (Leco, Australia) (Table 3.2).

**Water holding capacity for soils:** The maximum water holding capacity (MWHC) is a method used to determine the amount of water that a free draining soil can hold. Soil was dried (50 g) overnight at 105˚C and recording the weight. Soil was then placed in a small container fitted with fine-meshed gauze at the base, and allowing it to stand in a tray of water level with the top on the container. Soils were left for four hours before removing from the tray and re-weighing. The MWHC is the difference between the weight of the dry soil and the saturated soil and expressed as a percentage (Smart *et al.*, 2004).

<table>
<thead>
<tr>
<th>Soil</th>
<th>Maximum water holding capacity (%)</th>
<th>pH</th>
<th>Amorphous Al-oxides (mg kg⁻¹)</th>
<th>Amorphous Fe-oxides (mg kg⁻¹)</th>
<th>Cation exchange capacity (cmol·kg⁻¹)</th>
<th>Total carbon (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Halkett</td>
<td>56</td>
<td>5.6</td>
<td>95</td>
<td>176</td>
<td>8</td>
<td>3.1</td>
</tr>
<tr>
<td>Summit</td>
<td>67</td>
<td>5.6</td>
<td>525</td>
<td>696</td>
<td>22</td>
<td>7.4</td>
</tr>
<tr>
<td>Wakanui</td>
<td>65</td>
<td>6.1</td>
<td>144</td>
<td>313</td>
<td>16</td>
<td>3.5</td>
</tr>
<tr>
<td>Biosolids</td>
<td>&gt;100</td>
<td>8.3</td>
<td>na</td>
<td>na</td>
<td>44</td>
<td>40.1</td>
</tr>
</tbody>
</table>

na = not analysed
Table 3.3 Calculated values of total base saturation (TBS), exchangeable bases (EB) and percentage of base saturation (BS) for three New Zealand soils and biosolids sourced from the Bromley wastewater treatment plant used in experiments described in Chapters 4 and 5.

<table>
<thead>
<tr>
<th>Soil</th>
<th>TBS (%)</th>
<th>Exchangeable bases (cmol(_c) kg(^{-1}))</th>
<th>Base saturation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Ca</td>
<td>Mg</td>
</tr>
<tr>
<td>Halkett</td>
<td>67</td>
<td>4.5</td>
<td>0.34</td>
</tr>
<tr>
<td>Summit</td>
<td>38</td>
<td>4.3</td>
<td>2.58</td>
</tr>
<tr>
<td>Wakanui</td>
<td>68</td>
<td>7.7</td>
<td>1.37</td>
</tr>
<tr>
<td>Biosolids</td>
<td>65</td>
<td>16.5</td>
<td>8.31</td>
</tr>
</tbody>
</table>

Table 3.4 Results of the soil texture analysis determined for three New Zealand soils used in experiments described in Chapters 4 and 5.

<table>
<thead>
<tr>
<th>Soil</th>
<th>Particle size (µm) reported in volume %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.06 – 2</td>
</tr>
<tr>
<td>Halkett</td>
<td>0.47</td>
</tr>
<tr>
<td>Summit</td>
<td>21.80</td>
</tr>
<tr>
<td>Wakanui</td>
<td>3.85</td>
</tr>
</tbody>
</table>

3.3 Plant and soil analyses using AAS, GFAAS and ICP-OES

All the metal concentrations in the extraction methods described below were analysed using a GBC Avanta Atomic Absorption Spectrophotometer (AAS), Graphite Furnace Atomic Absorption Spectrophotometer (GFAAS) GBC System 3000 and GBC 909AA with PAL 3000, or an Inductively Coupled Plasma Optical Emissions Spectrophotometer (ICP-OES) Varian 720-ES fitted with a SPS-3 auto-sampler and ultrasonic nebuliser.
3.3.1 Analysis of plant samples

Ryegrass and wheat from the seed and germination bioassays were prepared for analysis using the method described in Gray et al. (1999a) where 0.5 g of prepared plant sample was digested in 10 ml of 69% HNO₃ (Aristar BDB grade) and heated on a digestion block (DK Heating 42 place digestion block, VELP, Italy) for 6 hours (up to 140° C). All plant samples were analysed for total Cd, Cu, Ni and Zn using ICP-OES. A reference standard (1573a Tomatoes Leaves, U.S. Department of Commerce, National Institute of Standards and Technology, Gaitherburg, MD 20899) was used during batches of analyses to check the accuracy of the results.

3.3.2 Analysis of soil samples for estimates of metal availability

3.3.2.1 Total recoverable metals

Total recoverable Cd, Cu, Ni and Zn was determined using methods described in Kovacs et al. (2000), where 1 g of prepared soil is left overnight in 5 ml of 69% HNO₃ (Aristar BDB grade). The following day, 5 ml of 50% H₂O₂ was added to the pre-digested soils before placement on a 42 place digestion block (up to 120°C) for 4.5 hours. Samples were then filtered through a Whatman filter No. 42 filter paper and made up to 25 ml with deionised water. Samples from the first wheat seedling bioassay (Chapter 4) were analysed using AAS and GFAAS. The remainder of experimental samples were analysed by ICP-OES. A reference standard (2711 Montana Soil, Moderately Elevated Trace Element Concentrations, U.S. Department of Commerce, National Institute of Standards and Technology, Gaitherburg, MD 20899) was used during batches of analyses to check the accuracy of the results.
3.3.2.2 **EDTA extractions**

A solution of 0.04 M ethylene diamine tetra-acetic acid disodium salt (EDTA), adjusted to pH 6.0 with 0.1 M NaOH and was prepared according to McLaren *et al.* (1984). EDTA solution (25 ml) was added to 10 g of prepared soil, shaken for 2 hours then centrifuged at 15,000 rpm for 10 minutes. Samples were filtered through a Whatman No. 42 filter paper then analysed by AAS (first wheat seedling experiment, Chapter 4) and ICP-OES (remainder of experimental samples).

3.3.2.3 **Calcium nitrate (CaNO$_3$)$_2$ extractions**

A solution of 0.05M Ca(NO$_3$)$_2$ was prepared according to Gray *et al.* (1999a). Prepared Ca(NO$_3$)$_2$ (30 ml) solution was added to 5 g of prepared soil, shaken for 2 hours then centrifuged at 15,000 rpm for 10 minutes. Samples were filtered through a Whatman No. 42 filter paper then analysed by ICP-OES.

3.3.2.4 **Soil solution extractions**

Soil solution was extracted from prepared lysimeter soils and soils used in the seed and germination bioassay experiments using Rhizon soil moisture samplers for soil solution extractions (MOM-type Rhizons; Eijkelkamp Agrisearch Equipment, Giesbeek, Netherlands). Soils were wetted to between 90 ± 10 % of their MWHC, left for 24 hours to equilibrate before being fitted with the soil moisture probes (Figure 3.2). The pH measurements of the solution extracted was measured and each sample was analysed for dissolved organic carbon (DOC), anions (Br$^-$, Cl$^-$, NO$_3^-$, NO$_2^-$, PO$_4^{3-}$, SO$_4^{2-}$), cations (Ca, K, Mg, Na, NH$_4^+$) and trace metals (Cd, Cu, Ni, Zn). Total organic carbon was analysed using a Shimadzu Total Organic Carbon Analyser (TOC-5000A, Shimadzu, Australia), anions and cations were analysed using Dionex DX-120 Suppressed Ion Exchange Chromatograph (Dionex Corporation, California, U.S.A.),
Flow Injection Analyser (FOSS FIAstar 5000 triple channel analyser, Foss Tecator, Hoganas, Sweden) and ICP-OES. Cadmium, Cu, Ni and Zn were analysed using ICP-OES.

Figure 3.2  Soil solution extraction using Rhizon MOM type probes from soils used in the wheat seedling bioassays described in Chapters 4 and 6
3.3.2.5 Diffusive gradient in thin-films and DIFS analyses

Analysis by diffusive gradient in thin-films (DGT) was undertaken according to the methodology described by Zhang et al. (1998). Diffusive and chelex resin gels were made from a gel preparation solution, which consists of a 2% DGT gel cross-linker (DGT Research Ltd, Lancaster, United Kingdom) mixed with an acrylamide solution (40%) and double deionised water. For the diffusive gels the gel preparation solution was mixed with ammonium persulphate solution (10%), followed by a solution of N,N,N,N-Tetramethylethylene diamine (TEMED, 99%). The prepared diffusive gel solution was pipetted into acid washed glass plates held 0.8 mm apart. Diffusive gel moulds were then placed in an oven set at 45°C for 1 hour. Set diffusive gel moulds were then removed, washed in 1 L of MQ water, and then left to hydrate for 4 hours in a fresh 1 L beaker of MQ water. Hydrated diffusive gels were washed an additional two times with fresh MQ water before being cut into size (25 mm) and stored in 0.01 M NaNO₃ solution until required.

The chelating resin was prepared using an ion exchange resin (Chelex-100), that had been pre-soaked in MQ water for 1 hour. Excess water was then decanted off the resin and 4 g of hydrated resin was added to the prepared gel solution and mixed thoroughly before a solution of ammonium persulphate solution (10%) was added followed by a solution of TEMED (99%). The resin gel mixture was then pipetted into acid washed glass plates held 0.4 apart and then placed in an oven set at 45°C for 1 hour. Set chelating gels were then removed and left to hydrate in 0.5 L of MQ water. Water was changed twice (every 4 hours), before gels were removed for cutting of moulds. Cut moulds (25 mm) were placed in 0.01 M NaNO₃ solution until required.

The assembled DGT device consisted of a plastic backing plate, followed by a chelating resin gel (0.4 mm thick), a diffusive gel (0.8 mm thick) and a mixed cellulosic ester membrane filter 0.45µm thick (Advantec, Japan) held in place by a plastic cap with a 17 mm window. Wetted soil (paste consistency) from each pot was smeared using a stainless steel spatula on the exposed filter window of the DGT device before placement onto the soils (Figure 3.2). DGT devices were left in the saturated soil for 24 hours. The resin gel layer was then removed from the DGT device and metals extracted into 10 ml 1M HNO₃ prior to analysis using ICP-OES. The concentration at
the soil-diffusive layer interface \( (C_{DGT}) \) and the effective concentration \( (C_E) \) were calculated using equations 1-3:

The mass of the metals accumulated by the resin gel was calculated using Equation 1:

\[
M = \frac{C_e(V_{HNO_3} + V_{gel})}{f_e}
\]

Equation 1

where \( C_e \) is the metal concentration in the 1M HNO\(_3\), \( V_{HNO_3} \) is the volume of HNO\(_3\), \( V_{gel} \) is the volume of gel and \( f_e \) is the elution factor for metals.

The time averaged concentration at the soil-diffusive layer interface, \( C_{DGT} \), was calculated from Equation 2:

\[
C_{DGT} = \frac{M\Delta g}{D\Delta t A}
\]

Equation 2

where \( \Delta g \) is the diffusion layer thickness, \( D \) is the diffusion coefficient of the metal in the diffusion layer at a given temperature, \( t \) is the deployment time (s) and \( A \) is the area in membrane exposed to the soil.

Effective concentration, \( (C_E) \), was calculated from Equation 3:

\[
C_E = \frac{C_{DGT}}{R_{DIFF}}
\]

Equation 3

where \( R_{DIFF} \) was calculated using the 2D-DIFS modelling programme (Harper et al., 2000) from soil density, porosity, and the diffusion coefficients for each of the metals in water and soil. The value \( R_{DIFF} \) represents the ratio of the mean interfacial concentration due to resupply by diffusion only to the initial or bulk solution concentration.
Figure 3.3   Washing of DGT device with de-ionised water after 24 hour placement on soil wetted to 90 ± 10% MWHC and allowed to equilibrate. Photo B shows removal of filter and diffusive gel, and photo C shows placement of resin gel in 10 ml of 0.01 M HNO₃.

3.3.2.6 Speciation and solid-solution phase modelling using WHAM 6.0

Soil solution speciation and solid-solution partitioning of Cd, Cu, Ni and Zn was done using WHAM (Windermere Humic Aqueous Model) version 6.0 (Tipping, 1994; 1998). The WHAM version 6 modelling programme is a discrete site and electrostatic model for simulating cation binding to humic substances. Electrostatic accumulations of ions adjacent to the molecular surfaces (non-specific binding), is simulated with Donnan-type expressions. Complexation reactions with inorganic ligands are also accounted for. WHAM 6.0 also includes a surface complexation model (SCM) with parameters for ion-binding to oxides of aluminium, iron. Manganese and silicon, and a model for cation exchange on clay.

Input parameters into the model for soil solution speciation were soil solution concentrations of Cd, Cu, Ni and Zn, major anions, (Br⁻, Cl⁻, NO₃⁻, NO₂⁻, PO₄³⁻, SO₄²⁻) and cations (Ca, K, Mg, Na, NH₄⁺). Soil solution pH, temperature (assigned value of 278 K), pCO₂ (assigned value of 0.0003 atm) were also required. Fulvic acid in solution was assumed to be 65% (Tipping et al., 2002) of the calculated amount of DOC.
Solid phase input parameters for modelling solid phase and soil solution partitioning consisted of total soil concentrations of Cd, Cu, Ni and Zn; major anions (Cl\(^-\), Nitrate-N, Nitrite-N, PO\(_4\)\(^{3-}\), SO\(_4\)\(^{2-}\)), and major cations (Ca\(^{2+}\), K\(^+\), Mg\(^{2+}\), Na\(^+\), NH\(_4\)\(^+\)), temperature, fulvic acid and pH as determined from soil solution extractions. Clay content was determined from textural analyses (Table 3.4), Al and Fe oxides using the oxalic acid extract methods (Table 3.2), particulate humic acid and fulvic acid calculated from the total soil carbon content (Table 3.2), which is assumed to be a 50:50 distribution of both, humic acid and fulvic acid (Stevenson et al., 1984; Stevenson, 1994).
Chapter 4
Evaluating the Effects of Biosolids Amendment on the Bioavailability of Cd, Cu, Ni and Zn in Soils Maintained Under Controlled Environmental Conditions

4.1 Introduction

Incubation of soils and germination of plants under controlled environmental conditions have been commonly used to investigate soil process dynamics, efficacy of soil amendments (e.g. biosolids), and changes in chemistry of metal contaminated soils (Amery et al., 2007; Brown et al., 2005; Buekers et al., 2008; Dussault et al., 2008; Öncel et al., 2000; Rooney et al., 2007a).

Biosolids and composts have been used extensively as soil amendments to reduce the bioavailability and phytotoxicity of metals in contaminated soils (Nwachukwu and Pulford, 2009; van Herwijnen et al., 2007b). The hypothesis for the use of these soil amendments is that added organic matter may induce sorption or precipitation reactions for metals, resulting in increased metal retention on the solid phase and thus a reduction in the metal fraction available for plant uptake (Nwachukwu and Pulford, 2009). The success of organic amendments is dependent upon the type of soil and source of the organic matter (Brown et al., 2005; Navas et al., 1999; Ruttens et al., 2006; Santibanez et al., 2008; van Herwijnen et al., 2007a; 2007b).

In some instances, a positive environmental effect of reduced metal availability resulting from organic amendments can also coincide with an unexpected effect of increased metal solubility. Amending soils with biosolids provides additional dissolved organic carbon (DOC) to the system. The presence of DOC, particularly humic acids, can mobilise soil nutrients and metals via decreased pH and chelation by soluble organic ligands as well as reduce the availability of metals by complexation (Antoniadis and Alloway, 2002b; Khan et al., 2006).

Increases of metal bioavailability as a result of additional DOC can occur in a variety of ways, including extra sorption sites provided by biosolids may be lost rapidly as a result of microbial
decomposition (Hooda and Alloway, 1994a; McBride, 1995); the acidic groups contained in DOC such as carboxyl and phenolic OH functional groups decrease pH, thereby increasing metal solubility (Khan et al., 2006; Marschner and Kalbitz, 2003) and HA competing for free metal ions to form soluble complexes, thereby reducing metal adsorption onto soil surfaces and retaining these bioavailable organo-metal complexes in soil solution (Antoniadis and Alloway, 2002b; Ashworth and Alloway, 2004). Thus, while it is often hypothesized that organic-rich amendments will reduce metal bioavailability this is not always the case (Antoniadis and Alloway, 2002b; van Herwijnen et al., 2007a; van Herwijnen et al., 2007b). Therefore, further work is needed to elucidate the impact of biosolids, on heavy metal bioavailability in agricultural environments.

Obtaining an effective method to measure metal bioavailability in soils has been the subject of much research and is still inconclusive (Hamon et al., 1997; Hooda, 2007; Zhang and Young, 2005; Zhang et al., 2001). In particular, studies investigating the efficacy of metal bioavailability predictions have not yet established the effect of organic amendments on the validity of assays for metal bioavailability in soils. Therefore, the experiments in this study were used to determine the effects of organic amendments on the validity of metal bioavailability assays.

The main objective of this study was to examine the effects of biosolids amendment on the bioavailability of Cd, Cu, Ni and Zn in contrasting soil types placed in controlled environmental conditions. This was achieved using wheat (Triticum aestivum) as the model plant system. Plants were grown in three soils spiked with increasing levels of metal salts which had been incubated for two and 24 weeks.

Metal bioavailability was determined by comparing shoot metal concentrations with six different assays of soil metal extractability. Results of metal bioavailability assessments were compared to establish which assay was most successful across soils and amendments. The data from this study was used to assist in the validation of bioavailability measures to predict plant concentrations of Cd, Cu, Ni and Zn in a diverse range of New Zealand soil types, which is presented in Chapter 6.
4.2 Methods

4.2.1 Set-up of incubation trials

Approximately 90 kg each (three 25 L volume buckets) of Halkett (HK), Summit (SM) and Wakanui (WK) soils (Table 3.1) were collected from the top 10 cm of each soil profile. Each soil was divided equally into 90 portions immediately after collection, with each portion weighing $3.0 \pm 0.1$ kg, (three replicates per treatment and 10 treatments per soil). Soil portions were placed in 2 L plastic containers, and each portion received a single application of combined increasing concentrations of Cd, Cu, Ni and Zn or one of Cd only (Table 4.1). A treatment of Cd only was included as the considerably higher concentrations of Cu, Ni and Zn added may mask any effects that could result from the addition of Cd.

Metals were added as hydrated metal sulphates. Biosolids, as received, were sourced from the Christchurch City Council treatment works at Bromley and added to half of the metal treated soils at a rate equivalent to 400 kg N ha$^{-1}$ (fresh biosolids collected for this study comprised on average 5.6% of N). The rate of biosolids application was based on the maximum rate of application to agricultural soils as outlined in the guidelines regarding the safe application of biosolids to land (NZWWA, 2003). Cadmium and Ni concentrations were also based on current biosolids guideline levels (NZWWA, 2003). Copper and Zn concentrations were chosen based on previous complimentary work by Environmental Science and Research (Ltd) and the Centre for Soil and Environmental Quality, Lincoln University (McLaren and Clucas, 2001; Speir et al., 2007).

Fresh treated soil portions were watered with de-ionised water to field capacity. Soils were then placed in an incubator at $25 \pm 2^\circ$C in darkness and mixed at two week intervals. Subsamples of the treated soils were removed from the incubators after two weeks and 24 weeks for use in the seedling bioassay experiments.
Table 4.1  Soil treatments for incubation experiments. Metal were added in the form of hydrated sulphates and biosolids were applied at a rate equivalent to 400 kg N ha⁻¹.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Abbreviations</th>
<th>Cd added (mg kg⁻¹)</th>
<th>Cu added (mg kg⁻¹)</th>
<th>Ni added (mg kg⁻¹)</th>
<th>Zn added (mg kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>C</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Biosolids</td>
<td>B</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Biosolids + low metals</td>
<td>BLM</td>
<td>1</td>
<td>30</td>
<td>50</td>
<td>70</td>
</tr>
<tr>
<td>Low metals</td>
<td>LM</td>
<td>1</td>
<td>30</td>
<td>50</td>
<td>70</td>
</tr>
<tr>
<td>Biosolids + medium metals</td>
<td>BMM</td>
<td>5</td>
<td>200</td>
<td>150</td>
<td>300</td>
</tr>
<tr>
<td>Medium metals</td>
<td>MM</td>
<td>5</td>
<td>200</td>
<td>150</td>
<td>300</td>
</tr>
<tr>
<td>Biosolids + high metals</td>
<td>BHM</td>
<td>10</td>
<td>750</td>
<td>300</td>
<td>1000</td>
</tr>
<tr>
<td>High metals</td>
<td>HM</td>
<td>10</td>
<td>750</td>
<td>300</td>
<td>1000</td>
</tr>
<tr>
<td>Biosolids + Cd only</td>
<td>BCd</td>
<td>10</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cd only</td>
<td>Cd</td>
<td>10</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

4.2.2 Seedling bioassay experiments

Seedling emergence experiments were undertaken according to the methods in Smart et al. (2004). Full descriptions of plant and soil sample preparation and analytical methods were provided in section 3.3.

Before use in the seedling bioassay, samples of incubated treated soils were air dried at 23°C, ground then sieved through a 2 mm stainless steel sieve. The dried and sieved soil was weighed 200 ± 5 g into 90, 250 ml plastic pots and wetted to 50% of the soil's maximum water holding capacity (MWHC), and left to equilibrate for 24 hours. Twenty-two seeds of bread wheat (Triticum aestivum sourced from Luisetti Seed Grain Merchant, Rangiora, New Zealand) were
sown into each pot of soil. The pots were then placed in controlled light (PAR of 400 µmol m\(^{-2}\) s\(^{-1}\)) growth cabinets at 21 °C (day) and 15 °C (night) on a 12 hour light/dark cycle for 20 days. After five days, cotyledons visible above the soil surface were counted as having emerged. On the 5\(^{th}\) day emerged seedlings were then thinned to 10 plants per pot and 5 ml of nutrient solution (Ruakura solution, Smart, et al. (2004)) was added on days five, 10 and 15 after thinning. Pots were weighed daily and watered with deionised water as necessary to ensure that soils were maintained at 50% MWHC for the duration of the experiment. On day 20, seedlings were removed from the growth cabinets and photographed to record any visual differences in treatment responses. Seedlings were then harvested, washed thoroughly using deionised water and fresh weights recorded for each pot. Wheat shoots were placed overnight in a drying oven at 45 ± 1 °C, removed and weighed to record dry weight per pot. Dried plants were cut with stainless steel scissors into lengths of <0.5cm before being acid digested using 10 ml of concentrated HNO\(_3\) per sample and placed on a heating block cycle (section 3.3.1). Digested plant samples were then analysed for total Cd, Cu, Ni and Zn (section 3.3).

Extraction of soil solution and diffusive gradient in thin films (DGT) placement (sections 3.3.2.4 and 3.3.2.5) were undertaken simultaneously on the remaining soil in the pots after plant harvest. Soils were also analysed for total, EDTA extractable, and Ca(NO\(_3\))\(_2\) extractable metal concentrations (sections 3.3.2.2 and 3.3.2.3). Soil pH and soil solution pH were also measured (section 3.2). Concentrations of DOC, major anions and cations in the soil solution were also measured (section 3.3.2). Soil extraction samples were analysed for total Cd, Cu, Ni and Zn using ICP-OES, AA and GFAAS (section 3.3.2). Salinity of the soil solution was defined as the sum of Cl\(^-\), Na\(^+\) and SO\(_4^{2-}\) concentrations (Taiz et al., 1998).

Speciation and free ion activity of metals in soil solution were determined using WHAM 6.0. (Tipping, 1998). Effective concentration (C\(_E\)) was calculated from metal fluxes measured using DGT devices placed on soils and the supplementary modelling freeware 2D DIFS (Harper et al., 2000; Sochaczewski et al., 2007). Full calculations and descriptions of DGT placement, and speciation modelling using WHAM 6.0., were given in sections 3.3.2.5 and 3.3.2.6.
4.2.3 Derivation of critical levels of metals in plant tissues

Critical levels (CL) of Cd, Cu, Ni and Zn in wheat shoot tissue were obtained from Macnicol and Beckett (1985) and used to compare against plant results. Critical levels are based on a statistically significant 10 % reduction in dry weight yield.

4.2.4 Statistical analyses

Comparisons of plant metal concentrations between biosolid and non-biosolid amended soils, incubation times and treatment concentrations were analysed using paired t-tests in Sigma Plot 8.02 (SPSS, UK). Linear regressions for shoot metal concentrations and soil measured of bioavailability also used Sigma Plot 8.02. Correlations between variables and differences between metal treated soils amended and unamended with biosolids were determined using grouped and separate linear regression analyses performed in GenStat 11 (VSN International, UK).

4.3 Results

4.3.1 Changes in soil chemistry

4.3.1.1 Major cations, dissolved organic carbon and pH

Comparisons between biosolids amended and unamended metal spiked soils revealed significant increases in concentrations of dissolved organic carbon (DOC), soil solution salinity, and Ca and Mg ions in soil solution as a result of the addition of biosolids, irrespective of metal treatment concentrations (Table 4.2). Overall, the increased length of soil incubation time resulted in a significant decrease in soil and soil solution pH, as well as an increase in salinity levels of the soil solution after 24 weeks (Table 4.3).
Table 4.2  Significance levels for comparisons of general soil parameters between biosolids amended and unamended metal spiked soils (n = 180). Differences were considered significant at $P<0.05$ and highlighted in bold.

<table>
<thead>
<tr>
<th>Variable</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil pH</td>
<td>0.768</td>
</tr>
<tr>
<td>Soil solution pH</td>
<td>0.196</td>
</tr>
<tr>
<td>DOC (mg L$^{-1}$)</td>
<td>0.001</td>
</tr>
<tr>
<td>Salinity of soil solution (mg L$^{-1}$)</td>
<td>0.002</td>
</tr>
<tr>
<td>Soil solution Ca concentration (mg L$^{-1}$)</td>
<td>0.027</td>
</tr>
<tr>
<td>Soil solution Mg concentration (mg L$^{-1}$)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Table 4.3  Significance levels for comparisons of soil parameters between all treated soils sampled after two weeks and 24 weeks of incubation, n = 90. Differences were considered significant at $P<0.05$ and highlighted in bold.

<table>
<thead>
<tr>
<th>Variable</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil pH</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Soil solution pH</td>
<td>0.009</td>
</tr>
<tr>
<td>DOC (mg L$^{-1}$)</td>
<td>0.24</td>
</tr>
<tr>
<td>Salinity of soil solution (mg L$^{-1}$)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Soil solution Ca concentration (mg L$^{-1}$)</td>
<td>0.65</td>
</tr>
<tr>
<td>Soil solution Mg concentration (mg L$^{-1}$)</td>
<td>0.35</td>
</tr>
</tbody>
</table>

4.3.1.2 Metal extractability

Results from pooled soil data showed that increased metal rate resulted in an increase in metal extractability (Appendix A). The presence of biosolids significantly increased the total amount of Cu and Zn in the soils (Table 4.4). With the exception of DGT results for Zn, which showed a significant decrease in effective concentration ($C_E$), the presence of biosolids had no significant effect on EDTA extractable, Ca(NO$_3$)$_2$ extractable or soil solution metal concentrations, or on
DGT measured metal solubility and free ion activity (Table 4.4). In contrast, the longer soil incubation time of 24 weeks resulted in significant increases in the availability of Cd, Ni and Zn but not Cu compared to an incubation period of two weeks (Table 4.5). These results show that biosolids amendment did not alter the bioavailability of these metals as measured by five of the assays, irrespective of soils or treatment concentrations, but that the bioavailability of these metals did significantly increase with soil incubation time.

Table 4.4  Significance values for comparisons of six potential measures of metal bioavailability between biosolids amended and unamended metal spiked soils (log transformed data, n = 180). Differences were considered significant at $P<0.05$ and highlighted in bold.

<table>
<thead>
<tr>
<th>Method</th>
<th>Cd</th>
<th>Cu</th>
<th>Ni</th>
<th>Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total metal</td>
<td>0.090</td>
<td>&lt;0.001</td>
<td>0.060</td>
<td>0.002</td>
</tr>
<tr>
<td>EDTA</td>
<td>0.190</td>
<td>0.940</td>
<td>0.818</td>
<td>0.433</td>
</tr>
<tr>
<td>Ca(NO$_3$)$_2$</td>
<td>0.760</td>
<td>0.700</td>
<td>0.409</td>
<td>0.415</td>
</tr>
<tr>
<td>Soil solution</td>
<td>0.320</td>
<td>0.853</td>
<td>0.289</td>
<td>0.855</td>
</tr>
<tr>
<td>DGT</td>
<td>0.320</td>
<td>0.344</td>
<td>0.667</td>
<td>0.033</td>
</tr>
<tr>
<td>Free ion activity</td>
<td>0.320</td>
<td>0.335</td>
<td>0.785</td>
<td>0.816</td>
</tr>
</tbody>
</table>

Table 4.5  Significance levels for comparisons of six potential assays of metal bioavailability between treated soils incubated for two weeks and soils incubated for 24 weeks (log transformed data, n = 90). *Total metal was only measured on one occasion (24 weeks incubated soils). Differences were considered significant at $P<0.05$ and highlighted in bold.

<table>
<thead>
<tr>
<th>Method</th>
<th>Cd</th>
<th>Cu</th>
<th>Ni</th>
<th>Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total metal</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>EDTA</td>
<td>0.98</td>
<td>0.97</td>
<td>0.78</td>
<td>0.22</td>
</tr>
<tr>
<td>Ca(NO$_3$)$_2$</td>
<td>0.28</td>
<td>0.95</td>
<td>0.64</td>
<td>0.008</td>
</tr>
<tr>
<td>Soil solution</td>
<td>0.002</td>
<td>0.128</td>
<td>0.005</td>
<td>0.002</td>
</tr>
<tr>
<td>DGT</td>
<td>&lt;0.001</td>
<td>0.113</td>
<td>0.006</td>
<td>0.001</td>
</tr>
<tr>
<td>Free ion activity</td>
<td>0.001</td>
<td>0.98</td>
<td>0.003</td>
<td>0.001</td>
</tr>
</tbody>
</table>
4.3.2 Responses of plants

Over the course of the experiment, the typical changes in appearance of the seedlings were increased chlorosis, discolouration and relative stunting of plants with increasing metal treatment concentrations. These symptoms of phytotoxicity also increased in severity over the length of the experiment. Plants grown in the control pots showed no signs of discolouration or necrosis for the duration of the experiment (Figure 4.1). However, chlorosis and necrosis of some leaf tips were seen in plants grown in two of the soils (HK and SM). There were no observable changes in plant appearances for treatments B, BLM and LM across all soils. Plants grown in biosolids and medium metal treatment began to show signs of leaf discolouration (Figure 4.2). Signs of reduced shoot size first appeared in BMM treated HK soil (Figure 4.2) with symptoms of stunting, chlorosis, discolouration and necrosis were most severe in plants grown in treatments BHM and HM (Figure 4.3).

![Figure 4.1](image)

Figure 4.1  A. Appearance of wheat seedlings after 20 days of growth in untreated (control) soil previously incubated for six months at 50% of maximum water holding capacity. Scale is 10 centimetres.
Figure 4.2  A. Appearance of wheat seedlings after 20 days of growth in all three soils treated with a combination of biosolids and 5, 200, 150 and 300 mg kg$^{-1}$ of Cd, Cu, Ni and Zn (biosolids and medium metals), and incubated for 24 weeks at field capacity. B. Close-up of seedlings grown in HK soil showing first signs of chlorosis in some of the leaves as a result of soil treatment. Scale is 10 centimetres.
Figure 4.3  A. Appearance of wheat seedlings after 20 days of growth in all three soils treated with a combination of biosolids and 10, 750, 300 and 1000 mg kg$^{-1}$ of Cd, Cu, Ni and Zn, and incubated for 24 weeks at field capacity. B. Appearance of seedlings in all three soils grown in 10, 750, 300 and 1000 mg kg$^{-1}$ of Cd, Cu, Ni and Zn without the addition of biosolids. Scale is 10 centimetres.
4.3.2.1 Estimations of metal bioavailability

Log-log relationships between pooled shoot metal concentrations and the six measures of metal extractability from pooled soil results were significant ($P < 0.001$) for all four metals.

**Cadmium:** Both EDTA and Ca(NO$_3$)$_2$ extractable Cd gave the strongest correlations of the six extraction methods with shoot Cd concentrations ($r^2 = 0.63$ and 0.62 respectively, Figure 4.4B and C). The spread of data for Ca(NO$_3$)$_2$ concentrations lower than 0.01 mg kg$^{-1}$ was most likely indicative of natural attenuation in the soils (Figure 4.4C). A distinctive clustering of data for soil solution results was observed which is most likely attributed to the Cd only treatments (Figure 4.4D)

**Copper:** Even though, all six extraction methods gave significant relationships with shoot Cu concentrations, (Figure 4.5) the strength of the correlations for each were poor with the highest recorded correlation obtained for total Cu ($r^2 = 0.15$, Figure 4.5A) and the lowest obtained for soil solution Cu ($r^2 = 0.06$, Figure 4.5D)

**Nickel:** Calcium nitrate extractable Ni gave the strongest correlation with shoot Ni concentrations ($r^2 = 0.73$, Figure 4.6C) with soil solution Ni and Ni$^{2+}$ activity yielding the next strongest and very similar correlations ($r^2 = 0.66$ and 0.67 respectively, Figure 4.6D and F).

**Zinc:** As with Cd and Ni, Ca(NO$_3$)$_2$ extractable Zn yielded the strongest correlation of the six extraction methods with shoot Zn concentrations ($r^2 = 0.55$, Figure 4.7C). In comparison, soil solution Zn and Zn$^{2+}$ gave the poorest correlations ($r^2 = 0.33$ for both, Figure 4.7D and F).
Figure 4.4  Relationships determined between shoot Cd concentrations and Cd concentrations in soil obtained using various methods: (A) Total Cd, (B) EDTA-extractable Cd, (C) Ca(NO$_3$)$_2$ extractable Cd, (D) soil solution, (E) effective concentration, (F) free ion activity. A linear regression line is fitted for each plot. n = 175.
Figure 4.5  Relationships determined between shoot Cu concentrations and Cu concentrations in soil obtained using various methods: (A) Total Cu, (B) EDTA-extractable Cu, (C) Ca(NO₃)₂ extractable Cu, (D) soil solution, (E) effective concentration, (F) free ion activity. A linear regression line is fitted for each plot. n = 175.
Figure 4.6  Relationships determined between shoot Ni concentrations and Ni concentrations in soils obtained using various methods: (A) Total Ni, (B) EDTA-extractable Ni, (C) Ca(NO₃)₂ extractable Ni, (D) soil solution, (E) effective concentration, (F) free ion activity. A linear regression line is fitted for each plot. n = 175.
Figure 4.7  Relationships determined between shoot Zn concentrations and concentrations of Zn in the soil obtained using various methods: (A) Total Zn, (B) EDTA-extractable Zn, (C) Ca(NO₃)₂ extractable Zn, (D) soil solution, (E) effective concentration, (F) free ion activity. A linear regression line is fitted for each plot. n = 175.
Comparisons of the coefficients of determinations for all assays of bioavailability and shoot metal concentrations, revealed that Ca(NO₃)₂ extractable metals gave the strongest relationship for shoot and soil data overall, closely followed by EDTA extractable metals. Cadmium, Cu and Zn concentrations in the soil solution gave the poorest correlations with corresponding shoot metal concentrations. However, for Ni the relationship determined between soil solution Ni and shoot Ni concentrations was second highest and similar to the r² value obtained for Ni²⁺ activity and shoot Ni concentrations (Figure 4.6). Relationship strengths for C_E were similar and strongest for Ni and Zn and shoot concentrations (Figure 4.6E and 4.7E). However these r² values were considerably lower than Ca(NO₃)₂ extractable and EDTA extractable correlations (Figure 4.6 B-C and 4.7B-C).

### 4.3.2.2 Dry matter production

Dry matter production significantly increased (P< 0.001) as soil incubation time increased (Figure 4.8). Dry weight appeared to decrease with increasing metal concentrations but the comparison of pooled treatment results for biosolids amended metal spiked soils gave no significant differences in dry weight (P = 0.08) due to the presence of biosolids. However, further analysis of the relationship between dry weight and Ca(NO₃)₂ extractable Cd, Cu, Ni and Zn concentrations revealed that Cu and Ni significantly decreased dry matter production irrespective of the presence of biosolids, while Cd and Zn only significantly decreased dry matter production in the presence of biosolids (Figure 4.9). Correlations were strongest for relationships between dry weight and extractable metal concentrations in metal treated soils amended with biosolids (Figure 4.9)
Figure 4.8 Mean dry weights of wheat seedlings grown in biosolids amended and unamended metal treated soils incubated for two weeks and 24 weeks at field capacity. Bars denote the SEM, n = 89 for both trials.
Figure 4.9  A-D. Relationships determined between plant dry weight and corresponding Ca(NO₃)₂ extractable metal concentrations in biosolids amended and unamended metal treated soils, (A) = Cd, (B) = Cu, (C) = Ni, (D) = Zn, n = 179.

Other soil chemistry variables, such as pH, major cations and the salinity of the soil solution, were explored for significant correlations and of these it was found that only dry weight production correlated significantly with salinity ($P < 0.001$). Furthermore, comparisons with correlation strengths of metals and dry weights, and salinity and dry weights, revealed that salinity gave the highest correlation for pooled soil data ($r^2 = 0.28$) and that every 100 mg L⁻¹
An increase in salinity resulted in a 5% reduction in dry weight production (Figure 4.10). Further examination of the relationship between salinity and dry weight revealed that the addition of biosolids significantly decreased the overall dry weight production \((P = 0.017)\) compared to the unamended metal spike soils.

![Figure 4.10](image)

**Figure 4.10** Relationship determined between plant dry weight and the salinity of the soil solution, \(n = 179\).

These results indicate that while the addition of biosolids did not significantly alter the effect that each of the four metals had on dry weight production \((P = 0.426, 0.171, 0.231\) and 0.189 for Cd, Cu, Ni and Zn respectively), it did result in significant change in the gradients for Cd and Cu, i.e. biosolids altered the relationship between these metals and dry weight \((Cd, P = 0.004\) and for Cu, \(P = 0.030)\). Although the strongest influence exerted on dry weight production resulted from an increase in amounts of salinity as a consequence of biosolids amendment rather than additional metal loading from either biosolids or metal salt application (Table 4.2).
4.3.2.3 Metal concentrations in wheat shoots

Soil incubation time had a significant effect ($P < 0.001$) on wheat shoot metal concentration and generally metal concentrations were lower in plants grown in soils incubated for 24 weeks (Table 4.6). There were also significant differences ($P < 0.05$) in shoot metal concentrations between soil types (Appendix A).

**Cadmium:** The critical level (CL) for wheat of 43 mg kg$^{-1}$ for Cd was not reached at either sampling time (Figure 4.11) and incubation time had no significant effect on overall shoot Cd concentrations ($P = 0.12$). Comparisons of shoot Cd concentrations in plants grown in biosolids amended metal spiked soils to unamended metal spiked soils revealed a significant decrease in shoot Cd concentrations in plants grown in biosolids amended soils for both soil incubation times (Table 4.6). This significant decrease in shoot Cd concentration in the presence of biosolids was observed across all levels of metal treatment concentrations (Table 4.7).

**Copper:** Concentrations of Cu in the shoots were significantly higher in plants grown in soils incubated for two weeks ($P < 0.001$) compared with plants grown in soils incubated for 24 weeks. The CL for wheat of 17 mg kg$^{-1}$ for Cu was only significantly ($P < 0.05$) exceeded in plants grown in the two week incubated BHM and HM treated soils (Figure 4.12). The presence of biosolids resulted in significant increases in shoot Cu concentrations in plants grown in soils incubated for two weeks (Table 4.6). However, this significant effect was not observed in plants grown in soil incubated for 24 weeks (Table 4.6). Moreover, comparisons of shoot Cu concentrations between biosolids amended and unamended metal spiked soil at each treatment level revealed no significant differences ($P > 0.05$, Table 4.7).

**Nickel:** Nickel concentrations in the shoots generally increased with increasing treatment concentrations and the CL for wheat of 46 mg kg$^{-1}$ was exceeded in treatments HM and BHM for both soil incubation periods (Figure 4.13). Overall soil incubation time resulted in no significant differences in shoot Ni concentrations ($P = 0.90$) and amendment with biosolids did not produce a significant effect on shoot Ni concentrations at either soil incubation period (Table 4.6). However, at the lowest level of metal treatment concentration the presence of biosolids did
significantly increase shoot Ni concentrations (Table 4.7), although this increase was relatively small (Figure 4.13).

**Zinc:** Plants grown in soils incubated for two weeks had significantly higher shoot Zn concentrations ($P = 0.009$) compared with plants grown in soils incubated for 24 weeks. The CL for shoot Zn concentrations in wheat (224 mg kg$^{-1}$) was exceeded in the highest metal treatment at both soil incubation periods (Figure 4.14). The addition of biosolids to metal spiked soils did not significantly impact on overall shoot concentrations (Table 4.6). Furthermore, comparisons at individual metal treatment levels revealed no significant differences in Zn shoot concentrations (Table 4.7).

![Figure 4.11](image_url)  
**Figure 4.11**  Mean Cd concentrations in wheat shoot tissue (mg kg$^{-1}$ dry weight) grown in biosolids amended and unamended metal spiked soils incubated for two weeks and 24 weeks. The critical level (CL) for Cd as depicted by the red line is 43 mg kg$^{-1}$ (Macnicol and Beckett, 1985). Bars denote the SEM, $n = 89$ for both trials.
Figure 4.12  Mean Cu concentrations in wheat shoots (mg kg\(^{-1}\) dry weight) grown in biosolids amended and unamended metal spiked soils incubated for 2 weeks and 24 weeks. The critical levels (CL) for Cu as depicted by the red line, is 17 mg kg\(^{-1}\) (Macnicol and Beckett, 1985). Bars denote the SEM, n = 89 for both trials.
Figure 4.13  Mean Ni concentrations in wheat shoots (mg kg^{-1} dry weight) grown in biosolids amended and unamended metal spiked soils incubated for 2 weeks and 24 weeks. The critical levels (CL) for Ni as depicted by the red line, is 46 mg kg^{-1} (Macnicol and Beckett, 1985). Bars denote the SEM, n = 89 for both trials.

Figure 4.14  Mean Zn concentrations in wheat shoot tissue (mg kg^{-1} dry weight) grown in biosolids amended and unamended metal spiked soils incubated for 2 weeks and 24 weeks. The critical levels (CL) for Zn as depicted by the red line, is 224 mg kg^{-1} (Macnicol and Beckett, 1985). Bars denote the SEM, n = 89 for both trials.
Table 4.6  (A) Significance levels for comparisons between shoot metal concentrations in plants grown in biosolids-amended and unamended metal spiked soils (log transformed data, n = 35). (B) Significance levels for comparisons between two and 24 weeks incubation (log transformed data, n = 35). Differences were considered significant at \(P<0.05\) and highlighted in bold.

<table>
<thead>
<tr>
<th></th>
<th>Cd</th>
<th>Cu</th>
<th>Ni</th>
<th>Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Two weeks incubation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cd</td>
<td>0.003</td>
<td></td>
<td>0.390</td>
<td>0.388</td>
</tr>
<tr>
<td>Cu</td>
<td>0.024</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ni</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zn</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>24 weeks incubation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cd</td>
<td>0.042</td>
<td></td>
<td>0.368</td>
<td>0.449</td>
</tr>
<tr>
<td>Cu</td>
<td>0.368</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ni</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zn</td>
<td></td>
<td></td>
<td></td>
<td>0.415</td>
</tr>
</tbody>
</table>

(B) Comparisons between soil incubation times (two weeks vs 24 weeks)

<table>
<thead>
<tr>
<th></th>
<th>Cd</th>
<th>Cu</th>
<th>Ni</th>
<th>Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Biosolids amended metal spiked soils</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cd</td>
<td>0.229</td>
<td>&lt;0.001</td>
<td>0.877</td>
<td>0.008</td>
</tr>
<tr>
<td>Cu</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ni</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zn</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Unamended metal spiked soils</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cd</td>
<td>0.695</td>
<td>&lt;0.001</td>
<td>0.112</td>
<td>0.013</td>
</tr>
<tr>
<td>Cu</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ni</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zn</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 4.7  Significance levels for comparisons between shoot metal concentrations in plants grown in biosolids-amended and unamended metal treated soils, in order of increasing metal treatment levels (log transformed data, n = 70). Differences were considered significant at \(P<0.05\) and highlighted in bold.

<table>
<thead>
<tr>
<th>Metal treatment concentrations</th>
<th>Cd</th>
<th>Cu</th>
<th>Ni</th>
<th>Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td>BLM vs LM</td>
<td>&lt;0.001</td>
<td>0.062</td>
<td>&lt;0.001</td>
<td>0.234</td>
</tr>
<tr>
<td>BMM vs MM</td>
<td>&lt;0.001</td>
<td>0.062</td>
<td>0.954</td>
<td>0.234</td>
</tr>
<tr>
<td>BHM vs HM</td>
<td>&lt;0.001</td>
<td>0.062</td>
<td>0.954</td>
<td>0.234</td>
</tr>
<tr>
<td>BCd vs Cd</td>
<td>0.001</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
4.3.3 Effects of biosolids amendments on relationships between soil extraction methods and metal concentrations in shoots

The assessment of relationship strengths between six different extraction methods of metals from soils and total metal concentrations in plants (Figure 4.4 – 4.7) revealed that Ca(NO₃)₂ extractable metals gave the best estimate of bioavailability. Therefore, Ca(NO₃)₂ extractable metal concentrations were used to explore any potential impacts that amendment of metal spiked soils with biosolids may have on correlation strengths of bioavailability estimates.

There were no significance differences in relationships determined between extractable metal concentrations and shoot metal concentrations in the presence or absence of biosolids for Cd, Cu, Ni or Zn (Figure 4.15). A linear regression analysis of the grouped data revealed that for Cd, the addition of biosolids significantly reduced overall shoot Cd concentrations ($P = 0.023$, Figure 4.15A). This significant result for the reduction of Cd concentration in shoots in the presence of biosolids also supports findings presented in section 4.3.2.3 (Table 4.6 and 4.7). Comparisons made of the gradients of Cd bioavailability estimates between biosolids amended soils and unamended soils revealed no significant changes ($P = 0.625$), indicating that the relationship between Cd concentrations in shoots and Ca(NO₃)₂ extractable Cd in the presence or absence of biosolids were not significantly different. Consequently this result provides evidence that Cd bioavailability as measured by Ca(NO₃)₂ is not dependent on the presence or absence of biosolids.

For Cu, Ni and Zn, the addition of biosolids did not significantly alter Cu, Ni or Zn shoot concentrations ($P = 0.389$, $P = 0.576$, $P = 0.598$ for Cu, Ni and Zn respectively). These findings are also supported by results presented in section 4.3.5.3 (Table 4.6 and 4.7). Furthermore, there were no significant differences found between the gradients of biosolids amended soils and unamended soils for either Cu (Figure 4.15B), Ni (Figure 4.15C) or Zn (Figure 4.15D). These results are consistent with the findings for Cd, that Cu, Ni and Zn bioavailability as measured by Ca(NO₃)₂ is not dependent on substrate type.
Previously described results for the $C_E$ of Zn in biosolids amended soils and unamended soils revealed significant difference between the groups (Table 4.4). Hence, the relationship between concentrations of Zn in shoots and $C_E$ were compared for biosolids amended and unamended metal spiked soils (Figure 4.16). For both biosolids amended and unamended metal spiked soils there was a significant relationship ($P<0.001$). Comparisons made between the gradients for biosolids amended and unamended soils yielded no significant difference ($P = 0.423$). These
findings confirm that the presence of biosolids did not alter the relationship between metal concentrations in shoots and $C_{\text{E}}$-Zn and thus, providing further validation that soil estimates of metal bioavailability are independent of biosolids amendments.

![Graph showing relationship between shoot Zn concentration and effective concentration as affected by amendment with biosolids, n = 71 (Biosolids + MS) and n = 72 (MS).](image)

Figure 4.16  Relationship between shoot Zn concentration and effective concentration as affected by amendment with biosolids, n = 71 (Biosolids + MS) and n = 72 (MS).

4.4 Discussion

4.4.1 Influence of biosolids on the availability of metals in soils

The addition of biosolids significantly (Table 4.2 and 4.4) increased the concentration of DOC, salinity, Ca and Mg in soil solution as well as increasing the total amount of soil Cu and Zn. These differences resulted from the higher concentrations of these parameters present in biosolids than levels found naturally in the soils used in this study (section 3.2, Appendix A). The amendment of metal spiked soils with biosolids did not significantly affect metal availability as measured by the six methods assessed (Table 4.4). The partitioning of metals in the biosolids amended and unamended metal
spiked soils were also compared and these results concurred with the findings of the soil extractability methods (Appendix A).

While addition of biosolids did not alter the extractability of these metals, soil incubation time did significantly increase the availability of Cd, Ni and Zn, which may be attributed to the significant (Table 4.3 and 4.5) decrease in soil and soil solution pH that was also observed. Traditionally, soil organic matter decomposition is considered one of the main contributing factors to decrease in soil pH (Helyar and Porter, 1989). This along with microbial and chemical degradation of biosolids would most likely contribute to the decrease in pH as well as the production of significantly higher levels of DOC (Table 4.2). However the degradation of biosolids alone did not contribute to the decrease in pH (Table 4.2). Thus a combination of soil organic matter decomposition, biosolids decomposition and adsorption to soil particulate matter that results in release of H⁺ are the mostly likely factors that resulted in the decrease in pH.

The decrease in pH after 24 weeks of incubation is likely to have been the main factor contributing to the increase in dissolved Cd, Ni and Zn concentrations (Table 4.5) and several studies have linked the solubility of Cd, Ni and Zn to the pH of the soil (Gray and McLaren, 2006; McBride et al., 1997a; Sauve et al., 2000a; Stephan et al., 2008). Another possible cause for increased concentrations of dissolved Cd, Ni and Zn, is the presence of increased amounts of DOC. Corresponding increases in the concentration of these metals in the solution phase and DOC has been found in a number of studies (Amery et al., 2007; Antonladis et al., 2008; Ashworth and Alloway, 2004; Doig and Liber, 2007; McBride et al.; 1997b; van Herwijnen et al., 2007a). Overall DOC concentrations did not change with increased incubation time, although the presence of biosolids did result in an increase in DOC compared to unamended soils. The pH of the soil and soil solution decreased with increased incubation time, while biosolids amendment of soils did not result in any pH changes. Increased Cd, Ni and Zn in solution with incubation time cannot directly be attributed to DOC it must therefore be attributed mainly to the change in pH.

Interestingly, the percentage of Cu and Ni present in the dissolved phase as modelled by WHAM 6.0. were predicted to be higher in the soil incubated for 24 weeks than soil incubated for two weeks. Actual measurements of Cu and in particular Ni,
directly measured after two and 24 weeks of incubation revealed that Cu and Ni were present in lower concentrations after 24 weeks of incubation compared with concentrations measured after two week of incubation (Appendix A). Although no direct explanation for the modelled and measured discrepancies can be deduced from this study, two recent studies by Amery et al. (2007; 2008) demonstrated that the mobilising potential of Cu depends upon the degree of humification of dissolved organic matter (DOM). This finding may also partly explain the difference in measured and modelled Ni (Amery et al., 2007). Nickel is similar to Cu in that it has strong affinity for binding to organic matter (Sauve, et al., 2000), and so the degree of aromaticity may also contribute to the increased mobilisation of Ni as no relationship between total DOC and an increase in dissolved Ni concentrations was observed.

Unlike Cu and Ni, Cd affinity for DOC is rather small as Cd adsorbs weakly to organic matter, clays and oxides unless the pH is greater than 6 (Degryse et al., 2007). As a majority of the pH values were below 6, DOC would have had little influence in the solubility of Cd. While incubation time clearly had an effect on the solubility of Cd (Table 4.5), the presence of biosolids did not (Table 4.4). Thus it appears that the solubility of Cd is more strongly influenced by pH than DOC, and this relationship has been demonstrated by many studies (Antoniadis and Alloway, 2002b; Collins et al., 2003b; Degryse et al., 2007; Gray and McLaren, 2006; McBride et al., 1997a Meers et al.; 2005; Shi et al., 2007). Likewise, the same relationship with pH and solubility can be described for Zn (Antoniadis et al., 2008).

4.4.2 Responses of plants grown in metal spiked soils in the presence and absence of biosolids

4.4.1.1 Dry weight reduction

Examination of relationships between soil variables revealed two factors controlling the production of dry matter, namely salinity of the soil solution and, concentrations of Cu and Ni in the shoots (Figure 4.9 and 4.10). Of these two factors, salinity had the strongest relationship with dry weight decreases (Figure 4.10). While increased incubation time resulted in an increase in salinity (Table 4.3), salinity was
significantly higher in soils amended with biosolids irrespective of the incubation period (Table 4.2). The appearance and texture of plants grown in soils containing both medium to high levels of metals and biosolids, suggested that plants were under severe stress either as result of toxic concentrations of metals or osmotic pressures, or both (Figure 4.2 and 4.3). While it was expected that degrees of phytotoxicity would be exhibited by plants grown in the medium to high metal treatments, the osmotic stress induced by the presence of biosolids was unforeseen. Biosolids used in this study contained higher concentrations of exchangeable Na, K, Ca and Mg than were measured in the unamended soils used in this study (Table 3.3). In addition, metal salt dissolution would most likely have contributed to the overall increase in salinity in all amended soils. While dry matter production was most strongly influenced by salinity levels in the current study, the concentrations measured in the soil solution were well below levels considered saline (40 mM of NaCl which generates an osmotic pressure of approximately 0.2 Mpa) for soils (Munns and Tester, 2008).

Concentrations of Cu, Ni and Zn were present above critical thresholds in plants grown in the highest metal treatments, irrespective of the presence of biosolids (Figure 4.12 and 4.14). While Cu, Ni and Zn concentrations in shoots exceeded the CL in one or both incubated soil periods, only Cu and Ni gave significant correlations with dry weight reduction in the absence of biosolids (Figure 4.9B and C). The morphological symptoms of interveinal discolouration and leaf chlorosis, observed in the plants were highly suggestive of metal toxicity (Figure 4.2 and 4.3), where the appearance of interveinal foliar chlorosis is commonly linked to initial stages of Cu toxicity (Reichman, 2002), and interveinal chlorosis of leaves is often ascribed to Ni toxicity (Molas and Baran, 2004). However, further analysis would be needed to quantify and separate the effects that Cu or Ni had on plant growth.

### 4.4.1.2 Metal concentrations in shoots

Overall, Cd concentrations in shoots were reduced in plants grown in metal spiked soils amended with biosolids compared to unamended metal spiked soils. This outcome is in disagreement with the non-significant changes found between Cd soil extractability in biosolids amended and unamended soils (Table 4.4). Increased DOC,
salinity, Ca and Mg were the only significant changes in soil chemistry observed between amended and unamended metal spiked soils. Correlations between these variables and Cd concentrations in the shoots were only significant for DOC, but this relationship was poor \( (P = 0.019, r^2 = 0.06, \text{Appendix A}) \) and salinity, which was also poorly correlated \( (P < 0.001, r^2 = 0.19, \text{Appendix A}) \). Thus no strong direct relationship could be inferred from these soil chemistry parameters and reduced plant Cd uptake. While findings in this study appear to support the retention of Cd in biosolids amended soils, limitations in the experimental set-up and analyses make it difficult to accurately attribute the mechanism(s) by which Cd retention is occurring. Studies on the availability and uptake of Cd in plants have identified pH and DOC as the major influential factors controlling soil availability (Collins et al., 2003b; Gray et al., 1999b; 2003; McLaughlin et al., 2006). As pH did not significantly change with the addition of biosolids, this can not be directly attributed to the reduction in Cd concentrations in shoots measured in this study. From these results two possible mechanisms may be responsible for this effect:

- Dissolved organic matter sourced from biosolids reducing the availability of Cd in soil solution to the plants via complexation of free Cd\(^{2+}\) and weakly sorbed Cd (Merrington and Smernik, 2004);

- Addition of contaminants and salts in biosolids (i.e. Zn and Ca ions) which competitively inhibit the uptake of Cd by plants. This mechanism assumes that metal uptake by plants is mediated by a generic, divalent cation transporter and, many studies have confirmed that this is the most likely transport mechanism for micronutrient uptake (Reid et al., 2003a).

The addition of biosolids did not result in significant changes in Cu, Ni (with the exception of the low treatment) and Zn concentrations in the shoots compared to unamended treatments (Table 4.6 and 4.7), which is in agreement with the non-significant changes found in the soil extractability/solubility of these metals (Table 4.4). However, increased soil incubation time did result in significant reductions in Cu and Zn concentrations in the shoots (Table 4.6). The reduction of Cu and Zn concentration in shoots may be attributed to increased complexation of free metal ions by DOC, although estimations of speciation distributions between the two soil incubation periods do not clearly correspond to this hypothesis (Appendix A).
Complexation of metals by DOC could partly account for the decrease in shoot concentrations Cu and Zn, although competitive uptake as result of increased salinity in the soil solution with increased incubation time (Table 4.2) could also explain the reduction in Cu and Zn shoot concentrations. Further work would be required to elucidate the mechanism(s) responsible for the reduction in Cu and Zn shoot concentrations and the relationship with soil incubation time.

4.3.2 Estimates of metal bioavailability

Pooled soil and plant data revealed that Ca(NO$_3$)$_2$ extracted concentrations of Cd, Ni and Zn gave the strongest relationship with plant shoot concentrations (Figure 4.4 – 4.7). Correlation strengths for ETDA and total metal concentrations were generally second highest, while soil solution, $C_E$ and free ion activity were often the weakest correlations. Of the four metals, Ca(NO$_3$)$_2$ extractable Ni and corresponding shoot concentrations revealed the highest correlation ($r^2 = 0.73$). Nickel results for the remaining five extraction methods were also the most closely correlated (Figure 4.6). Results for Ni indicate that plant uptake of Ni is strongly indicative of the amount of Ni extracted from the soil using the six methods assessed, particularly 0.05 M 0f Ca(NO$_3$)$_2$. Hence the solubility of Ni as measured by Ca(NO$_3$)$_2$ extraction can reliably predict the degree of phytotoxicity and growth limitations resulting from excess amounts present in soils. The robustness of this method is further validated by the fact that the correlation was obtained across a range of soils, metal concentrations as in the presence and absence of biosolids.

Coefficient of determination values between extractable amounts of Cu from the soil and shoot Cu concentrations were poor in comparison to Cd, Ni and Zn. Copper is an essential trace element whose concentration is strongly regulated by the plant and is known to accumulate in, or around the roots, thus correlation between shoot Cu concentrations and soil Cu extractability tests are often poor as shoot concentrations will typically be unreflective of the extractability or solubility of Cu (Menzies et al., 2007). Therefore, root concentrations may provide stronger relationships with extractable Cu and this relationship has been suggested as an alternative to shoot concentrations (Chaignon et al., 2003). Other studies examining the success of predictions of Cu uptake by wheat using methods such as DGT and free ion activity
also found that these yielded poor relationships and that total Cu gave the best fit (Menzies et al., 2007; Nolan et al., 2005)

Responses for Cd and Zn were similar with Ca(NO₃)₂ and EDTA extractable concentrations providing the best relationships with shoot concentrations \(r^2 = 0.54\) and \(r^2 = 0.63\), Figure 4.4 and Figure 4.7). These results indicate that shoot accumulation is moderately responsive to the availability of Cd and Zn as measured by Ca(NO₃)₂ and EDTA. However, from a predictive perspective, correlation results for Cd and Zn methods need improving and this may be achieved by either including soluble inorganic and/or organic complexes of these metals, or sorption suppression by competing cations (Antonladis and Tsadilas, 2007; Degryse et al., 2009b). The overall success of Ca(NO₃)₂ to predict shoot accumulation of Cd, Ni and Zn does support the theory that weak salt extractants, such as Ca(NO₃)₂ are based on the assumption that bioavailable trace elements are located on the mineral surface and can be easily displaced by other desorbing cations such as Ca (Andrews et al., 1996; McLaughlin et al., 2006).

Bioavailability estimated by \(C_E\) and free ion activity typically provided the poorest correlations with shoot concentrations (although results for Ni\(^{2+}\) activity were reasonable). This result may be partly explained by plant uptake of metal complexes and/or the dissociation of these complexes at the plant root-soil interface, which are not accurately accounted for by these methods. Furthermore, a study by Berkelaar and Hale (2003) found that plants either take up the complexes, or by alleviation of diffusional constraints for uptake of the free ion, through dissociation of labile metal complexes in the diffusive boundary. Also, in another experiment using the same wheat variety the relationship between \(C_E\) and free ion activity of Cd, Cu, Ni and Zn showed that the uptake of these metals was not only controlled by the free ion form, but it is also dependent on the concentrations of metal ligand complexes (Degryse et al., 2006a). These poor relationships could also be attributed to the large variations in measured concentrations that can arise when quantifying very small pools of metals in a complex and dynamic system, which would make obtaining a strong correlation difficult.

The success of these tests were not influenced by biosolids amendments which was evident in the examination of the relationship between Ca(NO₃)₂, the \(C_E\) of metals and
shoot concentrations (Figure 4.15 and 4.16). This result concurs with some recent studies (McBride et al., 2009; Menzies et al., 2007), which provides evidence to the hypothesis that the bioavailable metals, especially Ni are located on mineral surfaces (Andrews et al., 1996; McLaughlin et al., 1999).

4.5 Conclusions

Treating and incubating three contrasting soil types with metal salts in the presence and absence of biosolids provided a unique opportunity to observe the overarching effects of biosolids on soils and plants and the interaction between the two, with respect to metal chemistry. The addition of biosolids did not significantly alter the availability of Cd, Cu, Ni and Zn as determined by EDTA, Ca(NO₃)₂, soil solution, DGT and free ion activity. However, the presence of biosolids did result in significant increases of salinity, DOC, total Cu and Zn, as well as concentrations of Ca and Mg. Dry matter production was adversely affected by increased levels of salinity, which in turn was significantly influenced by the presence of biosolids. Concentrations of Cu and Ni also negatively impacted on dry weight however these were not as strongly correlated as levels of salinity were with dry weight. Concentrations of Cd in shoots were significantly lower in biosolids amended soils, irrespective of soil incubation time.

From a predictive perspective, the best overall measure of Cd, Zn and especially Ni concentrations in wheat plants was Ca(NO₃)₂-extractable soil metal. Measures of soil solution, Cₑ and free ion activity were poorly correlated with Cd and Zn shoot concentrations. Of the four metals, Ni consistently yielded the strongest correlations with all methods, while predictions of Cu concentrations in shoots were typically poor. This suggests that while Ni concentrations in wheat shoots are highly reflective of Ni solubility, shoot concentrations of Cu are non-responsive to the availability of Cu. Examination of the relationships between metal shoot concentrations and measures of metal availability for biosolids amended and unamended metal spiked soils revealed that the addition of biosolids did not alter the success of these tests. As this study was carried out on a range of soils, with varying metal concentrations and
biosolids, these findings support the conclusion that Ca(NO₃)₂ extraction is a robust method to predict Cd and Ni bioavailability.
Chapter 5
Evaluating the Effects of Biosolids and Metal Salt Applications on the Bioavailability of Cd, Cu, Ni and Zn in Soils Managed Under Field Conditions.

5.1 Introduction

Field studies using intact soil monolith lysimeters have been used to study the effects of soil amendments on the plant uptake and mobility of nutrients and non essential trace elements (Antoniadis, 2008; Deram et al., 2006; Goss and Ehlers, 2009; McLaren et al., 2003; 2004; 2005; Ruttens et al., 2006). Previous work carried out by Lincoln University and Environmental Science and Research Ltd. have used lysimeters as well as field trials to study the effects of amending soils with biosolids and metal salts on bioavailability (McLaren et al., 2003; 2004; 2005; Speir et al., 2004). One main advantage of conducting field trials over environmentally controlled pot trials is that soil and pasture are studied under more natural conditions, thereby potentially increasing the applicability to soil use and management practices (Goss and Ehlers, 2009).

The same rationale for examining the efficacy of biosolids amendment on metal contaminated soils outlined in Chapter 4 was employed in this study. However, the study in Chapter 4 was carried out under controlled environmental conditions in pots, while the study described in this chapter has been modified to examine the effects of biosolids amendments, metal salt applications, and time on soil chemistry, metal availability and plant responses grown in free draining soils managed under field conditions.

The main objective of this study was to examine the effects of biosolids amendment and metal salt applications on the bioavailability of Cd, Cu, Ni and Zn in contrasting soil types managed under field conditions over a 24-month period. The data generated was also used to assess the validity of measures developed to predict shoot
concentrations of Cd, Cu, Ni and Zn in a diverse range of New Zealand soil types, which is presented in Chapter 6.

5.2 Methods

5.2.1 Set-up of lysimeter experiment and treatment of soils

Twenty one lysimeters of approximately 20 cm diameter and 30 cm depth were collected from each of the Halkett (HK), Summit (SM) and Wakanui (WK) (Table 3.1). The design of the lysimeter castings and the methods of soil sampling were undertaken according to methods referenced in Cameron et al. (1992) and McLaren et al. (2003; 2004; 2005). Sixty three lysimeters in total were transported back to Lincoln University and placed randomly around a dug-out trench, located at the Lincoln University field laboratory (Figure 5.1). Lysimeters were housed in-ground to provide thermal insulation and natural environmental exposure.
Figure 5.1  Map view and photo of field lysimeter set-up. Locations of soil and treatments within the trench are also shown.

5.2.2 Soil treatments

In October 2006, the top 10 cm of each soil monolith was removed and thoroughly mixed with a single application of combined increasing concentrations of Cd, Cu, Ni and Zn and one of Cd only (Table 5.1). As with the treatment applications in Chapter 4, metals were added as hydrated metal sulphates. Biosolids, as received, were
sourced from the Christchurch City Council treatment works at Bromley and added to untreated soils and also to the lowest metal treatment concentration only at a rate equivalent to 400 kg N ha\(^{-1}\) (fresh biosolids collected for this study comprised on average 5.6% of N). The rate of biosolids application was based on the maximum rate of application to agricultural soils as outlined in the guidelines regarding the safe application of biosolids to land (NZWWA, 2003). Cadmium and Ni concentrations chosen were also based on current biosolids guideline levels (NZWWA, 2003). Copper and Zn concentrations were chosen based on previous complimentary work by Environmental Science and Research Ltd. and Lincoln University (McLaren and Clucas, 2001; Speir \textit{et al.}, 2007). Ryegrass (\textit{Lolium perenne}, sourced from Luisetti Seed Grain Merchant, Rangiora, New Zealand) was sown immediately after treatment application. During January to March, 40 mm of water was added five times per week to maintain plant growth.

Table 5.1  Soil treatments for field lysimeter experiment. Metals were added in the form of sulphates and biosolids were applied at a rate equivalent to 400 kg N ha\(^{-1}\)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Abbreviations</th>
<th>Cd added (mg kg(^{-1}))</th>
<th>Cu added (mg kg(^{-1}))</th>
<th>Ni added (mg kg(^{-1}))</th>
<th>Zn added (mg kg(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>C</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Biosolids</td>
<td>B</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Biosolids + low metals</td>
<td>BLM</td>
<td>1</td>
<td>30</td>
<td>50</td>
<td>70</td>
</tr>
<tr>
<td>Low metals</td>
<td>LM</td>
<td>1</td>
<td>30</td>
<td>50</td>
<td>70</td>
</tr>
<tr>
<td>Medium metals</td>
<td>MM</td>
<td>5</td>
<td>200</td>
<td>150</td>
<td>300</td>
</tr>
<tr>
<td>High metals</td>
<td>HM</td>
<td>10</td>
<td>750</td>
<td>300</td>
<td>1000</td>
</tr>
<tr>
<td>Cd only</td>
<td>Cd</td>
<td>10</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
5.2.3 Harvesting of ryegrass and sampling of soil

Ryegrass was harvested six months after treatment applications and thereafter at six-monthly intervals. A mixture of commercial grade superphosphate at a rate equivalent to 0.078 kg m\(^{-2}\) and urea at a rate equivalent to 0.042 kg m\(^{-2}\) was applied to the soil monoliths after each harvest. Immediately after harvest, the top 10 cm of the soil was sub-sampled (one core per lysimeter) using a steel corer, approximately 2.5 cm in diameter and 15 cm long. Sampled soil was air dried at 25\(^\circ\)C and then passed through a 2 mm sieve prior to analysis.

5.2.4 Analysis of plant and soil samples

Fresh plant cuttings were weighed to record the fresh weights. Plant cuttings were dried at 45\(^\circ\)C removed and re-weighed. Dried plants were ground into small pieces using an A10 Yellowline plant grinder (IKA Ltd. Germany) then digested using 10 ml of concentrated HNO\(_3\) and placed on a heating block cycle (section 3.3.1). Digested plant samples were then analysed for total Cd, Cu, Ni and Zn using ICP-OES (section 3.3.1).

Soils were also analysed for total, EDTA extractable, and Ca(NO\(_3\))\(_2\) extractable metal concentrations (section 3.3.2.2 and 3.3.2.3). Extraction of soil solution and diffusive gradient in thin films (DGT) placement (section 3.3.2.4 and 3.3.2.5) were undertaken simultaneously on the remaining soil which had been placed in plastic pots and wetted to 90 ± 10 % of the soils maximum water holding capacity and left to equilibrate for 24 hours (section 3.2). Soil pH and soil solution pH were also measured (section 3.2). Concentrations of DOC, major anions and cations in the soil solution were also measured (section 3.3.2.4). Salinity of the soil solution was defined as the sum of Cl\(^-\), Na\(^+\) and SO\(_4^{2-}\) concentrations (Taiz \textit{et al.}, 1998).

Speciation and free ion activity of metals in soil solution were determined using WHAM 6.0. (Tipping, 1998). Effective concentration (C\(_E\)) was calculated from metal
fluxes measured using DGT devices placed on soils and the supplementary modelling freeware 2D DIFS (Harper et al., 2000; Sochaczewski et al., 2007). A full description of the calculations and methods involved in DGT –DIFS and speciation modelling using WHAM 6.0. were given in sections 3.3.2.5 and 3.3.2.6. All soil extractions were analysed using ICP-OES (section 3.3.2).

5.2.5 Derivation of critical levels of metals in plant tissues

Critical levels (CL) of Cd, Cu, Ni and Z in ryegrass shoot tissue used to compare against plant results in this chapter were obtained from Macnicol and Beckett (1985). Critical levels are based on a statistically significant 10 % reduction in dry matter yield.

5.2.6 Statistical analyses

Comparisons of shoot metal concentrations, soil chemistry and assays of bioavailability between biosolids and non-biosolids amended soils used paired t-tests (Sigma Plot 8.02, SPSS, UK). Linear regressions were also carried out using Sigma Plot 8.02. Differences in correlation determination values between biosolids amended soils and unamended soils used grouped and separate linear regression analyses performed in GenStat 11 (VSN International, UK). The effect of time on metal availability and shoot metal concentrations used a generalised linear model performed in S-Plus 4.5 (Mathsoft, U.S.A.).
5.3 Results

5.3.1 Changes in soil chemistry

5.3.1.1 Dissolved organic carbon, pH and major anions and cations

Comparisons between biosolids amended (BLM) and unamended (LM) metal spiked soils revealed significant increases in concentrations of DOC, Ca and Mg in soil solution as well as an increase in salinity in soils amended with biosolids (Table 5.2). All soil solution variables shown in Table 5.2 changed significantly with time (Table 5.4). There were significant decreases in soil pH, soil solution pH and DOC, and of these, soil solution pH showed the highest overall rate of decrease (Table 5.3 and 5.4). In contrast, soil solution salinity and concentrations of Ca and Mg increased with time, of which Ca in soil solution showed the highest rate of increase (Table 5.3 and 5.4).

Table 5.2 Significance levels for comparison s of general soil parameters between pooled results for biosolids-amended (BLM) and unamended (LM) metal spiked soils (n = 27). Differences were considered significant at \( P<0.05 \) and highlighted in bold (*data log\(_{10}\)-transformed).

<table>
<thead>
<tr>
<th>Variable</th>
<th>( P )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil pH</td>
<td>0.564</td>
</tr>
<tr>
<td>Soil solution pH</td>
<td>0.091</td>
</tr>
<tr>
<td>DOC (mg L(^{-1}))</td>
<td>0.030</td>
</tr>
<tr>
<td>Salinity of soil solution (mg L(^{-1})) *</td>
<td>0.007</td>
</tr>
<tr>
<td>Soil solution Ca concentration (mg L(^{-1})) *</td>
<td>0.008</td>
</tr>
<tr>
<td>Soil solution Mg concentration (mg L(^{-1})) *</td>
<td>0.027</td>
</tr>
</tbody>
</table>
Table 5.3  Mean soil chemistry characteristics from pooled treated soils, sampled six months, 12 months and 24 months after treatment application (values are means, n = 63).

<table>
<thead>
<tr>
<th>Variable</th>
<th>6 months</th>
<th>12 months</th>
<th>24 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil pH</td>
<td>5.59</td>
<td>5.71</td>
<td>5.19</td>
</tr>
<tr>
<td>Soil solution pH</td>
<td>5.36</td>
<td>5.28</td>
<td>4.53</td>
</tr>
<tr>
<td>DOC (mg L⁻¹)</td>
<td>143.3</td>
<td>224.8</td>
<td>101.4</td>
</tr>
<tr>
<td>Salinity of soil solution (mg L⁻¹)</td>
<td>62.6</td>
<td>75.7</td>
<td>127.3</td>
</tr>
<tr>
<td>Soil solution Ca concentration (mg L⁻¹)</td>
<td>27.8</td>
<td>53.2</td>
<td>113.5</td>
</tr>
<tr>
<td>Soil solution Mg concentration (mg L⁻¹)</td>
<td>4.54</td>
<td>9.54</td>
<td>16.58</td>
</tr>
</tbody>
</table>

Table 5.4  Effect of time calculated from residual deviance (variation due to treatment effects removed) on soil chemistry characteristics. Effects were considered significant at $P<0.05$ and highlighted in bold (*data log₁₀-transformed). $P$-value for the T distribution is also included.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Residual deviance</th>
<th>Degrees of freedom</th>
<th>$P$-value</th>
<th>Gradient</th>
<th>$P$-value for T distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil pH</td>
<td>34.85</td>
<td>181</td>
<td>&lt;0.001</td>
<td>-0.200</td>
<td>0.841</td>
</tr>
<tr>
<td>Soil solution pH</td>
<td>64.72</td>
<td>181</td>
<td>&lt;0.001</td>
<td>-0.418</td>
<td>0.679</td>
</tr>
<tr>
<td>DOC (mg L⁻¹)*</td>
<td>74.58</td>
<td>181</td>
<td>0.013</td>
<td>-0.233</td>
<td>0.818</td>
</tr>
<tr>
<td>Salinity of soil solution (mg L⁻¹)*</td>
<td>60.23</td>
<td>181</td>
<td>&lt;0.001</td>
<td>0.362</td>
<td>0.717</td>
</tr>
<tr>
<td>Soil solution Ca concentration (mg L⁻¹)*</td>
<td>147.5</td>
<td>181</td>
<td>&lt;0.001</td>
<td>0.6599</td>
<td>0.515</td>
</tr>
<tr>
<td>Soil solution Mg concentration (mg L⁻¹)*</td>
<td>131.9</td>
<td>181</td>
<td>&lt;0.001</td>
<td>0.609</td>
<td>0.543</td>
</tr>
</tbody>
</table>

5.3.1.2  Metal extractability

Results from the pooled data showed that the presence of biosolids had a significant effect on the concentration of metal extracted using EDTA, $C_E$ and modelled free ion activity (Table 5.5). The addition of biosolids resulted in an increase in the EDTA-extractable Cd and Ni, $C_E$-Zn, as well as modelled Cu²⁺ activity (Table 5.5). In contrast the amount of $C_E$-Cd and $C_E$-Ni as measured by DGT decreased significantly in biosolids amended soils compared to unamended soils. Moreover, in the case of Ni,
the amount of Ni from the biosolids amended soils was less than half of the amount extracted from the unamended soils. The modelled activities of Ni$^{2+}$ and Zn$^{2+}$ also significantly decreased in the presence of biosolids (Table 5.5).

Table 5.5  Significance levels for comparisons of six potential measures of metal bioavailability between pooled sampling results for biosolids amended (BLM) and unamended (LM) metal spiked soils (log transformed data, n = 27). Differences were considered significant at $P<0.05$ and highlighted in bold.

<table>
<thead>
<tr>
<th>Method</th>
<th>Cd</th>
<th>Cu</th>
<th>Ni</th>
<th>Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total metal</td>
<td>0.754</td>
<td>0.626</td>
<td>0.993</td>
<td>0.525</td>
</tr>
<tr>
<td>EDTA</td>
<td><strong>0.013</strong></td>
<td>0.363</td>
<td><strong>0.001</strong></td>
<td>0.147</td>
</tr>
<tr>
<td>Ca(NO$_3$)$_2$</td>
<td>0.362</td>
<td>0.088</td>
<td>0.498</td>
<td>0.464</td>
</tr>
<tr>
<td>Soil solution</td>
<td>0.272</td>
<td>0.657</td>
<td>0.087</td>
<td>0.093</td>
</tr>
<tr>
<td>DGT</td>
<td><strong>0.030</strong></td>
<td>0.143</td>
<td><strong>0.025</strong></td>
<td><strong>0.037</strong></td>
</tr>
<tr>
<td>Free ion activity</td>
<td>0.869</td>
<td><strong>0.009</strong></td>
<td>0.038</td>
<td><strong>0.033</strong></td>
</tr>
</tbody>
</table>

**Cadmium:** Pooled soil results revealed overall there was little significant change in the availability of Cd from soil with time. Of the six measures of Cd extractability, only soil solution Cd and Cd$^{2+}$ activity significantly increased with time (Table 5.6 and 5.7), with Cd$^{2+}$ activity having a higher rate of increase than concentrations of Cd in solution.

**Copper:** As with Cd, there were only two significant effects of time on on Cu availability in the soil. C$_E$-Cu as measured by DGT and Cu$^{2+}$ activity increased significantly over time, with Cu$^{2+}$ activity showing the highest rate of increase (Table 5.8 and 5.9).

**Nickel:** Of the six measures of Ni availability in soil, only Ni$^{2+}$ activity increased significantly over time (Table 5.10 and 5.11).

**Zinc:** Compared with other metals tested Zn had the most significant changes in metal extractability and solubility with time (Table 5.12 and 5.13). Total-Zn and C$_E$-Zn both decreased significantly with time since treatment application, whilst soil solution-Zn and Zn$^{2+}$ activity increased significantly with time. Rates of increase in
Zn$^{2+}$ activity were similar to those of Cd$^{2+}$ activity (Table 5.7) and Ni$^{2+}$ activity (Table 5.11).

### Table 5.6  Means for six potential measures of Cd bioavailability taken from pooled soil results from each sampling occasion (n = 63 for each sampling occasion).

<table>
<thead>
<tr>
<th>Method</th>
<th>6 months</th>
<th>12 months</th>
<th>24 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Cd (mg kg$^{-1}$)</td>
<td>14.2</td>
<td>12.8</td>
<td>15.2</td>
</tr>
<tr>
<td>EDTA (mg kg$^{-1}$)</td>
<td>17.0</td>
<td>15.1</td>
<td>27.3</td>
</tr>
<tr>
<td>Ca(NO$_3$)$_2$(mg kg$^{-1}$)</td>
<td>7.173</td>
<td>6.130</td>
<td>6.37</td>
</tr>
<tr>
<td>Soil solution (mg L$^{-1}$)</td>
<td>1.33</td>
<td>0.17</td>
<td>0.43</td>
</tr>
<tr>
<td>DGT (mmol L$^{-1}$)</td>
<td>1.01</td>
<td>0.76</td>
<td>1.40</td>
</tr>
<tr>
<td>Cd$^{2+}$ activity (mol L$^{-1}$)</td>
<td>0.44 x 10$^{-6}$</td>
<td>0.62 x 10$^{-6}$</td>
<td>0.15 x 10$^{-5}$</td>
</tr>
</tbody>
</table>

### Table 5.7  Effect of time as calculated from residual deviance (variation due to treatment effects removed) on six measures of Cd bioavailability. Effects were considered significant at $P<0.05$ and highlighted in bold (*data log$_{10}$-transformed). $P$-value for the T distribution is also included.

<table>
<thead>
<tr>
<th>Method</th>
<th>Residual deviance</th>
<th>Degrees of freedom</th>
<th>$P$-value</th>
<th>Gradient</th>
<th>$P$-value for T-distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Cd (mg kg$^{-1}$)</td>
<td>52.46</td>
<td>181</td>
<td>0.427</td>
<td>0.029</td>
<td>0.976</td>
</tr>
<tr>
<td>EDTA (mg kg$^{-1}$)</td>
<td>423.8</td>
<td>181</td>
<td>0.104</td>
<td>0.021</td>
<td>0.983</td>
</tr>
<tr>
<td>Ca(NO$_3$)$_2$(mg kg$^{-1}$)</td>
<td>107.3</td>
<td>181</td>
<td>0.1214</td>
<td>1.489</td>
<td>0.138</td>
</tr>
<tr>
<td>Soil solution (mg L$^{-1}$)</td>
<td>132.4</td>
<td>181</td>
<td><strong>0.002</strong></td>
<td><strong>0.344</strong></td>
<td>0.731</td>
</tr>
<tr>
<td>DGT (mmol L$^{-1}$)</td>
<td>430.7</td>
<td>181</td>
<td>0.758</td>
<td>-0.027</td>
<td>0.978</td>
</tr>
<tr>
<td>Cd$^{2+}$ activity (mol L$^{-1}$)</td>
<td>941.6</td>
<td>181</td>
<td><strong>&lt;0.001</strong></td>
<td><strong>0.784</strong></td>
<td>0.434</td>
</tr>
</tbody>
</table>
Table 5.8 Means for six potential measures of Cu bioavailability taken from pooled soil results from each sampling occasion (n = 63 for each sampling occasion).

<table>
<thead>
<tr>
<th>Method</th>
<th>6 months</th>
<th>12 months</th>
<th>24 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total (mg kg⁻¹)</td>
<td>481.0</td>
<td>410.0</td>
<td>474.0</td>
</tr>
<tr>
<td>EDTA (mg kg⁻¹)</td>
<td>392.0</td>
<td>302.0</td>
<td>369.0</td>
</tr>
<tr>
<td>Ca(NO₃)₂ (mg kg⁻¹)</td>
<td>46.10</td>
<td>31.10</td>
<td>48.70</td>
</tr>
<tr>
<td>Soil solution (mg kg⁻¹)</td>
<td>3.51</td>
<td>1.72</td>
<td>5.03</td>
</tr>
<tr>
<td>DGT (mmol L⁻¹)</td>
<td>29.8</td>
<td>9.3</td>
<td>26.1</td>
</tr>
<tr>
<td>Cu²⁺ activity (mol L⁻¹)</td>
<td>1.90 x 10⁻⁵</td>
<td>2.56 x 10⁻⁶</td>
<td>3.09 x 10⁻⁵</td>
</tr>
</tbody>
</table>

Table 5.9 Effect of time as calculated from residual deviance (variation due to treatment effects removed) on six measures of Cu bioavailability. Effects were considered significant at \( P < 0.05 \) and highlighted in bold (*data log₁₀-transformed). \( P \)-value for the T distribution is also included.

<table>
<thead>
<tr>
<th>Method</th>
<th>Residual deviance</th>
<th>Degrees of freedom</th>
<th>( P )-value</th>
<th>Gradient ( P )-value for T-Distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total (mg kg⁻¹)</td>
<td>52.46</td>
<td>181</td>
<td>0.405</td>
<td>0.040</td>
</tr>
<tr>
<td>EDTA (mg kg⁻¹)</td>
<td>423.8</td>
<td>181</td>
<td>0.239</td>
<td>0.161</td>
</tr>
<tr>
<td>Ca(NO₃)₂ (mg kg⁻¹)</td>
<td>107.3</td>
<td>164</td>
<td>0.121</td>
<td>-0.119</td>
</tr>
<tr>
<td>Soil solution (mg kg⁻¹)</td>
<td>132.4</td>
<td>181</td>
<td>0.152</td>
<td>0.110</td>
</tr>
<tr>
<td>DGT (mmol L⁻¹)</td>
<td>430.7</td>
<td>181</td>
<td>&lt;0.001</td>
<td>0.689</td>
</tr>
<tr>
<td>Cu²⁺ activity (mol L⁻¹)</td>
<td>941.6</td>
<td>181</td>
<td>&lt;0.001</td>
<td>1.436</td>
</tr>
</tbody>
</table>

Table 5.10 Means for six potential measures of Ni bioavailability taken from pooled soil results for each sampling occasion (n = 63 for each sampling occasion).

<table>
<thead>
<tr>
<th>Method</th>
<th>6 months</th>
<th>12 months</th>
<th>24 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total (mg kg⁻¹)</td>
<td>70.3</td>
<td>65.4</td>
<td>54.4</td>
</tr>
<tr>
<td>EDTA (mg kg⁻¹)</td>
<td>76.2</td>
<td>58.5</td>
<td>61.5</td>
</tr>
<tr>
<td>Ca(NO₃)₂ (mg kg⁻¹)</td>
<td>46.6</td>
<td>39.8</td>
<td>31.2</td>
</tr>
<tr>
<td>Soil solution (mg L⁻¹)</td>
<td>2.59</td>
<td>2.22</td>
<td>4.13</td>
</tr>
<tr>
<td>DGT (mmol L⁻¹)</td>
<td>17.2</td>
<td>8.8</td>
<td>14.3</td>
</tr>
<tr>
<td>Ni²⁺ activity (mol L⁻¹)</td>
<td>2.45 x 10⁻⁵</td>
<td>1.98 x 10⁻⁵</td>
<td>3.24 x 10⁻⁵</td>
</tr>
</tbody>
</table>
Table 5.11  Effect of time as calculated from residual deviance (variation due to treatment effects removed) on six measures of Ni bioavailability. Effects were considered significant at \( P<0.05 \) and highlighted in bold (*data log10-transformed). \( P \)-value for the T distribution is also included.

<table>
<thead>
<tr>
<th>Method</th>
<th>Residual deviance</th>
<th>Degrees of freedom</th>
<th>( P )-value</th>
<th>Gradient</th>
<th>( P )-value for T-distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total (mg kg(^{-1}))</td>
<td>30.68</td>
<td>181</td>
<td>0.208</td>
<td>-0.046</td>
<td>0.963</td>
</tr>
<tr>
<td>EDTA (mg kg(^{-1}))</td>
<td>378.8</td>
<td>181</td>
<td>0.228</td>
<td>0.156</td>
<td>0.876</td>
</tr>
<tr>
<td>Ca(NO(_3))(_2) (mg kg(^{-1}))</td>
<td>142.1</td>
<td>171</td>
<td>0.053</td>
<td>-0.168</td>
<td>0.866</td>
</tr>
<tr>
<td>Soil solution (mg L(^{-1}))</td>
<td>167.3</td>
<td>168</td>
<td>0.056</td>
<td>0.180</td>
<td>0.857</td>
</tr>
<tr>
<td>DGT (mmol L(^{-1}))</td>
<td>153</td>
<td>178</td>
<td>0.088</td>
<td>0.143</td>
<td>0.886</td>
</tr>
<tr>
<td>Ni(^{2+}) activity (mol L(^{-1}))</td>
<td>298.6</td>
<td>180</td>
<td>(&lt;0.001)</td>
<td>0.742</td>
<td>0.459</td>
</tr>
</tbody>
</table>

Table 5.12  Means for six potential measures of Zn bioavailability taken from pooled soil results from each sampling occasion (\( n = 63 \) for each sampling occasion).

<table>
<thead>
<tr>
<th>Method</th>
<th>6 months</th>
<th>12 months</th>
<th>24 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total (mg kg(^{-1}))</td>
<td>209.6</td>
<td>184.0</td>
<td>167.3</td>
</tr>
<tr>
<td>EDTA (mg kg(^{-1}))</td>
<td>170.0</td>
<td>124.0</td>
<td>186.0</td>
</tr>
<tr>
<td>Ca(NO(_3))(_2) (mg kg(^{-1}))</td>
<td>95.0</td>
<td>124.0</td>
<td>186.0</td>
</tr>
<tr>
<td>Soil solution (mg L(^{-1}))</td>
<td>5.20</td>
<td>4.75</td>
<td>11.10</td>
</tr>
<tr>
<td>DGT (mmol L(^{-1}))</td>
<td>27.6</td>
<td>19.9</td>
<td>26.8</td>
</tr>
<tr>
<td>Zn(^{2+}) activity (mol L(^{-1}))</td>
<td>(3.88 \times 10^3)</td>
<td>(3.35 \times 10^3)</td>
<td>(7.65 \times 10^3)</td>
</tr>
</tbody>
</table>

Table 5.13  Effect of time calculated from residual deviance (variation due to treatment effects removed) on six measures of Zn bioavailability. Effects were considered significant at \( P<0.05 \) and highlighted in bold (*data log10-transformed). \( P \)-value for the T distribution is also included.

<table>
<thead>
<tr>
<th>Method</th>
<th>Residual deviance</th>
<th>Degrees of freedom</th>
<th>( P )-value</th>
<th>Gradient</th>
<th>( P )-value for T-distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total (mg kg(^{-1}))</td>
<td>23.63</td>
<td>181</td>
<td>0.039</td>
<td>-0.067</td>
<td>0.946</td>
</tr>
<tr>
<td>EDTA (mg kg(^{-1}))</td>
<td>225.8</td>
<td>181</td>
<td>0.009</td>
<td>0.261</td>
<td>0.764</td>
</tr>
<tr>
<td>Ca(NO(_3))(_2) (mg kg(^{-1}))</td>
<td>93.52</td>
<td>173</td>
<td>0.692</td>
<td>-0.027</td>
<td>0.878</td>
</tr>
<tr>
<td>Soil solution (mg L(^{-1}))</td>
<td>163</td>
<td>181</td>
<td>(0.031)</td>
<td>0.184</td>
<td>0.854</td>
</tr>
<tr>
<td>DGT (mmol L(^{-1}))</td>
<td>98.93</td>
<td>178</td>
<td>(&lt;0.001)</td>
<td>-0.927</td>
<td>0.355</td>
</tr>
<tr>
<td>Zn(^{2+}) activity (mol L(^{-1}))</td>
<td>245.2</td>
<td>178</td>
<td>(&lt;0.001)</td>
<td>0.774</td>
<td>0.439</td>
</tr>
</tbody>
</table>
Additionally, pooled soil solution compositions were taken 24 months after treatment application from 10 cm and 20 cm depths. Comparisons between the depths found that with change in depth there was no significant difference in DOC levels \( (P = 0.434) \), nor pH \( (P = 0.235) \). However, there was a 10-fold reduction in soil solution Cd, Cu, Zn as well as Ca and Mg at depth \( (P < 0.001) \), but no significant difference in Ni concentrations \( (P = 0.321) \). These results suggest that Ni in solution is more evenly distributed at least in the upper part (< 30 cm depth) of the soil horizon, while soluble Cd, Ca, Cu, Mg and Zn appear to largely be contained within the top 5 to 10 cm of the soil.

5.3.2 Responses of plants

Ryegrass growth in the treated lysimeters was well established by three months after treatment application in all but the two highest metal salt treatments (MM and HM). At the time of the six month harvest, ryegrass had yet to germinate in either MM or HM treatments across all soils (Figure 5.2). Subsequent harvestings of ryegrass from these treatments at 12, 18 and 24 months produced extremely low yields, and at 24 months there were still six lysimeters out of 63 in which ryegrass had still failed to germinate. In the MM and HM treated soils, eventual occurrence of grass growth in these lysimeters was restricted to the soil closest to the lysimeter edges. Ryegrass grown in the Cd-only treated lysimeters appeared to have shorter and thinner blades than those in the control, biosolids or low metal treated soils.
Figure 5.2  Appearance of ryegrass in two of the soil lysimeters six months after treatment application (MM = medium metal treatment).

5.3.2.1 Metal concentrations in ryegrass

A general comparison between the pooled data of ryegrass grown in biosolids amended and unamended metal treated soil over 24 months revealed that the addition of biosolids had no significant effect on the herbage concentrations of Cd ($P = 0.054$), Cu ($P = 0.126$), Ni ($P = 0.076$) or Zn ($P = 0.167$). Moreover, only plant concentrations of Ni (decreased) and Zn (increased) changed significantly with time ($P < 0.001$ for both Ni and Zn).

**Cadmium:** Concentrations of Cd generally increased with increasing treatment concentrations (Figure 5.3). However, the critical level (CL) for mature ryegrass of 30-35 mg kg$^{-1}$ was not exceeded at any stage of the trial. Mean Cd concentrations in shoots over the experiment were not significantly affected by the presence of biosolids. However, a comparisons of pooled data at 24 months revealed a significant ($P = 0.001$) decrease in Cd concentrations in shoots of plants grown in biosolids.
amended soils compared to unamended soils (Table 5.14). A comparison of Cd shoot concentrations over time revealed no significant changes ($P = 0.188$, Table 5.15).

**Copper:** There was little variation in Cu shoot concentrations with the CL for mature ryegrass of 30-35 mg kg$^{-1}$ only exceeded at the highest metal treatment level (Figure 5.4). Although the presence of biosolids had no significant effect on the shoot concentrations of Cu over the 24 month trial, significant ($P < 0.001$) decreases in Cu shoot concentration were observed at 18 and 24 months in grass grown in biosolids amended soils (Table 5.14). A comparison of Cu shoot concentrations over the length of the trial revealed no significant change with time ($P = 0.131$, Table 5.15).

**Nickel:** The CL range of 130-220 mg kg$^{-1}$ for mature ryegrass of Ni in shoots was exceeded in the two highest metal concentration treatments (Figure 5.5). The addition of biosolids had no significant effect on Ni shoot concentrations at any of the harvest times (Table 5.14). With the removal of variation due to treatment effects, results revealed that there was an overall significant ($P < 0.001$) decrease (0.65 mg kg$^{-1} = \text{antilog of } -0.185$, Table 5.15) in shoot Ni concentrations over the duration of the experiment.

**Zinc:** Generally shoot Zn concentrations increased with increasing treatment concentrations (Figure 5.6) and the CL range of 370-650 mg kg$^{-1}$ for mature ryegrass was only exceeded in the highest metal treatment. Although the presence of biosolids had no significant effect on the overall concentration of Zn in shoots, there was a significant decrease observed in shoot Zn concentrations at the 24 month harvest in ryegrass grown in biosolids amended soils compared with unamended soils ($P < 0.001$, Table 5.14). Analysis of the shoot Zn concentrations over time revealed significant increase in shoot concentrations of Zn ($P < 0.001$, 1.32 mg kg$^{-1} = \text{antilog of } 0.12$, Table 5.15).
Figure 5.3  Mean Cd concentrations in ryegrass shoot tissue (mg kg\(^{-1}\) dry weight) grown in biosolids amended and unamended metal spiked soils managed under field conditions over 24 months. The critical level (CL) for Cd as depicted by the red line is 30-35 mg kg\(^{-1}\) (Macnicol and Beckett, 1985). Bars denote the SEM, n = 45, 54, 56 and 59 for 6, 12, 18 and 24 months, respectively.
Figure 5.4  Mean Cu concentrations in ryegrass shoot tissue (mg kg⁻¹ dry weight) grown in biosolids amended and unamended metal spiked soils managed under field conditions over 24 months. The critical level (CL) for Cu as depicted by the red line is 30-35 mg kg⁻¹ (Macnicol and Beckett, 1985). Bars denote the SEM, n = 45, 54, 56 and 59 for 6, 12, 18 and 24 months, respectively.
Figure 5.5  Mean Ni concentrations in ryegrass shoot tissue (mg kg\(^{-1}\) dry weight) grown in biosolids amended and unamended metal spiked soils managed under field conditions over 24 months. The critical level (CL) for Ni as depicted by the red line is 130-220 mg kg\(^{-1}\) (Macnicol and Beckett, 1985). Bars denote the SEM, \(n = 45, 54, 56\) and 59 for 6, 12, 18 and 24 months, respectively.
Figure 5.6  Mean Zn concentrations in ryegrass shoot tissue (mg kg\(^{-1}\) dry weight) grown in biosolids amended and unamended metal spiked soils managed under field conditions over 24 months. The critical level (CL) for Zn as depicted by the red line is 370-560 mg kg\(^{-1}\) (Macnicol and Beckett, 1985). Bars denote the SEM, n = 45, 54, 56 and 59 for 6, 12, 18 and 24 months, respectively.

Table 5.14  Significance levels for comparisons between shoot metal concentrations in plants grown in biosolids-amended (BLM) and unamended (LM) metal spiked soils harvested after treatment application (log transformed data, n = 45, 54, 56 and 59 for 6, 12, 18 and 24 months, respectively). Differences were considered significant at \(P<0.05\) and highlighted in bold.
Table 5.15  Effect of time calculated from residual deviance (variation due to treatment effects removed) on plant uptake of metals. Effects were considered significant at $P<0.05$ and highlighted in bold (*data log10-transformed). $P$-value for the T distribution is also included.

<table>
<thead>
<tr>
<th>Metal</th>
<th>Residual deviance</th>
<th>Degrees of freedom</th>
<th>$P$-value</th>
<th>Gradient</th>
<th>$P$-value for T-Distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cd</td>
<td>135.2</td>
<td>206</td>
<td>0.188</td>
<td>-0.069</td>
<td>0.968</td>
</tr>
<tr>
<td>Cu</td>
<td>108.9</td>
<td>214</td>
<td>0.131</td>
<td>-0.041</td>
<td>0.967</td>
</tr>
<tr>
<td>Ni</td>
<td>84.12</td>
<td>207</td>
<td>&lt;0.001</td>
<td>-0.185</td>
<td>0.853</td>
</tr>
<tr>
<td>Zn</td>
<td>39.33</td>
<td>206</td>
<td>&lt;0.001</td>
<td>0.120</td>
<td>0.904</td>
</tr>
</tbody>
</table>

5.3.2.2  Estimations of metal availability

Log-log relationships determined between pooled shoot metal concentrations and the six measures of metal availability taken from pooled soil results were significant ($P < 0.001$) for all four metals.

**Cadmium:** Total-Cd and soil solution Cd gave the strongest correlations with shoot Cd concentration of the six methods assessed ($r^2 = 0.73$ and 0.71 respectively, Figure 5.7A and D). The poorest correlation obtained was for EDTA-extractable and Ca(NO$_3$)$_2$-extractable Cd ($r^2 = 0.28$ and 0.34 respectively Figure 5.7B and C) and shoot Cd concentration. Effective concentrations of Cd and Cd$^{2+}$ activity gave very similar correlations with Cd shoot concentrations ($r^2 = 0.65$ and 0.64, respectively, Figure 5.7E and F).

**Copper:** Correlations for Cu were the poorest of all metals (Figure 5.8). The highest $r^2$ value obtained between Cu shoot concentrations and assay of Cu bioavailability was for Ca(NO$_3$)$_2$-extractable Cu ($r^2 = 0.59$, Figure 5.8C), followed by total-Cu and soil solution-Cu ($r^2 = 0.49$ for both measures, Figure 5.8and B). Of the six methods, Cu$^{2+}$ activity gave the poorest correlation with shoot concentrations ($r^2 = 0.07$, Figure 5.8F).

**Nickel:** Correlations for extractable and soluble Ni and shoot Ni concentrations were the strongest of the four metals (Figure 5.9). Of these, Ca(NO$_3$)$_2$-extractable Ni gave the strongest correlation ($r^2 = 0.87$, Figure 5.9C) closely followed by CE-Ni, soil solution Ni and total Ni ($r^2 = 0.84$, 0.83 and 0.82 respectively, Figure 5.9E, D and A). EDTA-extractable Ni yielded the poorest correlation result with $r^2 = 0.44$ (Figure 5.9B).
**Zinc:** Effective concentration of Zn gave the strongest correlation with shoot Zn concentrations ($r^2 = 0.76$, Figure 5.10E), which was closely followed by Zn$^{2+}$ activity, soil solution Zn and Ca(NO$_3$)$_2$-extractable Zn ($r^2 = 0.68$, 0.68 and 0.63 respectively, Figure 5.10F, D and C). As with Ni and Cd, EDTA extractable Zn gave the poorest correlation result with shoot Zn concentrations ($r^2 = 0.35$, Figure 5.10B).
Figure 5.7 Relationships determined between soil extractable Cd and shoot Cd concentration using various extractants; (A) Total Cd, (B) EDTA-extractable Cd (C) Ca(NO₃)₂ extractable Cd, (D) soil solution, (E) effective concentration, (F) free ion activity. A linear regression line is fitted for each plot. n = 157.
Figure 5.8  Relationships determined between soil extractable Cu and shoot Cu concentration using various methods; (A) Total Cu, (B) EDTA-extractable Cu (C) Ca(NO₃)₂ extractable Cu, (D) soil solution, (E) effective concentration, (F) free ion activity. A linear regression line is fitted for each plot. n = 157.
Figure 5.9  Relationships determined between soil extractable Ni and shoot Ni concentration using various extractants; (A) Total Ni, (B) EDTA-extractable Ni (C) Ca(NO₃)₂ extractable Ni, (D) soil solution, (E) effective concentration, (F) free ion activity. A linear regression line is fitted for each plot. n = 157.
Figure 5.10 Relationships determined between soil extractable Zn and shoot Zn concentration using various extractants; (A) Total Zn, (B) EDTA-extractable Zn (C) Ca(NO$_3$)$_2$ extractable Zn, (D) soil solution, (E) effective concentration, (F) free ion activity. A linear regression line is fitted for each plot. $n = 157$. 
5.3.2.3 Effects of biosolids amendments on relationships between soil extraction methods and metal concentration in plants

Evaluations of the correlation strengths determined between six potential measures of bioavailability and shoot metal concentrations (Figure 5.7-5.10) revealed that soil solution concentrations of metals gave the best overall estimate of bioavailability. However results for Ca(NO₃)₂ extractable metals, EDTA-extractable metals, effective solution concentrations and free ion activities of metals were also used to explore any potential impacts that the addition of biosolids may have on the relationships obtained from pooled BLM and LM treatments.

**Cadmium**: Only two of the five methods gave overall significant relationships with shoot Cd concentrations (Table 5.16), namely soil solution and Cd²⁺ activity ($P = 0.018$ for both methods). Further analyses of gradients for all five methods revealed that biosolids did not affect the relationship determined between the measures of bioavailable Cd and shoot Cd concentrations (Table 5.16).

**Copper**: None of the five methods assessed for results from treatments BLM and LM gave significant relationships between the estimates of bioavailable metal concentrations and Cu concentrations in shoots (Table 5.17). Moreover, gradient analyses of the five methods revealed the addition of biosolids had no significant effect on the relationships determined between bioavailable Cu and shoot Cu concentrations (Table 5.17).

**Nickel**: Relationships determined between the overall available Ni concentrations and shoot concentrations of Ni were not significant (Table 5.18). Analyses of the gradients revealed that the addition of biosolids significantly affected the relationships determined between both soil solution Ni and Ni²⁺ activity and concentrations of Ni in shoots ($P = 0.024$ and $0.006$ for soil solution Ni and Ni²⁺ activity respectively). Also the presence of biosolids significantly ($P = 0.047$) decreased the plant uptake of Ni in soil solution (Table 5.18).

**Zinc**: As with Cu, none of the five methods for estimating bioavailability gave significant relationships with shoot concentrations of Zn (Table 5.19). Analyses of the gradients also revealed that as with Cd and Cu the presence of biosolids does not significantly impact upon the relationship determined between shoot metal
concentrations and measures of bioavailability. Hence these results indicate that bioavailability of Cd, Cu and Zn as measured by these methods are not dependent upon substrate type.

Table 5.16  Significance levels obtained from grouped regression analyses for relationships determined between Cd concentrations in ryegrass and five potential measures of Cd availability in biosolids-amended (BLM) and unamended (LM) metal spiked soils. Differences were considered significant at $P<0.05$ and highlighted in bold ($n = 27$).

<table>
<thead>
<tr>
<th>Method</th>
<th>Overall $P$ value</th>
<th>Presence of biosolids ($P$-value)</th>
<th>Gradient difference ($P$-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EDTA</td>
<td>0.063</td>
<td>0.194</td>
<td>0.187</td>
</tr>
<tr>
<td>Ca(NO$_3$)$_2$</td>
<td>0.437</td>
<td>0.117</td>
<td>0.799</td>
</tr>
<tr>
<td>Soil solution</td>
<td>0.018 ($r^2 = 0.00$)</td>
<td>0.376</td>
<td>0.293</td>
</tr>
<tr>
<td>DGT</td>
<td>0.312</td>
<td>0.227</td>
<td>0.910</td>
</tr>
<tr>
<td>Cd$^{2+}$ activity</td>
<td>0.018 ($r^2 = 0.00$)</td>
<td>0.317</td>
<td>0.860</td>
</tr>
</tbody>
</table>

Table 5.17  Significance levels obtained from grouped regression analyses for relationships determined between Cu concentrations in ryegrass and five potential measures of Cu availability in biosolids-amended (BLM) and unamended (LM) metal spiked soils. Differences were considered significant at $P<0.05$ and highlighted in bold ($n = 27$).

<table>
<thead>
<tr>
<th>Method</th>
<th>Overall $P$ value</th>
<th>Presence of biosolids ($P$-value)</th>
<th>Gradient difference ($P$-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EDTA</td>
<td>0.149</td>
<td>0.919</td>
<td>0.940</td>
</tr>
<tr>
<td>Ca(NO$_3$)$_2$</td>
<td>0.769</td>
<td>0.822</td>
<td>0.293</td>
</tr>
<tr>
<td>Soil solution</td>
<td>0.491</td>
<td>0.783</td>
<td>0.918</td>
</tr>
<tr>
<td>DGT</td>
<td>0.233</td>
<td>0.640</td>
<td>0.739</td>
</tr>
<tr>
<td>Cu$^{2+}$ activity</td>
<td>0.134</td>
<td>0.659</td>
<td>0.733</td>
</tr>
</tbody>
</table>
Table 5.18  Significance levels obtained from grouped regression analyses for relationships determined between Ni concentrations in ryegrass and five potential measures of Ni availability in biosolids-amended (BLM) and unamended (LM) metal spiked soils. Differences were considered significant at $P<0.05$ and highlighted in bold ($n = 27$).

<table>
<thead>
<tr>
<th>Method</th>
<th>Overall $P$ value</th>
<th>Presence of biosolids $(P$-value)</th>
<th>Gradient difference $(P$-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EDTA</td>
<td>0.243</td>
<td>0.149</td>
<td>0.270</td>
</tr>
<tr>
<td>Ca(NO$_3$)$_2$</td>
<td>0.791</td>
<td>0.135</td>
<td>0.822</td>
</tr>
<tr>
<td>Soil solution</td>
<td>0.110</td>
<td>0.047</td>
<td>0.024</td>
</tr>
<tr>
<td>DGT</td>
<td>0.448</td>
<td>0.185</td>
<td>0.173</td>
</tr>
<tr>
<td>Ni$^{2+}$ activity</td>
<td>0.171</td>
<td>0.057</td>
<td>0.006</td>
</tr>
</tbody>
</table>

Table 5.19  Significance levels obtained from grouped regression analyses for relationships determined between Zn concentrations in ryegrass and five potential measures of Zn availability in biosolids-amended (BLM) and unamended (LM) metal spiked soils. Differences were considered significant at $P<0.05$ and highlighted in bold ($n = 27$).

<table>
<thead>
<tr>
<th>Method</th>
<th>Overall $P$ value</th>
<th>Presence of biosolids $(P$-value)</th>
<th>Gradient differences $(P$-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EDTA</td>
<td>0.164</td>
<td>0.547</td>
<td>0.559</td>
</tr>
<tr>
<td>Ca(NO$_3$)$_2$</td>
<td>0.323</td>
<td>0.324</td>
<td>0.534</td>
</tr>
<tr>
<td>Soil solution</td>
<td>0.369</td>
<td>0.285</td>
<td>0.849</td>
</tr>
<tr>
<td>DGT</td>
<td>0.322</td>
<td>0.343</td>
<td>0.421</td>
</tr>
<tr>
<td>Zn$^{2+}$ activity</td>
<td>0.296</td>
<td>0.278</td>
<td>0.573</td>
</tr>
</tbody>
</table>

5.4 Discussion

5.4.1 Influence of biosolids and time after treatment application on metal availability in soils

The addition of biosolids significantly increased the concentrations of DOC, salinity, Ca and Mg in soil solution as well as assays of bioavailability of all four metals. These results for increased DOC, salinity, Ca and Mg in soil solution concur with findings in Chapter 4. The length of time following treatment application had the most
effect on soil chemistry (Table 5.3). Over the duration of the field trial the pH of the soil and soil solution significantly decreased with time as did DOC (Table 5.2 and 5.4). Similar trends of decreasing pH and DOC with time have also been found in longer term field trials involving biosolids applications (Walter et al., 2002).

The increase of DOC in biosolids amended soils compared with unamended soils is most likely attributed to the degradation of biosolids (Table 5.2). Although the decomposition of biosolids provides extra DOC, leaching and mineralisation can lead to the reduction of DOC in soil solution with time (Amery et al., 2008; Antonidas and Alloway, 2002b), which may largely explain the results observed in this study.

The decomposition of organic matter has generally been accepted as a major cause of decreasing pH in soils amended with organic-rich composts (Helyar and Porter, 1989), However, in more recent studies describing the effects of organic soil amendments on soil chemistry Butterly et al. (2008) and Nwachaukwu and Pulford (2009) demonstrated that pH changes in soil amended with organic-rich material appear largely dependent on the type of organic material used. However, as the pH of the soil or soil solution was not significantly affected in the presence of biosolids (Table 5.2) a more likely explanation is that a combination of three of the following lead to decreasing soil pH overtime:

- decomposition of soil organic matter,
- plant activities (release of H⁺ as a response to the uptake of other nutrient cations);
- changes in the cation exchange capacity status of the soils.

Biosolids contain significantly ($P<0.001$) higher amounts of Ca, Mg and salinity levels than the soils resulting in higher levels of these in the biosolids amended soils compared with the unamended soils. While pH and DOC decreased with time, Ca, Mg and salinity of the soil solution increased with time (Table 5.2 and 5.4). Thus biosolids and length of time since treatment application resulted in increased amounts of Ca, Mg and salinity in the soil solution. Also six-monthly applications of superphosphate fertiliser after each harvest would have contributed to overall higher Ca levels. Increases in exchangeable Ca and in particular Mg with time can also be
attributed to the displacement of these cations (via competition from other cations) from exchange sites within the soil.

Biosolids amendment of metal spiked soils resulted in an increase in Cu$^{2+}$ activity and also a decrease in the activities of Ni$^{2+}$ and Zn$^{2+}$ compared with unamended soils (Table 5.5). However, over the 24 month course of the field trial all four of the metal activities increased (Table 5.7, 5.9, 5.11 and 5.13). Along with increasing in free ion activities of each metal, the amount of Cd and Zn in soil solution also increased. This increase in Cd and Zn in soil solution could also be a results of the lowering of pH over time and this relationship between Cd and Zn solubility and soil pH has been demonstrated by many studies (Degryse *et al.*, 2007; Gray and McLaren, 2006; Gray *et al.*, 1999b; McBride *et al.*, 1997; Nwachukwu and Pulford, 2009; Smith, 1994). The decrease in pH result in less available binding sites on soil colloids and clay materials for cations such as Zn and Cd, therefore leading to an increase of these metals in the solution phase (McBride, 1994).

The increase in free ion activities also coincided with general increasing soil solution metal concentrations. However, leaching, organic complexation, precipitation, adsorption and plant uptake of cations and anions can decrease the ionic strength of solution which could result in an increase of activities (Antoniadis and Alloway, 2002a; 2003a).

Of the four metals Zn was the only metal that revealed a significant loss in total Zn from the top 10cm of the soil profile with time and a decrease in C$_E$-Zn was also measured (Table 5.13). The loss of total Zn from the soil is most likely attributed to Zn leaching through the soil profile of the free-draining lysimeters. Losses of total soil Zn from the top of the soil profile has also been observed in a number of studies where biosolids were applied to soils managed under field conditions resulted in considerable movement of Zn through the soil profile (Antoniadis, 2008; Antoniadis and Alloway, 2002a; 2003a; McLaren *et al.*, 2003; Speir *et al.*, 2003b; Ruttens *et al.*, 2006). Additionally, it was found that Zn was the most mobile of the metals examined out of Cd, Cu, Ni and Zn and these finding are overall in agreement with results described in this study. An increase in EDTA-extractable Zn with time was also observed and it may in part be attributed to the increased amount of Zn in more strongly complexed forms. This effect has also been described by Ashworth and
Alloway (2004) and Walter et al. (2002) as a consequence of increased soil contact time. This could also explain the increase in EDTA-extractable Cd and Ni observed in this study. Although, these type of complexation reactions are more pronounced at higher pH (Buekers et al., 2008).

Diffusive gradient in thin film method has alternatively been used to describe the degree of metal resupplied from the solid phase to the soil solution i.e the kinetics of metal resupply from solid phase to the soil solution (Zhang and Young, 2005; Zhang et al., 2004). Moreover, the rate of desorption measured by DGT, found that Cd and Zn rates are considerably faster than rates of Ni and Cu (Ernstberger et al., 2005) and this finding appeared similar to observations made in this study (Table 5.7, 5.11 and 5.13). Thus a decrease in the $C_E$ of Cd, Ni and Zn in soil amended with biosolids compared with unamended soils could be indicative of a decrease in the re-supply rate of these metals from the solid phase to the soil solution. This decrease in the resupply rate is most likely attributed to the presence of additional organic matter from biosolids which would increase the amount of binding sites available for cations (Kovarikova et al., 2007). In a study of Cd sorption in biosolids, Merrington and Smernik (2004) found that Cd sorption appeared to be dominated by the inorganic fraction, which may also contribute to the decrease in $C_E$-Cd observed in this study.

5.4.2 Responses of plants grown in biosolids amended and unamended metal spiked soils

5.4.2.1 Presence of biosolids

Comparisons at the 18 and 24 month harvests yielded significant decreases in Cd, Cu and Zn shoot concentrations in soils amended with biosolids compared to unamended metal spiked soils (Table 5.14). The results for Cd agree with the significant changes in $C_E$-Cd (decrease, $P = 0.030$), indicative of reduced plant available Cd. Although there was an increase in EDTA extractable Cd (increase, $P = 0.013$) observed, this assay weakly correlated with shoot Cd concentration ($r^2 = 0.28$) and thus EDTA-extractable Cd is a poor measure of plant available Cd.
Findings for shoot concentrations of Cu and Zn in plants grown in biosolids amended soils compared with unamended soils (Table 5.14) are not reflected in the CE-Zn and Cu$^{2+}$ activity increases observed in the presence of biosolids (Table 5.5). However, no assay of Cu bioavailability was able to significantly predict greater than 50% of Cu concentrations in shoots, hence it is not surprising that shoot concentrations of Cu and assays of Cu bioavailability are in disagreement. Although CE-Zn did give a good estimate of Zn concentrations in shoots ($r^2 = 0.76$) using pooled data. It is likely differences between shoot concentrations of Zn in plants grown in biosolids amended soils compared with unamended soils at the 24 month harvest is more a result of time. Time since treatment application has been shown to significantly change bioavailable pools of Zn (Table 5.13).

In Chapter 4, DOC, salinity, exchangeable Ca and Mg were found in higher concentrations in biosolids amended soils compared with unamended soils, although no strong relationship was found between these variables and shoot concentrations of Cd. However, results for decrease shoot concentrations of Cd in the presence of biosolids did support the theory that biosolids have the capacity to retain Cd (Nwachukwu and Pulford, 2008; Ruttens et al., 2006; van Herwijnen et al., 2007a). This theory may partially account for the difference in shoot concentrations of Cd in plants grown in biosolids amended soils compared with unamended soils at the 24 month harvest occasion (Table 5.14). However, the absence of such significant results from previous harvest occasions (Table 5.14) makes it more likely that changes in shoot concentrations of Cd and also Cu, Ni and Zn is most likely a result of time than the presence of biosolids.

### 5.4.2.2 Change in shoot metal concentrations over time

Shoot concentrations of Ni and Zn were the only metals that showed significant changes over time (Table 5.15). While concentrations of Zn in ryegrass increased on average by 1.32 mg kg$^{-1}$ over 24 months Ni concentrations in shoots decreased by 0.65 mg kg$^{-1}$. However, there was considerably more variation in the data for Ni than there was for Zn (Table 5.11 and 5.13). This result for Ni concentrations in shoots is in disagreement with extractable and soluble Ni results, where the only significant
change was in Ni$^{2+}$ activity which increased with time (Table 5.11). Although assessment of concentrations of Ca(NO$_3$)$_2$ extracted Ni and soil solution Ni did not yield significant results, p-values were only marginally higher than 0.05 ($P = 0.053$ and 0.056 for Ca(NO$_3$)$_2$ and soil solution Ni respectively). Significant results for soil solution Zn and Zn$^{2+}$ activity coincided with an increase in Zn uptake by ryegrass over 24 months (Table 5.13 and 5.15) but were in contrast to total Zn in the top 10 cm of the soil profile, which decreased significantly with time as did the $C_E$ of Zn (Table 5.13). These results suggest that the concentration of Zn in solution and free ion Zn is more related to the bioavailable fraction than total Zn or $C_E$-Zn. This result also coincides with a decrease in pH over time, which has been shown to significantly influence soil solution to plant transfer of Zn in ryegrass grown in metal contaminated soil (Hough et al., 2005).

Given that five of the soil assay of Ni bioavailability were able to predict greater than 75% of Ni concentrations in shoots (Figure 9), the discrepancy between assays of Ni bioavailability and plant uptake of Ni over time (Table 5.11 and 5.15) could be accounted for in two ways, namely cation competition and the species of Ni in solution. Species of Ni in solution (e.g. Ni complexed with soluble organic ligands), and competition from other cations (Meers et al., 2009) could limit Ni uptake (Chaney et al., 2008), even though soluble forms of Ni would be increasing with time. Analyses of the soil solution results certainly confirmed increases in Ca, Mg, Cu and Zn overtime (Table 5.3, 5.9 and 5.12). Although this study did not differentiate between soluble organic ligand types, in particular it has been demonstrated in other studies that Ni speciation in the presence of different types of dissolved organic carbon has a major impact on the availability of Ni (Antoniadis and Alloway, 2002b; Ashworth and Alloway, 2004; Doig and Liber, 2007; Molas and Baran, 2004; Nolan et al., 2009; Rooney et al., 2007b). In particular, it has been demonstrated that even when the solubility of Ni increases this does not necessarily translate into increased bioavailability or uptake by plants (Antoniadis and Alloway, 2002b; Rooney et al., 2007b).
5.4.3 Estimates of metal bioavailability

Findings for Ni in this study were in agreement with results from Chapter 4, indicating that shoot concentrations of Ni are highly indicative of the concentration of Ni present in solution and Ni weakly sorbed to soil particles. Hence, the solubility of Ni as measured by Ca(NO₃)₂ extraction can reliably predict the plant availability and phytotoxicity resulting from excess Ni in soil. The validity of Ca(NO₃)₂ extractable Ni is further demonstrated given that the robustness of the assay was determined despite confounding factors such as:

- use of contrasting soils spiked with metal salts in the presence and absence of biosolids;
- managed under controlled and field conditions;
- use of two different plant species.

The range of data in the literature regarding the success of a neutral salt extraction such as Ca(NO₃)₂ in measuring the bioavailable soil fraction is limited. Although Ca(NO₃)₂ extraction has provided the most useful indication of metal bioavailability across a range of soils and metal concentrations (Meers et al., 2009; Menzies et al., 2007; Wang et al., 2009).

The relationships for Cd and Zn concentrations in shoots and soil solution, Cₑ and free ion activities were similar to each other and these methods provided the best relationships (Figure 5.7 and Figure 5.10). The similarity in behaviour of these metals was also observed in Chapter 4. However, correlations for Ca(NO₃)₂-extractable Cd and shoot Cd concentrations were higher in the wheat seedling experiment (r² = 0.54), than with Cd concentration in ryegrass (r² = 0.34).

Comparing the correlation results for Zn in this study, with those obtained in the wheat seedling experiment, revealed a stronger relationship with ryegrass concentration and Ca(NO₃)₂ extracted Zn, with Cₑ-Zn, soil solution and Zn²⁺ activity also able to significantly describe greater variation in ryegrass shoot concentrations than wheat grown under controlled environments. These results suggest that ryegrass uptake of Cd and Zn in a field managed system is more related to Cd and Zn in
solution. Moreover the similar coefficient of determination values obtained for Cd and Zn concentrations in shoots and free ion activity ($r^2 = 0.64$ and $r^2 = 0.68$ for Cd and Zn, respectively), suggests that free ion Cd and Zn the most available component present in the soil solution. Stronger relationships between $C_E$-Zn and shoot Zn concentrations in rye grass ($r^2 = 0.76$) than wheat ($r^2 = 0.43$) suggests a greater demand for Zn with increased growth in the longer running field trial compared to the pot trial, as there would be a greater need to replenish soil solution Zn as it is taken up by the plants. Coefficient of determination values for $C_E$-Cd were generally lower than for $C_E$-Zn, which suggest competitive sorption may also partly explain the $C_E$ results, and this has also been demonstrated by Antonladis and Tsadilas (2007) where Zn was more strongly bound than Cd and Ni, with the order of reduced sorption being $Zn > Ni > Cd$. However, internal detoxification of Cd by the plant may also account for the poorer relationship between Cd measures of bioavailability and Cd concentrations in shoots compared with Zn which is an essential micronutrient (Reid et al., 2003a).

Relationships between measured soil concentrations of Cu and plant concentrations of Cu were the poorest of all the metals assessed (Figure 5.8). Results obtained in the wheat seedling experiment (Chapter 4) were also in agreement with this finding. The strongest relationship obtained for Cu was provided by Ca(NO$_3$)$_2$ extractable Cu ($r^2 = 0.59$), while in the wheat experiment, total Cu provided the strongest relationship ($r^2 = 0.15$). Overall, relationships determined between extracted Cu and plant concentrations were higher in results obtained from the field lysimeter trial. The lack of strong relationships obtained between extractable/soluble Cu and plant concentrations across soil types was noted by Menzies et al. (2007) and Nolan et al. (2005). Zhang et al. (2001) showed that stronger relationships can be obtained between the $C_E$ of Cu and plant uptake ($r^2 = 0.95$), although this result was obtained from a smaller dataset than this study. However a known hyperaccumulating plant species was used in this study and soils were only contaminated with Cu. Further comparisons between assays of Cu bioavailability and plant uptake are explored in Chapter 6.

Interestingly, when relationships were determined between shoot concentrations of Cd and corresponding assays of Cd bioavailability in biosolids amended soils (BLM) and unamended soil (LM), only soil solution and free ion activity for Cd revealed
significant correlations (Table 5.16). Additionally, the relationships determined between these assays and shoot concentrations of Cd were not significantly affected by the presence of biosolids (Table 5.16). Conversely, McLaughlin et al. (2006) compared the solubility of Cd present in biosolids matrix versus soil spiked by Cd salts and found that the solubility of Cd was found to be equal or greater in soils when present bound in biosolids matrix than as Cd in a hydrated salt form which disagrees with findings in this study.

The presence of biosolids did significantly change the relationship determined between Ni concentrations in shoots and corresponding bioavailability assays of Ni in solution and Ni$^{2+}$ (Table 5.18). These findings suggest that in biosolids amended soils, Ni uptake by ryegrass is lower from these measured bioavailable fractions compared to plant uptake of Ni from unamended soils. This trend was not observed in the wheat in Chapter 4, where a larger range of Ni concentrations in soils were used to determine relationships between extraction assay and plant concentrations. However, the lack of significant correlations for relationships between extracted Ni and corresponding concentrations in shoots may also be due to the limited range of Ni concentrations present in the soil and shoots. Although the robustness of these bioavailability measures in this experiment, appear dependent on the presence or absence of biosolids it is difficult to ascertain the effect of biosolids amendment has on correlation strengths from data with a limited range of values.

The relationship between EDTA, Ca(NO$_3$)$_2$ and the $C_E$ of each metal and shoot metal concentrations were not significantly different in the presence of biosolids. Given the overall success of Ca(NO$_3$)$_2$ as a predictor of shoot metal concentration in this experiment and the wheat seedling experiment, it appears that this method is the most robust for predicting metal bioavailability across contrasting soil properties, varying metal concentrations and presence of biosolids. This lends support to the hypothesis that bioavailable metals are located on mineral surfaces and these can be displaced by competing cations (Andrews et al., 1996; McLaughlin et al., 1999).
5.4.4 Conclusions

Applying a treatment regime of increasing concentrations of metal salts in the presence and absence of biosolids to undisturbed soil monolith lysimeters of three contrasting soil types provided two important research opportunities:

- to examine the combined effects of biosolids amendment and metal salt applications on the soil chemistry and bioavailability of Cd, Cu, Ni and Zn in contrasting soil types managed under field conditions over a 24-month period;

- to provide a field comparison to the wheat seedling experiment carried out under controlled environmental condition as described in Chapter 4.

The addition of biosolids significantly increased the amount of DOC, salinity, Ca and Mg in solution and these results concurred with findings in Chapter 4. Biosolids also altered the extractability and solubility of Cd, Cu, Ni and Zn, although this was assay dependent, with Ni yielding the greatest number of significant differences, and CE and free ion activity yielding the greatest number of significant differences of the six methods used. Time had a larger overall influence on soil chemistry and metal bioavailability, with decreases in pH and DOC, and increases in salinity, Ca and Mg in soil solution. The free ion activities of each of the metals also increased with time, as did soil solution Cd and Zn, and the CE of Cu. Results for Zn suggested that a significant amount of Zn leaches through the soil profile with time. These findings suggest that length of experiments may alter the strength of the relationships between measures of metal bioavailability and shoot metal concentrations and thus experiments carried out over shorter time periods may not be appropriate use as models of longer term contamination studies.

Overall the addition of biosolids did not result in any significant changes in metal uptake by plants and only shoot concentrations of Ni and Zn significantly changed with time. The decrease in Ni concentration over time is most likely attributed to competition from other cations (i.e. Ca, Mg, Na and Zn). While increased Zn uptake may be linked to increased plant nutrient requirements a decrease pH could also result
in increased bioavailable forms of Zn and also competitive cation effects resulting from relatively high concentrations of Zn present in the soils.

Relationships determined between metal concentrations in soil solution and shoot metal concentrations showed the overall highest coefficient of determinations. However, in the presence of biosolids the relationship between shoot Ni concentration and Ni in soil solution was significantly affected as was the relationship between shoot Ni concentrations and Ni$^{2+}$ activity. From a predictive perspective, the best overall bioavailable measure of Cd, Cu, Zn and especially Ni concentrations determined from a range of soils containing a range of metals in the presence and absence of biosolids was Ca(NO$_3$)$_2$ extractable soil metal. Additionally, relationships determined between Ca(NO$_3$)$_2$ extractable soil metal and ryegrass concentrations in this study were stronger than those obtained in Chapter 4.
Chapter 6
Evaluating Methods for Estimating the Bioavailability of Metals Using a Range of New Zealand Soils Amended with Different Rates of Biosolids and Metal Salts

6.1 Introduction

Numerous studies and several reviews on concepts and methods to accurately assess metal bioavailability and toxicity in soils have attempted, with some success, to link assays of metal extractability and solubility to plant tissue concentrations (Andrews et al., 1996; Basar, 2009; Degryse et al., 2009a; Lehto et al., 2006c; Lofts et al., 2007; McBride et al., 2009; McLaughlin et al., 2000; Meers et al., 2008; Menzies et al., 2007). A few studies have met with some success but in general results from these predictions have been largely inconclusive (Hamon et al. 1997; Hooda 2007; Zhang and Young 2005; Zhang et al. 2001).

Studies investigating the efficacy of metal bioavailability predictions have yet to establish the impact of organic amendments on the validity of these tests, especially across a range of soils. Part of the problem stems from inconsistencies in experimentation and insufficient datasets (Menzies et al., 2007). Moreover, very few studies have attempted to resolve the additional effect that different plant species may have on the outcome of these bioavailability measures (Almas et al., 2006; Datta and Young, 2005; Gray and McLaren, 2005; Lehto et al., 2006a).

The concentration of metal in the solution phase is thought of as the most bioavailable metal fraction with the amount sorbed onto the solid phase controlling the amount in solution (Degryse et al., 2009a; McBride et al., 1997a). While the free ion concentration is usually considered to be the major determinant species of bioavailability (Degryse et al., 2009a; 2009b), there is increasing interest in how low affinity ligands may affect metal bioavailability and toxicity (Antunes et al., 2006; Arnold et al., 2007; Stacey et al., 2008).
Several techniques used to estimate the bioavailability of metals are based on the hypothesis that free ion and isotopically exchangeable metal fractions (those weakly bound to soil particles) reasonably represent the phytoavailable metal pool (McLaughlin et al., 1999; Meers et al., 2009; Menzies et al., 2007; Nolan et al., 2005; Zhao et al., 2006). Methods developed to estimate the bioavailable metal fraction have to consider factors that influence the size of the bioavailable metal pool, e.g. soil type, pH, dissolved organic carbon (DOC) forms and concentration, type and concentration of inorganic ligands, presence of competing cations, rhizosphere processes, plant physiology etc (Alloway, 1995b; Basar, 2009; Gregory, 2006; Jones et al., 2004; McBride, 1994; Sauve, 2003), in order for them to accurately predict bioavailability. Because of the confounding factors it is unlikely that any given method will accommodate all possible factors and scenarios. The validity and strength of metal bioavailability predictions in soils is dependent on the suitability of the method used and the response of the biological model system being studied.

The main objective of this study was to assess the overall strength of the relationships determined between metal concentrations in plants and various measures of Cd, Cu, Ni and Zn solubility for a range of New Zealand soils amended with biosolids and/or combinations of metal salts. This was achieved by combining the data from the studies described in Chapters 4 and 5 with published and unpublished data form Environmental Science and Research Ltd. (ESR) in collaboration with Lincoln University.

In Chapters 4 and 5, wheat seedlings (*Triticum aestivum*) and perennial ryegrass (*Lolium perenne*) were used as the model plant systems. Both plant species were grown in three soil types spiked with increasing levels of metal salts, to which biosolids had been added to half of the metal treated soils at a rate equivalent to 400 kg N ha\(^{-1}\) (Chapter 4), and to the lowest metal salt concentration treatment only (Chapter 5).

This data was combined with data from the following field trials:

1. A seven-year field lysimeter study located at Lincoln University, Porirua and Hamilton in which biosolids have been applied annually from 2002 – 2008, to five soil types (different to soil types used in Chapters 4 and 5) at rates of 0,
200 and 800 kg N ha\(^{-1}\) in wet, composted and pelletised forms. Ryegrass (*Lolium perenne*) was re-sown annually on these plots (McLaren, 2005);

2. A pasture (*Lolium perenne*) field trial established at Lincoln University where Cu, Ni and Zn spiked biosolids were applied in wet form at a rate 400 kg N ha\(^{-1}\) in 1997 and 1998; in 2003 these plots were subdivided into unlimed and limed treatments (McLaren, 2007);

3. Three pasture (*Lolium perenne*) field trials that involved addition of increasing rates of Cu and Zn were established in 2006 and 2007 and are located at Lincoln University, Nelson, South Island, New Zealand, and Hamilton, North Island, New Zealand (unpublished data);

4. A forest (*Pinus radiata*) field trial established at Doyles Block, Canterbury, involving addition of increasing concentrations of Cu, Ni and Zn spiked biosolids at a rate equivalent to 400 kg N ha\(^{-1}\) in 1998 and 1999 (McLaren *et al.*, 2007).

Metal bioavailability in the studies by ESR and Lincoln University were determined by relating shoot concentrations to six different measures of bioavailability in a similar manner to Chapters 4 and 5. Correlations strengths from relationships determined between pooled soil and plant results were compared to establish which of these tests were most successful at predicting shoot concentrations. Differences between plant species relationships and assays of metal availability were also assessed. Comparisons between coefficients of determinations obtained for smaller soil treatment groups are discussed.
6.2 Methods

The data used in this Chapter comes from a range of previously conducted but closely related trials. The results from these trials have been pooled along with results from Chapters 4 and 5 to gain information on the effects of broad ranges of soil types, plant species and presence of biosolids has on assays of metal bioavailability.

6.2.1 Set up of ESR and Lincoln University trials

All plant and soil samples were sampled, prepared and analysed according to methods previously described in Chapters 3, 4 and 5. A brief description of the trials by ESR and Lincoln University are given below.

6.2.1.1 Field lysimeter study to assess the effects of annual biosolids application to land

A lysimeter trial was established in 2002, and housed at Lincoln University (Figure 6.1), ESR Porirua and Landcare Research, Hamilton in which biosolids as received in composted and pelletised forms, were applied to five soil types (Table 3.1) annually at increasing rates of 0, 200 and 800 kg N ha\(^{-1}\).

Lysimeters were set-up according to Cameron et al. (1992). Each lysimeter was 50 cm in diameter and 70 cm deep. The procedures involved with the treatment and sampling of the lysimeters were described in detail by McLaren (2005). Pasture ryegrass was sown every year after biosolids application and is harvested once herbage has attained a height of 10cm. Harvested plant material was dried, weighed and ground for analysis (section 3.3.1). Soils were sub-sampled annually prior to biosolids application and analysed for total, EDTA-extractable, Ca(NO\(_3\))\(_2\) extractable metals (section 3.3.2).
In 2007, a wheat seedling bioassay (section 4.2.2) was carried out on soils sampled in October 2006. Metal concentrations in soil solution, DGT measured fluxes of metals and modeled free metal ion activities were also determined along with total, EDTA-extractable and Ca(NO$_3$)$_2$ extractable metals.

Figure 6.1. Lysimeter set-up at Lincoln University to assess the effects on leachate quality, soil chemistry and herbage uptake of metals from annual application of biosolids.

6.2.1.2 Pasture field trial to assess the effects of biosolids spiked with metal salts on soil properties and plant uptake of metals

A pasture field trial assessing the effects of metal spiked biosolids was established in 1997 and 1998 at Lincoln University. Full details of the incorporation of metals into the biosolids was given in McLaren and Clucas (2001). In 1997 and 1998, applications of metal spiked biosolids were applied, at a rate equivalent to 400 kg N ha$^{-1}$ yr$^{-1}$ to approximately 1 m$^2$ plots. Pastures were sown following biosolids incorporation into the soil. Pastures were sampled twice per year for analysis. Soil samples were collected annually and analysed for total, EDTA-extractable and Ca(NO$_3$)$_2$-extractable metals (section 3.3.2). In 2003 these plots were subdivided into
unlimed and limed treatments and in 2006 a wheat seedling bioassay experiment (section 4.2.2) was conducted on soil sampled from the field trial. Metal concentrations in soil solution, DGT measured fluxes of metals and modeled free metal ion activities were also determined along with total, EDTA-extractable and Ca(NO₃)₂ extractable metals. Both the field trial and wheat bioassay data are used in the analyses for this chapter.

### 6.2.1.3 Pasture field trials to assess the effects of metal salt applications on soil chemistry and plant uptake

Three pasture field trials were established in 2006 and 2007 at Lincoln University, Nelson, South Island, New Zealand (Figure 6.2) and Hamilton, North Island, New Zealand. All localities are shown in Figure 3.1. Increasing amounts of Cu (5, 20, 50, 120, 300, 750 and 2000 mg kg⁻¹) and Zn (10, 30, 70, 160, 400, 1000 and 3000 mg kg⁻¹) in the form of hydrated metal sulphates incorporated into the top 10 cm of the soil at the commencement of the trial. Each plot was approximately 1 m² in size.

Soils from each site were sampled twice per year between 2006 and 2007, and a wheat seedling bioassay (section 4.2.2) was carried out on the soils sampled in addition to analyses of herbage pasture. Sampled soils were analysed for total, EDTA-extractable, Ca(NO₃)₂-extractable concentrations in soil solution, DGT measured fluxes of metals and modelled metal free ion activities (section 3.3.2).
Figure 6.2  Photograph of biosolids spiked with metal salts field trial, Top photo: Lincoln University trial set up in 1997, Bottom photo: metal salt trial set-up in 2006, Nelson, South Island, New Zealand.

6.2.1.4 Addition of metal spiked biosolids to forest soils

A forest field trial was established at Doyles Block in Canterbury, which involved the addition of Cu (at 50, 100, 150 and 200 mg kg$^{-1}$), Ni (at 15, 30, 45 and 60 mg kg$^{-1}$) and Zn (at 100, 200, 300 and 400 mg kg$^{-1}$) spiked biosolids at a rate equivalent to 400 kg N ha$^{-1}$ in 1998 and 1999. Details of the incorporation of the metal salts into the biosolids were described in full in McLaren and Clucas (2001). Samples of soils were taken from the site six years after treatment application, wetted to 80% ± 5% field moisture capacity and incubated for two years at a constant 25°C. A wheat seedling bioassay (Chapter 4) was carried out on the incubated soils. Seedlings were harvested on day 20 of the experiment, prepared and analysed for total metals (section 4.2.2). Soils were analysed for total, EDTA-extractable, Ca(NO$_3$)$_2$-extractable metals, metal
concentrations in soil solution, DGT measured fluxes of metals and modeled metal free ion activities (section 3.3.2).

6.2.2 Statistical analyses

Linear regression analyses used Sigma Plot 8.02 (Sigma Plot 8.02, SPSS, UK). Correlations between variables and gradient differences between wheat and ryegrass were determined using grouped and separate linear regression analyses performed in GenStat 11 (VSN International, UK). Coefficient of determination values ($r^2$) less than 0.50 were considered poor measures of fit between variables.
6.3 Results

6.3.1 Effects of metal spiking of biosolids and soils on estimates of metal bioavailability

The range of shoot data variation that could be significantly ($P < 0.05$) explained by the six assays of bioavailability in smaller more specific datasets was assessed by subdividing the pooled plant and soil data into distinct but closely related groups. Groupings were based on the soil treatment of no amendments (control), and different combinations of biosolids and metal salts. Subdivided groups are listed below and names in brackets were used in the subsequent description of results:

- control soils (control)
- soils having received an application of biosolids only (biosolids only)
- soils treated with metal spiked biosolids (spiked biosolids)
- metal spiked soils amended with biosolids (biosolids and metals)
- soils spiked with metal salts only (metals only)

**Cadmium:** None of the methods tested could significantly explain more than 50% of variation in the data. Overall, Ca(NO$_3$)$_2$-extractable Cd (40%) and $C_E$-Cd (21%) described the greatest amount of Cd shoot variation across all groups. However this was only observed in two of the groups (Table 6.1). For the biosolids only group, just two of the methods tested showed significant correlations with shoot Cd concentration but accounted for less than 10% of the shoot Cd concentration (Table 6.1). None of the methods were able to significantly explain any variation of Cd shoot concentration in spiked biosolids. In biosolids and metals, and metals only soils, just half of the methods tested had significant correlations with shoot concentrations but could explain less than 20% variation in shoot Cd concentrations (Table 6.1).

**Copper:** Aside from the metal spiked biosolids group, no assay was able to describe greater than 50% of the variation in shoot Cu concentrations (Table 6.2). Just two methods significantly correlated with shoot Cu concentrations in the control and biosolids only group, but could account for less than 20% of the variation in shoot Cu concentrations (Table 6.2). While results obtained for biosolids and metals were
significant for four of the assays tested, these accounted for less than 10% of variation in Cu shoot concentration. Four of the six assays tested were able to explain greater than 50% of shoot Cu concentration variability in the metal spiked biosolids group, with EDTA extractable Cu having the highest $r^2$ value (0.63, Table 6.2). Correlation results for the metals only group were significant for all assays tested and had the second highest explanatory power to describe variation in shoot concentration of the assays tested compared to the metal spiked biosolids group (Table 6.2).

**Nickel:** Coefficients of determinations increased with increasing concentrations of Ni added to the soil. No method could explain greater than 50% of shoot Ni concentration variation in the control and biosolids only groups (Table 6.3). In the spiked biosolids group, all assays tested significantly correlated with shoot Ni concentration but only (Ca(NO$_3$)$_2$)-extractable Ni accounted for more than 50% of Ni shoot concentration variability. In the biosolids and metals, four of the six assays could describe greater than 50% of shoot Ni concentration variability, with (Ca(NO$_3$)$_2$)-extractable Ni having the highest $r^2$ value (0.81). In the metals only group, all assays gave significant correlations and could account for more than 50% of shoot Ni concentration, with (Ca(NO$_3$)$_2$)-extractable Ni able to describe 81% of the variation in Ni shoot concentration.

**Zinc:** The number of significant relationships and $r^2$ values increased with increased Zn concentrations across soil groups (Table 6.4). Only two of the assays in the control and biosolids only groups significantly correlated with shoot concentrations but accounted for less than 50% of shoot Zn variability. For spiked biosolids, biosolids and metals, and metals only, all methods significantly correlated with shoot concentration (Table 6.4). Five of the six assays tested could explain more than 50% of the shoot Zn concentration variability in spiked biosolids group, with EDTA extractable Zn describing 87% of variability in shoot Zn concentration. In biosolids and metals, none of the assays tested could account for greater than 50% of variation in shoot Zn concentration. In the metals only group all methods could describe more than 50% of shoot Zn concentration, with total Zn, Ca(NO$_3$)$_2$ extractable Zn and CE-Zn accounting for 70% of shoot Zn concentration variability.
Table 6.1  Coefficient of determination ($r^2$) for each soil amendment group from relationships determined between plant concentration and six potential measures of Cd bioavailability. The highest scoring significant $r^2$ value ($P<0.05$) for each group is highlighted in red. All data was log10 transformed for analysis. * $P$-values were greater than 0.05.

<table>
<thead>
<tr>
<th>Method</th>
<th>Control (n = 61)</th>
<th>Biosolids only (n = 94)</th>
<th>Spiked biosolids (n = 23)</th>
<th>Biosolids and metals (n = 96)</th>
<th>Metals only (n = 148)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>0.04*</td>
<td>0.00*</td>
<td>0.00*</td>
<td>0.03*</td>
<td>0.01*</td>
</tr>
<tr>
<td>EDTA</td>
<td>0.19 ($P&lt;0.001$)</td>
<td>0.07 ($P = 0.006$)</td>
<td>0.05*</td>
<td>0.00*</td>
<td>0.00*</td>
</tr>
<tr>
<td>Ca(NO₃)₂</td>
<td>0.40 ($P&lt;0.001$)</td>
<td>0.01*</td>
<td>0.05*</td>
<td>0.00*</td>
<td>0.00*</td>
</tr>
<tr>
<td>Soil solution</td>
<td>0.05*</td>
<td>0.00*</td>
<td>0.09*</td>
<td>0.08 ($P = 0.003$)</td>
<td>0.08 ($P&lt;0.001$)</td>
</tr>
<tr>
<td>DGT</td>
<td>0.00*</td>
<td>0.00*</td>
<td>0.00*</td>
<td>0.21 ($P&lt;0.001$)</td>
<td>0.15 ($P&lt;0.001$)</td>
</tr>
<tr>
<td>Free ion activity</td>
<td>0.00*</td>
<td>0.04 ($P = 0.04$)</td>
<td>0.10*</td>
<td>0.07 ($P=0.004$)</td>
<td>0.05 ($P=0.003$)</td>
</tr>
</tbody>
</table>

Table 6.2  Coefficient of determination ($r^2$) for each soil amendment group from relationships determined between plant concentration and six potential measures of Cu bioavailability. The highest scoring significant $r^2$ value ($P<0.05$) for each group is highlighted in red. All data was log10 transformed for analysis. * $P$-values were greater than 0.05.

<table>
<thead>
<tr>
<th>Method</th>
<th>Control (n = 82)</th>
<th>Biosolids only (n = 102)</th>
<th>Spiked biosolids (n = 106)</th>
<th>Biosolids and metals (n = 96)</th>
<th>Metals only (n = 237)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>0.00*</td>
<td>0.07 ($P = 0.003$)</td>
<td>0.57 ($P&lt;0.001$)</td>
<td>0.06 ($P = 0.005$)</td>
<td>0.41 ($P&lt;0.001$)</td>
</tr>
<tr>
<td>EDTA</td>
<td>0.00*</td>
<td>0.00*</td>
<td>0.63 ($P&lt;0.001$)</td>
<td>0.05 ($P = 0.014$)</td>
<td>0.30 ($P&lt;0.001$)</td>
</tr>
<tr>
<td>Ca(NO₃)₂</td>
<td>0.00*</td>
<td>0.00*</td>
<td>0.23 ($P&lt;0.001$)</td>
<td>0.03*</td>
<td>0.43 ($P&lt;0.001$)</td>
</tr>
<tr>
<td>Soil solution</td>
<td>0.00*</td>
<td>0.01 ($P&lt;0.001$)</td>
<td>0.52 ($P&lt;0.001$)</td>
<td>0.02*</td>
<td>0.32 ($P&lt;0.001$)</td>
</tr>
<tr>
<td>DGT</td>
<td>0.00*</td>
<td>0.00*</td>
<td>0.55 ($P&lt;0.001$)</td>
<td>0.03 ($P = 0.035$)</td>
<td>0.35 ($P&lt;0.001$)</td>
</tr>
<tr>
<td>Free ion activity</td>
<td>0.03*</td>
<td>0.18 ($P&lt;0.001$)</td>
<td>0.39 ($P&lt;0.001$)</td>
<td>0.01*</td>
<td>0.36 ($P&lt;0.001$)</td>
</tr>
</tbody>
</table>
Table 6.3  Coefficient of determination ($r^2$) for each soil amendment group from relationships determined between plant concentration and six potential measures of Ni bioavailability. The highest scoring significant $r^2$ value ($P<0.05$) for each group is highlighted in red. All data was log10 transformed for analysis. * $P$-values were greater than 0.05.

<table>
<thead>
<tr>
<th>Method</th>
<th>Control (n = 76)</th>
<th>Biosolids only (n = 91)</th>
<th>Spiked biosolids (n = 54)</th>
<th>Biosolids and metals (n = 91)</th>
<th>Metals only (n = 141)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>0.00*</td>
<td>0.00*</td>
<td>0.20 ($P&lt;0.001$)</td>
<td>0.67 ($P&lt;0.001$)</td>
<td>0.72 ($P&lt;0.001$)</td>
</tr>
<tr>
<td>EDTA</td>
<td>0.00*</td>
<td>0.01*</td>
<td>0.57 ($P&lt;0.001$)</td>
<td>0.65 ($P&lt;0.001$)</td>
<td>0.50 ($P&lt;0.001$)</td>
</tr>
<tr>
<td>Ca(NO$_3$)$_2$</td>
<td>0.07 ($P=0.009$)</td>
<td>0.07 ($P=0.005$)</td>
<td>0.31 ($P&lt;0.001$)</td>
<td>0.81 ($P&lt;0.001$)</td>
<td>0.81 ($P&lt;0.001$)</td>
</tr>
<tr>
<td>Soil solution</td>
<td>0.00*</td>
<td>0.00*</td>
<td>0.38 ($P&lt;0.001$)</td>
<td>0.61 ($P&lt;0.001$)</td>
<td>0.74 ($P&lt;0.001$)</td>
</tr>
<tr>
<td>DGT</td>
<td>0.00*</td>
<td>0.00*</td>
<td>0.30 ($P&lt;0.001$)</td>
<td>0.35 ($P&lt;0.001$)</td>
<td>0.57 ($P&lt;0.001$)</td>
</tr>
<tr>
<td>Free ion activity</td>
<td>0.09 ($P=0.003$)</td>
<td>0.00*</td>
<td>0.36 ($P&lt;0.001$)</td>
<td>0.43 ($P&lt;0.001$)</td>
<td>0.63 ($P&lt;0.001$)</td>
</tr>
</tbody>
</table>
Table 6.4  Coefficient of determination ($r^2$) for each soil amendment group from relationships determined between plant concentration and six potential measures of Zn bioavailability. The highest scoring significant $r^2$ value ($P<0.05$) for each group is highlighted in red. All data was log$_{10}$ transformed for analysis. * $P$-values were greater than 0.05.

<table>
<thead>
<tr>
<th>Method</th>
<th>Control (n = 84)</th>
<th>Biosolids only (n = 98)</th>
<th>Spiked biosolids (n = 38)</th>
<th>Biosolids and metals (n = 95)</th>
<th>Metals only (n = 297)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>0.16 ($P&lt;0.001$)</td>
<td>0.21 ($P&lt;0.001$)</td>
<td>0.47 ($P&lt;0.001$)</td>
<td>0.24 ($P&lt;0.001$)</td>
<td>0.70 ($P&lt;0.001$)</td>
</tr>
<tr>
<td>EDTA</td>
<td>0.02* ($P&lt;0.001$)</td>
<td>0.12 ($P&lt;0.001$)</td>
<td>0.87 ($P&lt;0.001$)</td>
<td>0.33 ($P&lt;0.001$)</td>
<td>0.62 ($P&lt;0.001$)</td>
</tr>
<tr>
<td>Ca(NO$_3$)$_2$</td>
<td>0.02* ($P&lt;0.001$)</td>
<td>0.46 ($P&lt;0.001$)</td>
<td>0.65 ($P&lt;0.001$)</td>
<td>0.36 ($P&lt;0.001$)</td>
<td>0.70 ($P&lt;0.001$)</td>
</tr>
<tr>
<td>Soil solution</td>
<td>0.00* ($P&lt;0.001$)</td>
<td>0.12 ($P&lt;0.001$)</td>
<td>0.62 ($P&lt;0.001$)</td>
<td>0.23 ($P&lt;0.001$)</td>
<td>0.64 ($P&lt;0.001$)</td>
</tr>
<tr>
<td>DGT</td>
<td>0.00* ($P&lt;0.001$)</td>
<td>0.00* ($P&lt;0.001$)</td>
<td>0.61 ($P&lt;0.001$)</td>
<td>0.33 ($P&lt;0.001$)</td>
<td>0.70 ($P&lt;0.001$)</td>
</tr>
<tr>
<td>Free ion activity</td>
<td>0.00* ($P&lt;0.001$)</td>
<td>0.16 ($P&lt;0.001$)</td>
<td>0.64 ($P&lt;0.001$)</td>
<td>0.25 ($P&lt;0.001$)</td>
<td>0.60 ($P&lt;0.001$)</td>
</tr>
</tbody>
</table>

6.3.2 Effects of plant species on estimates of bioavailability

Generally, concentrations of Cd and Zn were significantly ($P<0.001$) lower in ryegrass than wheat shoots, while Ni concentrations in ryegrass shoots were significantly higher than those measured in wheat shoots ($P<0.001$).

**Cadmium:** Comparisons of the relationships between shoot concentrations and the six potential measures of bioavailability for wheat and ryegrass (Figure 6.3) revealed significant differences between the plant species for each method assessed (Table 6.5). Wheat uptake was significantly higher in bioavailable fractions measured by total Cd, EDTA extraction, and Ca(NO$_3$)$_2$ extraction (Figure 6.3A-C and Table 6.5).
In contrast, ryegrass uptake of Cd was higher in bioavailable fractions measured by solution Cd, $C_E$ and Cd$^{2+}$ (Figure 6.3D-F and Table 6.5).

**Copper:** Relationships determined between shoot Cu concentration and six potential measures of bioavailability were significantly different between the two plant species, with most assays able to explain a greater amount of variation in the ryegrass data than in the wheat data (Figure 6.4 and Table 6.5). The difference between gradients revealed that Cu uptake by ryegrass was significantly less to that of wheat uptake of Cu across five of the assays of Cu bioavailability (Table 6.5).

**Nickel:** Apart from soil solution ($P = 0.273$), gradients for relationships between shoot Ni concentration and assays of Ni bioavailability differed significantly between the two plant species (Figure 6.5, and Table 6.5). Measures of Ni bioavailability were able to describe more variation in ryegrass, than in wheat (Figure 6.5).

**Zinc:** Only two of the six potential measures of bioavailability assessed revealed significant differences in the gradients obtained for the two plant species (Figure 6.6 and Table 6.5). Results showed that Zn uptake by ryegrass was less in bioavailable fractions as measured by EDTA-extraction and Ca(NO$_3$)$_2$ extraction than Zn uptake by wheat seedlings (Table 6.5).
Figure 6.3  Relationships determined between 20 day old wheat seedlings, pasture ryegrass concentrations and Cd concentrations obtained using various methods; (A) Total Cd, (B) EDTA-extractable Cd, (C) Ca(NO$_3$)$_2$ extractable Cd, (D) soil solution, (E) effective concentration, (F) free ion activity (n = 272 for wheat and n = 188 for ryegrass).
Figure 6.4  Relationships determined between 20 day old wheat seedlings, pasture ryegrass concentrations and Cu concentrations obtained using various methods; (A) Total Cu, (B) EDTA-extractable Cu, (C) Ca(NO₃)₂ extractable Cu, (D) soil solution, (E) effective concentration, (F) free ion activity for 20 day old wheat seedlings and ryegrass (n = 439 for wheat and n = 277 for ryegrass).
Figure 6.5  Relationships determined between 20 day old wheat seedlings, pasture ryegrass concentrations and Ni concentrations obtained using various methods; (A) Total Ni, (B) EDTA-extractable Ni (C) Ca(NO₃)₂ extractable Ni, (D) soil solution, (E) effective concentration, (F) free ion activity (n = 295 for wheat and n = 208 for ryegrass).
Figure 6.6  Relationships determined between 20 day old wheat seedlings, pasture ryegrass concentrations and Zn concentrations obtained using various methods; (A) Total Zn, (B) EDTA-extractable Zn (C) Ca(NO₃)₂ extractable Zn, (D) soil solution, (E) effective concentration, (F) free ion activity (n = 439 for wheat and n = 220 for ryegrass).

$y = 0.09 + 0.94x$, $r^2 = 0.63$, $p < 0.001$ (wheat)

$y = 3.42 + 0.29x$, $r^2 = 0.64$, $p < 0.001$ (ryegrass)

$y = 1.10 + 0.52x$, $r^2 = 0.67$, $p < 0.001$ (wheat)

$y = 1.21 + 0.35x$, $r^2 = 0.42$, $p < 0.001$ (ryegrass)

$y = 1.40 + 0.47x$, $r^2 = 0.70$, $p < 0.001$ (wheat)

$y = 1.30 + 0.39x$, $r^2 = 0.65$, $p < 0.001$ (ryegrass)

$y = 1.95 + 0.28x$, $r^2 = 0.49$, $p < 0.001$ (wheat)

$y = 3.48 + 0.28x$, $r^2 = 0.49$, $p < 0.001$ (ryegrass)

$y = 1.54 + 0.42x$, $r^2 = 0.78$, $p < 0.001$ (wheat)

$y = 1.54 + 0.35x$, $r^2 = 0.42$, $p < 0.001$ (ryegrass)

$y = 1.63 + 0.50x$, $r^2 = 0.65$, $p < 0.001$ (wheat)

$y = 1.82 + 0.39x$, $r^2 = 0.68$, $p < 0.001$ (ryegrass)

$y = 1.72 + 0.41x$, $r^2 = 0.58$, $p < 0.001$ (wheat)

$y = 1.54 + 0.42x$, $r^2 = 0.78$, $p < 0.001$ (wheat)
Table 6.5  Significance levels and differences between plant species as determined from comparisons of
relationships (gradients) between metal concentration in shoots of 20 day old wheat seedlings and
ryegrass, and six potential measures of metal bioavailability. All data was log<sub>10</sub> transformed for analysis.
Differences were considered significant at $P<0.05$ and highlighted in bold.

<table>
<thead>
<tr>
<th>Method</th>
<th>Method</th>
<th>$P$-value for gradient comparison</th>
<th>ryegrass deviation from wheat (reference gradient)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cadmium</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>0.002</td>
<td>-0.2126</td>
</tr>
<tr>
<td>EDTA</td>
<td></td>
<td>&lt;0.001</td>
<td>-0.3602</td>
</tr>
<tr>
<td>Ca(NO$_3$)$_2$</td>
<td></td>
<td>&lt;0.001</td>
<td>-0.2211</td>
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<tr>
<td>Soil solution</td>
<td></td>
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</tr>
<tr>
<td>DGT</td>
<td></td>
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<td>0.1478</td>
</tr>
<tr>
<td>Free ion activity</td>
<td></td>
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<td>0.2509</td>
</tr>
<tr>
<td>Copper</td>
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<td></td>
<td></td>
</tr>
<tr>
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<tr>
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<tr>
<td>Total</td>
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<tr>
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<td>Free ion activity</td>
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<td>-0.0971</td>
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<tr>
<td>Zinc</td>
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<td></td>
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</tr>
<tr>
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<td>Free ion activity</td>
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</tbody>
</table>
6.3.3 Estimations of bioavailability using pooled plant species and soil data

Log-log relationships determined between pooled plant metal concentrations and the six measures of metal bioavailability for pooled soil results were significant for all four metals ($P < 0.001$).

**Cadmium:** No assay tested was able to describe greater than 50% of the variability in shoot Cd concentrations (Figure 6.7). Effective Cd concentrations and total Cd gave the strongest relationship with shoot Cd concentrations of the six methods tested ($r^2 = 0.47$ and $r^2 = 0.42$, respectively Figure 6.7E and A). Ethylene diamine tetra-acetic acid disodium salt - extractable Cd and $\text{Ca(NO}_3\text{)}_2$-extractable Cd gave similar correlations to each other ($r^2 = 0.33$ and $r= 0.31$, respectively Figure 6.7B and C), while soil solution Cd and Cd$^{2+}$ gave the poorest correlations ($r^2 = 0.19$ and $r^2 = 0.1$, respectively Figure 6.7D and F).

**Copper:** Overall, relationships between Cu concentrations in shoots and measures of availability were the poorest of the four metals assessed in this Chapter with none able to explain greater than 50% of variability in shoot Cu concentration (Figure 6.8). Of the assays tested, total Cu gave the strongest correlation with Cu shoot concentration ($r^2 = 0.34$, Figure 6.8A). Results for EDTA-extractable, $\text{Ca(NO}_3\text{)}_2$-extractable, soil solution, $C_E$ and Cu$^{2+}$ had very similar results to each other ( $r^2 = 0.26 – 0.30$ Figure 6.8B-F).

**Nickel:** Of the four metals examined, Ni generally gave the strongest relationships between shoot Ni concentration and the six methods assessed (Figure 6.9). Calcium nitrate extractable Ni gave the strongest correlation with shoot Ni concentration ($r^2 = 0.74$ Figure 6.9C). Total and soil solution Ni had similar correlation responses to each other ($r^2 = 0.67$ and $r^2 = 0.64$, respectively Figure 6.9A and D). While Ni$^{2+}$ activity and $C_E$ had the poorest relationship with shoot Ni concentration ($r^2 = 0.55$ and $r^2 = 0.54$, respectively Figure 6.9F and E).

**Zinc:** As with Ni, $\text{Ca(NO}_3\text{)}_2$ gave the highest correlation with Zn concentrations in shoots ($r^2 = 0.60$ Figure 6.10C), followed by $C_E$ ($r^2 = 0.60$ Figure 6.10E). Correlation
for determinations between EDTA-extractable and soil solution Zn concentrations were similar ($r^2 = 0.57$ and $r^2 = 0.56$, respectively Figure 6.10B and D), while total and Zn$^{2+}$ activity had the same $r^2$ values as each other ($r^2 = 0.52$ Figure 6.10A and F).
Figure 6.7 Relationships determined between shoot Cd concentration and soil Cd concentrations obtained using various methods; (A) Total Cd, (B) EDTA-extractable Cd, (C) Ca(NO₃)₂ extractable Cd, (D) soil solution, (E) effective concentration, (F) free ion activity. A linear regression line is fitted for each plot. n = 399.
Figure 6.8  Relationships determined between shoot Cu concentration and soil Cu concentrations obtained using various methods; (A) Total Cu, (B) EDTA-extractable Cu, (C) Ca(NO₃)₂ extractable Cu, (D) soil solution, (E) effective concentration, (F) free ion activity. A linear regression line is fitted for each plot. n = 592.
Figure 6.9  Relationships determined between shoot Ni concentration and soil Ni concentrations obtained using various methods; (A) Total Ni, (B) EDTA-extractable Ni, (C) Ca(NO₃)₂-extractable Ni, (D) soil solution, (E) effective concentration, (F) free ion activity. A linear regression line is fitted for each plot. n = 425.
Figure 6.10  Relationships determined between shoot Zn concentration and soil Zn concentrations obtained using various methods; (A) Total Zn, (B) EDTA-extractable Zn, (C) Ca(NO$_3$)$_2$ extractable Zn, (D) soil solution, (E) effective concentration, (F) free ion activity. A linear regression line is fitted for each plot. n = 594
6.4 Discussion

6.4.1 Effects of metal spiking of biosolids and soils on estimates of bioavailability

Pooled plant and soil data were subdivided into more specific datasets based on differences in biosolids and metal salt treatments. The purpose of this was to assess the degree of variability in shoot metal concentration that each test could significantly explain \((P<0.05)\) in smaller more defined datasets. Variations in shoot metal concentrations described by each test in these smaller groups were also compared with correlation results from pooled plant and soil data.

There were marked differences in the relative abilities of the assays to account for shoot metal concentrations between the groups and metals. For Cd, bioavailability assays were unable to describe greater than 50% of variation in shoot concentrations. Results for Cu revealed that measures of bioavailability were also unable to describe greater than 50% of variation in shoot Cu concentrations, with the exception of soils treated with metal spiked biosolids. While measures of Ni and Zn bioavailability were able to explain greater than 50% shoot metal concentration, but only in groups containing metal salts.

Explanatory ranges for Cd for the separate groups were between 4% and 40% (Table 6.1) for all methods tested and were considerably lower in range compared to pooled plant and soil data (16% to 47%, Figure 6.7). Effective Cd concentration in the pooled data could describe the greatest amount of variability in shoot Cd concentration (47%). However when assessed in the smaller groups, \(C_E\)-Cd was able to describe less than half of the variability in shoot Cd concentration (15% to 21%), and only in soil spiked with Cd salts. In soils having received no biosolids or metal salts, \(\text{Ca(NO}_3\text{)}_2\) and EDTA extractable Cd could describe between 19% and 40% shoot Cd concentration. This range is closer to that obtained from the pooled plant and soil data (30% and 33% for \(\text{Ca(NO}_3\text{)}_2\) and EDTA extractable Cd, respectively). These results suggest that the presence of Cd as a metal contaminant strongly determines the descriptive range of bioavailability measures such as DGT. While in unamended soils methods such as \(\text{Ca(NO}_3\text{)}_2\) and EDTA extractable Cd more accurately reflect plant available Cd.
Explanatory ranges for variation in shoot Cu concentrations were highest in soils amended with metal spiked biosolids and measures of Cu soil bioavailability were able to describe between 23% and 63% of shoot Cu concentration (Table 6.2). Soils spiked with Cu salts gave similar ranges (32% to 43%) for the six methods assessed, compared with the ranges determined for pooled plant and soil data (26% to 34%, Figure 6.8). Of these tests EDTA extractable Cu and CE-Cu accounted for the greatest amount of shoot Cu concentration in soils amended with metal spiked biosolids (55% to 63%), which were higher than soils amended with Cu salts (30% to 35%) and also results for pooled plant and soil data (26% to 27%, Figure 6.8). Thus it appears that the presence of Cu as Cu salts strongly influences the explanatory range of these methods assessed. Moreover, metal salts incorporated into the biosolids matrix results in an increase in the bioavailability and plant uptake of Cu.

For Ni the degree of variability in shoot Ni concentrations described by the assays tested increased with the addition of Ni salts (Table 6.3). In soils spiked with Ni salts (biosolids and metals, and metals only), almost all of the methods assessed could account for greater than 50% of the variability in shoot Ni concentrations. However in soils that received metal spiked biosolids, only EDTA-extractable Ni was able to describe greater than 50% of variability in shoot Ni concentration. When the descriptive ranges of the smaller groups were compared to the relationships determined between pooled plant and soil data (Figure 6.5), soils that had been treated with Ni salts gave similar ranges to those obtained from pooled results. The presence of biosolids including the spiking of biosolids with metal salts appeared to decrease the descriptive power of the assays tested. Hence, these results suggest the presence of Ni as a metal salt in soils strongly influences the outcome of these bioavailability assays with Ca(NO₃)₂-extractable Ni and soil solution Ni having the highest correlation with shoot Ni concentrations (between 61% and 81% for soil solution Ni and Ca(NO₃)₂-extractable Ni, respectively).

The number of significant relationships for shoot Zn concentration and assays of Zn bioavailability were higher across the five groups compared to Cd, Cu and Ni (Table 6.4). However, the general trend of increasing descriptive range of these tests with the presence of Zn in soils as Zn salts was similar to that of the other three metals. Of the
groups compared, metal spiked biosolids gave correlations similar to those obtained from the pooled plant and soil data (Figure 6.6). Results from assays on soils treated with metal salts only, could explain a higher degree of variability in shoot Zn concentrations than from plants grown in metal spiked biosolids, as well as relationships determined using pooled plant and soil results. However, only in two of the groups (metal spiked biosolids and metal salts only) were any assays able to describe greater than 50% of variability in shoot Zn concentration, and of these tests Ca(NO$_3$)$_2$-extractable Zn and CE-Zn were the most robust measures of Zn bioavailability (61% to 70%, Table 6.4). Thus it appears that the power of assays of bioavailability is influenced by the concentrations of Zn in biosolids with higher concentrations of Zn showing greater descriptive ranges.

The ability of each of these methods to significantly predict metal concentrations in shoots appeared to be largely influenced by the concentration of metal present in the biosolids and soil (i.e. the more metal present the higher $r^2$ value). The findings in this study suggest that three factors need to be considered when assessing the robustness of any method in estimating bioavailability:

- The range of variability that each assay of bioavailability can describe across a range of soils and soil amendments, i.e. an assay that presents a narrow range (e.g. 60% to 70%) may be more robust than an assay that provides a wider descriptive range (e.g. 40% to 75%);

- total metal concentration and likely form of metal (e.g. mostly bound to organic anions, or sorbed to organic matter) which would depend on the nature of the study, e.g. heavily contaminated soil, or assessment of naturally occurring elevated metal concentrations in serpentine soils;

- if the metal is known to accumulate around the roots or translocate to the shoots of the plants.

The appropriateness of methods used to estimate bioavailability have been discussed in a few recent studies (Basar, 2009; Degryse et al., 2009a; 2009b; McBride et al., 2009; Meers et al., 2007; Meers et al., 2009; Menzies et al., 2007; Nolan et al., 2005). McBride et al. (2009), concluded that chemically non-aggressive neutral salts, such as CaCl$_2$ may be the most appropriate extractants where phytotoxicity is the concern in
metal contaminated soils as these methods appear more responsive to soil properties affecting chemical lability of the metals. These findings certainly concur with the findings of this chapter and that of Chapters 4 and 5, in that Ca(NO₃)₂ gave the strongest relationship across a range of soils and amendments, especially for Ni. Certainly methods that considered the response of the solid phase reservoir of metals (i.e. Ca(NO₃)₂ and DGT) correlated better in general, and this has also been discussed in three previous studies (Degryse et al., 2009b; Lehto et al., 2006b; McBride et al., 2009). Although the predictive strength of these methods, especially DGT, is dependent on diffusive transport of the element from soil to the plant roots being rate-limited, competing cations are not affecting plant uptake of the metal in question and, and labile complexes are not taken up by the plant if diffusion is not limiting (Degryse et al., 2009b).

6.4.2 Effects of plant species on estimates of bioavailability

There are two major confounding factors in this assessment of plant species effects on estimates of bioavailability that may limit the interpretation of the results:

- plant maturity, i.e. 20 day-old wheat seedlings versus mature ryegrass;
- controlled environment versus field conditions.

In general assays of bioavailability were able to explain more variation in ryegrass shoot metal concentrations than wheat shoot metal concentrations. The relationships determined for both plant shoot metal concentrations and six measures of metal bioavailability were significantly different between species of plant for most of the assays tested. Overall shoot concentrations of Cd and Zn were significantly higher in wheat, while shoot concentrations of Ni was significantly higher in ryegrass. No assay was able to describe more than 50% variation in Cu shoot concentration data for either plant species.

Cadmium in soil solution and Ce-Cd appeared to be a dominant source of plant available Cd for ryegrass grown under field conditions (Table 6.5). When the explanatory ranges for each plant species were compared to relationships obtained for
pooled plant and soil data for Cd (Figure 6.7), pooled correlation values were generally lower, or similar to that obtained for wheat.

This trend was also observed with Ni concentrations in ryegrass shoots in that bioavailable Ni as measured by $C_E$ was the dominant source of plant available Ni compared with wheat grown in pots. However, wheat also showed similar relationships with assays of Ni bioavailability to ryegrass. Comparing the individual species relationships with assays of bioavailability to the overall relationship determined for pooled plant and soil data revealed that pooled coefficient of determination values (Figure 6.9) were more closely aligned with relationships determined for wheat.

Plant species relationships with Zn and assays of Zn bioavailability were the least different of all the metals assessed. Only pools of Zn as measured by EDTA and Ca(NO$_3$)$_2$, were significantly different with wheat having an overall higher uptake of Zn from available pools as measured by these methods. Relationships determined between pooled plant and soil data and six measures of Zn bioavailability appear more closely aligned with correlation values obtained using wheat. However, the degree to which these tests can significantly describe shoot Cu and Zn variability for the two species of plant are not as robust or consistent as they are for Cd and Ni, which suggests that either plant (or trial) may adequately describe variation in shoot Zn concentration.

Differences in plant species uptake of metals has been documented in several studies (Almas et al., 2006; Chen et al., 2009; Dunbar et al., 2003; Gray and McLaren 2005; Hamon et al., 1997). For example, in a pot trial carried out by Almas et al. (2006) using two species of plants, ryegrass, (*Lolium multiflorum*), and spinach (*Spinacia oleracea*) grown in metal contaminated soils in a controlled environment, good correlations were found between ryegrass Zn and $C_E$-Zn as measured by DGT ($r^2 = 0.96$ for Zn), while no significant correlation was found for $C_E$-Zn and spinach Zn concentrations. Moreover, Gray and McLaren (2005) found that even within the ryegrass genus, there was a significant amount of intraspecies variation in herbage metal concentrations in plants grown in the same contaminated soil. Another successful relationship between plant Zn concentration and $C_E$-Zn was also observed in lettuce (*Lactuca sativa*) grown in contaminated agricultural soils, but a less robust
relationship was observed with Cd concentrations in the leaves and $C_E$ Cd as measured by DGT (Cornu and Denaix, 2006).

Results from this study concur with findings reported by Almas et al. (2006) and Cornu and Denaix (2006) which suggests that the strength of potential measures of metal availability is species dependent. Additionally, relationships between availability measurements and plant concentrations would likely be affected by plant health as metal uptake generally decreases in plants affected by metal toxicity, whereas most measures of metal solubility are not limited by a physiological response. Other factors to consider regarding the differences of relationships determined between plant metal concentrations and measures of metal availability are:

- sources of available metals other than the resupply of metals weakly bound to the solid phase of the soil, e.g. metals bound to low affinity ligands;

- metal species other than the free ion are being taken up by plants, e.g. CdCl$^+$ (Degryse et al., 2006a; Degryse et al., 2006b; Weggler et al., 2004);

- competitive uptake of other divalent cations such as Ca, Mg (Kalis et al., 2006);

- the possibility that certain species may specifically exclude the uptake of some divalent cations, or internal detoxification by the plant, e.g. by binding Cd to phytochelatins, also the lack of translocation from the root to the shoot (Reid et al., 2003a);

- rhizosphere processes (including root morphology) that may alter the availability of the metal, such as the exudation of low molecular weight organic compounds combined with alteration of pH (Gregory, 2006; Jones et al., 2004; Lu et al., 2007).

The power of measures of bioavailability to predict shoot concentrations were significantly different for ryegrass and wheat. Overall assays were able to describe more variation in ryegrass shoot data than for wheat, with Ca(NO$_3$)$_2$, DGT and soil solution being the most successful measure of Cd, Ni and Zn concentration in
ryegrass shoots. However, when comparing relationships obtained from pooled plant and soil data, these were more closely aligned with relationships obtained with 20 day-old wheat seedlings. In summary, results suggest that the use of a wheat seedling assay may not adequately reflect metal bioavailability in longer term studies of metal contamination and plant uptake. Therefore the use of wheat seedling assays as model systems carried out in controlled environmental conditions to evaluate metal bioavailability may need to be standardised against mature plant models managed under field conditions.

6.4.3 Evaluation of six potential measurements of bioavailability using pooled plant species and soil data

In total, 12 soils were amended with different combinations and rates of biosolids and metal salts and two species of plant (wheat and ryegrass) were chosen as the biological model systems to evaluate the response of plants to soil amendments from studies carried out over several years. Although this comparison of pooled data from across several studies provided the opportunity to determine the robustness of these methods it also introduced several confounding factors that can potentially affect the predictive strength of these assays and the interpretation of results. Such factors were described in previous sections and included: field experiments versus controlled environment; wheat versus ryegrass responses, and mature plants versus 20 day old seedlings.

The range of predictive strengths for Cd bioavailability found in this study, are also similar to those described previously in the literature. Studies assessing the predictive capabilities of methods (i.e. DGT, speciation models, free ion concentration, EDTA-extraction and weak salt solutions) to determine shoot Cd concentrations also found a range of coefficient determinations for Cd as measured by these methods (Almas, et al., 2006; Gray et al., 2004; Menzies, et al., 2007; Nolan, et al., 2005). The robustness of these potential measures of bioavailability appeared dependent on the supply of the metal (Almas, et al., 2006; Menzies, et al., 2007; Nolan, et al., 2005) and the plant species involved (Almas et al., 2006).
In this study, no method assessed could explain more than 50% of variability in shoot Cd concentration. However, \( C_E \text{-Cd} \) was able to explain the most variation in shoot Cd concentration (47%). The inability of any of the methods assessed to significantly predict greater than 50% of the variability for Cd could be due to the relatively low concentrations of Cd present in the soil with respect to other cations such as Ca, Cu, Mg, Ni and Zn. This situation could have lead to the competitive uptake of cations present in higher concentrations via non-specific cation pathways found in plant roots thereby limiting uptake of cations present in much lower concentrations, such as Cd (Reid et al., 2003a). Another factor that may have affected the ability of methods such as DGT to explain greater than 50% of variability in shoot Cd concentration are labile complexes of Cd not accounted for by DGT that may be taken up by plants (Degryse et al., 2009b). Moreover, unlike Cu, Ni and Zn, Cd is a non-essential element (Alloway, 1995a; Kiekens and Cottenie, 1985; Sloan et al., 1997) and may be actively excluded from shoots by the plant, and thus affect the amount of shoot Cd concentration variation described by the methods assessed.

Several studies assessing the predictive power of assays of metal bioavailability have found that predictions of Cu uptake by plants from soils by the various measures of available Cu are typically poor \( (r^2 < 0.5) \) (Basar 2009; Nolan et al., 2005; Menzies et al., 2007), except for total Cu (Nolan et al., 2005; Menzies et al., 2007), which is in agreement with the predictive results obtained in study. This result is unsurprising as plants typically exert strong physiological controls on the translocation of Cu from the roots to the shoots (Reid et al., 2003a). Zhang et al. (2001) were able to predict 95% of the variability in plant Cu using DGT in a pot trial using 29 soils and Smith’s pepperwort \( (Lepidium heterophyllum) \) as the biological response model. However, the soils used in the study were contaminated mostly with Cu and the concentrations present in the soils were more than 8 times higher \( (> 8000 \text{ mg kg}^{-1}) \) than Cu concentrations present in any of the treated soils used in this study (highest treatment application was 1000 mg kg\(^{-1}\)). In addition, \( L. \text{ heterophyllum} \) is also known to be an effective accumulator of Cu (Nolan et al., 2005) and thus does not tightly regulate Cu translocation from the roots to the shoots as in non-accumulating plant species.

Bioavailability measures of Ni and Zn were the most successful of the four metals in determining shoot metal concentrations. The validity of 0.05 M Ca(NO\(_3\))\(_2\) extraction as a reasonable predictive measure of Ni and Zn bioavailability is evident given that
these results were obtained from a range of New Zealand soils, biosolids and metal concentrations and two plant species. The success of neutral salt extractants such as such as 0.05 M of Ca(NO₃)₂ as an indicator of metal availability has been briefly described by Menzies *et al.* (2007) and Meers *et al.* (2009) These extractants were developed on the theory that bioavailable metals are located on soil mineral surfaces and these can be displaced by competing cations (Andrews *et al.*, 1996; McLaughlin *et al.*, 1999).

### 6.5 Conclusions

The predictive powers of each bioavailability method used in this study rely on the theory that the concentration of metal acquired will reasonably reflect the concentrations found in plant shoots. Although there was a considerable amount of variation in correlation strengths of Ca(NO₃)₂-extractable metals, this method significantly accounted for the most variation in shoot metal concentrations observed for Ni (74%) and Zn (65%). Total metal, EDTA extraction and DGT had the lowest explanatory range (33 – 34%) and were able to explain greater than 50% of the shoot concentration variation for Ni and Zn, but were on average lower in their predictive strength for Ni and Zn than for Ca(NO₃)₂-extractable Ni and Zn. Correlation results for Cd and especially Cu were weak and none of the methods assessed could explain greater than 50% of variability, with the lowest predictive capability obtained using Cd²⁺ activity ($r^2 = 0.16$).

The responses of wheat and ryegrass as assessed using six potential measures of bioavailability were consistently different. Concentrations of Cd and Zn were generally higher in wheat than in ryegrass, while concentrations of Ni were typically higher in ryegrass. Apart from Cu, the methods assessed were able to significantly explain a greater amount of variability for ryegrass data than for wheat, which suggests a greater predictive capability for assessments involving ryegrass. Unsurprising, shoot concentrations of each metal were different for wheat and ryegrass. Concentrations of metals in wheat shoots were generally higher in relation to larger sized pools of available metals as described by EDTA, Ca(NO₃)₂ and soil
solution, whereas ryegrass shoot concentrations of metals were generally higher in relation to the much smaller pools of exchangeable metals, as measured by DGT. Hence this suggests that longer growth periods had a greater demand on the soil system.

Interpreting the relationships between shoot metal concentrations and assays of metal bioavailability in five soil treatment groups that share similarities for the purpose of assessing method validity is complicated. However the findings of this study suggest that two factors need to be considered when assessing a method’s robustness and appropriateness in estimating bioavailability:

- range of $r^2$ values for the method across a range of soils and soil amendment;
- the total soil concentration of the metal and whether that metal is known to accumulate, or translocate to the tops of the plants.

Methods that consider the biologically relevant amount of metal supplied from the solid phase, appear to provide a more robust measure of bioavailability and this study had proven this to be the case for Zn and especially Ni, where the validity of these methods were determined using two species, soil types and amendment regimes.
Chapter 7
General Discussion, Conclusions and Approaches for Further Studies

7.1 Summary

It is widely accepted that metal bioavailability is important in providing accurate environmental risk assessments of metal contaminated soils. However there is still uncertainty regarding mechanisms that control the pathway of metals from the soil to the biological endpoint (i.e. leafy shoots) (Palmer and Guerinot 2009; Reid et al., 2003a). Commonly, the concentration of metal considered bioavailable is often defined as the fraction of metal extracted from soil using a chemical reagent, based on correlation with plant shoot metal concentrations (Menzies et al., 2007). In most cases bioavailability is largely a measure of metal solubility. Existing techniques have been developed and/or modified for use in soils, which have attempted to provide a more inclusive or, targeted approach in determining bioavailability. These include:

- the development and modification of the DGT method which simulates plant uptake of metals through diffusion via the roots (Lehto et al., 2006a; Zhang et al., 2001). The DGT method is used in conjunction with the DIFS modelling programme that considers the replenishment capacity of the solid phase to resupply the amount depleted in soil solution (Harper et al., 2000);

- assemblage models that determine free ion activity and speciation in soil solution (WHAM) (Degryse et al., 2009a; Tipping et al., 2003b);

- neutral salt solutions with ionic strengths similar to that of soil solution that saturate the system with competing cations, thereby displacing the fraction of metal considered readily available for plant uptake (Andrews et al., 1996; Meers et al., 2009; Menzies et al., 2007).
However, there are insufficient datasets that span confounding factors such as soil properties, metal concentrations, soil amendments and plant species to rigorously verify these methods.

The overall aim of this thesis was to assess and validate measures of bioavailability of Cd, Cu, Ni and Zn across various soils amended with metals and biosolids. This was achieved by assessing six potential measures of bioavailability in three distinct, but complimentary chapters:

- Chapter 4 assessed the effects of biosolids amendment on the bioavailability of Cd, Cu, Ni and Zn in three metal spiked soils under controlled environmental conditions, using wheat as the model plant system;
- Chapter 5 assessed the effects of biosolids and metal salt amendment on the bioavailability of Cd, Cu, Ni in the same three soils used in Chapter 4; managed under field conditions, using ryegrass as the model plant system;
- Chapter 6 assessed the overall strength of the relationships between shoot metal concentrations in wheat and ryegrass and potential measures of Cd, Cu, Ni and Zn bioavailability from 12 New Zealand soils, including those soils used in Chapters 4 and 5, amended with different combinations of biosolids and metal salts.

### 7.2 Estimates of bioavailability

A total of 12 soils were amended with different combinations of biosolids and metal salts. Two species of plant (wheat and ryegrass) were chosen as the biological model systems to evaluate the response of plants to treated soils from data gathered between 2002 and 2008.

Of the four metals examined, Ni was found to have the strongest relationship between measures of bioavailability and shoot concentrations (Table 7.1). Results indicated that shoot concentrations of Ni were highly indicative of the concentration of Ni present in soil solution and Ni weakly sorbed to soil particles. Of the six methods
assessed, Ca(NO₃)₂ was the most robust assay and could predict 73% to 87% of the variability in Ni concentrations in the shoots of both species. However, all methods assessed were relatively good predictors of bioavailable Ni and could explain greater than 50% of shoot responses (Table 7.1).
Table 7.1  Range of significant ($P<0.05$) coefficients of determination for six potential measures of bioavailability assessed in three related studies. Total variation for each method and metal is derived from the highest $r^2$ value minus the lowest $r^2$ value.

### Chapter 4: Pot trials using wheat

<table>
<thead>
<tr>
<th>Method</th>
<th>Cd</th>
<th>Cu</th>
<th>Ni</th>
<th>Zn</th>
<th>Total variation (method)</th>
</tr>
</thead>
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<tr>
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<td>15%</td>
<td>63%</td>
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<td>48%</td>
</tr>
<tr>
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<td>63%</td>
<td>13%</td>
<td>60%</td>
<td>54%</td>
<td>50%</td>
</tr>
<tr>
<td>Ca(NO$_3$)$_2$</td>
<td>62%</td>
<td>10%</td>
<td>73%</td>
<td>55%</td>
<td>63%</td>
</tr>
<tr>
<td>Soil solution</td>
<td>30%</td>
<td>6%</td>
<td>66%</td>
<td>33%</td>
<td>60%</td>
</tr>
<tr>
<td>DGT</td>
<td>26%</td>
<td>13%</td>
<td>44%</td>
<td>43%</td>
<td>30%</td>
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<tr>
<td>Free ion activity</td>
<td>30%</td>
<td>11%</td>
<td>67%</td>
<td>33%</td>
<td>56%</td>
</tr>
<tr>
<td>Total variation (metal)</td>
<td>37%</td>
<td>9%</td>
<td>29%</td>
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### Chapter 5: Field lysimeter trial using ryegrass

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<td>Ca(NO$_3$)$_2$</td>
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<td>59%</td>
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<tr>
<td>Soil solution</td>
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<td>49%</td>
<td>83%</td>
<td>68%</td>
<td>34%</td>
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<tr>
<td>DGT</td>
<td>65%</td>
<td>25%</td>
<td>84%</td>
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<td>59%</td>
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<tr>
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<td>7%</td>
<td>75%</td>
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<td>Total variation (metal)</td>
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<td>52%</td>
<td>43%</td>
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### Chapter 6: Combined Lincoln University and ESR trials

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<th>Zn</th>
<th>Total variation (method)</th>
</tr>
</thead>
<tbody>
<tr>
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<td>52%</td>
<td>33%</td>
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<tr>
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<td>22%</td>
<td>56%</td>
<td>52%</td>
<td>34%</td>
</tr>
<tr>
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<td>30%</td>
<td>30%</td>
<td>74%</td>
<td>65%</td>
<td>44%</td>
</tr>
<tr>
<td>Soil solution</td>
<td>19%</td>
<td>29%</td>
<td>64%</td>
<td>56%</td>
<td>45%</td>
</tr>
<tr>
<td>DGT</td>
<td>47%</td>
<td>26%</td>
<td>54%</td>
<td>60%</td>
<td>34%</td>
</tr>
<tr>
<td>Free ion activity</td>
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<td>39%</td>
</tr>
<tr>
<td>Total variation (metal)</td>
<td>31%</td>
<td>12%</td>
<td>18%</td>
<td>13%</td>
<td></td>
</tr>
</tbody>
</table>
The robustness of Ca(NO₃)₂ extraction for estimating Zn bioavailability was unable to describe the same degree of variability in shoot metal concentration as was shown for Ni. However, results from the pot trial did give a higher degree of predictive power than results from the field trial (55% versus 43% for each respectively, Table 7.1). Although in Chapter 6, where ESR studies were combined with results presented in Chapters 4 and 5, the predictive power of Ca(NO₃)₂-extractable Zn increased to 65%. The overall strengths of the six measures of bioavailability to predicted Zn concentration in shoots were stronger in the field lysimeter trials than in the pot trials, with soil solution Zn, C₆-Zn and Zn²⁺ activity significantly explaining 68% to 76% of the variations in shoot Zn concentrations (Table 7.1). The difference between the experiments in ability to predict bioavailable Zn most likely reflects three major confounding factors:

- field studies versus controlled environments (i.e. leaching of cations and anions through the soil profile);
- wheat versus ryegrass;
- 20 day old seedlings versus mature plants.

These factors can alter the effect that the presence of competitive cations, or complexes, that these tests that may overcompensate for, or complexes that are (un)available for plant uptake (Almas et al., 2006; Degryse et al., 2006b; 2009b). For example, the trials in pots were not free draining and hence, would retain many of the cations and anions (both inorganic and organic) that were lost as a result of drainage that occurred in the field trial. The retention of soil solution in pots could also allow a greater amount of interaction between components in the solution and exchange with the solid phase of the soil. Also, seedlings have different nutritional requirements to more mature plants and in longer term trials, the most available fraction (i.e. soil solution) would have been depleted within the first few months.

The results for Cd were generally similar to Zn and were explain very similar percentages of variation (50.5% versus 50.1% for Cd and Zn respectively). Additionally results from the lysimeter study could explain the greatest amounts of shoot Cd variation compared to Cd results from pot trials (Table 7.1). However, results from the combined studies (Chapter 6) revealed an overall decrease in the
predictive capabilities of all methods assessed compared to results from field trials, with no assay being able to describe more than 50% of shoot Cd variability (Figure 6.7). The lower predictive power of the soils tests to describe Cd variability in Chapter 6 compared with Chapters 4 and 5 is most likely due to the Cd data mostly being from pot trials where ranges of Cd concentrations in the soil were low compared with other metals. Comparitively low soil concentrations of Cd compared with Cu, Ni and Zn would result in greater concentrations of those metals present in higher concentrations being taken up by plants, because of the effect of mass action. This result also suggests that competing cations such as Ca and Zn may also affect the outcome of these tests with respect to Cd as these would be present in much higher concentrations.

None of the methods assessed could explain more than 50% of shoot Cu variability, although total Cu and soil solution Cu results from the field lysimeter trial did result in correlation of $r^2 = 0.49$ for both methods. Although some studies have shown good fits with potential measures of bioavailability and Cu uptake by plants (Zhang et al., 2001), the majority of research has found poor relationships between potential bioavailability measure and shoot Cu concentrations (Menzies et al., 2007). The reason for this is that Cu in soil solution is strongly complexed by organic matter, hence most Cu is considered unavailable for plant uptake, and the uptake of Cu to shoots from roots is strongly regulated by plant metabolism of nonaccumulating plants.

The overall efficacy of the different methods for assessing bioavailability appeared to be largely dependent on the metal itself (i.e. Ni was a good candidate for solubility based tests, while Cu was not), the extent of metal contamination (higher and greater range of concentrations of metal in the soil gave stronger relationships with shoot metal concentrations), the presence of competitive cations and complexing anions (i.e. Ca, Mg competing with Cd and Zn for uptake, and DOC complexing with Cu and Ni making Cu and Ni less/more available for plant uptake), and the general condition of the plant.
7.3 Influence of biosolids on metal bioavailability

Chapters 4 (pot trial) and 5 (field trial) explored the potential effects that biosolids amendment might have on the bioavailability of Cd, Cu, Ni and Zn. In the pot trial using wheat, all metal salt treatments had corresponding biosolids amendments, while in the field trial using ryegrass, only the lowest concentration of metal salt treatments was amended with biosolids. For both studies the addition of biosolids had a marked effect on soil chemistry, with increases in DOC, Ca, Mg and salinity observed in the soil solution. Also in the pot trial, an increase in total soil Cu and Zn was observed, largely owing to the ubiquitous presence of Cu and Zn in biosolids. Increased DOC, Ca, Mg and salinity were correlated with increased soil incubation time (pot trial) and length of time since treatment application (field trial).

In the pot trial, the presence of biosolids did not significantly affect the extractability or solubility of any of the metals as measured by five of the soil tests (EDTA, Ca(NO₃)₂, soil solution, DGT and free ion activity, Table 4.4). The relationship determined between shoot metal concentrations and soil assays of metal bioavailability were also not affected by the presence of biosolids. This result suggests that the presence of biosolids did not increase the potential bioavailability of the four metals.

Results from the field trial showed that in the presence of biosolids increased EDTA-extractable Cd, Cu²⁺ activity, EDTA-extractable Ni and Cₑ-Zn, but decreased Cₑ-Cd, Cₑ-Ni, Ni²⁺ activity and Zn²⁺ activity (Table 5.5). The relationships determined between shoot Cd and Ni concentrations and assays of Cd and Ni bioavailability were also significantly different in the presence of biosolids (Table 5.16 and 5.18). Three possible explanations could account for these results:

- influence of time on the system (six months versus 24 months) resulted in increased sorption of Cd and Ni into the biosolids matrix as observed by the increase in EDTA-extractable Cd and Ni, and the decrease in the readily available fraction of Cd and Ni (Cₑ and free ion activity);

- greater plant demand on the system as a result of longer growth period (e.g. the removal of readily available Zn), with the presence of biosolids providing an additional supply of Zn to replenish the amount of Zn in solution (increase in Cₑ-Zn);
• leaching of cations (Ca and Zn), anions and DOC through the profile would remove ions and DOC from the top 10 cm of the soil profile, leading to a decrease in ionic strength of the soil solution that may result in an increase in Cu\(^{2+}\) activity.

### 7.4 Plant responses

In the pot trial, a significant reduction in dry weight was recorded in soils amended with biosolids. While several factors, including the toxic effects of metals were explored, results revealed that salinity of the soil and to a lesser extent Cu and Ni concentrations within the shoots was controlling the production of dry matter (Figure 4.9, 4.12 and 4.13). It was expected that symptoms of phytotoxicity would be exhibited by plants grown in the highest metal treatments, although the osmotic stress induced by the presence of biosolids was unexpected, with the dissolution of metal salts having also contributed to the overall osmotic stress experienced by the plants. Changes in dry weight were not determined in the lysimeter study, as only a representative plant sample was collected at each harvest and so comparisons were not made between the pot and field trials.

The presence of biosolids resulted in a decrease in Cd concentration in wheat in the pot trial (Table 4.6 and 4.7). However this effect was only observed at the 24 month harvest period in the lysimeter trial (Table 5.14). Although the significance level for the 18 month harvest period in the field trial was only just greater than 0.05 (\(P = 0.057\)). Even though Cd results from the lysimeter trial were less conclusive than from the pot trial, inferences based on soil chemistry results from both trials suggest that the presence of biosolids decreases plant available Cd, and from these two mechanisms may be responsible for this effect:

• increased concentration of DOC (Table 4.2 and 5.2) reducing the availability of Cd by complexation;

• presence of cations such as Ca and Mg(Table 4.2 and 5.2) that would competitively inhibit the uptake of Cd by plants.

In the pot trial, increased soil incubation time resulted in the significant decrease in Cu concentration in wheat, while generally Zn concentration in wheat increased significantly (Table 4.6). The same result for Zn concentration in ryegrass was also observed in the field trial, while overall Ni concentration in ryegrass decreased with time in the field trial. It was...
inferred from soil chemistry results that complexation by soil organic matter (Cu) and DOC (Cd and Ni), and an increased demand from the plant system (Zn) with increased availability (in the case of the pot trial) were responsible for these observed trends.

Chapter 6 compared the two plant species at different stages of growth and their relationship to potential measures of bioavailability. The relationships of wheat and ryegrass were consistently significantly different from each other. Overall concentrations of Cd and Zn were significantly higher in wheat than in ryegrass, while Ni was present in significantly higher concentrations in ryegrass. In relation to Cu responses, ryegrass had significantly lower shoot concentrations of Cu than wheat as determined by relationships between \( \text{Ca(NO}_3\text{)}_2 \), soil solution Cu and \( C_E \)-Cu and shoot Cu, although regression values for Cu were typically poor \((r^2 <0.45)\). Coefficients of determinations between shoot concentrations and potential measures of metal bioavailability were higher for ryegrass than for wheat. In addition, results suggested that metal concentrations measured by 0.05 M of \( \text{Ca(NO}_3\text{)}_2 \), extraction, \( C_E \) and metals in soil solution were the dominant pools of available Cd and Ni for ryegrass, while available pools of Zn as measured by EDTA and \( \text{Ca(NO}_3\text{)}_2 \) were a dominant source of Zn for wheat grown in pots compared to than for ryegrass. This suggests that longer growth periods (i.e. ryegrass in the field trial) deplete the most readily available sources of metals, which then result in these metals having to be replenished from the solid phase.

### 7.5 Conclusions

There are many extractive techniques used to determine the bioavailable metal fraction in soils but currently there is no consensus as to the most appropriate measure of metal bioavailability. The predictive power that these methods have are based on hypotheses that the amount of metal present in solution along with varying amounts of metal extracted from the solid phase of the soil will reasonably reflect concentrations in the above ground plant tissues. Investigation of six potential measures of bioavailability across soils with contrasting properties, range of metal concentrations, presence of biosolids and two plant species carried out in this study represented a rigorous assessment of this topic. The degree to which these tests could significantly explain the variability in shoot metal concentrations appeared to be metal specific, with the concentration of metal in the soil, competing cations and the presence of DOC influencing the availability. Of these tests \( \text{Ca(NO}_3\text{)}_2 \) provided the most robust measure of Cd, Zn and especially Ni bioavailability with results for Ni able to account for
more than 80% of the variability in shoot Ni concentrations in the lysimeter field trial. Thus Ca(NO$_3$)$_2$ provided the most useful indication of bioavailability across soils, soil amendments and plant species for Cd, Ni and Zn.

Results for other measures of Ni availability yielded the strongest correlations overall, suggesting that the solubility of Ni is highly indicative of shoot concentrations. On the other hand, results for measures of bioavailable Cd, Zn and especially Cu were less indicative of shoot concentrations. Relationships between shoot concentrations of Cd and Zn and corresponding soil concentrations require further investigations. Results for Cu were expected as assays of Cu solubility are almost always poorly correlated with shoot concentrations, hence tests based on the extractability and solubility of metals are not robust measures of predicting phytotoxic concentrations of Cu in soils. The addition of biosolids did not alter the outcome of these bioavailability tests in the pot trials presented in Chapter 4 and results presented in Chapter 6 suggest that it is the range of metal concentrations present in the soil and biosolids that have a greater influence on $r^2$ values, rather than simply the presence of biosolids. Findings presented in Chapter 4 also indicated that the presence of biosolids in a particular treatment appeared to decrease the concentration of Cd in shoots, although the exact mechanism(s) responsible were inferred but not determined. While species differ in their uptake of metals from soils, results from this study suggest that both species access the same bioavailable pool of metals. Also the disparity between wheat and ryegrass is highly likely to be reflective of the different growth periods (i.e. 20 days versus 24 months), which would exert different demands on the system. Hence standardising seedling assays against mature plant models may be necessary for bioavailability assessments carried out on longer term studies of metal contamination.

7.6 Approaches for further studies

The findings of the research presented in this thesis suggest further studies in the following areas:

- identification and quantification of organic anions excreted by the plant roots – are some more prevalent than others? and do certain organic anions have a greater affinity for metals than others? Would these alter the bioavailability of Cd, Cu and Zn? By quantifying these organic anions and their affinity for metals improve the
strength and consistency of the relationship between soil measures of Cd, Cu and Zn and plant concentrations;

- the use of root Cu concentration instead of shoot Cu concentration as a biological endpoint.

- does root morphology impact upon metal uptake? Different species of plants have different root systems, would this alter metal uptake?

- does soil microbial activity affect metal bioavailability? if so are there specific communities associated with plant species, especially commercial crops, that can be identified?

- do mycorrhizae species affect metal bioavailability during their acquisition of P from soil minerals?
References


Biosolid Research Program - how it came about, and what has it discovered? Water Practice & Technology 2:art 88.


Warne M.S.J., Heemsbergen D., Stevens D., McLaughlin M., Cozens G., Whatmuff M., Broos K., Barry G., Bell M., Nash D., Pritchard D., Penney N. (2008b) Modelling the
toxicity of copper and zinc salts to wheat in 14 soils. Environmental Toxicology and Chemistry 27:786-792.


## Appendix A. Soil chemistry, metal extractability and speciation modelling results for Chapter 4

Table A1  Background concentrations (mg kg$^{-1}$) of Cd, Cu, Ni and Zn in the three soils in the presence and absence of biosolids.

<table>
<thead>
<tr>
<th>Soils</th>
<th>treatment</th>
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<th>Cu</th>
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<th>Zn</th>
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Figure A1 Comparison of dissolved organic carbon (DOC) and pH in soil solution extracted from treated soils at two weeks and six months incubation time. Error bars denote SEM.
Figure A2   Comparison of Ca(NO$_3$)$_2$, EDTA and total recoverable Cd in soils at two weeks and six month incubation times. Error bars denote SEM.
Figure A3  Comparison of Ca(NO₃)₂, EDTA and total recoverable Cu in soils at two weeks and six month incubation times. Error bars denote SEM. Total Cu is only shown with the EDTA extractable results. Note the difference in y-axis for Ca(NO₃)₂ extractable results.
Figure A4 Comparison of Ca(NO₃)₂, EDTA and total recoverable Ni in soils at two weeks and six month incubation times. Error bars denote SEM.
Figure A5  Comparison of Ca(NO₃)₂, EDTA and total recoverable Zn in treated soils at two weeks and six month incubation times. Error bars denote SEM.
Figure A6 Comparison of Cd concentrations and pH changes in soil solution extracted from treated soils at two weeks and six month incubation times. Note the variation of the y-axis for Halkett soil graphs. Error bars denote SEM.
Figure A7  Comparison of Cu concentrations and pH changes in soil solution extracted from treated soils at two weeks and six month incubation times. Note the variation of the y axis for the Halkett soil graphs. Error bars denote SEM.
Figure A8 Comparison of Ni concentrations and pH changes in soil solution extracted from treated soils at two weeks and six month incubation times. Error bars denote SEM.
Figure A9 Comparison of Zn concentrations and pH changes in soil solution extracted from treated soils at two weeks and six month incubation times. Note the variations of both y-axes of the Halkett soil graphs. Error bars denote SEM.
Figure A10  Partitioning co-efficient (KD) for biosolids and non-biosolids amended metal spiked soils (A) results for Cd, (B) results for Cu and, (C) results for Ni. All relationships were significant (P<0.0001), n = 71 (Biosolids + MS) and n = 72 (MS).
Figure A11  Mean % of Cd species present in soil solution as colloidal fulvic acid bound (FA), simple ion (Cd\(^{2+}\)), CdSO\(_4\), and remaining inorganic species (CdOH\(^+\), Cd(OH)\(_2\), CdCl\(^-\), CdHCO\(_3\)^-, CdCO\(_3\)^2-, Cd(CO\(_3\))\(_2\)). NB: for the two week graphs Cd was below detection limits for untreated HK and SM, as well as the biosolids treatment for HK.
Figure A12  Mean percentage of Cd distribution on organic matter (particulate HA and FA), Fe and Al oxides, clay, and present in the aqueous phase for HK, SM and WK soils incubated for two weeks and six months.
Figure A13  Mean % of Cu species present in soil solution as colloidal fulvic acid bound (FA), simple ion (Cu\(^{2+}\)), CuSO\(_4\) and remaining inorganic species (CuOH\(^+\), Cu(OH)\(_2\), CdCl\(^+\), CuHCO\(_3\)^-, Cu(CO\(_3\))^\(_2\)^-) for HK, SM and WK soils incubated for two weeks and six months.
Figure A14  Mean percentage of Cu distribution on organic matter (particulate HA and FA), Fe and Al oxides, clay, and in the aqueous phase for HK, SM and WK soils incubated for two weeks and six months
Figure A15  Mean % of Ni species present in soil solution as colloidal fulvic acid bound (FA), simple ion (Ni$^{2+}$), NiSO$_4$ and remaining inorganic species (NiOH, Ni(OH)$_2$, NiCl, NiHCO$_3^-$, NiCO$_3^-$) for HK, SM and WK soils incubated for two weeks and six months.
Figure A16  Mean percentage of modeled Ni distribution on the organic matter (particulate HA and FA), Fe and Al oxides, clay, and in the aqueous phases for HK, SM and WK soils incubated for two weeks and six months.
Figure A17  Mean % of Zn species present in soil solution as colloidal fulvic acid bound (FA), simple ion (Zn\(^{2+}\)), ZnSO\(_4\) and remaining inorganic species (ZnOH\(^+\), Zn(OH)\(_2\), ZnCl\(^+\), ZnHCO\(_3\)^+, ZnCO\(_3\)) for HK, SM and WK soils incubated for two weeks and six months.
Figure A18  Mean percentage of Zn distribution on organic matter (particulate HA and FA), Fe and Al oxides, clay, and in the aqueous phase for HK, SM and WK soils incubated for two weeks and six months.
Appendix B. Plant results for Chapter 4

Figure A19  Mean germination rates and dry weights of wheat seedlings grown in biosolids amended and unamended metal treated soils incubated for two weeks and 24 weeks at field capacity. Bars denote the SEM, n = 89 for both trials.
Figure A20  Mean Cd concentrations for in wheat shoot tissue (mg kg⁻¹ dry weight) grown in biosolids ammended and unamended metal spiked soils incubated for two weeks and 24 weeks. The critical level (CL) for Cd as depicted by the red line is 43 mg kg⁻¹ (Macnicol and Beckett, 1985). Bars denote the SEM, n = 89 for both trials.

Abbreviation key
C = Control
B = Biosolids only
BLM = Biosolids + low metals
LM = Low metals
BMM = Biosolids + medium metals
MM = Medium metals
BHM = Biosolids + high metals
HM = High metals
BCd = Biosolids + Cd only
Cd = Cd only
Figure A21  Mean Cu concentrations in wheat shoots (mg kg$^{-1}$ dry weight) grown in biosolids amended and unamended metal spiked soils incubated for 2 weeks and 24 weeks. The critical levels (CL) for Cu as depicted by the red line, is 17 mg kg$^{-1}$ (Macnicol and Beckett, 1985). Bars denote the SEM, n = 89 for both trials.
Figure A22  Mean Ni concentrations in wheat shoots (mg kg\(^{-1}\) dry weight) grown in biosolids amended and unamended metal spiked soils incubated for 2 weeks and 24 weeks. The critical levels (CL) for Ni as depicted by the red line, is 46 mg kg\(^{-1}\) (Macnicol and Beckett, 1985). Bars denote the SEM, n = 89 for both trials.
Figure A23  Mean Zn concentrations in wheat shoot tissue (mg kg\(^{-1}\) dry weight) grown in biosolids amended and unamended metal spiked soils incubated for 2 weeks and 24 weeks. The critical levels (CL) for Zn as depicted by the red line, is 224 mg kg\(^{-1}\) (Macnicol and Beckett, 1985). Bars denote the SEM, n = 89 for both trials.
Appendix C. Regression and ANOVA results for DOC, salinity and shoot Cd concentrations for Chapter 4

Regression analysis for the relationship determined between DOC and shoot Cd concentrations

\[
R = 0.27966490 \quad \text{Rsqr} = 0.07821246 \quad \text{Adj Rsqr} = 0.06465676
\]

Standard Error of Estimate = 0.3952

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Regression analysis for the relationship determined between soil solution salinity and shoot Cd concentrations

\[
R = 0.44867972 \quad \text{Rsqr} = 0.20131349 \quad \text{Adj Rsqr} = 0.18956810
\]

Standard Error of Estimate = 0.3679

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