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Copper and zinc dynamics and bioavailability in soils amended with biosolids

A thesis presented in partial fulfilment of the
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Abstract

Global sewage sludge (biosolids) production is increasing as a result of rapidly growing human population and ensuing industrial activities. Land application of this waste is becoming a serious environmental issue because the high levels of heavy metals in biosolids can upset soil microbial activity and nutrient balance when the waste is added to forest or agricultural lands. It is widely accepted that bioavailability, rather than total soil concentration, is more important when assessing the risk associated with metal contamination. The bioavailability of a heavy metal is dependent on the chemical nature of the metal, the chemical, physical and biological properties of biosolids that contain the metal and of soil that receives the biosolids. It also depends on the interaction of the biosolids with soil, plants and soil microorganisms. The overall aim of this thesis was to assess the bioavailability of biosolids-derived Cu and Zn and the comparative effects of these metals on plant and soil microbial activity, with special attention to mycorrhiza, and the effects of application of lime and Al dross as ameliorants for the reduction of bioavailability of these two metals.

Biosolids were collected from the Palmerston North City Council Waste Water Treatment Plant (PNCCWTP) sludge lagoon in Palmerston North, New Zealand. Because the metal concentrations were low, for research purposes these biosolids were spiked separately with three levels of Cu (to give final concentrations of 50, 150 and 250 mg/kg soil) and Zn (to give final concentrations of 150, 450 and 750 mg/kg soil) added as metal sulphate salts. The biosolids were anaerobically incubated, and it was found that a shorter period of equilibration (2 months) than the previously used 6–9 months was sufficient for Cu and Zn to be fully incorporated into the biosolids matrix. As biosolids in New Zealand are currently applied to forest lands, two important forest plants, poplar and pine, were considered for the study in this thesis. The effects of elevated concentrations of Cu and Zn in a soil amended with metal spiked biosolids on poplar plants were investigated in a 147 day glasshouse pot trial. The findings of this trial showed that at the same total soil metal concentration, biosolids-derived Cu was more

toxic than Zn to soil microorganisms, whereas Zn was more toxic to poplar and ECM fungi. In a similar glasshouse study lasting 312 days with pine, Cu did not show a phytotoxic effect, but Zn was phytotoxic to pine. However, both metals were toxic to microorganisms and neither metal influenced ECM fungi colony development. The currently recommended maximum metal concentration limits for New Zealand soils of 100 mg/kg for Cu and 300 mg/kg for Zn appear to be high for both metals with respect to soil microbial activity, but low for Cu and high for Zn with respect to poplar and pine growth.

A laboratory incubation trial with Cu- and Zn- spiked biosolids added to eight soils representing the major Soil Groups collected from across the North Island of New Zealand (biosolids added to give final soil concentrations of 150 mg/kg for Zn or 450 mg/kg for Cu), showed that crystalline Fe oxide was the dominant factor explaining 90% of the variability in exchangeable Cu. For Zn, clay content and pH were the controlling soil factors that together explained 73% of the variability in exchangeable Zn. An increased content of crystalline Fe oxide increased the soil exchangeable Cu concentration. Decreasing pH and increasing clay content increased the soil exchangeable Zn concentration.

The effect of lime (0.1 and 0.5%), Al dross (2 and 6%) and a combination of lime and Al dross (0.1% lime+2% Al dross) as soil amendments for the amelioration of the toxic effect of biosolids-derived Cu (144 mg/kg in soil) and Zn (417 mg/kg in soil) on microorganisms and poplar were also studied. Results revealed that application of lime and Al dross (pH 10) as a mixture (0.1% lime+2% Al dross) was able to ameliorate Cu and Zn phytotoxicity, and significantly increase the DM yield of poplar, ECM fungi population and microbial activity in the Cu and Zn contaminated soils.

The findings of the studies in this thesis are applicable to environmental regulations with respect to heavy metal limits that seek to protect agricultural and forest land, human and animal health, and soil and drinking water quality, in scenarios where biosolids are applied to soil.

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Chapter 1

Literature review and research objectives

1.1 Introduction

1.1.1 Biosolids, definition, disposal and use

Domestic waste or sewage is a combination of human feces, urine, and graywater. Graywater is the liquid (aqueous) waste that results from washing, bathing, and meal preparation. Biosolids is defined as the solid or semisolid residue generated during the treatment of domestic sewage in a waste water treatment plant (USEPA 2005). Biosolids is also defined as the primary organic solid product yielded by municipal waste water treatment processes that can be beneficially recycled (NRC 2002; USEPA 1995).

The production of biosolids around the world is increasing with increasing population and industrial development. This waste needs to be disposed off safely, without any adverse effect on the environment. Biosolids can have beneficial effects to land as a source of nutrients (Martinez *et al.* 2003; Shober *et al.* 2003) and organic matter (Caravaca *et al.* 2002), that can improve the physical, chemical and biological properties of the soil, and hence, can be used as a soil amendment in agriculture and forestry. However, biosolids can also contain heavy metals in potentially toxic, readily available forms in most cases, and pathogens which may be harmful to flora and fauna, and excess levels of nutrients such as nitrate and phosphate which may cause eutrophication in water bodies (Singh and Agrawal 2008). This chapter reviews the literature on heavy metals in biosolids, their dynamics and their effects on the terrestrial environment. This chapter also reviews the common methods that are used to reduce ecotoxicologic effects of heavy metals. Based on this review, important research gaps on this subject area

are identified and a set of objectives for the studies reported in this thesis are presented.

1.1.2 Biosolids production in New Zealand and world–wide

Disposal of biosolids is a major problem in many countries and, consequently, it is subject to numerous rules and regulations. In New Zealand, approximately 250 public wastewater treatment plants treat domestic sewage from about 80% of the country's population of 4 million people, as well as trade wastes from industry (NZWWA 2003). About 55% of the population is connected to 25 high–rate treatment plants (treat more than 55,000 tons of dry solids per year), many of which perform full secondary treatment of biosolids. The annual production of municipal biosolids in some developed countries is presented in Table 1.1

Table 1.1 Annual production of biosolids (NZWWA 2003)

| Countries | Production of biosolids | |
|--------------------|---------------------------------|------------------------------|
| | (million tons of dry solids/yr) | (kg dry solids/person/year)* |
| New Zealand | 0.077 | 18 |
| US | 5.6 | 18 |
| Australia | 0.3 | 15 |
| European Community | 8 | 16 |

* Approximate estimation made by dividing biosolids production/yr by the population (internet search) in the country.

1.2 Heavy metals in biosolids

The heavy metals in raw sewage come from manures and commercial fertilizers, human wastes, metal pipes, household products, businesses and industries. The majority of metals in raw sewage are transferred to biosolids during treatment (Figure 1.1). However, significant quantities may be lost from the treated effluent

depending on the solubility of the metal concerned. In general, a treatment plant has primary and secondary treatment systems. Primary sludge is the result of primary treatment (settleable and floatable solids are removed from the waste flow). Secondary treatment of sewage usually refers to application of a specific treatment process like aerobic biological digestion to reduce the amount of organic polluting material remaining in solution after settling (achieved through primary treatment). During secondary treatment biodegradable dissolved colloidal solids are removed. The residue usually has 5–20 % solids and this is transferred to settling lagoons to separate sludge from water. Water is recycled and discharged into river systems and sludge is passed through further treatment before land disposal (Thiele 2009). Figure 1.1 shows a simplified outline of biosolids treatment procedure in a treatment plant.

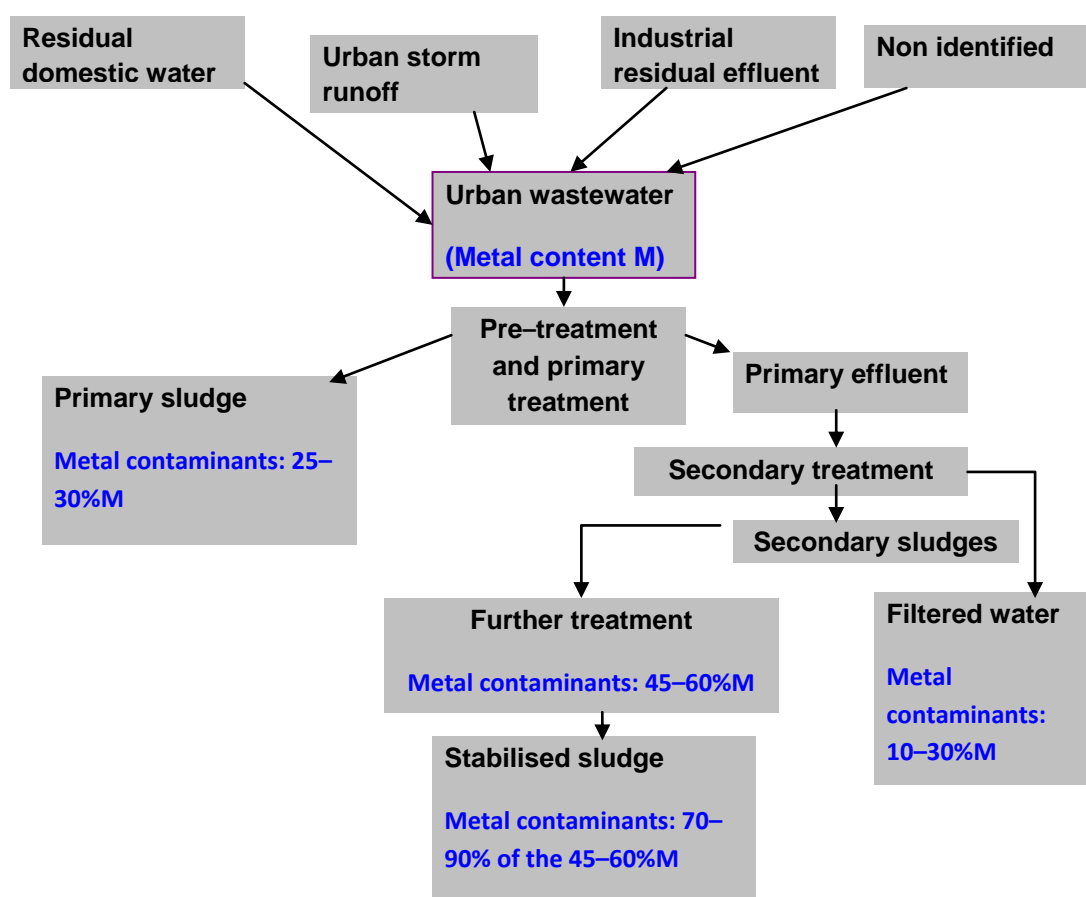


Figure 1.1 Origin and fate of metals during treatment of wastewater (ADEME 1995)

In many countries, biosolids are applied to land after removal of metals (Environment–Protection–Authority 1996; NRC 2002; NZWWA 2003). However, many developing countries apply biosolids to land or dispose of the waste in landfills without any pre–treatment to remove heavy metals (Gwenzi and Munondo 2008). In such situations the heavy metals present in biosolids can be potentially toxic to plants and microorganisms, can also leach from the soil and may contaminate ground water if metals are present at a high concentration. Biosolids typically contain much higher concentrations of metals such as Cu, Zn, Ni, Mo, Cd and Pb than the soils to which they are applied (McBride *et al.* 2004). Table 1.2 shows the contaminant levels of some heavy metals in biosolids in different countries, as well as the concentration ranges of metals in Australian and New Zealand agricultural soils. Analysis of these data suggests that copper (Cu) and zinc (Zn) are the heavy metals found at the highest concentration in most biosolids. This observation has been reported previously (Heemsbergen *et al.* 2010; Oliver *et al.* 2004; Singh and Agrawal 2008). Therefore, in this review and thesis special emphasis will be placed on these two heavy metals.

Table 1.2 Heavy metal concentrations (mg/kg dry weight) in biosolids (*and soils) from selected studies in different countries

| Country | Cu | Zn | Ni | Cd | Cr | Pb | Mn | As | References |
|-----------|-----------|----------|---------|----------|----------|--------|------|------|--|
| Aus/NZ* | 0.4–200 | 2–250 | 1–500 | 0.02–2.0 | 2–700 | 5–80 | | 1–20 | |
| NZ | 443 | 1154 | 72 | 1 | 1203 | 170 | 343 | | McLaren <i>et al.</i> (2007) |
| Thailand | 801 | 1326 | | 1 | | | 2621 | | Parkpain <i>et al.</i> (1998) |
| Spain | 174 | 445 | | 1 | | | | | Martinez <i>et al.</i> (2003) |
| India | 280–543 | 870–1510 | 192–293 | 41–54 | 102–8110 | 91–129 | | | Pathak <i>et al.</i> (2008); Singh <i>et al.</i> (2004) |
| China | 131–395 | 783–3096 | 49–96 | 6–13 | 46–78 | 58–109 | | | Dai <i>et al.</i> (2007) |
| Poland | 178 | 2080 | 48 | 12 | 141 | 209 | | | Stuczynski <i>et al.</i> (2007) |
| Australia | 203 –2190 | 323–2335 | | 2–21 | | 35–587 | | | Oliver <i>et al.</i> (2004) |
| Canada | 1300 | 760 | 68 | 6 | 91 | 96 | | | Bright and Healey (2003) |
| UK | 435 | 1519 | 64 | | | 183 | | | Gove <i>et al.</i> (2001) |
| USA | 306 | 736 | 14 | 1 | 51 | 117 | | | Farfel <i>et al.</i> (2005) |

*Heavy metal concentration (mg/kg dry weight) in Australian (Barry 1997; McLaughlin *et al.* 2000) and New Zealand (McLaughlin *et al.* 2000) agricultural soils

1.3 Biosolids disposal methods

Biosolids must be recycled or disposed somewhere in the environment. Disposal of this waste is becoming a serious environmental issue. Prior to 1990, ocean disposal was the preferred method in the world's coastal cities (Odegaard *et al.* 2002). The United States outlawed the ocean disposal of biosolids with the 'Ocean Dumping Ban Act of 1988' (USEPA 1988). Europe and Australia then enacted similar bans (Campbell 1998; Gibbs *et al.* 2006). With the prohibition of ocean biosolids disposal, the use of biosolids as a soil amendment (soil conditioners or fertilisers), or disposal through land filling or incineration to reduce the volume of biosolids, has become more widely practiced (Table 1.3). However, these disposal methods have some disadvantages and therefore certain restrictions have been placed on their use (Table 1.4). These restrictions are carefully considered in the decision making process for selecting an appropriate disposal method for a particular situation. Land filling and land application of biosolids are suggested to be the most economical disposal methods (Singh and Agrawal (2008).

Table 1.3 Methods of disposal of biosolids in selected countries

| Country | Description on usage of total production* | Reference |
|-----------|---|-----------------------------------|
| NZ | Forests (7.5), Agricultural land (7.5) Land filling (85) | NZWWA (2003) |
| UK | Agriculture land (62), Incineration (17), Land reclamation (11), Compost (7), Land fill (1) | Clarke (2006) |
| USA | Land application to; agriculture (40.2), domestic gardens (7.2), public parks (5.4), reclamation (5.4), forests (1.8), Land filling (17), Incineration (22) | NRC (2002) |
| Australia | Agricultural land (35), Landfill (30), Composting, forestry, land rehabilitation or incineration (35) | AWA (2009) |
| EU | Agriculture land (37), Land fill (40), Incineration (11), Forestry, silviculture, land reclamation (12) | Fytli and Zabaniotou (2008) |

* % of usage is given within the parenthesis

1.3.1 Incineration

Incineration is the most attractive disposal method (Khiari *et al.* 2004), however, this technique is not commonly practiced in New Zealand due to the associated high cost (Horswell *et al.* 2006). The main reason for the attraction of this method is that incineration results in a large reduction of biosolids volume; 10 % of the initial quantity, and thermal destruction of any toxic organic constituents (Vesilind and Ramsey 1996). Malerius and Werther (2003) indicated that approximately 30% of the solids remain as ash after incineration. Variations in the percentage of ash depend on the components of biosolids and the method used for incineration. This ash is generally desposed as land fill, and in some cases can be considered as highly toxic because of a higher metal concentration in the ash relative to the original biosolids. However, combustion of biosolids ensures a higher stability therefore a lower bioavailability of heavy metals in the ash compared with the parent biosolids (Kaminsky and Kummer 1989). The associated reduction in volume leads to low cost of transport to the site of disposal. One of the major constraints in the widespread use of incineration is public concern about possible harmful emissions from the incinerator smoke stack (Fytli and Zabaniotou 2008). For example, inorganic particulate matter (PM) can be formed during the incineration of biosolids (Seames *et al.* 2002). PM emissions from biosolids can be greater than those released from coal or even other biomasses, because of the specific properties of inorganic elements within biosolids (Zhang *et al.* 2008). Zhang *et al.* (2007) found that PM emissions from biosolids were dominated by volatile and semi-volatile elements including Br, Cl, P, S, Na, K, Zn, As, Cu, Mn and Ni.

1.3.2 Landfill Disposal

Biosolids can be mixed or layered with other solid waste and buried. Land filling of biosolids usually occurs where agricultural lands for recycling are not readily available or the quality of the biosolids does not meet the standard for recycling. According to New Zealand Waste Water Association statistics, in New Zealand,

landfill disposal is not presently encouraged mainly due to cost and social concerns (NZWWA 2003).

Table 1.4 Restrictions on biosolids handling methods (Fytili and Zabaniotou 2008)

| Biosolids handling method | Restrictions |
|---|---|
| Incineration | <ul style="list-style-type: none"> ▪ Permits for building an incineration plant ▪ Possibilities of co-incineration such as PM emissions may contain volatile elements. ▪ Costs (including costs for treatment of flue gases and ashes) |
| Landfill | <ul style="list-style-type: none"> ▪ Decomposition of organic components in the biosolids may lead higher metal availability ▪ Costs based on fees ▪ Transport costs ▪ Scarcity of land ▪ Permits for new land fill areas ▪ Recycling requirements (i.e. pathogens) |
| Land application (Agricultural and forest use) | <ul style="list-style-type: none"> ▪ Components (metals, toxic organics, pathogens) in Biosolids ▪ Non-acceptance from food industry and public ▪ Technical restrictions (handling of the biosolids, etc.) |
| Product recovery from biosolids (e.g. fertilisers) | <ul style="list-style-type: none"> ▪ Acceptance from users of the biosolids products (market considerations) ▪ Needs of resources for product recovery (chemicals, energy, costs, etc.) ▪ Restrictions due to technical problems |

1.3.3 Land application

Biosolids are an excellent source of essential plant nutrients and organic matter, which improve soil structure, water holding capacity and microbial activity

(Brown *et al.* 2003; Fornes *et al.* 2009; Martinez *et al.* 2002; Ramulu 2001). Application of biosolids to land is receiving greater acceptance from wastewater managers in New Zealand (Magesan and Wang 2003). Globally this appears to be the most popular method of disposal (Table 1.3).

However, biosolids may also contain toxic, persistent and bioaccumulative heavy metals and toxic organic compounds. Many researchers have demonstrated that the heavy metals present in biosolids can have an adverse effect on soil microbial process and microbial populations (Fernandes *et al.* 2005a; 2005b; Kandeler *et al.* 2000; Kao *et al.* 2006). In addition, biosolids can contain pathogenic microorganisms, depending on the degree of treatment they have received. Consequently, there are both environmental and public health risks associated with the application of biosolids to land. Therefore, before application to the land, biosolids should be treated to reduce the concentration of these contaminants. A careful assessment of the characteristics of biosolids is required prior to land application of biosolids. The chemical composition of biosolids is an important issue in developing recommendations for the rates of biosolids application on agricultural land, and in reducing the risks of groundwater contamination (McLaren *et al.* 2003; 2004; 2005).

1.4 Soil limits for biosolids–derived heavy metals

Each country has developed guidelines for the safe application of biosolids to land based on land usage. Internationally, heavy metals have been the principal impetus behind regulations governing the land application of biosolids because of their potential toxicity and persistence in soil. Some countries regulate pathogen content, and a few regulate organic contaminants (NZWWA 2003). Table 1.5 shows the soil limits and biosolids classification by contaminant level for heavy metals in New Zealand. These limits are based on the total concentration of metals in biosolids. Guidelines based on total concentrations are not reliable; because only a fraction of the metals within the biosolids matrix are bioavailable. Therefore, in the opinion of some commentators, to make the regulations more environmentally relevant, guidelines should be expressed in terms of bioavailable

metal fractions rather than the total metal concentration (McLaughlin *et al.* 2000). Determination of the chemical forms and concentration in biosolids is therefore essential to determine the potential biotoxicity of metals in biosolids.

Table 1.5 Soil limits and biosolids classification by contaminant levels (mg/kg dry weight) in New Zealand (NZWWA 2003)

| Metals | Soil limit conc. | Biosolids limits | | |
|--------|------------------|---------------------|----------------|---------------------|
| | | Grade A* max. conc. | | Grade B* max. conc. |
| | | until 31/12/12 | after 31/12/12 | |
| As | 20 | 20 | 20 | 30 |
| Cd | 1 | 3 | 1 | 10 |
| Cr | 600 | 600 | 600 | 1500 |
| Cu | 100 | 300 | 100 | 1250 |
| Pb | 300 | 300 | 300 | 300 |
| Hg | 1 | 2 | 1 | 7.5 |
| Ni | 60 | 60 | 60 | 135 |
| Zn | 300 | 300 | 300 | 1500 |

* Grade A biosolids are defined as essentially free of pathogens; Grade B biosolids are defined as biosolids with a potentially significant pathogen content

1.5 Chemical forms of heavy metals in biosolids and soils

Heavy metals can be present in both the solid phase and liquid phase of biosolids. Excessively high concentrations of the metals in biosolids have to be removed by chemical methods such as ion-exchange (Lee *et al.* 2006), and electrokinetic treatment (Virkutyte *et al.* 2005) before the biosolids can be applied to agricultural lands. Ion-exchange recovers heavy metal species from solution or solids by direct contact with ion-exchange resins. In the electrokinetic treatment method, a low-level current induces, via electro-migration and electro-osmosis, the mobility of heavy metals, thus removing charged pollutants from the

contaminated matrices. The technique to be used and the efficiency of removal of heavy metals by these methods depends on the chemical form and concentration of the metal species in the biosolids. Knowledge of the chemical forms of metals in biosolids can also provide information on the immediate effect residual metals will have when applied to soils, such as mobility and biotoxicity. Numerous studies have shown that a substantial fraction of heavy metals in biosolids applied to soils can be lost from the topsoil over years (Gasco *et al.* 2005; Gove *et al.* 2001; Keller *et al.* 2002; McLaren *et al.* 2005; 2004). Evaluation of the total metal concentration through analysis of a strong acid digest solution (Kovács *et al.* 2000) of biosolids may be useful as a gross index of contamination, but it provides little insight into the potential mobility and bioavailability of metals under field conditions (Su and Wong 2004).

1.5.1 Solid phase

The chemical forms of heavy metals in the solid phase of biosolids and soils are studied using chemical fractionation schemes capable of recovering metals from each separate chemical component in biosolid and soils. Sequential extractions are recognized as the most simple and best available methods for gaining valuable information on the bioavailability and mobility of heavy metals in biosolids, and help predict the behaviour of heavy metal in soil if biosolids are to be used as a soil amendment (Fuentes *et al.* 2004).

Several sequential extraction schemes have been presented in literature (Ma and Rao 1997; Tessier *et al.* 1979; Tokalioglu and Kartal 2002). The procedure of Tessier *et al.* (1979) is one of the most thoroughly researched and widely used procedures to evaluate the efficiency of metal sequential extraction, and the use and limits of this procedure has also been reviewed (McLaren and Clucas 2001; Shrivastava and Banerjee 2004). McLaren and Clucas (2001) have modified the method developed by Tessier *et al.* (1979) into a convenient and practical form (Table 1.6). This procedure involves the sequential chemical extraction of most heavy metals into one of five fractions: soluble plus exchangeable, specifically sorbed, oxide bound, organic bound (also sulfides), and residual. The chemical

nature and bioavailability of each of these fractions are described in the following sections.

Table 1.6 Sequential Extraction procedure (McLaren and Clucas 2001; Tessier *et al.* 1979)

| Step | Nominal fraction | Extractant | Conditions; extraction procedure |
|------|-------------------------------|--|---|
| 1 | Soluble plus exchangeable | 1 M $\text{Mg}(\text{NO}_3)_2$, pH 7.0 | 1 g biosolids, 8 mL extractants; 1 h shaking |
| 2 | Specifically sorbed | 1 M NaOAc, adjusted to pH 5.0 with acetic acid | Residue from step 1 shaken for 5 h with 8 mL extractant |
| 3 | Oxide bound | 0.4 M $\text{NH}_2\text{OH} \cdot \text{HCl}$ in 25% (v/v) acetic acid | Residue from step 2 heated for 6 h at 96°C with 20 mL extractant |
| 4 | Organic bound (also sulfides) | H_2O_2 adjusted to pH 2 with HNO_3 | Residue from step 3 heated at 85°C for 2 h with 8 mL extractant then 3 mL extractant added and heated for further 3 h |
| 5 | Residual | H_2O_2 (30%)/ HNO_3 (65%) digestion | Residue from step 4 dried and 0.5 g sub sample heated for 30 min. at 60°C with 5 mL HNO_3 and again heated for 270 min. at 120°C with H_2O_2 (Kovács <i>et al.</i> 2000) |

1.5.1.1 Soluble and exchangeable metal ions

The concentration of heavy metals in the soluble fraction generally constitutes the smallest of all fractions. However, as the soluble phase interacts with the biota of the soil, it is very important in terms of risk assessment. Metal ions held electrostatically onto ion exchange sites in soil are described as exchangeable ions. Exchange sites comprise the negatively charged surfaces of clays and iron,

aluminium and manganese oxides, and also the functional groups of organic matter. Exchangeable metals are non-specifically adsorbed to the exchange sites and therefore they are labile.

1.5.1.2 Specifically sorbed

Specific sorption of metal is the formation of stable complexes between metal ions and specific functional groups at the surface of organic and inorganic soil colloids. Cu and Zn can be specifically sorbed by layer silicate clays, hydrous oxides of Fe, Mn, and Al, and organic matter. In specific sorption, ions are held much more strongly by the surface charge or even unchanged neutral sites (McBride 1981) and therefore they are less labile than exchangeable metal ions.

1.5.1.3 Iron and manganese oxides

Metals sorbed onto Fe, and Mn oxides may ultimately be occluded by further growth of the oxides. These oxides exist as nodules, concretions, cement between particles or simply as a coating on particles and are excellent scavengers for heavy metals. The redox potential and pH of the soil influence the bioavailability of metals in this fraction.

1.5.1.4 Organic matter

Heavy metals may be bound to various forms of organic matter such as living organisms and detritus (humates, fulvates). Metals form very strong complexes with organic colloids. Comparatively, Cu has a higher binding affinity for organic matter than Zn (McBride 1994; Mesquita and Carranca 2005). Organic complex formation occurs with various functional groups like carboxyl, hydroxyl, and phenol in humic substances. Many researchers have concluded that this complexation is also a form of specific sorption (Kabata-Pendias and Pendias 2001; McLaren *et al.* 2003).

1.5.1.5 *Residual fraction*

The residual solids contain mainly primary and secondary soil minerals, which may hold heavy metals within their crystal structure. These metals are not expected to be released in solution over a reasonable time period under the conditions normally encountered in soil. The residual fraction may also contain recalcitrant organic-bound metal compounds. The metals present in the residual fraction are considered recalcitrant and not readily available (Tandy *et al.* 2009a; Tandy *et al.* 2009b)

McLaren and Clucas (2001) using the method proposed by Tessier *et al.* (1979) found that Cu in biosolids is mostly associated with the organic bound and residual fractions, with very little associated with the soluble plus exchangeable, specifically sorbed, or oxide bound fractions. A similar investigation for Zn showed a higher proportion of this metal was associated with the oxide fraction followed by organic and specifically sorbed fractions.

Elliott *et al.* (1990) also proposed a five-step chemical fractionation method based on the work of Tessier *et al.* (1979) that can be used to characterise the partitioning of heavy metals in biosolids. The modified method separates the metals into exchangeable, dilute acid-extractable, Fe-Mn oxide bound, organically bound, and residual fractions. García-Delgado *et al.* (2007) used this modified fractionation method and found that Cd, Ni and Zn were observed to be in the exchangeable fraction of the biosolids at up to 35% of the total metal content, whereas Cu and Pb were distributed in the organically bound fraction at up to 25% and 60%, respectively. These values were agreeable with McLaren and Clucas (2001) for Zn and Ni but varied for Cu.

Similarly, Shrivastava and Banerjee (2004) modified the sequential extraction method proposed by Tessier *et al.* (1979) into five fractions: exchangeable phase; oxidisable phase (organic matter); acid soluble phase (carbonates); reducible phase (Fe/Mn oxides and hydroxides); and the residual fraction (silicate and detrital materials). They ranked the chemical forms of 6 metals (Cu, Zn, Pb, Ni,

Cr, and Cd) in both biosolids and biosolid-amended soils and found that the percentage of Cu in biosolids decreased in the order, residual > acid soluble > oxidisable > reducible > exchangeable, and for Zn in biosolids the order was residual > reducible > acid soluble > oxidisable > exchangeable. The chemical partitioning order for Cu in biosolid-amended soil was ranked as residual > oxidisable > acid soluble > reducible > exchangeable and for Zn the order was residual > reducible > acid soluble > oxidisable > exchangeable. Metal fractionation in biosolids can be changed as biosolids are incorporated into the soil (McBride *et al.* 1999; Richards *et al.* 2000) due to the influence of soil physical and chemical characteristics.

1.5.2 Liquid phase

Metals in soil pore water may be present as free ions or as complexes with inorganic (e.g. Cl^- , SO_4^{2-} , CO_3^{2-}) or organic (dissolved organic matter–DOM) ligands (Cancès *et al.* 2003). The association of heavy metals in solution with ligands is strongly pH dependant. McLaren and Clucas (2001) reported that the addition of increasing rates of inorganic Zn to biosolids reduced the pH and increased Zn availability in the liquid phase, but increasing Cu rates had no effect on pH or the concentration of Cu in the liquid phase. They suggested that the reason for the higher Zn in liquid phase was due to higher application of Zn exceeding the capacity of biosolids to incorporate the Zn into the biosolids matrix. In addition, the lower pH effected treatment would have reduced the sorption capacity of biosolids Zn-treated relative to Cu and increased exchange of Zn into solution. In contrast, the lower concentration of Cu in solution was due to the stronger sorption of Cu onto the biosolids solid phase, and also due to increasing Cu additions not changing the soil pH values.

Depending on the element, the free ion may be the dominant species in the liquid phase, or may account for only a minor fraction of the total metal in liquid phase. For instance, Cd and Zn are usually mainly present as free ions in soil solution, whereas Cu is mainly complexed with DOM in soil solution (Khoshgoftarmanesh *et al.* 2006; Nolan *et al.* 2003b).

1.5.2.1 *Measurements*

Analytical techniques to measure or estimate the ‘free ion’ concentration of metals in solution include dialysis techniques such as Donnan dialysis, potentiometric techniques (ion-selective electrodes, ISE), voltammetric techniques, resin exchange methods, and chromatographic techniques (Nolan *et al.* 2003a). Many of these methods have been reviewed by Zhang and Young (2005). Dialysis techniques use semi permeable membranes that allow only certain species to cross, depending on size and/or charge. Donnan dialysis uses a small-volume acceptor (receiving) solution to minimize errors resulting from perturbation of the chemical equilibrium in solutions with limited buffering capacity for heavy metals.

1.5.2.2 *Model predictions*

A number of computer-based speciation models have been generated using geochemical programs, such as GEOCHEM (Mattigod and Sposito 1979) ECOSAT (Weng *et al.* 2001), WHAM (Model VI) (Centre for Ecology and Hydrology 2002) or ORCHESTRA (Schroder *et al.* 2005). These mechanistic models can be used to describe metal partitioning between soil and solution, or to calculate the solution speciation only (Nolan *et al.* 2003b; Weng *et al.* 2002). These models are conceptually attractive, but they require extensive input information, which is often not available in routine research. Some chemical speciation models are disadvantaged by the need for humic-and fulvic-metal stability constants, which are difficult to define and vary from soil to soil (Nolan *et al.* 2003a). In spite of these limitations, studies have shown that speciation model predictions of metal ion concentration in solution are closely related to measured concentrations (Benedetti 2006).

GEOCHEM and WHAM (Model VI) chemical equilibrium computer programs are commonly used to estimate metal speciation in contaminated soil ‘pore waters’ (Degryse *et al.* 2009; Nolan *et al.* 2003b; Tipping *et al.* 2003). Nolan *et al.* (2003b) determined the free Cu^{2+} , Zn^{2+} , Cd^{2+} , and Pb^{2+} species in soil pore waters

from 15 agricultural and 12 long-term metal-contaminated soils in Australia and USA. They reported that for the contaminated soils, the equilibrium speciation programs GEOCHEM and WHAM (Model VI) provided reasonable estimates of free Zn^{2+} species in comparison to the measured species. The models predicted stronger binding of Cu^{2+} to DOC and hence a lower concentration of free Cu^{2+} as compared with the observed species. They also reported that the binding of Cu^{2+} to DOC predicted by WHAM (Model VI) was much stronger than that predicted by GEOCHEM. Tipping *et al.* (2003) calibrated WHAM (Model VI) with a large number of published data on measured metal ion concentrations, and found that the predicted free metal ion concentrations were in reasonable agreement with corresponding values obtained through measuring solution metal concentrations. Because the free ionic form of Cu and Zn and their complexes can be more easily predicted by WHAM (Model VI) than other methods, WHAM was selected to modelling solution speciation for the present study.

1.6 Fate of biosolids-derived heavy metals in soil

The bioavailability of heavy metals in soil treated with biosolids depends on the chemical forms of the metals in the original biosolids, and the dynamics of these metals in soil. In this section, Cu and Zn reactions in soil, their chemical forms, and their dynamics are discussed.

Copper and Zn when added to soil undergo many reactions depending on the nature and properties of the soil. The dynamic equilibrium of these metals with other soil components is governed by various interactions between the soil solid, liquid, and gaseous phases, and microbes, as is illustrated by Figure 1.2. The bioavailability of metals depends on their chemical speciation and is determined by the physical and chemical properties of the soil, such as soil particle size distribution, organic matter content of the soil, clay mineralogy, salinity, concentration of other metals in solution, pH and redox potential (Table 1.7). As discussed under section 1.5, the chemical forms of heavy metals in soil can be studied by sequential fractionation.

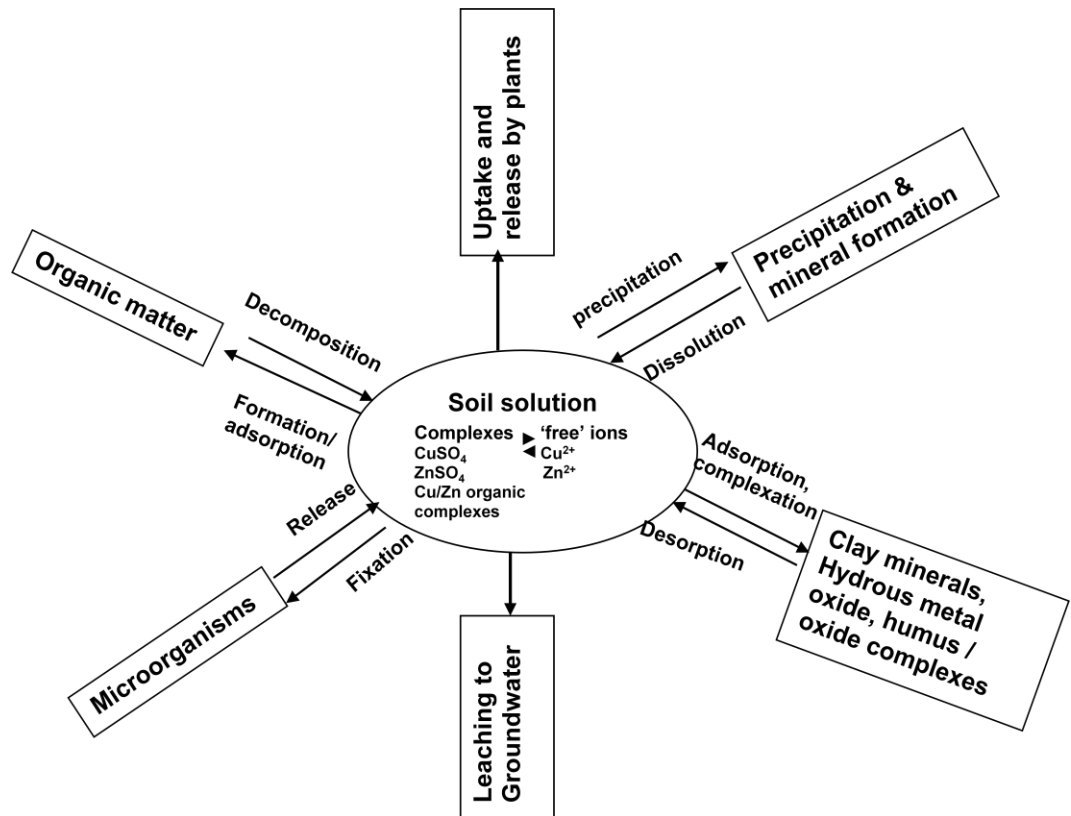


Figure 1.2 Dynamics of Cu and Zn reactions in soil (Adriano 2001; Kabata-Pendias and Pendias 2001; Loganathan *et al.* 2008)

Copper and Zn in the soluble and exchangeable, specifically sorbed and organic bound soil fractions and in the liquid phase are in equilibrium with each other and are immediately or slowly available for plant uptake in the short term. Oxide and residual forms of Cu and Zn are relatively unavailable for plant uptake (Adriano 2001). Most common mobile Cu and Zn species in soil are generally in the form of free and complexed ions in the soil solution, but several other ionic species that occur as non-specifically and specifically adsorbed cations can also be easily mobilised in soil.

Table 1.7 Soil properties influencing the dynamics of biosolids–derived Cu and Zn

| Soil property | Cu and Zn availability | References |
|--------------------------------|---|---|
| pH | Increased Cu and Zn availability with decreasing pH (decreased organic complexation of soluble metal; decrease soil adsorption). | Adriano (2001); Cavallaro and McBride (1984); McBride <i>et al.</i> (1997); McBride and Bouldin (1984) |
| Dissolved organic carbon (DOC) | Cu is mainly complexed with DOC in acidic and alkaline soils and for Zn mainly complexed in alkaline soils. Affinity order: Cu~Pb>>Zn~Cd>Ni>Co | Alcacio <i>et al.</i> (2001); Ashworth and Alloway (2008); Fotovat and Naidu (1998); Martinez <i>et al.</i> (2003);McBride <i>et al.</i> (1997) |
| Fe, Mn, and Al oxides | Relatively stronger affinity for Cu ²⁺ than Zn ²⁺ Affinity order: Cu > Pb > Zn > Co > Cd | Bertsch and Seaman (1999); McBride and Blasiak (1979) |
| Organic matter | Cu affinity for soil organic matter is higher. Affinity order: Cu > Ni > Pb > Co > Zn > Mn. Increase organic matter content, decrease soluble Cu and Zn concentration. | Alcacio <i>et al.</i> (2001); Alloway 1995; Chukwuma <i>et al.</i> (2010); McBride <i>et al.</i> (1997) |
| Clay minerals | Decrease of solution concentration due to sorption; affinity order: | Adriano (2001) |
| goethite | Cu>Pb>Zn> Cd> Co> Ni>Mn | Schwertmann and Taylor (1989). Ikhsan <i>et al.</i> (1999) |
| hematite | Pb >Cu>Zn> Cd> Co> Ni>Mn | |
| kaolinite | pH at which 50% of metal adsorption decreases in the order: Pb<Cu<Zn=Co<Mn | |
| Other ions in solution | Ca decreases solution Cu concentration (Cu concentration increase in DOC—especially in alkaline soils). Ca increase solution Zn concentration (competitive adsorption). At lower pH values, adsorption of Zn ²⁺ can be reduced by competing cations. | Degryse <i>et al.</i> (2009); Romkens and Salomons (1998); Zhao and Selim (2010) |
| CEC | Increase CEC decrease Cu and Zn availability in soils due to increase specific sorption of Cu | Chukwuma <i>et al.</i> (2010); Wu <i>et al.</i> (2000) |

1.6.1 Soil properties that influence the availability of Cu and Zn in soil

Many studies have been conducted to evaluate the influence of soil properties on the comparative solubilities of both Cu and Zn. Sims (1986) conducted a greenhouse experiment with soils having 4 different textures (pH range 4–7.7) to measure Mn, Cu, and Zn uptake by wheat. He found that exchangeable Zn was higher when soil pH<5.2, while at pH>5.2 more Zn was complexed with the organic and Fe–oxide fractions. They also observed that the majority of soil Cu was in the organic fraction and pH had limited influence for the pH range of 4–7.7. Gray and McLaren (2006) analysed 202 soil samples from various sites in New Zealand that had different types and amounts of heavy metal contamination, and found that the total soil Cu was significantly and positively correlated with the soluble Cu concentration. They also concluded that an increase in soil pH and the concentration of amorphous extractable Fe, and decrease in total Zn concentration were all associated with a significant reduction in the soluble Zn concentration. McBride *et al.* (1997) found that for 31 metal contaminated soils from different parts of Europe, the measured soluble Cu concentration was significantly and positively correlated to total soil Cu concentration but not to other soil properties such as organic matter and pH. For Zn, reducing soil pH and increasing total Zn concentration significantly increased the solution Zn concentration. However, for another group of soils, McBride *et al.* (1997) found that, along with increasing total Cu, both reducing pH and increasing organic matter content were associated with a significantly increased soluble Cu concentration. Zhao *et al.* (1999) found that the soil solution Cu concentration in 72 biosolids–amended soils was increased with increasing total or EDTA extractable soil Cu and DOC, and decreasing soil pH. For Zn, they found that increasing organic carbon and total or EDTA extractable soil Zn concentration, and decreasing soil pH, were significantly associated with an increased soluble Zn concentration in soils.

Alloway (1995) concluded that clay minerals, organic matter and pH are likely to be the most important factors affecting Zn adsorption by soils. Zinc tends to be

highly available in soils with low pH, and pH highly influences mobility of Zn in comparison to other soil properties (Degryse *et al.* 2009). Chukwuma *et al.* (2010) investigated Zn availability in relation to selected soil properties in a crude-oil-polluted eutric tropofluvent soil in Southeastern Nigeria, and concluded that reducing pH and increasing effective CEC were the most dominant soil properties that control an increase in the soil solution Zn concentration. Wu *et al.* (2000) proposed that the order of soil properties regulating the activity of the Zn^{2+} ion in soil solution is $\text{CEC} > \text{pH} > \text{Ca}^{2+} > \text{DOC}$. Wu and Aasen (1994) proposed regression models for the prediction of soil Zn availability at different pH points (range 5–8) in a barley field, and concluded that soil Zn availability was significantly reduced at pH above 6.5. They also stated that below this point, the pH effect becomes gradually less important, but, in the low pH range, the soil Zn availability is mainly dependent on total soil Zn concentration.

Interaction of Cu with DOC is an important physicochemical process affecting Cu mobility in soils. Zhou and Wong (2001) conducted a study to investigate the effects of DOC on the sorption of Cu to an acidic sandy loam soil and a calcareous clay loam soil and found that the Cu sorption capacity decreased markedly with an increase in DOC for both soils. In addition, the sorption of Cu increased with an increase in pH for both soils without the addition of DOC, while Cu sorption in the presence of DOC was decreased with an increase in pH for $\text{pH} > 6.8$. This implied that Cu availability in soil solution is more influenced by DOC than pH. The results obtained for Cu in this study are consistent with the findings of Ashworth and Alloway (2008). They conducted batch experiments in a heavily biosolids-amended clay loam soil in UK and showed that the solubility of Cu showed a strong positive relationship ($r^2=0.99$) to the DOC, particularly at high pH (>5.97). Similarly, Yin *et al.* (2002) concluded that an increase in pH strongly increases the binding of free Cu^{2+} to the solid phase of soil, but often has only a minor effect on the total solution concentration, because the pH increase also enhances complexation of Cu^{2+} with DOC. Burton *et al.* (2003) conducted a laboratory study with biosolids leachate-derived Cu and Zn in humic-gley soil and found that the DOC substantially depressed Cu and Zn sorption.

Fotovat and Naidu (1998) conducted a laboratory incubation study to examine the influence of basic cations on the concentration of Cu, Zn, Cd and DOC in soil solution, for two acidic (pH 5.3–5.8) and two alkaline (7.4–8.7) soils sampled in Australia. They observed a decrease in the concentrations of Zn in alkaline sodic soils and Cu in both acidic and alkaline sodic soils. They concluded that metal solubility may be related to the decrease in DOC as increasing saturated cation concentrations which lead flocculation of DOC in these soils.

The review of literature presented above and in Table 1.7 suggests that soil solution Cu and Zn concentrations are influenced by pH, organic matter content, clay minerals, DOC and the total soil metal concentration.

1.7 Soil Cu and Zn availability to plants

Bioavailability defines the relationship between the concentration of a contaminant in soil and the amount of the contaminant that actually enters biological receptors, and thus may cause harm (Reichenberg and Mayer 2006). Therefore, any deficiency or excess of bioavailable metals may damage plant growth. Copper and zinc are essential metals for the growth and development of plants. Removal of Cu by crops is negligible when compared to its content in soil. An average cereal crop removes about 20 to 30 g Cu/ha/yr, whereas forest biomass removes about 40 g Cu/ha/yr (Kabata-Pendias and Pendias 2001). Bell *et al.* (2004) reported that in the high yielding cereals (e.g. rice, wheat), mean Zn removal rate is about 250 g/ha/yr in the grain of the cereal plants. Billett *et al.* (1991) reported that for forest plants the mean Zn accumulation rate is on average 114 g/ha/yr. Zublena (1991) summarized the nutrients removal by crops in North Carolina and reported that the removal rates of Cu and Zn for various grain crops ranged from 5 to 27 g Cu /ha/yr and 14–136 g Zn/ha/yr. Sakal (2000) in a field experiment on rice–wheat–sorghum sequence in calcareous soil after 10 cropping cycles observed that average removal of Cu ranges from 71–82 g Cu/ha/yr and for Zn, it was 148–186 Zn/ha/yr.

1.7.1 Cu and Zn deficiency

Low available concentrations of Cu and Zn in soil can reduce crop DM yields and quality, sometimes without obvious symptoms of deficiency. Alloway (2008a) reported that generally in plants, deficiency of Cu and Zn causes pollen sterility, reduced grain weight and number, inhibited photosynthesis, poor protein content and quality, poor root growth and increased disease susceptibility. Copper deficiency most commonly occurs in cereals, especially wheat, barley and oats on organic-rich soils and sandy soils, although it also occurs on calcareous soils, especially where the organic matter content is relatively high (Alloway 2005; Sinclair and Edwards 2008). The crop species most susceptible to Zn deficiency include: maize, rice, beans, cotton, flax, coffee, citrus and apple. However, other less susceptible crops, such as wheat, can also be severely affected by deficiency where the available Zn content of soils is extremely low (Alloway 2009; 2008b). Alloway (2008b) reported that calcareous, sandy, strongly weathered deep tropical soils, saline and sodic (salt-affected) soils, vertisols and gleysols are common soil types that show Zn deficiency. Around 50% of the agricultural land in both India and China is Zn-deficient, and crops on these deficient soils respond well to Zn fertilizer (Singh 2008; Singh *et al.* 2005; Zou *et al.* 2008).

1.7.2 Cu and Zn toxicity

At elevated soil concentration, Cu and Zn can be toxic to plants. Signs of toxicity may include stunting, chlorosis and reduced yields. Table 1.8 presents the deficiency and toxicity limits of Cu and Zn in mature leaves and plant tissues of various plant species. Variations in the data can be explained through the influence of different soil, crop, and climatic factors and the way the experiments are conducted.

Rusk *et al.* (2004) reported that Cu and Zn toxicity affects the biological N fixation process in the roots of legumes, so that legume plants and adjacent species show poor growth due to nitrogen deficiency. Adverse effects of Cu and Zn present at high concentration in soil contaminated by biosolids were found on

numbers of rhizobium species (*R. leguminosarum* bv. *viciae* and *trifolii*), the microsymbionts for beans and white clover, during analysis of a long-term field experiment in Nottinghamshire, UK (Chaudri *et al.* 2000a). They found that the lowest concentration affecting rhizobium activity was 250 mg/kg for Cu and 90 mg/kg for Zn, where the control soil Cu and Zn concentrations were 9 and 44 mg/kg soil respectively. McGrath *et al.* (1988) reported a 50% reduction in nitrogen fixation of white clover in a pot trial on biosolids amended (20 years ago) soil at metal levels of 99 mg Cu/kg soil and 334 mg Zn/kg soil where the control soil Cu and Zn concentrations were 24 and 94 mg/kg soil respectively.

Table 1.8 Approximate concentrations of Cu and Zn in mature leaf tissue generalized for various plant species (mg/kg dry weight)

| | Cu | Zn | References |
|----------------------------------|--------|---------|---|
| Deficient | 2–5 | 10–20 | } Adriano (2001); Alloway (2008a) |
| Sufficient | 5–30 | 27–150 | |
| Toxic | 20–100 | 100–400 | |
| Tolerable in agronomic crops | 5–20 | 50–100 | Kabata-Pendias and Pendias (2001); Macnicol and Beckett (1985) |
| Toxic in sensitive plant species | 15–20 | 150–200 | Kabata-Pendias and Pendias (2001) |
| For 10% yield loss* | 10–30 | 100–500 | Macnicol and Beckett (1985) |

Fuentes *et al.* (2007a) observed little or no evidence of Cu or Zn phytotoxicity in a field trial with one year old Aleppo pine seedlings (*Pinus halepensis*) grown in three Mediterranean forest soils (marl, limestone and sandstone) amended with biosolids (60 Mg/ha) to yield soil concentrations of 2098 mg Cu/kg and 5377 mg Zn/kg under semiarid conditions. Davis and Carlton-Smith (1984) conducted a pot trial with perennial ryegrass on a sandy loam soil enriched with biosolids (pH 7.0) at 5 levels of Cu and Zn (0, 125, 250, 500, 1000, 1500 mg Cu or Zn/kg soil) and reported that the phytotoxic concentrations of total Cu and Zn in soil to ryegrass in terms of yield reduction were 105 mg/kg and 319 mg/kg respectively.

The Environment Protection Authority (1996) and Smith (1996), after reviewing the literature on the phytotoxicity of biosolid-borne heavy metals, concluded that phytotoxicity has only been observed in glasshouse pot or field trials when biosolids with high metal concentration (>70 mg Cu/kg and >200 mg Zn/kg) are added to soils at high rates or when soils are acidic ($\text{pH} < 5.5$). It has been argued that soluble salts should not be used to simulate the effects of biosolid-borne heavy metals in soils because soluble inorganic Cu and Zn salts contribute high levels of free metal ions to soil solution, which have much higher plant bioavailability than would be expected for 'real' biosolids where metals are bound to the biosolids matrix at equilibrium with the parent soil chemistry (Logan and Chaney 1983; Sommers *et al.* 1987). Recently, Heemsbergen *et al.* (2010) compared the bioavailability of Cu and Zn in biosolids with those of metal salts (sulphates) in the same soils using twelve Australian field trials. The results showed that bioavailability for Zn was similar in biosolid and salt treatments, but for Cu, the results were inconclusive.

There is no evidence from long-term trials that soils are able to convert a large portion of the metals added in biosolids to insoluble, unavailable forms (Udom *et al.* 2004). Nor is there evidence of increased availability of metals with time, but this possibility cannot be completely discounted. However, Beckett and Davis (1978) suggested a possible 'time bomb effect' might eventually occur whereby plant uptake and leaching of heavy metals could increase due to decomposition of organic matter and release of sorbed Cu and Zn. The 'time bomb effect' was tested by Oliver *et al.* (2006) who conducted a study to determine the organic and mineral fractions of Cu in biosolids collected from 3 treatment plants in Australia and incubated at 60% water holding capacity and $21\text{--}30^{\circ}\text{C}$ for 21 months. The control was setup as the air-dried biosolids kept in sealed containers. One of the 3 biosolids sample showed a higher association of Cu with the inorganic fraction of the biosolids and the 'solid-solution distribution coefficient (K_d)' was constant throughout the experiment. They suggested that for biosolids collected from this location, the sorption capacity lost from the organic fraction was not significant, and the biosolids mineral fractions shielded the environment from metal releases

upon decomposition of organic matter. In contrast, the organic fraction was found to dominate Cu sorption for the biosolids collected from the other 2 treatment plants, and the K_d (solid–solution distribution coefficient) for Cu was much lower in samples incubated for 21 months than non–incubated samples. They concluded that the increasing soluble Cu with time in the 2 biosolids may be due to degradation of organic matter over time. They also recommended that further studies should be conducted to determine at what point the loss of sorption capacity becomes important in regard to the retention of metals within the biosolid matrix.

1.7.3 Soil tests for bioavailability

The relative plant availability or phytoavailability of heavy metals depends on the forms and the concentration of the metal species in soil solution (Table 1.9).

Table 1.9 Relative phytoavailability of different species of metals in soils (Kabata-Pendias and Pendias 2001)

| Metal species | Phytoavailability |
|--|---|
| Simple or complex cations in solution phase | High |
| Exchangeable cations in organic and inorganic complexes | Medium |
| Chelated cations | Slight |
| Metal compounds precipitated on soil particles | Available after dissolution |
| Metal bound or fixed inside organic substances | Available after decomposition |
| Metal bound or fixed inside mineral particles (primary or secondary soil minerals) | Available after weathering and/or decomposition |

A number of extraction methods are used to estimate the phytoavailability of heavy metals, such as extraction with solutions of acid (HCl, HNO₃, *aqua regia*), chelating agents (EDTA, DTPA), buffered solutions (Acid Ammonium Acetate buffer), and unbuffered salt solutions (CaCl₂, MgCl₂, NaNO₃, NH₄NO₃). Acid extractants, depending on the strength, can extract various proportions of the total amounts of heavy metal in soil. The metal concentration extracted depends on the type of acid used and soil tested. Chelating agents and buffered salt solutions are believed to extract potentially mobile portions of metal in soil (Kabata-Pendias and Pendias 2001). EDTA is a well-known strong chelating agent widely used to predict plant-available heavy metal concentrations in soil (Alvarez *et al.* 2006; Saifullah *et al.* 2009). EDTA has been reported to remove metals that are organically bound, occluded in oxides, and associated with secondary clay minerals (Paya-perez *et al.* 1993). DTPA is another chelating agent commonly used to extract Cu and Zn from soil to estimate plant-available metal concentrations (Hammer and Keller 2002; Kabata-Pendias and Pendias 2001; Pastor *et al.* 2007). The amount of heavy metal extracted by such methods gives an idea of the index of potential toxicity or deficiency of metals, but interpretation of the results depends on consideration of the extractant used and the soil tested. For example, DTPA was developed for use on neutral to alkaline soils, and the relevance of the bioavailability index to plant under these conditions has been questioned (Hammer and Keller 2002). Degryse *et al.* (2004) suggested that EDTA extraction appears to be the most appropriate choice to approximate the labile metal content, when used at concentrations ranging between approximately 50 to 100 mmol EDTA per kg for any soil type. A lower concentration may not completely extract the labile amount, whereas a higher concentration will possibly release considerable amounts of the non-labile metal pool.

Some other techniques like electro-dialysis, solution diffusion and diffusive gradient in thin film (DGT) have been also proposed. Electro-dialysis is a membrane process, during which ions are transported through a semi permeable membrane, under the influence of an electric potential. Cation-selective membranes are negatively charged polyelectrolytes, which reject negatively

charged ions and allow positively charged ions to flow through. By placing multiple membranes in a row, which alternately allow positively or negatively charged ions to flow through, the ions can be removed from soil. In the solution–diffusion technique, ions pass into the membrane material and then diffuse through the membrane down a concentration gradient.

DGT has recently been developed and has been shown to have promise as a quantitative measure for the bioavailability of metals in soils (Nolan *et al.* 2005; Zhang *et al.* 2001). The DGT device consists of a plastic assembly containing a layer of chelating resin gel, overlain by a layer of hydro gel and a protective filter through which ions can freely diffuse. They are deployed in a soil for a given period of time during which metal ions accumulate in the resin layer. The mass of accumulated metal in the resin layer is determined by dissolving the resin layer in nitric acid solution and then analysing the resulting solution for constituent metal concentration. Like plants, DGT locally lowers the metal concentration in the soil solution and by dissociation and desorption mechanism the metals redistribute between the liquid and solid phases of soil. The technique is based on kinetic rather than equilibrium principles, and can be used to measure the ‘effective concentration’, C_E , which includes a contribution from both the soil solution and its enhancement by supply from the solid phase.

It follows from the above discussion that since a number of soil parameters and climatic factors have a significant impact on the absorption of heavy metals by roots, any method used has to be related to a given soil and plant (Kabata-Pendias 2004). Table 1.10 and Table 1.11 present approximate lower and upper critical concentration limits of bioavailable Cu and Zn in soil for optimum plant growth as indicated by various extraction methods.

Table 1.10 Lower critical concentrations in soil extracts (mg/kg DM) (Alloway 2008a; Brennan *et al.* 1993; Srivastava and Gupta 1996)

| Soil extractant | Cu | Zn |
|---|---|---------|
| Total (<i>aqua regia</i>)* | 5–10 | 10 |
| 0.005 M DTPA + 0.01 M TEA (pH 7.3) | 0.2 | 0.5–1.5 |
| 0.05 M EDTA (pH 7) (mg/L)** | 1 (sand) 1.6 (loam) 2.5 (>10% OM) | 1–1.5 |
| 1M HCl | 2.0 | 1.5 |
| 0.5 M NH ₄ Ac + 0.02 M EDTA (pH 4.65) | 0.3–0.8 | 0.6–1.4 |
| 0.05 M HCl + .0125 M H ₂ SO ₄ | – | 0.5–3 |

* Tentative indicator value of low total Zn concentrations likely to be related to primary deficiency ** Concentration in soil extract

Table 1.11 Upper critical concentrations in soil extract (mg/kg DM)

| Soil extractant | Cu | Zn | References |
|---|---------------|----------|----------------------------|
| Total (<i>aqua regia</i>) | 20–30 | } | Walsh <i>et al.</i> (1972) |
| 0.005 M DTPA | 20 | | |
| 0.2 M EDTA (mg/L)* | 15 | | |
| 0.5 M NH ₄ Ac + 0.02 M EDTA (pH 7) | 16 | | Mathur and Levesque (1983) |
| 0.1 M HCl | 20 | 250–1000 | Walsh <i>et al.</i> (1972) |
| 1M NH ₄ Ac | Sandy soil 25 | | Delas (1984) |
| (pH 6) Clay soil | 100 | | |

* Concentration in soil extract

1.7.4 Zn and Cu availability to pine and poplar

In New Zealand, biosolids are permitted to be applied only to forest plantations. Pine and poplar are important forest trees in New Zealand. Therefore literature on the use of biosolids as a soil amendment for the production of these plant species is reviewed here. Poplar has also been indicated as an efficient plant to remediate metal-contaminated sites (Peuke and Rennenberg 2005; Robinson *et al.* 2000; Sebastiani *et al.* 2004). Therefore, the response of these trees to increased concentrations of heavy metal in soil is discussed in this section. Table 1.12 shows some selected studies for Cu and Zn phytotoxicity for pines and poplar under different experimental conditions.

1.7.4.1 Pine

Fuentes *et al.* (2007a) investigated the response of Aleppo pine (*Pinus halepensis*) seedlings to soil amended with biosolids that were enriched with Cu (2–44 mg/kg soil), Ni (6–35 mg/kg soil) and Zn (10–122 mg/kg soil). For this study pine was planted in three Mediterranean forest soils set up in lysimeters for 8 months. They reported that higher levels of metal in soil (24–44 mg Cu/kg, 12–35 mg Ni/kg and 64–122 mg Zn/kg) slightly increased plant metal concentrations but the concentrations were always below the phytotoxic levels, and that Cu, Zn or Ni amendments had no significant effect on seedling growth. In another study, Larchevêque *et al.* (2006) carried out a field experiment where compost amendment (equally mixed biosolids, green waste and pine bark) was used to improve survival and growth of planted tree seedlings of native species (Holly oak Aleppo pine and Stone pine) in a calcareous soil. The initial Cu and Zn total concentrations in the compost amended soil were 20–30 mg Cu/kg soil and 60–100 mg Zn/kg soil and they reported that the compost amendment increased EDTA extractable Cu and Zn concentrations in the soils to 3.5 and 13 mg/kg, respectively, when compared to control soil values of 1.7 and 3 for Cu and Zn one year after planting. Foliar Cu and Zn concentration in the seedlings however, remained similar for all treatments (5.6–6.6 mg Cu/kg DM and 75–93 mg Zn/kg DM).

Table 1.12 Selected studies on metal influence on forest plants

| Plant | Metal added | Media used | Critical conc. of media (μM) /soil to plant | Phytotoxic range (mg/kg) | References |
|---|--|---------------------------------------|---|---|--------------------------------------|
| Aleppo pine (<i>Pinus halepensis</i>), Mastic (<i>Pistacia lentiscus</i>), Prickly Juniper (<i>Juniperus oxycedrus</i>), Evergreen buckthorn (<i>Rhamnus alaternus</i>) | Cu 0– 4 Zn 0.073– 100 (μM) | Hydroponic silica sand culture | | Cu 1.7 to 4.1 Zn 25 to 128 (shoot) | Fuentes <i>et al.</i> (2007b) |
| Aleppo pine (<i>Pinus halepensis</i>) | Cu 1.6– 44 Zn 10– 122 (mg/kg) | soils from marl, limestone& sandstone | | Below phytotoxic level | Fuentes <i>et al.</i> (2007a) |
| Stone pine (<i>Pinus pinea</i>), Maritime pine (<i>Pinus pinaster</i>) | Cu 0.12– 5 (μM) | Solution culture | 5 (completely inhibit root growth), 1 (cell membrane damage) | 1–5 μM | Arduini <i>et al.</i> (1995) |
| Whit poplar (<i>Populus alba</i>) | Zn 0– 4000 Cu 5– 500 (μM) | Solution culture | Zn: 500–1000 (transiently toxic), 2000–4000 (higher toxic) Cu: > 25 (browsing leaves), >100 (rooting inhibition) | – | Franchin <i>et al.</i> (2007) |
| Black poplar (<i>Populus nigra</i>) | Cu 50– 850 (mg/kg) | Pot trial with contaminated soil | | 220–650 limited tolerance. >650 tolerance breakdown | Stobrawa and Lorenc-Plucinska (2008) |
| Radiata pine (<i>Pinus radiata</i>) | Cu 0.03– 3 (μM) | Solution culture | >3 root and shoot growth decline | | Gorgé <i>et al.</i> (1985) |

Hartley *et al.* (1999) reported that higher concentrations of Zn were found in the shoots (178–304 mg/kg) and roots (407–648 mg/kg) of 6–10 months old Scots pine (*Pinus sylvestris*) seedlings grown in pots (soils without biosolids amended) for 6 months than was found in the soil (100 mg Zn/kg) in which the plants were grown, reflecting a 2.5 and 5 fold concentration increase over soil levels for shoots and roots respectively. They did not observe any significant difference in pine shoot or root Cu concentrations (they didn't report the value) compared to the soil Cu concentration (13 mg Cu/kg soil). Moreover, both Cu and Zn at these soil levels significantly reduced shoot growth of pine seedlings relative to control suggesting that the Cu and/or Zn concentrations in shoot may be phytotoxic to the pine seedlings. Boardman *et al.* (1997) reviewed a range of Australasian literature on forest plants for the critical concentration levels of important major and minor elements at different stages of growth. Table 1.13 presents the critical concentrations of Cu and Zn in pine at important growth stages.

Table 1.13 Critical concentrations of Cu and Zn (mg/kg DM) in pine at different growth stages (Boardman *et al.* 1997)

| Metal | Stage | Deficient | Marginal | Adequate | Toxic | Country |
|-------|----------------------|-----------|----------|----------|-------|------------------------------|
| Zn | Seedling | <6 | 11–12 | | | Australia/NZ |
| Cu | Juvenile/ Mature | <2 | 2.1–2.3 | 2.4–9 | >40 | Various |
| Zn | Juvenile/ Mature* | <11 | 11–13 | 14–60 | >200 | Australia/NZ/South Africa |

* Adequate Zn range is 14–64 and >200 is defined as 'high' concentration for mature pine

1.7.4.2 *Poplar*

Knowledge of the physiological mechanisms controlling heavy metal accumulation and tolerance in poplar plants is still in its infancy (Arisi *et al.* 2000; Di Baccio *et al.* 2005; 2003; Koprivova *et al.* 2002; Tognetti *et al.* 2004). Factors such as the extent of metal tolerance and uptake are highly variable among poplar species and clones, due to abundant genetic variation throughout the genus

(Brunner *et al.* 2004). Comparative studies on metal accumulation and growth have demonstrated that different clones respond differently to heavy metal exposure for the same contaminated media (e.g. industrial waste) (Sebastiani *et al.* 2004; Tognetti *et al.* 2004). Borghi *et al.* (2007) found that Cu at concentrations equal to or higher than 100 μM supplied in Hoagland's solution in an aerated hydroponic system reduced growth, chlorophyll content and photosynthetic activity of poplar plants (Clone: *adda*) and this metal was mainly accumulated in the root system. Borghi *et al.* (2008) showed different responses to Cu in terms of tolerance and metal partitioning by two poplar clones: *P. x canadensis* accumulated Cu in roots, displaying features sought in plants suitable for phytostabilization, while White poplar (*P. alba*) accumulated the metal in leaves, like an indicator species (plant used to indicate phytotoxicity of metal concentration in soil). Sebastiani *et al.* (2004) indicated that metal in the biomass of poplar plants grown in soil amended with Zn and Cu-contaminated industrial waste at rates of 102 mg Cu/kg and 5040 mg Zn/kg, was partitioned differently between roots and shoots. Zinc was actively transported and accumulated in leaves, while Cu was almost entirely confined to roots. Castiglione *et al.* (2007) reported that poplar (clone: Villafranca) cuttings grown in ZnSO_4 solution showed reduced adventitious root formation and leaf chlorosis at a Zn concentration of 0.5–4 mM.

Stobrawa and Lorenc-Plucinska (2008) conducted a field study on the effect of different concentrations of Cu in contaminated soils (63–1132 mg Cu/soil) on poplar growth in Poland, and based on the relationship between soil metal concentration and poplar growth, they suggested total soil threshold Cu toxicity concentrations for different levels of Cu tolerance (Table 1.14).

Table 1.14 Proposed thresholds of soil Cu concentrations for tolerance by poplar (Stobrawa and Lorenc-Plucinska 2008)

| Threshold of Cu toxicity (mg Cu /kg soil) | Status of tolerance |
|---|------------------------|
| < 220 | Full tolerance |
| 220–650 | Limited tolerance |
| >650 | Breakdown of tolerance |

The reported literature shows that in general, the critical Cu concentration for phytotoxicity is lower than that for Zn for both pine and poplar. This indicates Cu is more toxic than Zn.

1.7.5 Rhizosphere

The rhizosphere is the fraction of soil that interacts with plant roots. The rhizosphere is defined as the few millimetres of soil surrounding the plant roots that is influenced by the activity of soil microorganisms and plant roots. The boundary between the rhizosphere and bulk soil is therefore difficult to differentiate. Soil characteristics, properties of the root system, nutritional status of the plants, and climatic conditions can be considered as factors influencing the dimension of the rhizosphere. The rhizosphere can be functionally defined as a highly dynamic, plant-driven microenvironment that is characterised by feedback loops of interactions between root processes, soil characteristics, and the dynamics of the associated microbial population (Barea *et al.* 2005).

Adriano (2001) indicated that rhizosphere soil is very different from bulk soil in terms of pH, redox potential, mineral composition, and organic matter cycling and composition. These parameters also largely control the bioavailability of heavy metals, and thus bioavailability may be different in the bulk soil in comparison to the rhizosphere soil. The pH in the rhizosphere may be very different from the bulk soil (Liangpeng *et al.* 2007). The principal reason for this difference is thought to be the unequal uptake of cations and anions by plant roots (Hedley *et al.* 1983). Uptake of an excess of cations over anions leads to H^+ exudation by roots, whereas uptake of an excess of anions leads to OH^- or HCO_3^- exudation. Due to the oxidative action of plant roots, speciation of metals in the rhizosphere can also be expected to differ from the bulk soil (Jassogne *et al.* 2009). Root-induced changes of the redox potential in the rhizosphere may change the speciation of the metals within millimeters of plant roots (Tao *et al.* 2003) as redox potential (Eh) is negatively related to pH.

The organic matter content of rhizosphere soil can also differ from that of bulk soil. Plant roots release a considerable amount of organic material, including water soluble exudates and water insoluble compounds into soil (Naidu and Kim 2008; Tao *et al.* 2003). Many of these materials are readily degraded by soil microorganisms, but their continuous release maintains the organic matter concentration of the rhizosphere at higher levels than in bulk soil. The higher organic matter concentration in the rhizosphere may influence the bioavailability of Cu and Zn.

1.7.6 Mycorrhizae

The majority of plants have mycorrhizal fungi associated with them. There are two main types of mycorrhizae. They are endomycorrhizae, where the fungus colonizes the interior of host plant root cells (e.g. ericoid and arbuscular (AM) mycorrhizae), and ectomycorrhizae (ECM), where the fungus is located outside the plant root cells. They have quite different host plant relationships and are thus largely confined to different ecosystems. For example, ectomycorrhizae are found in most forest trees (Liu *et al.* 2008). As both pine and poplar are important tree species in New Zealand and have ECM associated with their roots, literature on mycorrhiza/tree relationships is reviewed in this section.

The formation and function of mycorrhizal relationships are affected by various conditions such as soil composition, moisture, temperature, pH, cation exchange capacity, and also by anthropogenic stress factors including soil compaction, metals and pesticides (Entry *et al.* 2002). Mycorrhizal fungi are ecologically significant because they form relationships in and on the roots of a host plant in a symbiotic association. The host plant provides the fungus with soluble carbon sources, and the fungus provides the host plant with an increased capacity to absorb water and nutrients from the soil. They may also promote other advantages to plants, such as improved soil–plant water relations, enhanced resistance against certain pathogens, and enhanced tolerance to salt and metal stress (Bais *et al.* 2006; Barea *et al.* 2005).

Several studies have shown that mycorrhizae can reduce heavy metal uptake by plants (Tullio *et al.* 2003). Metal tolerance of mycorrhizal plants may be dependent on the host plant, fungal tolerance and compatibility between them (Entry *et al.* 2002). The mechanisms of tolerance in mycorrhiza are varied and include; binding of the metal by proteins, extracellular polymers or to the cell wall, compartmentation inside cells, formation of insoluble metal sulphides, decreased uptake, enhanced export from cells, and volatilization (Ferrol *et al.* 2009; Gadd 2010). Fomina *et al.* (2005) reported that mycorrhizal fungi are involved in proton-promoted and ligand-promoted metal mobilization from mineral sources, metal immobilization within biomass, and extracellular precipitation of mycogenic metal oxalates. Christie *et al.* (2004) reported that arbuscular mycorrhizae depressed translocation of zinc to shoots of host plants in soils that were moderately polluted with zinc through binding the metal in mycorrhizal structures, and thereby immobilizing the metal in the mycorrhizosphere. A copper-adapted ECM (*Suillus luteus*) provided protection against copper toxicity in pine seedlings exposed to an elevated copper concentration, and it was proposed that the metal-adapted ECM-pine combination might be suitable for large-scale land reclamation of phytotoxic-heavy metal contaminated and industrial land (Adriaensen *et al.* 2005). Krupa and Kozdroj (2004) reported that ectomycorrhizal fungi persistently fixed Cd (II) and Pb (II), and formed an efficient biological barrier that reduced movement of these metals into birch tissues. Gonzalez-Chavez *et al.* (2004) hypothesised that naturally-occurring soil organic compounds can stabilize potentially toxic metals such as copper, cadmium, lead and manganese, and showed that the insoluble glycoprotein glomalin, produced abundantly on the hyphae of arbuscular mycorrhizal fungi, reduced heavy metal toxic effects in sorghum grown in a glasshouse study.

Mycorrhizal associations may also be used for metal cleanup in the general area of phytoremediation. Mycorrhiza may enhance phytoextraction directly or indirectly by increasing plant biomass, and some studies have shown increased plant accumulation of metals, especially when the plants were inoculated with mycorrhiza isolated from metal polluted environments (Bissonnette *et al.* 2010;

Göhre and Paszkowski 2006). Tree–ECM associations are ideal target organisms for this application because key ECM host species readily colonize polluted soils of interest. Birch (*Betula*) species, for example, show an ability to rapidly colonise highly–polluted industrial soils (Cairney and Meharg 1999; Hartley *et al.* 1997a). Importantly, their mycorrhizae also proliferate under such conditions. In addition, other tree species, such as Scots pine (*Pinus sylvestris*), loblolly pine (*Pinus taeda*) and willow (*Salix*) spp., also reportedly self–seed at contaminated sites and maintain functional ECM associations (Cairney and Meharg 1999; Meharg and Cairney 2000). The potential impact of mycorrhizal fungi on bioremediation may be conditional and dependent on the metal tolerance of fungal strains, their mycorrhizal status, and the nutritional status of contaminated soils (Meharg 2003).

Although mycorrhizal fungi are tolerant to moderate levels of metal pollution, and in some cases increase mycorrhizal colonization, they can sometimes be affected by the presence of high metal concentrations in soil. Many studies on the effect of a high concentration of metals on mycorrhizae have been conducted on mycorrhizae isolates culture (Hartley *et al.* 1997b; Jacquot-Plumey *et al.* 2003; Takacs and Voros 2003). Many such studies were reviewed by Gadd (1993). Based on studies on different ECM isolates, Hartley *et al.* (1997a) reported that the EC₅₀ Zn concentration value (effective concentration which inhibits ECM growth by 50%) for Zn toxicity depended on the type of ECM isolates (Table 1.15). Similar EC₅₀ values for Cu are not available in the literature.

Table 1.15 Zn²⁺ concentration at EC₅₀ for ECM isolates (Hartley *et al.* 1997a)

| ECM isolates | Zn ²⁺ concentration (μM) at EC ₅₀ |
|---|---|
| Red pine mushroom (<i>Lactarius deliciosus</i>) | 100 ± 45 |
| brown roll-rim (<i>Paxillus involutus</i>) | 188 ± 36 |
| Velvet Bolete (<i>Suillus variegates</i>) | 343 ± 51 |
| Weeping Bolete (<i>S. granulatus</i>) | 336 ± 46 |
| Slippery Jack (<i>S. luteus</i>) | 284 ± 41 |

1.8 Copper and zinc toxicity to microorganisms

Soil microorganisms tend to be more vulnerable to Cu and Zn toxicity than higher plants because they have less well developed homeostatic defense mechanisms (Alloway 2008a). Adverse effects of Zn on soil microbial processes have been reported even in soils where Zn has no toxic effect on plants (McLaughlin and Smolders 2001). Table 1.16 presents selected references on the critical Cu and Zn concentrations affecting microbial activity. Many studies related to the toxicity of metals on enzyme activity in soils (such as dehydrogenase activity) have been reported in literature (Carrasco *et al.* 2006; Chaperon and Sauvé 2007; Chaudhuri *et al.* 2003). This has led to the use of enzyme assays as indicators of soil pollution.

Nitrification for example is an important soil process catalysed by microorganism that is highly sensitive to heavy metals. Rusk *et al.* (2004) reported that nitrification after 17 months for a Zn contaminated soil (spiked with ZnSO₄ to soil, 890 mg total Zn/kg) was 4 times lower than that in an uncontaminated soil (30 mg total Zn/kg). They also found that the EC₅₀ value (effective Zn concentration to inhibit 50% of bacterial activity) for Zn was 210 mg/kg in uncontaminated soil at the initial stage of experiment. However, the EC₅₀ was increased to 850 mg Zn/kg for bacterial communities that had been exposed to Zn contaminated soil for 17 months, indicating that significant adaptation of the bacterial community to Zn had occurred. Doelman (1986) conducted a laboratory experiment with metal sulphates added to 5 soil types and incubated for 18 months to measure the enzyme activities of microbes. He showed that the inhibition concentration for N-mineralization and soil respiration was >1000 mg/kg for Cu and Zn. He also reported that when the total soil Zn concentration was >10000 mg/kg, nitrification was inhibited, where as for Cu, the concentration for inhibiting nitrification was >1000 mg/kg. This review of the literature suggests that, for microorganisms, like plants, Zn in general is less toxic than Cu at the same metal concentration.

Table 1.16 Critical Cu and Zn concentrations affecting microbial activities (conc. unit mg/kg; exception (mg/L) indicated in table)

| Activity | Cu | Zn | References |
|--|---|---|----------------------------------|
| Bioluminescence response for bacterial biosensor (EC ₅₀) | – | 1.3 mg/L (litter solution), 3700 (total litter) | Horswell <i>et al.</i> (2006) |
| Bioluminescence response for fungal biosensor (EC ₅₀) | 0.12 mg/L (litter solution), 540 (total litter) | – | |
| Microbial biomass C (MBC) (Cu or Zn) | 690 (EC ₅₁), 197 (EC ₁₂) (total soil) | 705 (EC ₃₆), 429 (EC ₁₂) | Chander and Brookes (1993) |
| Microbial Biomass C (Cu and Zn both) | 197 (EC ₂₉), 262 (EC ₅₃) (total soil) | 367 (EC ₂₉), 427 (EC ₅₃) | |
| Sulphatase (EC ₂₀) –proportion to TOC* | 140 (Total soil), 2.6 (soil solution), 3 (free ion), 1.9 (activity) | 310,190, 170, 100 | Speir <i>et al.</i> (2007) |
| Phosphatase (EC ₂₀) | 48 (total soil), 3.3 (soil solution) | 330 (total) | |
| Microbial biomass C (EC ₂₀) – proportion to TOC* | 140 (total soil), 4 (soil solution) | 310 (total) | |
| Phosphatase | 959 (ED ₁₀), 2858(ED ₅₀) (total soil) | 36 (ED ₁₀), 2943 (ED ₅₀) | Doelman and Haanstra (1989) |
| Ectomycorrhizal fungi isolates (biomass weight) | – | 4.5–25.8 mg/L (ED ₅₀) (solution media) | Hartley <i>et al.</i> (1997b) |
| Arbuscular mycorrhizal fungal spores isolated | – | 0.005(Bosolids) 0.003 (FYM) (culture media) | Weissenhorn <i>et al.</i> (1994) |

*Sulphatase and MBC were expressed on the proportion of TOC measured in the same sample; ED–Effective dose

Chander and Brookes (1993) described the additive effect of Cu and Zn on soil microbial biomass. They found that a combination of Zn and Cu decreased the amount of microbial biomass at lower total soil metal concentrations (Cu and Zn) than was required when either metal was present singly, suggesting the effects were additive. They also reported that the biomass carbon content of soil as a percentage of total soil organic carbon, in soils amended with biosolids spiked with higher rates of Cu or Zn (3000 and 2800 kg/ha DM respectively), whether singly or combined, was less than 50% of that of a soil amended with biosolids not treated with any Cu and Zn. Horswell *et al.* (2006) conducted a field trial in a pine forest in New Zealand using metal-spiked biosolids (Cu 50–200 mg/kg soil and Zn 100–400 mg/kg soil) as soil amendments in the plots. They found that bacterial bioluminescence response declined as water soluble Zn concentration in the litter (decomposing organic matter and an accumulation of woody debris on the surface of forest soil) was increased ($EC_{50}=1.3$ mg/L) (Table 1.16). Gibbs *et al.* (2006), however, reported that application of metals at a similar concentration as the Horswell *et al.* (2006) study (15–200 mg Cu/kg and 60–450 mg Zn/kg) to agricultural soils in UK using liquid biosolids annually for 4 years, had no effect on soil respiration rates, biomass carbon concentrations or MPN (most probable number) of clover *Rhizobium*.

1.8.1 Comparative effect of metal toxicity in plants, microorganisms and mycorrhiza

Metal effects on plants (Table 1.12) and microorganisms (Table 1.15) have been documented with many references. However the comparative effect of metal toxicity on microorganisms and plants quantified in the same study is lacking. In most studies concerning the metal tolerance of mycorrhizal plants, only the effect of the fungi on metal toxicity to plants has been considered. Possible plant protective effects against heavy metal toxicity to the fungus have not been studied. Soil microorganisms, including mycorrhizal fungi, are affected by the presence of high concentrations of metals in soil (Gadd 1993; Giller *et al.* 1998), but the threshold of phytotoxicity may not be at the same metal concentration

level as for plants. For example, McGrath *et al.* (1988) studied the growth and N₂-fixation of white clover and N₂-fixing bacterial activity in a pot trial by using 20 year-old metal contaminated soil (EDTA extractable Cu concentration of 15–98 mg/kg and 40–235 mg/kg for Zn). They concluded that white clover did not show phytotoxicity, but N₂-fixing bacteria were severely affected, and thereby N availability to the plant through N₂-fixation was reduced. Furthermore, nearly 4 years before this report, using the same soil, Brookes and McGrath (1984) found that the microbial biomass C and soil ATP concentrations were only about half those of the uncontaminated soils, and the application of N fertilizer to the white clover plants improved their DM yield. This confirms the N deficiency in the plants was due to the absence of N₂ fixing bacterial activity.

Using soil sampled from the trial of McGrath *et al.* (1988) (discussed in the preceding paragraph), Koomen and McGrath (1990) studied the mycorrhizal infection rate of white clover. Total metal concentrations in the metal-contaminated (Cu, Zn, Ni and Cd) biosolids-treated soil (pH 6.5) were 126 mg Cu/kg and 381 mg Zn/kg. They reported that there was no significant difference between the yields of clover grown in the contaminated soil relative to a control soil. However, there was a substantial difference between the mean percentage mycorrhizal infections of the roots; 60% in the control soil and only 1% in the metal contaminated soil. This shows that clover growth was not affected by the high metal concentration in the biosolids-treated soil, but mycorrhizal infection was significantly affected. These authors also compared the effect of inoculation of the contaminated soil with mycorrhiza against the same but non-inoculated soil. There were no significant differences between DM yields of clover plants grown in the inoculated and non-inoculated pots containing the metal contaminated soil. None of the plants from the inoculated or the non-inoculated biosolids-treated soil had any mycorrhizal infection in the roots. However the control treatment showed 25% higher mycorrhizal infection for the inoculated treatment relative to the non-inoculated treatment. They concluded that the effect of the metals (Cu, Zn, Ni and Cd) was detrimental to mycorrhiza, but was not significant to the plants.

Metal toxicity to mycorrhiza may sometimes be higher during symbiosis with plants, relative to pure culture. Jentschke *et al.* (1999) studied the effects of ectomycorrhizal colonization by bicoloured deceiver (*Laccaria bicolour*) and brown roll-rim (*Paxillus involutus*) on Cd toxicity in Norway spruce seedlings (*Picea abies* L.). Both mycorrhizal and non-mycorrhizal seedlings were exposed to 0 (control), 0.5 or 5 μM Cd in a sand culture system. In pure culture, without plant, both isolates showed higher Cd tolerance. However, in symbiosis with plant, the 5 μM Cd treatments showed decreased colonization by bicoloured deceiver.

1.9 Methods to reduce soil heavy metal toxicity

Soil remediation methods are commonly used to control the bioavailability of heavy metals to plants, to soil microorganisms, and to manage the risk of metal leaching to groundwater. The methods used to control metal toxicity (biological, physical and chemical) are based on 2 principles; contaminant removal, or immobilization by fixation (Adriano 2001; Rosa *et al.* 2002). Biological cleaning methods include phytoimmobilisation (phytostabilisation), whereas removal strategies include phytoextraction (Salt *et al.* 1995), phytodegradation (Anderson 1993), and phytovolatilization (Meagher *et al.* 1997). Electrokinetic treatment (Virkutyte *et al.* 2005) is a technological (physical) option to remove heavy metals from soil however, other treatments such as separation and washing, and frozen ground barriers, are also being practiced (Iskandar and Adriano 1997). Physical methods are not, however, cost effective. Many experiments have demonstrated that it is possible to use simple and cheap chemical immobilization methods to attenuate the exposure of plants and other soil organisms to heavy metals ((McBride and Martinez 2000; Vangronsveld *et al.* 2000)

For soils not heavily polluted, bioavailability can be reduced through the immobilization of the mobile fractions of metals in the soil, or through the leaching of easily soluble fractions to a secured environment (Echevarria *et al.* 1998). Moderately polluted soils can be partially remediated or managed by

growing plants to remove metals (phytoremediation) (Borghi *et al.* 2007). But for soils severely contaminated with metals, phytoremediation cannot be a remediation strategy (Kabata-Pendias and Pendias 2001) as excessive concentrations of bioavailable metal are toxic and will hinder plant growth. In this situation, chemical treatment processes are required to reduce the solubility of heavy metals through increasing soil pH (Basta and McGowen 2004; Uchimiya *et al.* 2010) or immobilization by way of a metal binding process (precipitation or specific adsorption) (Adriano 2001). Copper mobility in soil can be lowered by carbonates, phosphates and clays by chemi-sorption (Kabata-Pendias and Pendias 2001). The high diversity of Zn compounds in soils (Manceau *et al.* 2004) may seem to facilitate the choice of stabilizing amendments. Zn can precipitate with hydroxides, carbonates, phosphates, sulfides, molybdates and several other anions as well as form complexes with organic ligands (Kiekens 1995). Cation exchange and complexation by organic ligands have been suggested as the main Zn mobility controlling mechanisms in acidic soils, while Al, Mn and Fe oxides were of less importance (Kabata-Pendias and Pendias 2001).

1.9.1 Zeolite, iron oxide and lime as amendments to immobilise metals in soil

Numerous materials have been selected for both their lack of negative influence on key aspects of soil health, and their efficacy in decreasing the solubility and bioavailability of heavy metals in contaminated soils (McBride *et al.* 2000; Oste *et al.* 2002). Immobilization can be an effective remediation technique when inexpensive materials are used; many inexpensive additives have been identified for their potential to immobilize heavy metals in soils. Many of these are alkaline materials, for example lime (Khan and Jones 2009), zeolite (Moattar and Hayeripour 2004) fly ash (Ogut *et al.* 2009), and iron oxide (Chlopecka and Adriano 1996). The physical and chemical properties of these amendments have been reviewed ((Mench *et al.* 1998).

1.9.1.1 Zeolites

Zeolites are naturally occurring aluminosilicates, whose ion exchange capabilities have been recognised for a considerable length of time (Allen *et al.* 1983). These can be used to produce materials with high ion exchange capacities similar to organic ion exchange resins. The structure of a crystallised synthetic hydrated sodium aluminium silicate is based on the sharing of all of the oxygens linked by $(\text{SiO}_4)^{4-}$ and $(\text{AlO}_4)^{5-}$ tetrahedra in the form of a porous three dimensional solid (João and Michael 2000). Water molecules are loosely held in the pores and most zeolites can be reversibly dehydrated. Cations associated with the aluminosilicates to balance net negative charge can be readily exchanged (Christie *et al.* 2002). The very high CEC of Zeolites is considered to be the reason for their ability to strongly adsorb heavy metals, and to subsequently reduce metal bioavailability (Nissen *et al.* 2000). Many studies have demonstrated the ability of zeolites to reduce the uptake of Cs, Sr, Cu, Cd, Pb, and Zn by plants (Castaldi *et al.* 2005; Chander and Joergensen 2002; Querol *et al.* 2006).

Nissen *et al.* (2000) conducted an incubation and a glasshouse experiment to examine the effect of incorporating a synthetic zeolite (Zeolite P) at a range of concentrations (0.1–1.0 w/w) into an experimental horticultural compost, derived from Cu and Zn contaminated biosolids, and planted with ryegrass. They reported that addition of 0.5% and 1% zeolite significantly reduced labile Zn (60–70%) over a 90 days period, but for Cu they did not make any conclusion due to the low Cu concentration in the sample. They also reported that the overall amount of metal amended by ryegrass was reduced by about 35%, for both Cu and Zn in the amended composts as compared to the unamended control biosolids. Furthermore, they showed that the treatment incorporating 0.5% zeolite P was as effective as the 5% lime treatment, in terms of reduction of the labile zinc pool.

Zorpas *et al.* (2000) reported that as the amount of added natural zeolite (clinoptilolite) increased (to 25–30%) in a contaminated compost pile incubated

for 150 days, the concentration of bioavailable Cu and Zn in the final compost was decreased by 10–15% and 40–46% respectively.

1.9.1.2 Iron and aluminium oxides, fly ash and by-products

Iron oxides and oxyhydroxides occur naturally in soils. Kabata-Pendias and Pendias (2001) reported that iron oxide application to soils effectively reduced the bioavailability of Co, Ni and Zn. Like zeolites, iron oxides have very high adsorptive capacity for heavy metals. They can specifically adsorb heavy metals (Singh and Agrawal 2008). Peacock and Rimmer (2000), in a soil column dissolution experiment, compared the metal cation adsorption by iron oxide with red gypsum (a gypsum by-product rich in iron oxides) and found that metal cation adsorption affinity order was $\text{Pb} > \text{Cu} > \text{Ni} > \text{Zn} > \text{Cd}$ for the iron oxide material and $\text{Pb} > \text{Zn} > \text{Cd} > \text{Cu} > \text{Ni}$ for red gypsum.

The sorption behaviour of the clay mineral palygorskite has been studied by Álvarez-Ayuso and García-Sánchez (2003) with respect to Pb, Cu, Zn and Cd, during a four-week laboratory incubation study, in order to evaluate its applicability as a soil remediation technique. They found that the maximum sorption values were 17.4 mg/g for Cu and 7.11 mg/g for Zn at pH 5–6. A batch extraction study using the same experimental setup showed that the application of 4% palygorskite to a contaminated soil almost completely immobilised soluble metal concentrations of Cu and 60% of the Zn. The soluble + exchangeable Cu and Zn concentrations were reduced by 77% each. In addition, the metal mobility illustrated through the leaching study was reduced by 59% and 52% for Cu and Zn respectively, when 4% of palygorskite was applied to soil.

Su and Wong (2004) used a loamy acid soil amended with fly ash-stabilized biosolids to grow corn over a period of 42 days under greenhouse conditions, to evaluate the effect of coal fly ash on the availability of Cu, Zn, Ni and Cd in the biosolids. They found that increasing the fly ash amendment rate significantly reduced DTPA-extractable Cu and Zn concentrations ($r=0.99$, $P<0.01$).

Percentages of Cu and Zn in the residual fraction increased with an increase in the fly ash amendment rates ($r=0.99$, $P<0.01$).

Zhang *et al.* (2008a) conducted an incubation experiment with different ratios of biosolids, fly ash and alkaline mine tailings to evaluate the solubility of ions and heavy metals from stabilized biosolids. They reported that the Cu concentration in solution for all mixtures of treatment (25% biosolids, 50% fly ash, 25% alkaline mine tailings) was reduced from 47.7 to 15 $\mu\text{g/L}$ after 22 days of incubation and at this time reached a stable level. They concluded that application of coal fly ash to soil, that usually increases soil pH and the relative amount of carbonate, is an efficient measure to control Cu mobility. However, the results of a laboratory leaching study conducted for 30 months by Ciccu *et al.* (2003) reported that increasing the soil pH to above 8 through the application of coal fly ash (15% w/w) and red mud–gypsum (15% w/w) reduced Cu leaching by 50% and 10% over the untreated soil, respectively, whereas a combination of these amendments actually increased Cu leaching by 170% over the untreated soil. The mobility of Cu is usually low at slightly alkaline pH but can increase in highly alkaline conditions (>10) due to the formation of OH^- complexes. Although Fe, Al, Mn oxides are generally good sinks for Cu (Kabata-Pendias and Pendias 2001), their application to soil may not always lead to a desired reduction in heavy metal bioavailability. Hartley *et al.* (2004) tested several iron amendments: goethite, iron grit (Fe (0)), iron (II)/(III) sulfates combined with lime, but none of the treatments were efficient in the chemical stabilization of Cu bioavailability.

1.9.1.3 Lime

Many studies have shown that there is a decrease in plant uptake of heavy metals with application of limestone due to increasing pH (Basta and McGowen 2004). At high pH, many heavy metals, including Cu and Zn, are more strongly and specifically adsorbed to soil constituents or are precipitated as hydroxides or carbonates. Lime is a cheap and effective ameliorant for many metals, but repeated applications are required to maintain metal immobilization. Lime has

been applied in various form such as, CaCO_3 , $(\text{Ca,Mg})\text{CO}_3$, CaO , and $\text{Ca}(\text{OH})_2$ to not only increase soil pH and metal immobilization, but also to effect a complex change in total nutrient availability of the soil (Castaldi *et al.* 2005), an increase in total microbial activity (Bezdicek *et al.* 2003), and to substantial shift microbial population structure (Rangel–Castro *et al.* 2005). However care should be taken to not over–lime, because it will reduce the bioavailability of many other plant nutrients.

Gheshlaghi *et al.* (2008) conducted a glasshouse experiment to evaluate the effect of zeolite, iron waste (both 5 and 10% w/w), and a liming material (0.33 and 0.66% w/w) on Cu, Zn, and Ni phytoavailability to sunflower grown in two metal–contaminated biosolids–amended soils for 3 weeks. They reported that under the moderately acidic pH conditions of the original soils (pH 5.4–5.7), neither of the materials (zeolite or iron waste) had substantial effects on plant metal concentrations, and that the incorporation of zeolite into the soil resulted in a large decrease in plant yield (>60% reduction). However, in the presence of lime, both zeolite and iron waste materials reduced sunflower shoot Zn concentrations by 75–91%. Combinations of iron waste and lime decreased the soluble soil Zn concentration (>90% reduction) and increased plant yield (63% increase for highly contaminated soil). This resulted in remediation of Zn toxicity. However, there was little effect of any treatment on the Cu concentration in the sunflower plants.

1.10 Research justification

Based on the review presented as Chapter 1 of this thesis, it is clear that biosolids must be handled with the highest possible care during disposal. Land application, especially to forest plantation and to agricultural land is gaining increased popularity in many countries including New Zealand. In New Zealand, biosolids are currently permitted for application only to forest lands, but recently some forest lands have been converted into dairy farms. Heavy metals accumulated in forest lands may have serious direct environmental impact on plants and

microorganisms as well as possibly entering the food chain in such lands after land conversion.

Current biosolid application guidelines are based on total heavy metal concentrations in soil, but the concentration of the bioavailable forms of heavy metals, rather than the total metal concentration (McLaren and Clucas 2001), is proposed as a more suitable index to assess the biotoxicity of heavy metals. This is because not all metal species cause biotoxicity. In order to develop an index based on bioavailability, research information is required on the effect of variable concentrations of different metal species on soil microbiological activity and plant growth. This information can be obtained only through a better understanding of the dynamics of biosolids-derived heavy metals in soils, and their influence on soil microorganisms and plants.

Heavy metals applied to soil through biosolids may behave in a different manner compared to those applied as inorganic metal salts such as fertilizers and pesticides. The majority of research data on heavy-metal dynamics in soil are derived from research on inorganic metal salts applied to soils. More information is required on biosolids-derived heavy metal dynamics in soils.

Unlike in many countries, New Zealand biosolids generally have low heavy-metal concentrations due to low levels of industrial activity in this country. Therefore, for research purposes, biosolids need to be spiked with heavy metals prior to application to soil, as proposed by Gibbs *et al.* (2006) and McLaren and Clucas (2001). However, the time period to which the metals should be equilibrated to completely incorporate the metals into the biosolids matrix to simulate 'real-life' metal contaminations is not known. A periodic assessment of chemical forms of metal in the solid and liquid phases of the metal-spiked biosolids is necessary to determine the shortest time required for the metals to be incorporated into the biosolids matrix, and to ensure the valid use of metal-spiked biosolids in research trials. The first experiment of this thesis focussed on this aspect, with special emphasis on Cu and Zn, because these two heavy metals are

commonly found to be in very high concentrations in biosolids, including New Zealand biosolids. As biosolids are currently applied to forest lands, two important forest plants, pine and poplar, have been studied. As these two trees have ectomycorrhiza, the research has been designed to study the effect of Cu and Zn not only on plants but also on ectomycorrhiza, which to my knowledge has not been studied previously. Through reviewing the literature I found that there is very limited information available on the comparative toxicities of heavy metals to plants, soil and mycorrhizae in single trials. In order for regulatory agencies to set guidelines for the safe disposal of metal-contaminated biosolids to land, threshold heavy metal toxicity levels to plants and soil microorganisms need to be considered. This study provides data in support of the quantification of guideline levels.

1.11 Objective of the study

- 1 To amend biosolids with inorganic Cu and Zn salts and to determine the time it takes for the maximum quantity of metals to be incorporated into the biosolids matrix (Chapter 2).
- 2 To determine the Cu and Zn species concentration in biosolids that causes toxicity to microorganisms and to propose EC₅₀ values (Chapters 2, 3 and 4).
- 3 To determine the critical Cu and Zn species concentration in soils amended with biosolids that cause toxicity to poplar, pine and soil microorganisms, and to propose EC₅₀ values (Chapter 3 and 4).
- 4 To determine the effect of soil properties on the chemical fractionation and speciation of Cu and Zn in soil, using a range of contrasting New Zealand soils (8 in total) (Chapter 5).
- 5 To determine the chemical transformation of biosolids derived Cu and Zn added to soils (Chapters 3, 4 and 5).
- 6 To determine the amelioration effects of lime and an Al oxide waste on Cu and Zn toxicity to poplar, root mycorrhiza and other microorganisms (Chapter 6).

Chapter 2

Copper and zinc spiking of biosolids: Effect of incubation period on changes in metal chemical forms and bioavailability *

2.1 Introduction

Many biosolids have low concentrations of heavy metals, and therefore to determine the long-term cumulative effects of the low concentrations of heavy metals on soil, it is a common research practice to spike biosolids with heavy metal salts, and to study the ecotoxicological impacts of these metals on plants, the organic litter-dwelling microorganisms found in soils (Gibbs *et al.* 2006), and the potential risk of the heavy metals moving to ground waters (Gove *et al.* 2001; McLaren *et al.* 2004). New Zealand biosolids generally have low heavy metal concentrations (McLaren *et al.* 2007), due to low levels of industrial activity in this country. The key advantages of spiking media for research with metals are that impacts can be assessed immediately in the short-term, different levels of metal concentrations can be maintained, and an estimation of the individual effect of each metal is possible (McLaren and Clucas 2001; Speir *et al.* 2007). The major disadvantage of this experimental procedure is that the chemical nature of the metals in metal-spiked biosolids may be different to that in biosolids naturally contaminated with metals. Natural biosolids have passed through different treatment procedures and application of biosolids spiked with heavy metals to soil may not give similar effects as those metals already present in the sewage entering the treatment plant. Also, the application of a high concentration of metals causes a ‘sudden shock effect’ on soil organisms. To overcome these adverse effects, spiked biosolids are commonly anaerobically incubated for a time period to incorporate metal fractions into the sludge matrix. Previous studies have used 6–9 months as an equilibration period to incorporate the metals into the sludge matrix (Gibbs *et al.* 2006; McLaren and Clucas 2001; Speir *et al.* 2007). While it is

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possible that a much shorter period may be used to achieve this degree of metal incorporation, this theory has not been tested previously. A periodic assessment of the chemical forms of metal in the solid and liquid phases of the metal-spiked biosolids using an appropriate metal fractionation procedure is necessary to determine the shortest time required for the metals to be incorporated into the biosolids matrix.

Fractionation procedures are often criticised because of their complexity and difficulty in interpretation, arising from potential problems such as lack of specificity of extractants, and re-adsorption of metals during extraction. Nevertheless, providing that such limitations are recognised, sequential fractionations can provide extremely useful information on metal distribution in biosolids, particularly for comparative purposes such as changes with time. It should be noted, however, that the terms used to describe individual fractions (e.g. oxide bound, organic bound as described in Chapter 1) are not exact and should only be regarded as 'nominal' forms of metals. The chemical forms of metals as determined by the fractionation procedures can however provide information on the relative bioavailability and mobility of metals in soils (Fuentes *et al.* 2004).

This chapter examines the effect of three rates of copper (Cu) and zinc (Zn) applied separately to biosolids on the distribution of metal fractions in the solid phase, and metal species in the liquid phase of the biosolids, at four time intervals after application. The influence of the heavy metal fractions and species on microbial activity as quantified by dehydrogenase activity is also presented. Copper and Zn were selected for this study because these two heavy metals are commonly found to be in very high concentrations in biosolids (Oliver *et al.* 2004; Shrivastava and Banerjee 1998).

2.2 Materials and methods

2.2.1 Sampling and experimental setup

Anaerobically digested sewage sludge samples from the Palmerston North City Council Waste Water Treatment Plant sludge lagoon in New Zealand were collected in seven 20-L plastic cans to full capacity, and kept air tight in a shaded place (Figure 2.1). Fifteen days after collection, the sewage sludge samples (17 mg Cu/L and 73 mg Zn/L) were treated with 3 levels of Cu and Zn separately as metal sulphate salts ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ and $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$). The metal salts were added as sulphates because they were likely to be reduced to sulphides, which have been shown to be important forms of Cu, Zn and Cd in biosolids (Gibbs *et al.* 2006; Stover *et al.* 1976). Each of the sealed cans was vigorously shaken 5 times by hand, each shaking lasting one minute, followed by rolling of the cans on the ground to cause 10 rotations per can. The target metal concentrations (mg/L) in the metal spiked sludges were 586, 1355 and 2206 for Cu and 1179, 3837 and 5832 for Zn. These metal concentrations were selected so that when the contents in the cans were applied to soils at the rate of 200 kg N/ha (3% N in biosolids solid phase; 6.7 tonne dry solids/ha), the metal concentrations resulting in the experimental soils would be equivalent to 50, 150 and 250% of the soil metal concentration limit recommended by the New Zealand Water and Wastes Association (NZWWA 2003) (100 and 300 mg/kg for Cu and Zn respectively). One can, without Cu or Zn addition, was kept as a control.

The sealed cans were kept air tight and incubated for 117 days with frequent (twice a week) shaking by hand. Two subsamples (50 mL each) were collected from each container 0, 25, 55 and 117 days after metal addition for chemical and biological analysis. The first subsample at each time period was collected in a sterilised container, tightly sealed and stored at 4°C for dehydrogenase activity and biological oxygen demand (BOD) measurements. Gas pressure was released from each can at each of these sampling intervals.



a) Biosolids digester



b) Biosolids lagoon



c) Aeration lagoon



d) Plastic containers with metal spiked biosolids

Figure 2.1 (a,c) Palmerston North City Council Waste Water Treatment Plant and (b) biosolids lagoon where samples were collected and (d) stored after metal spiking

2.2.2 Chemical analysis

The second subsample from each collection was used for chemical analysis. Immediately after collection, pH and Eh were measured on a portion of this subsample using a Eutech Instruments CyberScan pH 310 meter fitted with a Ag/AgCl ORP reference electrode and a Pt electrode. A 255 mV reference standard (quinhydrone) was used to check the calibration of the ORP electrode and buffers at pH 5 and 8 were used to check the calibration of the pH electrode. The remaining portion of the subsample was divided equally into three tubes and centrifuged at 11953 g and 4°C for 30 min. The liquid phase was separated by filtration through a 0.45-μm millipore filter and stored at 4°C for further analysis. The solid portion was dried at 105°C overnight, weighed, and ring-ground to pass through a 250-μm sieve. Total Cu and Zn concentrations in the ground sample were determined using a wet digestion method described by Kovacs *et al.* (2000).

Dried biosolids sub samples (1 g) were initially digested with 5 mL 65% HNO_3 at 60°C for 30 minutes, and then digested with 5 mL 30% H_2O_2 at 120°C for 270 minutes. After digestion, the total solution was made up to 50 mL and then filtered through '640 W' filter paper.

Metal fractionation was conducted using a sequential extraction technique described by McLaren and Clucas (2001) and Tessier *et al.* (1979), as follows;

Soluble and exchangeable fraction (i): Dried biosolids sub samples (1 g) were extracted with 8 mL of 1 M $\text{Mg}(\text{NO}_3)_2$ at room temperature for 1 h with continuous 'end over end rotator' agitation.

Specifically sorbed fraction (ii): The residue from (i) was leached at room temperature with 8 mL of 1 M sodium acetate trihydrate (NaOAc) adjusted to pH 5 with acetic acid (HOAc). Continuous agitation with 'end over end rotator' was maintained for 5 hours.

Oxide bound fraction (iii): The residue from (ii) was extracted with 20 mL of 0.4 M hydroxylammonium chloride ($\text{NH}_2\text{OH}-\text{HCl}$) in 25% (v/v) HOAc measured pH 2. This was performed at 96°C for 6 hours with occasional agitation.

Organic bound fraction (iv): The residue from (iii) was extracted with 8 mL of 30% H_2O_2 adjusted to pH 2 with HNO_3 , and the mixture was heated to 85°C for 2 hours with occasional agitation. A second 3 mL aliquot of 30% H_2O_2 (pH 2 with HNO_3) was then added and the sample was heated at 85°C for 3 h with intermittent agitation.

Residual fraction (v): The residue from (iv) was digested using a wet digestion method (Kovács *et al.* 2000) according to the procedure described below for total metal analysis.

The "selective" extractions were conducted in centrifuge tubes (polypropylene, 50 mL) to minimize losses of solid material. Between each successive extraction, separation was done by centrifuging (Sorvall, Model RC2-B) at 10 000 rpm (11

953 g) for 30 min at 4⁰C. The supernatant was filtered through a 0.45 µm millipore filter and analysed for trace metals (see below). The residue was washed with 8 mL of deionised water, centrifugation for 30 minutes, the second supernatant was discarded. The volume of rinse water used was kept to a minimum to avoid excessive solubilisation of solid material.

The Cu concentration in each extract (both total and sequential) was determined by graphite furnace atomic absorption spectrometry (GFAAS) and the Zn concentration by flame atomic absorption spectrometry (FAAS).

In the biosolids liquid phase, the total Cu concentration was determined by GFAAS; Zn, Mg, Na, K and Ca concentrations were determined by FAAS. The liquid phase SO_4^{2-} and Cl^- concentrations were determined by ion chromatography, NO_3^- and NH_4^+ by an automated analysis (Kamphake *et al.* 1967) and dissolved organic carbon was measured on a Shimadzu TOC-5000 analyser (Wu *et al.* 1990). The speciation of Cu and Zn in the liquid phase of the biosolids was calculated using the Windermere Humic Aqueous Model (WHAM) (Centre for Ecology and Hydrology 2002), by inputting into the model the liquid phase cations (Ca, Mg, K, Na, NH_4^+), anions (SO_4^{2-} , Cl^- , NO_3^-), and dissolved organic carbon concentrations, and pH.

Biological oxygen demand (BOD) was determined in biosolids samples collected from the cans at 117 days after metal spiking using BODTrakTM ((BODTrakTM 1997). This measurement was made to investigate the degree of reducing conditions in the samples. Approximately 95 mL of fresh biosolids sample from each can was poured into BODTrack sample bottles. The bottles were then connected to the instrument's pressure sensors with an appropriate measurement range, and incubated at 28⁰C for 5 days. As microorganisms consume oxygen in the sample, the pressure in the bottle headspace drops, and this pressure change correlates directly to BOD. During the incubation a constant stirring was supplied with a magnetic stirrer to supply additional oxygen to the sample and to provide microorganisms with greater exposure to food. In addition, a few crystals of lithium hydroxide in the seal cup of each sample bottle were added to avoid the pressure created by CO₂ produced during oxidation of organic matter. This

pressure must be removed from the system to ensure that the pressure difference observed is proportional only to the amount of oxygen used. The BODTrack™ apparatus has a large graphic display that continuously updates results. The apparatus automatically ends the test and stores the results after the chosen test length.

2.2.3 Dehydrogenase activity

Dehydrogenase activity in this work was used as a measure of biological activity of the biosolids suspensions. Dehydrogenases are enzymes of respiration pathways of aerobic as well as of anaerobic microorganisms. The method of Chander and Brookes (1991) was adopted where 5 g biosolids suspension was mixed with 3 ml of 3% 2, 3, 5–triphenyltetra–zolium chloride (TTC) and 0.1 g CaCO_3 and incubated for 24 hr at 28°C. The triphenylformazan (TPF) formed in the reaction was extracted with methanol and its concentration quantified by measuring the absorbance in a spectrophotometer at 485 nm (DU– 640; Beckman, Krefeld, Germany). Biological activity is proportional to the intensity of absorbance.

2.2.4 Estimation of microbial population

A total microbial population count study was conducted by using the serial dilution plate method (Parkinson *et al.* 1971). Yeast extract agar and peptone–glucose–acid–agar medium were used to develop bacteria and fungi colonies respectively. These media, which are rich in nutrients, permit the recovery of a wide spectrum of bacteria, yeast and molds. The yeast extract agar was prepared by adding 15 g of agar, 1.0g of dextrose, 0.5 g of KNO_3 , 0.2 g of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.1 g of CaCl_2 , 0.1 g of NaCl , 0.01 g of FeCl_3 , 1.0 g of yeast extract, and 1 L of water into a sterilized Schott bottle which was subsequently autoclaved for 15 minutes at 121°C. Then, 1.0g of $\text{K}_2 \text{HPO}_4$ was added for each 1 L of medium and the pH was adjusted to 6.8 with HCl . Peptone–glucose– acid–agar medium was prepared by adding 1 g of KH_2PO_4 , 0.5 g of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 5 g of peptone, 10 g of glucose, 20 g of agar and 1 L of deionised water into a sterilized Schott bottle

which was subsequently autoclaved for 15 minutes at 121°C. Then, 10 ml of 0.5 N H₂SO₄ was added for each 1 L of medium.

A 10⁻¹ dilution was prepared by adding 10 ml of biosolids to 90 mL of sterile, deionised, distilled water. Decimal dilutions through 10⁻⁷ were prepared from the 10⁻¹ dilution (Dietrich and Lamar 1990). Three plates each were prepared on selective media at dilutions of 10⁻⁵, 10⁻⁶, and 10⁻⁷ for bacteria and 10⁻⁴, 10⁻⁵, and 10⁻⁶ for fungi, and then incubated in the dark at 28°C for 5 days.

2.2.5 Quality control measures

All chemicals used in the experiments were of analytical grade. Deionised water was used in preparing stock solutions and in each step of the leaching procedure. All glassware used for the experiments was previously soaked in 0.1 M HCl overnight, and rinsed with deionised water. The limit of detection for Cu in the GFAAS was 0.002 mg/L and for Zn in the FAAS was 0.2 mg/L. Accuracy of the measurements was assessed by analysing, in parallel with unknown samples, certified reference materials. For total metal analyses a river sediment US NBS–SRM–1645 (total Cu 109 mg/kg, Zn 1720 mg/kg) and a sewage sludge BCR CRM 145R (Cu 696 mg/kg, Zn 2122 mg/kg) from the commission of the European Community were used as standard reference materials. The mean Cu concentrations found for each of the reference materials were 95±4 mg/kg for the NBS material and 691±20 mg/kg for the BCR material. The corresponding mean Zn concentrations in the two reference materials were 1758±37 mg/kg and 2203±15 mg/kg, respectively. For metal fractionation, internal standards (Hort 1 and 2) were used in parallel with unknowns and the metal fraction concentrations obtained at each analysis were found to be within 90–105% of the mean values. Blanks were also analysed in parallel with unknowns and the concentrations obtained (<0.2–1.0% of the sample concentrations) were subtracted from the unknown sample values.

2.2.6 Data analysis

All results except for BOD and liquid phase solution ion speciation were presented as mean±standard error (n=3). Insufficient samples for chemical analysis precluded replications for BOD and ion speciation. Analysis of variance was conducted using the SAS[®] 9.1.2 (SAS Institute Inc 2004) statistical software package to determine whether there were significant differences (least significant difference at 5% probability level) in total metal concentrations and metal fractions between the times of biosolids incubation.

The dehydrogenase activity in the biosolids suspension was correlated with the metal concentration in the various solid phase fractions, and metal species concentration in the solution phase. To normalise the effect of incubation time, the data were transformed by expressing the values obtained for metal-amended biosolids as a percentage of the mean value for the unamended treatment. The data was analysed using SigmaPlot 10 (2006), a curve-fitting software package that can produce sigmoidal dose response curves. The equations used for the curve fitting were:

$$y = \frac{a}{1 + e^{-\left(\frac{x-c}{b}\right)}}$$

where y is the dehydrogenase activity; x is the log to the base 10 of the measured metal concentration; and a,b,c are constants.

and

$$y = d + \frac{a}{(x/b)^c}$$

where y is the dehydrogenase activity; x is the measured metal concentration ; and a, b, c, d are constants

2.3 Results and discussion

2.3.1 pH, Eh and BOD

Increased rates of Cu and Zn addition decreased the biosolids pH at all four times of sampling (Table 2.1). The decrease in pH is probably due to the metals chemically adsorbing to the organic and inorganic constituents in the biosolids, resulting in a release of protons (McBride 1994). McLaren and Clucas (2001) also reported that 6 months after application of Zn the pH of biosolids decreased. Even though a lower rate of Cu was added to the biosolids than Zn, the pH reduction was much higher for Cu than for Zn. This is probably due to a higher rate of Cu adsorption to biosolids than Zn (see Table 2.3).

Biosolids pH increased with time of incubation for the Cu treatments. This trend is due to the increased reducing conditions in the biosolids. It is known that inducing anaerobic conditions on acid soils leads to an increase in pH towards neutrality (McBride 1994) as observed when acidic paddy soils are flooded (Sun *et al.* 2007). A similar pH trend with time was not observed for Zn, probably because Zn concentrations in the biosolids were much higher than Cu concentrations (Table 2.3) resulting in toxicity to the microorganisms that cause the reduction, or perhaps to the fact that certain critical groups of microorganisms are more sensitive to Zn toxicity.

Increased rates of addition of Cu and Zn increased Eh (Table 2.1), probably because the metals at high concentrations reduced the activity of microorganisms, which resulted in reduced oxygen consumption. This is supported by the decrease in BOD observed as the metal concentration increased (Table 2.1). In any solid/liquid suspension, Eh is expected to be negatively related to pH (McBride 1994). Therefore, decrease in pH could be another reason for the increase in Eh with metal concentration. Although Eh increased with metal additions, the biosolids remained in the anaerobic range ($Eh < 350$ mV) (McBride 1994).

Table 2.1 pH, Eh and BOD (mean±s.e., n=3) of the biosolids

| Treatments | pH | | | | Eh (mV) | | | | BOD (mg /L) |
|------------|-----------|-----------|-----------|-----------|----------|----------|----------|-----------|-------------|
| Days | 0 | 25 | 55 | 117 | 0 | 25 | 55 | 117 | 117 |
| Control | 6.6 ± 0.1 | 6.6 ± 0.2 | 6.7 ± 0.2 | 7.9 ± 0.2 | −65 ± 5 | −62 ± 6 | −99 ± 7 | −124 ± 11 | 10550 |
| Cu1 | 6.2 ± 0.1 | 6.6 ± 0.2 | 6.7 ± 0.2 | 6.8 ± 0.2 | 213 ± 14 | −22 ± 2 | −89 ± 3 | −72 ± 3 | 10240 |
| Cu2 | 5.7 ± 0.1 | 6.3 ± 0.2 | 6.2 ± 0.1 | 6.6 ± 0.2 | 285 ± 16 | 102 ± 8 | −16 ± 1 | −99 ± 4 | 8200 |
| Cu3 | 4.8 ± 0.1 | 5.8 ± 0.1 | 5.9 ± 0.1 | 5.7 ± 0.1 | 323 ± 21 | 270 ± 13 | 215 ± 10 | 12 ± 1 | 1220 |
| Zn1 | 6.1 ± 0.2 | 6.3 ± 0.2 | 6.3 ± 0.1 | 6.3 ± 0.2 | 246 ± 14 | 66 ± 4 | 13 ± 1 | −39 ± 2 | 10320 |
| Zn2 | 5.9 ± 0.2 | 5.9 ± 0.1 | 6.0 ± 0.1 | 5.8 ± 0.1 | 260 ± 10 | 125 ± 10 | 76 ± 4 | 35 ± 2 | 1500 |
| Zn3 | 5.6 ± 0.1 | 5.8 ± 0.1 | 5.8 ± 0.1 | 5.7 ± 0.1 | 272 ± 14 | 134 ± 12 | 97 ± 3 | 52 ± 3 | 840 |

2.3.2 Metal fractionation in biosolids solid phase

The biosolids in the different cans consisted of 12–21% solids on a dry (105°C) weight basis. The total concentration of metals in the biosolids solid phase increased with the increasing rate of metals (Table 2.2). For each rate of metal addition there were statistically significant differences in metal concentrations between 0 and 25 days but not beyond 25 days. The difference in concentrations between 0 and 25 days is probably due to inadequate mixing of metal salts at time zero.

Table 2.2 Metal concentrations (mean \pm s.e., n=3) in solid biosolids

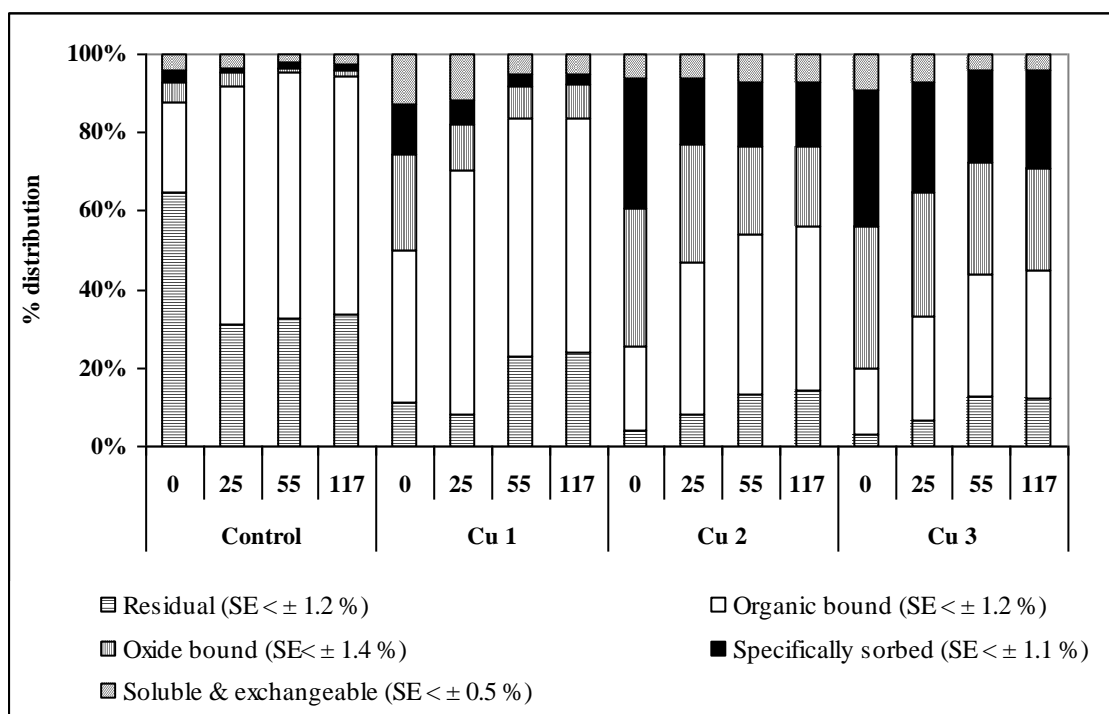
| Treatments | Cu (mg/kg) | | | |
|------------|------------------|-----------------|-----------------|-----------------|
| Days | 0 | 25 | 55 | 117 |
| Control | 86 \pm 14 | 146 \pm 19 | 148 \pm 1 | 143 \pm 2 |
| Cu1 | 3809 \pm 39 | 3957 \pm 87 | 3725 \pm 13 | 4048 \pm 35 |
| Cu2 | 8676 \pm 907 | 6620 \pm 502 | 6208 \pm 278 | 6371 \pm 262 |
| Cu3 | 12192 \pm 555 | 11554 \pm 621 | 11208 \pm 190 | 11477 \pm 296 |
| Treatments | Zn (mg/kg) | | | |
| Days | 0 | 25 | 55 | 117 |
| Control | 366 \pm 20 | 418 \pm 18 | 460 \pm 5 | 471 \pm 8 |
| Zn1 | 7821 \pm 977 | 5152 \pm 65 | 5347 \pm 106 | 5555 \pm 63 |
| Zn2 | 14666 \pm 184 | 14848 \pm 468 | 14892 \pm 189 | 15110 \pm 172 |
| Zn3 | 22378 \pm 1837 | 18500 \pm 827 | 18833 \pm 351 | 18629 \pm 441 |

The percentage distribution of Cu and Zn between the various solid phase fractions in both spiked and unspiked samples are shown in Figure 2.2. The sum total of all Cu and Zn fractions were equal to the total Cu and Zn concentrations, determined by acid digestion (Figure 2.3), indicating that the metal fractionation procedure fully recovered the metals in the biosolids. The fractionation data indicates that Cu in the unamended-biosolids (control) was primarily associated with the organic and residual fractions. Together these two fractions accounted for

85 to 95% of the total metal concentration in the control sample. However, with increased rate of Cu addition, these percentages decreased, probably because at high metal concentration the metal sorption sites of the organic fraction of the biosolids matrix were increasingly saturated, leaving fewer sites for sorption of additional Cu. Another possible reason for the decrease in Cu associated with the organic and residual fractions, with increasing Cu addition, is the decrease in pH. The decrease in pH would have reduced the adsorption capacity of biosolids (McBride 1994), and increased dissolution of minerals, complexes and co-precipitates containing Cu, (Oliver *et al.* 2004) leading to increased Cu concentrations in solution. Thus additional Cu was incorporated into the oxide and specifically adsorbed fractions, resulting in an increase in the concentration of these fractions at high rates of Cu addition. The percentage of Cu in the soluble and exchangeable fraction was low and ranged from 2 to 11% for control as well as spiked biosolids. This is consistent with the results of McLaren and Clucas (2001) who observed that less than 8% of the total Cu was recovered in the soluble and exchangeable fraction in their Cu-spiked treatments. The percentage of Cu in the organic and residual fractions increased with time due to an increased degree of metal incorporation into these components. The data, however, show that by day 55 the incorporation of Cu into the biosolids matrix was nearly complete. There were significant differences in the percentages of most of the metal fractions between the different sampling times up to day 55, but all fractions remained the same after 55 days. Analysis of variance showed that there were no significant differences in the percentages of Cu distributed among the five fractions between 55 and 117 days for all rates of Cu additions.

The relative distribution of Zn in the solid phase differed markedly from that of Cu (Figure 2.2). Unlike Cu, only a very small proportion of Zn was associated with organic matter. This is because Cu has a higher affinity for organic matter than Zn (McBride 1994). Zinc was associated mostly with the oxide (35–65 %), specifically bound (25–30%), and exchangeable (10–40%) fractions as also observed by McLaren and Clucas (2001).

(a)



(b)

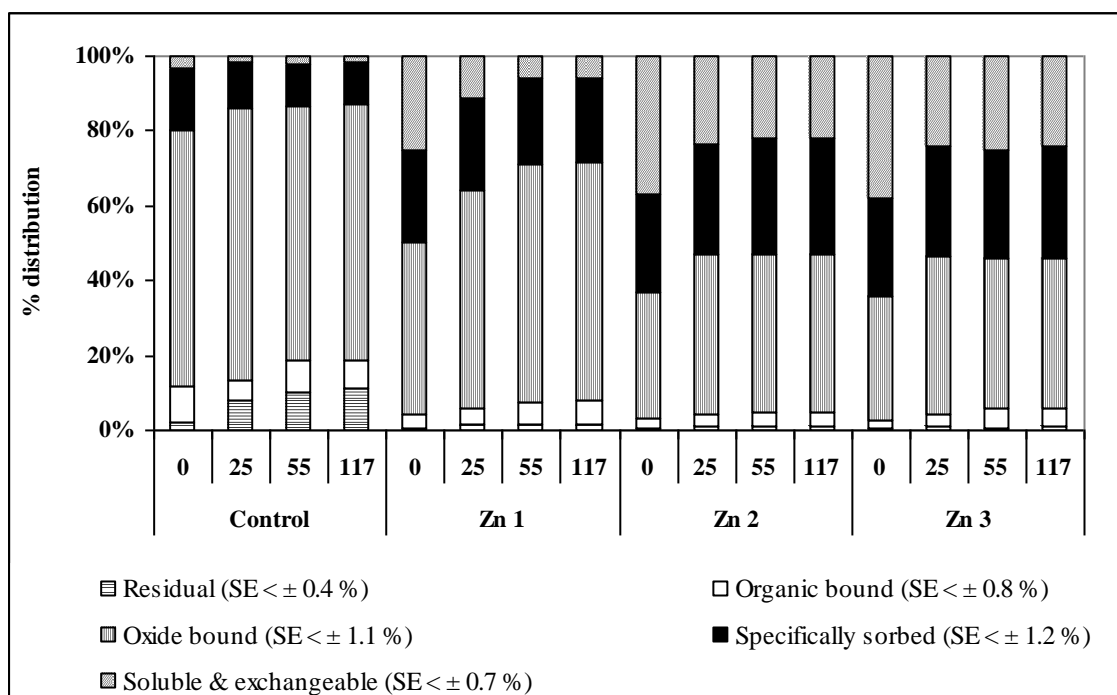


Figure 2.2 Percentage distribution of Cu fractions (a) and Zn fractions (b) in the solid phase of biosolids (SE=standard error for individual fractions, n=3)

The percentage of Zn associated with the oxide fraction decreased, and that with the soluble and exchangeable fraction increased, with the rate of Zn addition. At

high Zn concentrations the sorbing sites likely become saturated, leaving fewer sites available for further sorption, thereby a larger proportion of added Zn remains in the soluble and exchangeable fraction. The percentage of Zn associated with the oxide bound fraction increased from zero to 25 days while that with the soluble and exchangeable fraction decreased. Between day 25 and 117 Zn associated with these fractions was approximately the same for all Zn concentrations. These results show that Zn incorporation into the biosolids matrix, as observed for Cu, was nearly complete by day 55. Therefore in research practice, where biosolids are spiked with metals to simulate biosolids that are naturally contaminated with metals, a shorter period of equilibrium (2 months) than previously used (6–9 months) will be sufficient to ensure a high degree of incorporation of metals into the biosolids matrix.

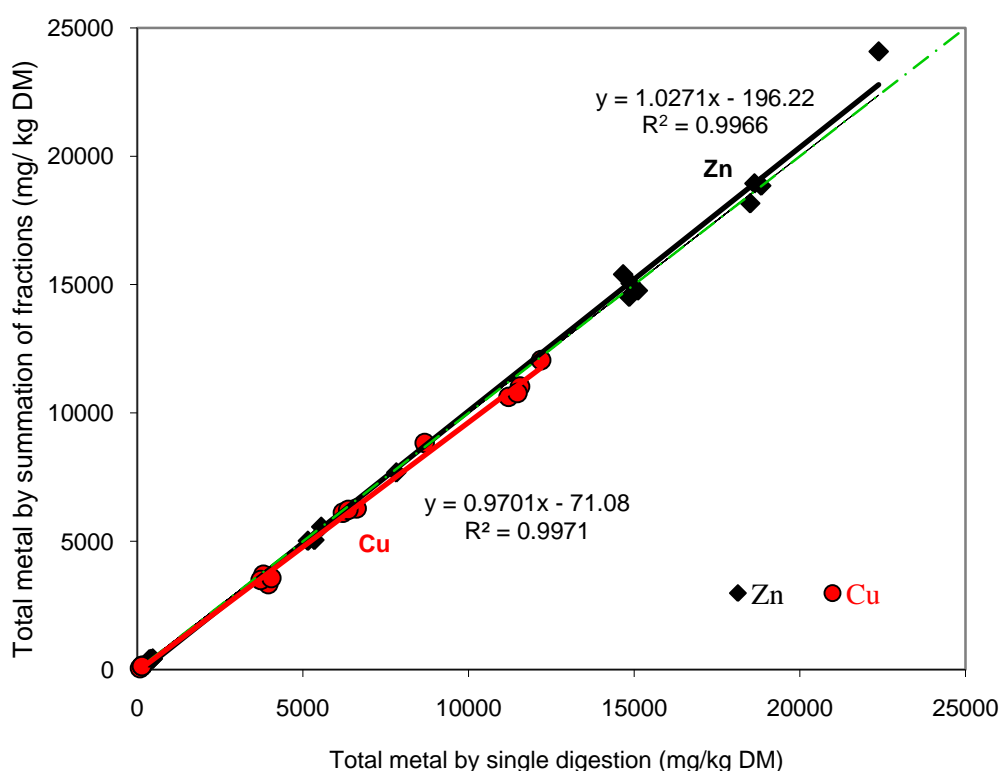


Figure 2.3 Relationship between the sum of all metal fraction concentrations and the total metal concentration determined by acid digestion

2.3.3 Metal speciation in the biosolids liquid phase

Table 2.3 Metals in liquid phase (0.45 μm) as a percentage of total metals (mean \pm s.e., n=3) in biosolids suspension

| Treatments | | % Cu | | | |
|------------|-----------------|------------------|------------------|------------------|--|
| Days | 0 | 25 | 55 | 117 | |
| Control | 0.25 \pm 0.01 | 0.07 \pm 0.01 | 0.03 \pm 0.004 | 0.04 \pm 0.005 | |
| Cu1 | 0.18 \pm 0.01 | 0.01 \pm 0.001 | 0.01 \pm 0.001 | 0.01 \pm 0.001 | |
| Cu2 | 0.82 \pm 0.04 | 0.05 \pm 0.01 | 0.03 \pm 0.002 | 0.02 \pm 0.001 | |
| Cu3 | 12.61 \pm 1.2 | 0.28 \pm 0.02 | 0.16 \pm 0.01 | 0.14 \pm 0.01 | |
| Treatments | | % Zn | | | |
| Days | 0 | 25 | 55 | 117 | |
| Control | 0.01 \pm 0.0 | 0.01 \pm 0.0 | 0.01 \pm 0.0 | 0 \pm 0.0 | |
| Zn1 | 6.60 \pm 0.6 | 0.89 \pm 0.04 | 0.11 \pm 0.01 | 0.07 \pm 0.01 | |
| Zn2 | 25.47 \pm 1.5 | 23.28 \pm 1.1 | 26.12 \pm 1.3 | 25.43 \pm 1.2 | |
| Zn3 | 36.82 \pm 1.8 | 34.98 \pm 1.6 | 36.94 \pm 1.8 | 36.62 \pm 1.7 | |

Table 2.3 shows the relative proportion of metal in solution as a percentage of the total metal content in biosolids at the four sampling times. The percentage of metal in solution increased with rates of metal additions, but decreased with time. The proportion of Cu in solution was much lower (0.01–13%) than that of Zn (0.07–37%). This is consistent with the solid phase fractionation results, which showed a higher proportion of Zn than Cu in the exchangeable and soluble fraction. The lower proportion of Cu in solution is due to the stronger sorption of Cu compared with Zn onto the biosolids solid phase fraction. For all rates of addition of Cu, the percentage of Cu in the liquid phase of biosolids significantly reduced from day zero to 25 but remained nearly constant thereafter. For the Zn1 treatment the percentage of Zn in the liquid phase also significantly reduced from day zero to 25 and remained constant thereafter. However, for the Zn2 and Zn3 treatments the percentage of Zn at each sampling time was constant. This data confirms the conclusion made for the solid phase fractionation data (Figure 2.2),

that an equilibration period of 55 days is sufficient to achieve almost complete incorporation of metals into the biosolids matrix.

Metal speciation in the liquid phase at different times after metal spiking, as determined by the WHAM software, is presented in Table 2.4. The data show that almost all Cu was predicted to be complexed with organic matter at the two lower rates of Cu addition (Cu1, Cu2). At the highest rate (Cu3) 93% of Cu was complexed with organic matter. Zinc in the solution phase was mainly present as Zn^{2+} (21–49%) and ZnSO_4 (17–59%). Zinc complexed to dissolved organic matter was 2–3% at the two highest levels of Zn addition, but 33–62% at the first level of addition. This lower affinity of Zn to organic matter related to Cu is consistent with the observations made for the distribution of the two metals in the solid phase fraction (Figure 2.2). The high percentage of ZnSO_4 species observed in solution is due to the use of sulphate salts of the metals in the spiking of biosolids.

Table 2.4 Percentage of metal species in the liquid phase of biosolids at different times after metal spiking^A

| Treatment | Cu^{2+} | | | CuSO_4 | | | Organic Cu | | |
|-----------|------------------|------|------|-----------------|------|------|------------|-----|-----|
| Days | 25 | 55 | 117 | 25 | 55 | 117 | 25 | 55 | 117 |
| Control | 0 | 0 | 0 | 0 | 0 | 0 | 100 | 100 | 100 |
| Cu1 | 0 | 0 | 0 | 0 | 0 | 0 | 100 | 100 | 100 |
| Cu2 | 0.07 | 0.01 | 0.01 | 0.04 | 0.01 | 0.01 | 100 | 100 | 100 |
| Cu3 | 10.52 | 3.95 | 3.60 | 7.74 | 3.11 | 2.83 | 81 | 93 | 93 |
| Treatment | Zn^{2+} | | | ZnSO_4 | | | Organic Zn | | |
| Days | 25 | 55 | 117 | 25 | 55 | 117 | 25 | 55 | 117 |
| Control | 0 | 0 | 0 | 0 | 0 | 0 | 100 | 100 | 100 |
| Zn1 | 38.3 | 22.9 | 21.1 | 28.8 | 18.0 | 16.5 | 33 | 59 | 62 |
| Zn2 | 48.7 | 45.7 | 45.5 | 48.5 | 51.4 | 51.0 | 3 | 3 | 3 |
| Zn3 | 48.8 | 39.0 | 38.9 | 49.3 | 58.6 | 58.8 | 2 | 2 | 2 |

^AMetal carbonates and chlorides are not shown as their concentrations are <0.1% of total metal. However, these species were included in the calculations of total metal percentage.

2.3.4 Total microbial population

There were no significant differences observed in bacterial population counts with increase in metal concentrations except for the first sampling conducted on the day 25, where the metals at the higher concentrations (at Cu2 for Cu but for Zn it was Zn3) significantly decreased the number of colonies (Figure 2.4). However at these higher concentrations, the bacterial population had increased again on day 55. Between day 55 and 117 bacterial population was approximately the same for all Cu and Zn levels and less than the population at control. It appeared that the metal amendment had a strong effect on the size of the microbial communities early in the incubation as a large part of the original bacterial community was not metal resistant. But, with time, the metal resistant bacteria species may have increased in population and this may be the reason for the absence of any effect of high rates of metal addition (Baath *et al.* 1998; Kelly *et al.* 1999).

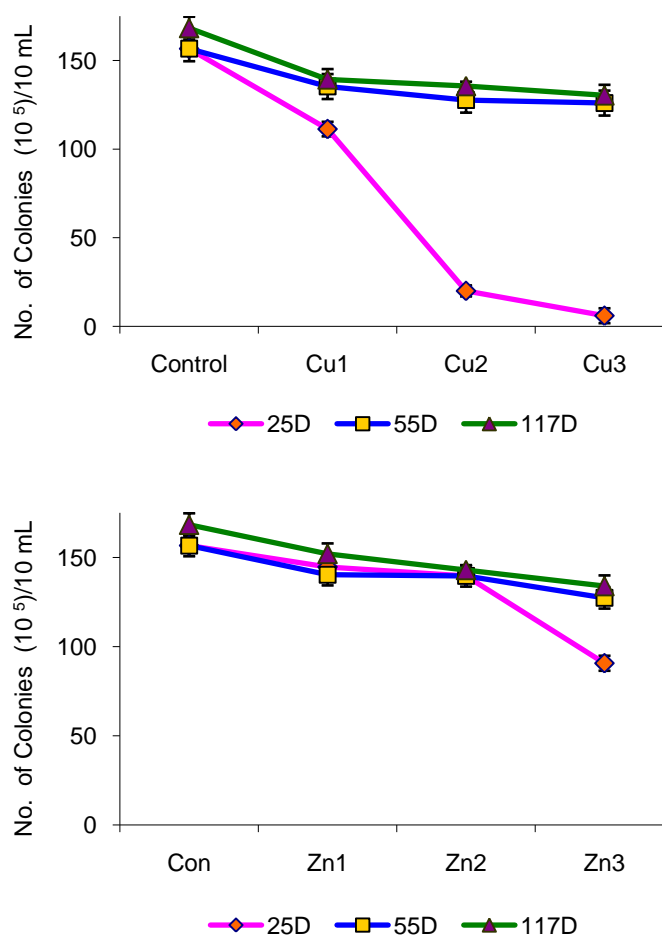


Figure 2.4 Bacteria counts in metal spiked biosolids (Solution + Solid phases)

Kelly *et al.* (1999) setup a laboratory experiment using a soil microcosm (anaerobic) amended with 6538 mg Zn/kg for 420 days and reported that at day 15 day of incubation, Zn amendment resulted in an 87% decrease in bacterial counts. By day 45, the bacterial counts had recovered and the population remained equivalent to the control microcosms for the rest of the experiment.

Fungal populations remained constant irrespective of metal levels and their counts were very small throughout the experiment (data not shown).

2.3.5 Dehydrogenase activity

Unlike the microbial population, dehydrogenase activity was significantly reduced by Cu and Zn additions at all three times of sampling (Figure 2.5). In the Cu treated biosolids the dehydrogenase activity reduction was significant up to Cu3 level except for 25th day. In contrast, for the Zn treated biosolids the greatest reduction was at the Zn2 level. This result is consistent with a dependence of Eh on microbial activity as discussed under section 2.3.1. The increased rates of addition of Cu and Zn increased Eh, probably because the metals at high concentrations reduced the activity of microorganisms. The time of sampling had a small or inconsistent effect on the dehydrogenase activity. Melo *et al.* (2007) reported that cadmium addition to sewage sludge reduced dehydrogenase activity and the degree of this effect varied at different times of incubation of the cadmium treated sludge. Because the time of sampling had an inconsistent effect on the absolute value of the dehydrogenase activity, the values were normalised by expressing the data as a percentage of the relevant control (no metal addition) value, so that all data for each metal could be analysed together, as for the method used by Speir *et al.* (2007). The normalised data were correlated with various solid and liquid phase metal concentrations. The liquid phase total metal concentration and solid phase soluble+exchangeable metal concentration showed the highest correlation. The dehydrogenase activity was therefore plotted against these parameters (Figure 2.6).

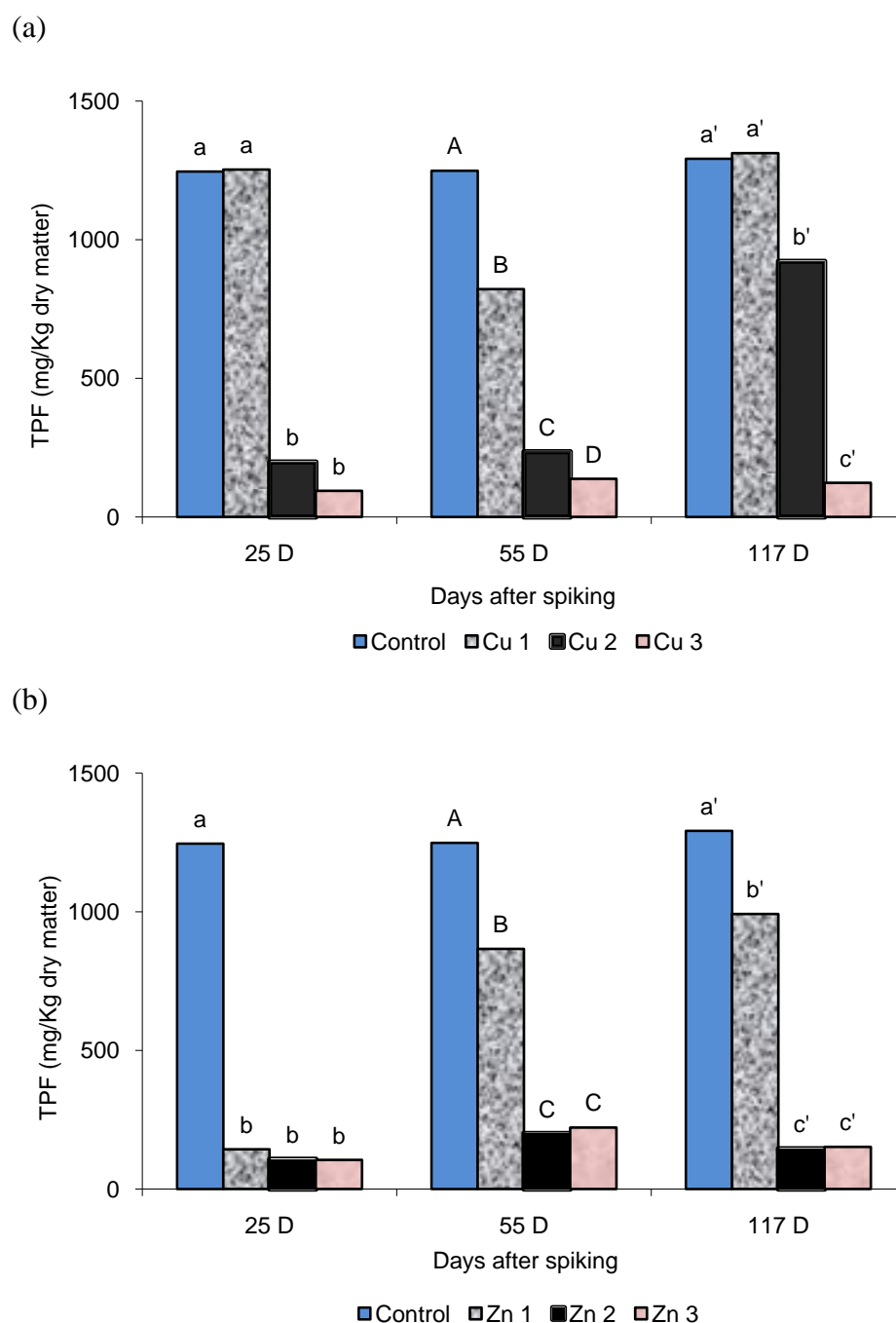


Figure 2.5 Dehydrogenase activity in biosolids spiked at different levels of Cu (a) and Zn (b) for different time intervals. Means Bars with different letters are significantly different ($p \leq 0.05$)

The study of Kelly *et al.* (1999) discussed in the previous section also found that the dehydrogenase activity of the Zn treated microcosm remained 93% lower than the controls throughout the experiment. Similar results were reported by Kozdroj (1995), in a laboratory study on the effect of Cu application to the soil at the rate of 5000 mg Cu/kg on bacterial population and activity. They reported a non-

significant effect of Cu addition on microbial plate count numbers but significant reduction in microbial activity, measured as arginine ammonification.

The concentration of metal in the soluble + exchangeable solid phase and in the total solution phase that corresponded to a 50% reduction in dehydrogenase activity (EC_{50}) was determined (calculated by assuming that dehydrogenase activity for the control treatment was 100%). The EC_{50} values for total solution phase Cu and Zn were 0.1 and 20 mg/L respectively, and for solid phase exchangeable Cu and Zn were 410 and 670 mg/kg respectively. The relationship between dehydrogenase activity and exchangeable Cu concentration was not as good as the other relationships described in Figure 2.6. An improved mathematical model is required to better explain this set of data and to calculate more accurately the EC_{50} value. The lower EC_{50} values for Cu compared with Zn indicate that Cu is more toxic to microorganisms than Zn on a unit concentration basis, as reported by others. Horswell *et al.* (2006) indicated in their bacterial and fungal bioluminescence studies on sewage sludge, that the EC_{50} value for water soluble Cu and Zn was 0.12 and 1.3 mg/L respectively. Speir *et al.* (2007) reported that the EC_{20} value for total soil Cu on sulphatase enzyme activity in biosolids treated soils was 140 mg /kg and the corresponding value for total soil Zn was 310 mg/kg.

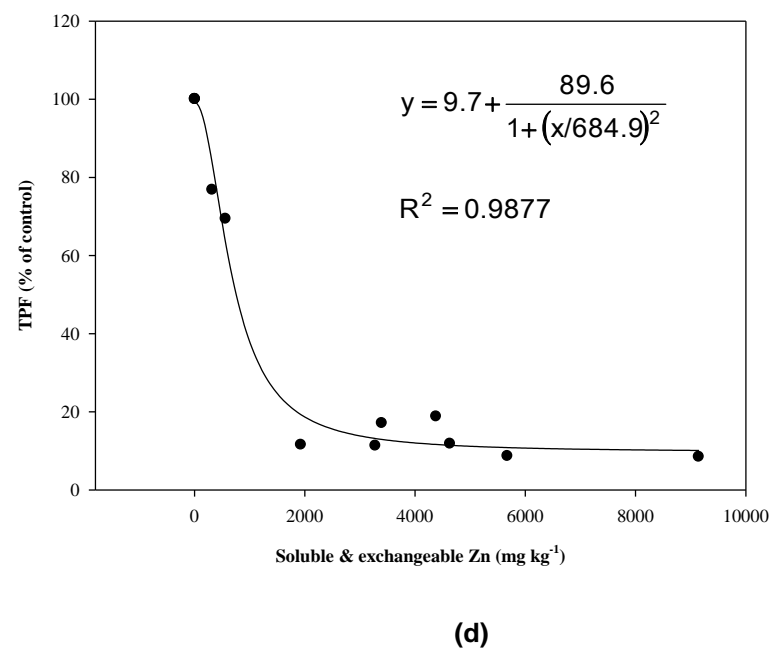
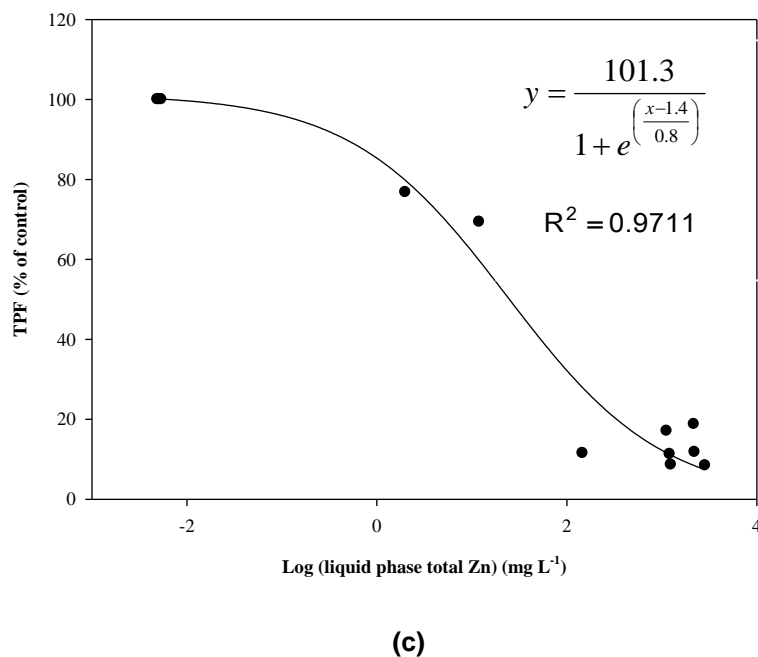
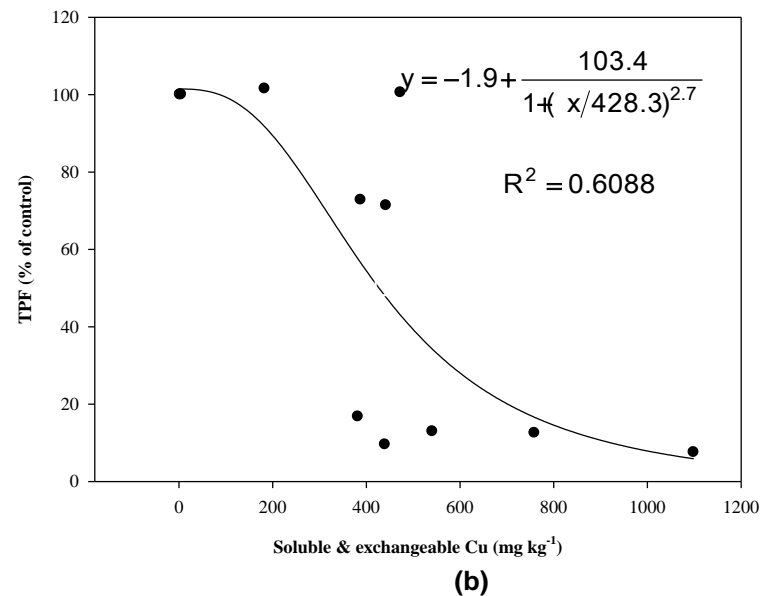
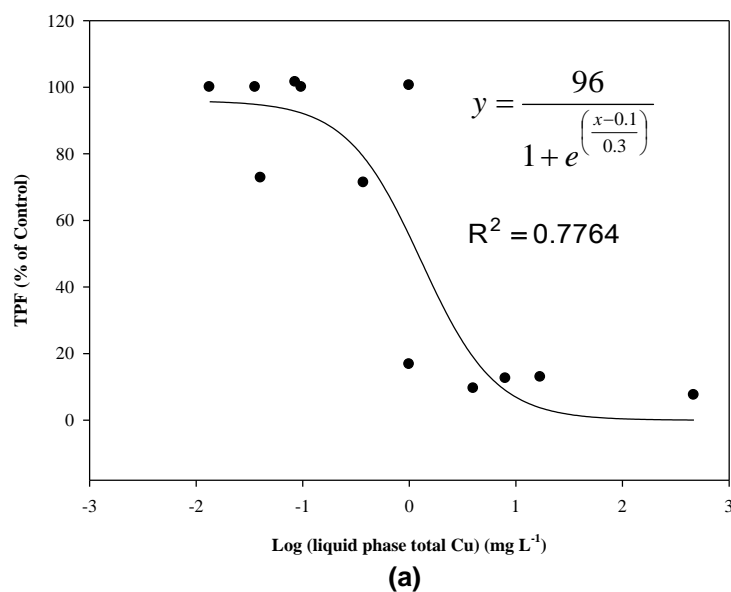


Figure 2.6 Relationship of dehydrogenase activity (TPF) with liquid phase Cu (a), exchangeable Cu (b), liquid phase Zn (c) and exchangeable Zn (d)

2.4 Conclusions

The relative concentration of Cu and Zn determined through the periodic fractionation of the solid phase and the speciation of the liquid phase of biosolids spiked with Cu and Zn salts, indicates that the metals were increasingly incorporated into the biosolids matrix. Fifty-five days after spiking of the biosolids the distribution of Cu and Zn into the various biosolids chemical fractions seems to have reached equilibrium. Very few free Cu ions (Cu^{2+}) in the liquid phase, and a very low proportion of Cu in the exchangeable fraction of solid phase, were detected. The majority of the Cu in the solid phase was found in the organic and residual fractions, while in the liquid phase, almost all Cu was organically bound. Zinc was associated mostly with the oxide, specifically bound, and exchangeable fractions. The proportion of Zn associated with organic matter in both the solid and solution phase was small.

The fractionation data for both metals in the solid phase showed that the metal incorporation into the biosolid matrix appears to be complete by day 55. The percentage of metal in the solution phase increased with the increased rate of metal additions but decreased up to 25 days and remained nearly constant thereafter. Therefore in research practice where biosolids are spiked with metals to simulate biosolids that are naturally contaminated a shorter period of equilibration (2 months) than previously used (6–9 months) may be sufficient to ensure a high degree of incorporation of metals into biosolids matrix.

Bacterial population density was affected by Cu and Zn addition during the initial stages of incubation, but with time there was no apparent affect of the metals. Fungi population was small in number and was not affected at anytime by the metals. High levels of Cu (>3725 mg/kg) and Zn (>5347 mg/kg) in this study markedly reduced dehydrogenase activity. The dehydrogenase activity in biosolids was reduced by 50% (EC_{50}) at a total solution phase Cu concentration of 0.1 mg/L and a solid phase exchangeable Cu concentration of 410 mg/kg; the corresponding EC_{50} values for Zn were 20 mg/L and 670 mg/kg, respectively.

Chapter 3

Comparative tolerance of poplar, mycorrhiza and microbial activity to copper and zinc toxicity in a biosolids–amended soil *

3.1 Introduction

In Chapter 2 the bioavailability of Cu and Zn in biosolids and its potential impacts on microbial activity were examined. As biosolids are commonly added to agricultural and forest lands it is important to study the bioavailability of these metals when biosolids are incorporated into soil. Bioavailability, plant uptake of the metals, and metal distribution in plants are largely controlled by the dynamics of biosolids–derived heavy metals in soils (McLaughlin *et al.* 2000; Oliver *et al.* 2004), root processes (Smith and Read 1997) and the rate of translocation from root to shoot (Borghi *et al.* 2008).

The bioconcentration factor (BCF), a ratio of metal concentration in plant shoots to metal concentration in soil, is a good indicator for metal uptake, translocation and accumulation in higher plants (Dos Santos Utmazian and Wenzel 2007; Unterbrunner *et al.* 2007). The BCF varies with the metal and plant species. For example, Mapanda *et al.* (2007) reported that the BCF for Cu in leafy vegetables was 0.13 and for Zn was 1.6, whereas in willow plants the corresponding values for BCF were 0.1 and 0.8 respectively (Vervaeke *et al.* 2003). In poplar species the BCF value for Zn varies from 1.8 to 5 (Unterbrunner *et al.* 2007).

In temperate forests, ectomycorrhiza (ECM) serve as a major organ for controlling nutrient uptake by trees, and it was estimated that up to 95% of the short roots in these trees contain ECM (Smith and Read 1997). Poplar is an important temperate forest species that is considered suitable for use in the phytoremediation of heavy

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metal contaminated soil, partly because of its high rate of biomass production and its high tolerance to heavy metals (Laureysens *et al.* 2005). Poplar is also of interest because of the relatively small size of its genome (Martin *et al.* 2004) and because of its application to systems of short rotation forestry (French *et al.* 2006). Metal uptake by poplar has been reported to be related to the degree of ectomycorrhizal infection of roots (Quoreshi and Khasa 2008). However, little is known regarding the symbiotic relationship between poplar and ECM under conditions of soil contamination with heavy metals, especially with Cu and Zn. Some studies show that the ECM fungal community is diverse for poplar growing on heavy metal contaminated soils (Krpata *et al.* 2008). Sell *et al.* (2005) showed that the association of poplar roots with specific mycorrhiza species (*Paxillus involutus*) leads to a highly significant increase of Cd concentration, in particular in the leaves (2.74 mg Cd/kg dry matter). When compared to the control (non inoculated plants) this was an enhancement of nearly 100%. The mycorrhiza species also significantly enhanced the translocation of Cd from the roots to the leaves, leading to a leaf:root concentration ratio of 0.32 compared to 0.20 for the control plants.

The objective of this chapter is to evaluate the bioavailability of Cu and Zn in soils amended with biosolids containing different Cu and Zn concentrations, and the toxicity effect of these metals on the growth and metal uptake by poplar with special attention paid to ectomycorrhizal and soil microbial activity. The hypothesis that total soil metal concentrations above the currently recommended maximum permissible levels of 100 and 300 mg/kg for Cu and Zn, respectively in New Zealand (NZWWA 2003) can cause toxicity to poplar and soil microorganisms was tested.

3.2 Materials and methods

3.2.1 Trial treatments and design

A glasshouse trial was conducted using poplar (*Populus deltoides* x *yunnanensis*) in pots containing soil (13 kg air-dried soil) that was amended with biosolids previously spiked with 3 levels of Cu or Zn separately as metal sulphate salts ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ and $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$) (as explained in Chapter 2). The control treatment was soil amended with biosolids not spiked with the metals. Soil and poplar cuttings for the pot experiment were taken from the 'Tree line nursery' plantation, a division of RST Environmental Solutions, Palmerston North, New Zealand. Soil was collected at 10–30 cm depth and is classified as a Manawatu fine sandy loam of the Recent Soil group ('Dystric Fluventic Eutrudept' in US Soil Taxonomic Classification as reported by Hewitt (1998). Prior to the trial, the soil was sieved (<5 mm), air dried, and stored for 2 months. One month prior to the trial, soil for each pot was spread in a large tray and a calculated volume of spiked biosolids was poured into the soil and hand mixed to ensure uniform and thorough mixing of biosolids with the soil. Total metal concentrations (mg/kg soil) in the treatments were 12 (control), 46 (Cu1), 137 (Cu2), 226 (Cu3) for Cu; 25 (control), 141 (Zn1), 433 (Zn2), 686 (Zn3) for Zn. These metal concentration ranges cover the soil metal concentration limits (100 and 300 mg/kg for Cu and Zn, respectively) recommended by the NZWWA (2003). The 7 treatments were replicated three times and arranged in a Randomized Complete Block Design (RCBD) in a glasshouse. Poplar cuttings (20 cm length) were previously grown to form roots in black plastic bags (200 mm x 100 mm) containing a mixture of soils obtained from the same poplar plantation (to provide a source of mycorrhizae hyphae) and potting mixture in the ratio of 1:1. The cuttings with roots were transplanted, one per pot, and regularly watered to maintain soil moisture at 80% 'pot field capacity'. The glasshouse temperature was maintained at $12 \pm 2^\circ\text{C}$ minimum (night) and $22 \pm 2^\circ\text{C}$ maximum (day). After 147 days of plant growth the experiment was concluded, and plant shoots, roots and mycorrhiza and soil samples from each pot were progressively collected. The following soil

parameters were measured: soil metal fraction (soluble and exchangeable, specifically sorbed, oxide, organic and residual fractions), soil solution total metal concentration, pH, and dehydrogenase activity in rhizosphere and bulk soils. The following plant parameters were measured: poplar foliar and root dry matter (DM) yields, mycorrhizae hyphal density, and shoot and root metal concentration.

3.2.2 Plant harvest and soil sampling

At harvest, aerial biomass was first collected. The root system of each plant was then carefully removed from each pot. Care was taken not to loosen rhizosphere soils from the root ball of each poplar tree. Initially, 10 equal sized root tips were cut and separated for mycorrhiza counting. The rest of the roots were carefully and gently shaken to collect bulk soil. Rhizosphere soil attached to the roots was collected by vigorously shaking the roots inside a bag (Liu *et al.* 2008). Both the bulk and rhizosphere soils were divided into three portions. One portion was used immediately after collection for pH measurement and soil solution extraction. The second portion was placed in sterilized containers tightly sealed and stored at 4°C for dehydrogenase activity measurements. The third portion was air dried, ground to pass through a 1 mm sieve and stored for metal fractionation analysis by means of a sequential extraction procedure (McLaren and Clucas 2001). The root and leaf portions of plants were separately collected, washed separately with de-ionised water (in order to remove the adhering soil as completely as possible) and dried at 60°C until 2 consecutive weights were equal. The total dry weight of each plant portion was recorded then ground using a Cyclotech 1093 Plant Mill equipped with a stainless steel blade and stored for chemical analysis.

3.2.3 Chemical analysis

Soil pH was measured (soil:water=1:2.5 w/w) using a Eutech Instruments CyberScan pH 310 meter. Solution buffers at pH 4 and 7 were used to check the calibration of the pH electrode. Moist samples of bulk soil were divided equally into 6 double centrifuge tubes and centrifuged at 11953 g and 4°C for 30 min.

The soil solutions were collected, filtered through a 0.45 µm millipore filter, and analysed for pH and Cu, Zn, basic cation and anion concentrations. The Cu concentration was determined by graphite furnace atomic absorption spectrometry (GFAAS); Zn, Mg, Na, K and Ca concentrations were determined by flame atomic absorption spectrometry (FAAS). The liquid phase SO_4^{2-} and Cl^- concentrations were determined by ion chromatography, NO_3^- and NH_4^+ by an automated analysis technique (Tecator 1983). Dissolved organic carbon was also measured, using a Shimadzu TOC-5000 analyzer (Wu *et al.* 1990). The speciation of Cu and Zn in the soil solution was calculated using the Windermere Humic Aqueous Model (WHAM) (Centre for Ecology and Hydrology 2002) by inputting into the model the liquid phase cations (Ca^+ , Mg^+ , K^+ , Na^+ , NH_4^+), anions (SO_4^{2-} , Cl^- , NO_3^-), and dissolved organic carbon concentrations, and pH.

Total Cu and Zn concentrations in the ground soil samples were determined using a wet digestion method described by Kovacs *et al.* (2000). Metal fractionation was conducted using a sequential extraction technique described by McLaren and Clucas (2001) and Tessier *et al.* (1979) as explained in Chapter 2. The Cu concentration in each extract was determined by GFAAS and the Zn concentration by FAAS.

Dried dry plant material (0.4 g) was pre-digested with 10 mL 65% HNO_3 for overnight. After the preliminary digestion, it was heated at 150°C for 1 hour (until traces of nitric acid disappear) and then the temperature was increased to 235°C for 30 minutes or until the total volume had been minimised to approximately 1 mL. The total digested solution was made up to a volume of 50 mL with deionised water and filtered through a 0.45 µm millipore filter, and the total metal concentrations determined by GFAAS for Cu and FAAS for Zn.

3.2.4 Dehydrogenase activity

Dehydrogenase activity was used as a measure of the biological activity of soil as this assay has been reported to be a suitable measure of microbial activity in soil

(Mills *et al.* 2006; Taylor *et al.* 2002). As dehydrogenase activity is not acting independently of the parent microbial cell as extracellular enzymes in soil, the measurement of this activity is a good overall indicator of microbial activity and the oxidation of soil organic matter (Obbard 2001; Pepper *et al.* 1995). The method of Chander and Brookes (1991) was adopted as described in Chapter 2.

3.2.5 Mycorrhiza counting

Plant roots were washed with tap water and subsamples were randomly taken for the estimation of ectomycorrhizal colonization. The roots were fixed in FAA (contains ethanol (70%), formaldehyde and acetic acid at a ratio of 90:5:5 by volume) and washed thoroughly before staining. The method of Giovannetti and Mosse (1980) for the measurement of vasicular arbuscular mycorrhizal (VAM) infection in roots was used in this study. This method was successfully used for both VAM and ectomycorrhiza infection of roots by Brundrett and Abbott (1994). Root samples were cleaned in 10% KOH and autoclaved for 30 minutes, washed in water thrice and soaked in 5% HCl for 1 minute, stained overnight in Trypan Blue stain (1 L glycerol, 950 mL distilled water, 50 mL acetic acid and 0.2 g trypan blue), and cut into 1 cm pieces from the root tip. From each root sample, ten 1cm pieces were randomly selected and arranged parallel to each other on a microscope slide with a cover slip. The mycorrhizal root colonization was determined microscopically at 100× magnification. Colonization was scored when hyphae were present per cm root length.

3.2.6 Quality control measures

All chemicals used in the experiments were of analytical grade. Reference samples of biosolids and soils and blanks were used as discussed in Chapter 2, to confirm the accuracy of biosolids and soil analyses. Reference plant samples (Wageningen plant standard (2004–3, No.2) obtained from The Netherlands was used to check the accuracy of plant analysis (the analytical values obtained were 94–106% of the reported values).

3.2.7 Data analysis

Analysis of variance and was conducted using SAS[®] 9.1.2 statistical software (SAS Institute Inc 2004) to determine whether there were significant differences (least significant difference at 5% probability level) in all parameters measured among treatments.

The dehydrogenase activity in soil was correlated with the metal concentrations in the various solid phase fractions and metal species concentrations in soil solution. Mathematical relationships were obtained between dehydrogenase activity and metal concentrations using the procedure described in Chapter 2. The equations used for the curve fitting were:

$$y = \frac{a}{1 + e^{-\left(\frac{x-c}{b}\right)}} \quad (1)$$

where y is the dehydrogenase activity; x is the measured metal concentration; and a,b,c are constants.

and

$$y = \frac{(1 + ax)}{(b + cx)} \quad (2)$$

where y is the dehydrogenase activity; x is the log to the base 10 of the measured metal concentration ; and a, b, c, d are constants

3.3 Results and discussion

3.3.1 Poplar yield and metal accumulation

Despite Cu being present at a concentration more than double the maximum permissible Cu concentration in soil set by NZWWA (2003) (Cu³ total soil concentration is 226 mg/kg, the maximum permissible concentration is 100

mg/kg), the overall poplar DM yield was not detrimentally affected. Increased Cu levels increased root DM yields but had no effect on leaf DM yield (Table 3.1). The concentrations of Cu in leaves and roots for all Cu treatments were significantly increased with every successive level of Cu applied. The leaf Cu concentrations were above the critical Cu concentration of 1–5 mg/kg generally considered the limit for Cu deficiency in plants (Robson and Reuter 1981) but below the phytotoxic range of 25–40 mg/kg as defined by Chaney (1989) for most plants. Stobrawa and Lorenc-Plucinska (2008) conducted a pot trial with poplar cuttings on Cu contaminated soils in Poland to measure the tolerance of this species to heavy metals and reported that up to a total soil Cu concentration of 220 mg/kg, Cu was not toxic to poplar. However, they did not report the concentration of plant-available Cu in their soil which is considered to be a better index of Cu phytotoxicity. Our results also showed that at a total soil Cu concentration (226 mg/kg) similar to that of Stobrawa and Lorenc-Plucinska (2008), poplar did not show any toxicity to Cu.

The effect of Zn on DM was different to that of Cu. Increasing the soil Zn level decreased root DM yield with every successive level of Zn applied. Leaf DM yield also significantly decreased with increasing Zn levels but only beyond the Zn1 treatment level (total soil Zn > 141 mg/kg). The root Zn concentration was significantly increased beyond the Zn1 treatment level. The leaf Zn concentration was significantly increased up to the Zn2 level but a further increase in leaf Zn concentration was not significant at the Zn3 level. The leaves for the Zn2 and Zn3 treatments showed severe yellowing and withering. The leaf Zn concentrations for the Zn2 and Zn3 treatments were much higher than the phytotoxic range (500–1500 mg Zn/kg DM) defined by Chaney (1989) confirming Zn phytotoxicity at these levels. Leaf and root DM yield reduction for Zn treatments at Zn2 and Zn3 (≥ 433 mg/kg) and severe damage of leaves visually observed at Zn2 and Zn3, indicate Zn toxicity at high levels of Zn addition. These results are in agreement with the maximum permissible Zn concentration in soil of 300 mg/kg set by NZWWA (2003). Above this concentration Zn is likely to become toxic to plants.

Table 3.1 Effect of Cu and Zn on soil pH, soil solution metal concentration, poplar metal concentration and DM yield

| Treatments | Total soil | pH | Soil solution | | | | | | | | BCF |
|------------|---------------|-----|---------------|----------------|--------|---------------------|--|----------|--|-------------------|--------|
| | metal | | metal | Dry matter (g) | | Metal conc. (mg/kg) | | | | Metal conc. ratio | |
| | Conc. (mg/kg) | | conc. (mg/L) | | | | | | | Leaf:Root | |
| | | | | Leaf | Root | Leaf | | Root | | | |
| Cu0 | 12 | 5.9 | 0.02 | 38.5 a | 46.3 b | 9.4 d | | 7.7 d | | 1.2 a | 0.8 a |
| Cu1 | 46 | 5.7 | 0.12 | 34.3 a | 50.8 b | 11.5 c | | 34.5 c | | 0.3 b | 0.2 b |
| Cu2 | 137 | 5.5 | 0.26 | 33.5 a | 63.7 b | 18.2 b | | 93.6 b | | 0.2 b | 0.1 c |
| Cu3 | 226 | 5.3 | 0.45 | 38.7 a | 90.7 a | 38.4 a | | 194.7 a | | 0.2 b | 0.2 b |
| Zn0 | 25 | 5.9 | 0.43 | 38.5 a | 46.3 a | 169.2 c | | 69.8 c | | 2.4 b | 6.8 b |
| Zn1 | 141 | 5.4 | 2.9 | 33.1 a | 32.5 b | 1408.5 b | | 356.5 c | | 4.0 a | 10.0 a |
| Zn2 | 433 | 4.9 | 78 | 23.5 b | 1.6 c | 3417.8 a | | 2251.4 b | | 1.5 bc | 7.9 a |
| Zn3 | 686 | 4.6 | 334 | 10.8 c | 1.6 c | 3755.6 a | | 4824.3 a | | 0.8 c | 5.5 b |

Values in columns followed by different letters are significantly different ($P \leq 0.05$)

The bioconcentration factor or BCF (defined as the ratio of the metal concentration in the leaf compared to that in the soil), was much lower for Cu than for Zn (Table 3.1). The BCF value for Cu was 0.1–0.8 compared with 5.5–10.0 for Zn. The BCF is a good indicator for biomagnifications of metals from soils to plants. The lower BCF values for Cu in comparison to Zn in poplar may be due to low plant availability of Cu in soil. The leaf to root concentration ratio was also lower for Cu than for Zn (Table 3.1). This shows that translocation of Cu from root to leaf was lower than that for Zn. In general, plants show poor ability to translocate Cu to the leaves (Todeschini *et al.* 2007). In plants associated with mycorrhiza this could possibly be due to the high efficiency of mycorrhizal accumulation of Cu (Huang *et al.* 2005). In rooted cuttings of poplar clones under hydroponic conditions, Cu was found to be accumulated in the roots from 200 to 12000 mg/kg when Cu concentrations in solution increased from 0.4 to 1000 μ M, while leaf concentrations remained in the range of 6–10 mg/kg (Borghi *et al.* 2007). Hassinen *et al.* (2009) reported that the maximum Cu and Zn concentrations in hybrid aspen (*Populus tremula* x *tremuloides*) leaves were 11 mg/kg (BCF 0.06) and 2500 mg/kg (BCF 4.8) respectively after growth in a coarse silt to silty-clay soil contaminated with Cu (180 mg/kg) and Zn (520 mg/kg). They also reported that the leaf to root ratio for Cu was much lower (0.6 to 1.1) than that for Zn (10.8 to 12.1), as was observed in our trial. Similarly, Castiglione *et al.* (2009) conducted a field trial with poplar clones on a heavily Cu (1300 mg/kg) and Zn (950 mg/kg) contaminated loam soil (pH 5.0 to 7.4) and reported that the BCF value for Cu was 0.1 to 0.3 and for Zn 1 to 2.5.

3.3.2 Metal concentration in soil solution

The total Cu concentrations (mg/L) in rhizosphere soil solution were 0.02, 0.12, 0.26 and 0.45 (0.31, 1.9, 4.1 and 7.1 μ M) for the control, Cu1, Cu2 and Cu3 treatments, respectively. The total Zn concentrations (mg/L) in the rhizosphere soil solution were 0.43, 2.9, 78 and 334 (6.6, 44.3, 1193 and 5107 μ M) for the control, Zn1, Zn2, and Zn3 treatments, respectively. The proportion of the total soil metal concentration that is in solution is much higher for Zn than for Cu

(Table 3.1). This is because Zn is more weakly adsorbed to soil than Cu (McBride 1994). This proportion remains nearly constant for Cu when the total soil Cu concentration increased, but for Zn this proportion markedly increased when the total Zn concentration increased. The likely reason for this is that the decrease of soil pH as the total soil metal concentration increased was higher for Zn than for Cu (Table 3.1). In acidic conditions, especially below pH 5.5, the solubility of Zn increases rapidly as pH decreases (McBride 1994).

Heavy metal effects on plants and microorganisms are closely related to their biological availability, which in turn are controlled by the metal ion species in the soil (Kabata-Pendias 1993). Metals exist in a number of different soluble and particulate forms of which free metal activity in solution has been shown to be the key factor in determining metal bioavailability and toxicity in most circumstances (Cancès *et al.* 2003; Parker and Pedler 1997). Therefore, soil solution Cu^{2+} and Zn^{2+} concentrations are proposed in this study as better indices of the bioavailability of Cu and Zn than total soil Cu and Zn concentrations. Using this approach, the effect of treatments on the soil solution Cu^{2+} and Zn^{2+} concentrations and their relationship to DM yield was investigated.

Table 3.2 Percentage of metal species in soil solution

| Treatment | M^{2+} | MHCO_3^+ | MSO_4^0 | MCl^+ | Organic M |
|-----------|-----------------|-------------------|------------------|----------------|--------------|
| Cu0 | 0.35 (0.03) | 0.03 (0.01) | 0.01 (0.00) | 0.00 (0.00) | 99.62 (0.04) |
| Cu1 | 0.90 (0.10) | 0.05 (0.01) | 0.01 (0.00) | 0.00 (0.00) | 99.35 (0.11) |
| Cu2 | 4.24 (0.28) | 0.14 (0.02) | 0.16 (0.03) | 0.00 (0.00) | 98.03 (0.84) |
| Cu3 | 7.11 (0.29) | 0.17 (0.03) | 0.49 (0.12) | 0.00 (0.00) | 94.59 (1.24) |
| Zn0 | 76.75 (1.27) | 0.22 (0.02) | 1.49 (0.02) | 0.04 (0.00) | 23.52 (1.31) |
| Zn1 | 81.70 (0.55) | 0.05 (0.00) | 10.65 (0.42) | 0.14 (0.01) | 10.98 (0.22) |
| Zn2 | 83.12 (1.03) | 0.02 (0.00) | 15.47 (1.21) | 0.14 (0.01) | 4.11 (0.17) |
| Zn3 | 83.99 (0.84) | 0.01 (0.00) | 15.14 (0.80) | 0.17 (0.01) | 3.54 (0.05) |

Values are means \pm standard error, n=3 (in parenthesis)

Rhizosphere soil solution metal speciation using the WHAM speciation model showed that the Cu^{2+} concentrations (μM) in the soil solution were 0.0008, 0.009, 0.04 and 0.27 for the control, Cu1, Cu2 and Cu3 treatments, respectively. There appears to be no published information on the critical soil solution Cu^{2+} concentrations for Cu deficiency and toxicity in poplar. Therefore, the values reported for another tree crop are used here to compare my results. The results of Gorge *et al.* (1985) indicate that there was an increase in the shoot and root weight of pine (*Pinus radiata*) seedlings grown in solution culture as the solution Cu concentration increased from 0.03 to 0.3 μM . However, at concentrations >3 μM a decline in root and shoot growth was reported. Similarly, Arduini *et al.* (1995) reported that Maritime Pine (*Pinus pinaster* Ait.) root elongation increased at a Cu concentration of 0.1 μM compared to at 0.012 μM , and decreased at 1 μM . Both these studies were conducted in dilute solution culture. Therefore, the Cu in solution was present mainly as the Cu^{2+} species and the solution Cu concentrations can be assumed to be equal to the Cu^{2+} concentration. Applying these criteria derived from studies on pines to the soil solution Cu^{2+} concentration calculated in our study, it appears that Cu was deficient for the poplar roots in the control, Cu1 and Cu2 treatment which had soil solution Cu^{2+} concentrations of 0.0008, 0.009 and 0.04 μM , respectively. This is consistent with the significant increase in poplar root growth observed for the Cu3 treatment compared with the control, Cu1 and Cu2 treatments. However, no toxic Cu^{2+} concentration can be assessed because the plants did not show any signs of Cu toxicity. Future trials on poplar need to test higher rates of Cu addition to determine the optimum and toxic rates of Cu and Cu^{2+} concentrations in soil solution for poplar growth.

The soil solution Zn^{2+} concentrations (μM) for the control, Zn1, Zn2 and Zn3 treatments were 5, 36, 991 and 4284 respectively. Comparison of percentages of the various metal species in soil solution showed that Cu was mostly associated with organic matter (94.6–99.6%) and Zn was present mostly as Zn^{2+} (76.8–84.0%) (Table 3.2). Because plant leaf DM yield was significantly decreased for the Zn2 and Zn3 treatment levels, the critical Zn^{2+} concentration in soil solution for Zn toxicity to poplar can be assessed, and this should lie between 36 μM (Zn1)

and 991 μM (Zn_2). Assuming 10% DM yield reduction from DM yield of control plant as an indicator of Zn toxicity, the critical Zn^{2+} concentration for this reduction was calculated to be 88.5 μM (Figure 3.1). This critical Zn^{2+} concentration for Zn toxicity is much higher than the critical Cu^{2+} concentration reported in literature (1 μM , Arduini *et al.* 1995) for Cu toxicity. Similar total Cu and Zn concentrations were present in Cu2 and Zn1 treatments. However, the Cu^{2+} concentration (0.04 μM) was much lower than the Zn^{2+} concentration (36 μM) at these levels of treatment. This is because a much higher percentage of Cu in soil solution was complexed to dissolved organic matter (95.6–99.6% organic Cu) compared to that of Zn (4–24% organic Zn) (Table 3.2). In addition, the relatively low soil solution Cu^{2+} concentration confirms that the lower BCF values for Cu in comparison to Zn in poplar (as reported in the previous section) was due to low plant availability of Cu in soil.

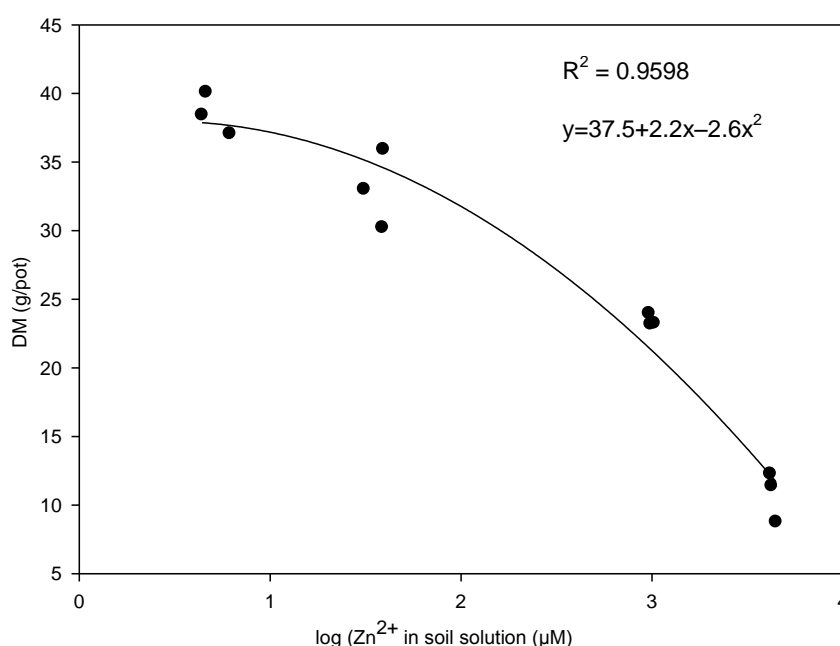


Figure 3.1 Relationship of poplar leaf DM with Zn^{2+} (μM) in soil solution

3.3.3 Metal fractions in soil solid phase

Results from the soil solid phase fractionation (Figure 3.2.) showed that the percentage of total Cu in the exchangeable fraction (6–9%) is low, and

significantly lower than that for Zn (12–55%). Compared with the exchangeable Cu fraction, a higher percentage of Cu was associated with the oxide (33–52%) and specifically adsorbed fraction (16–28%) of soil than Zn (25–49% and 5–15% for oxide and specifically adsorbed fraction, respectively). These effects were more pronounced at higher rates of metal addition. There were no significant differences observed in any of the fractions between bulk and rhizosphere soils.

The percentage of total Cu in the soluble+exchangeable soil fractions was low, and lower than that of Zn, indicating that not only is the bioavailability of solution Cu low, as discussed in the earlier section, but also that the bioavailability of the solid phase Cu is lower than that of Zn. This is supported by the metal uptake data (Table 3.1). McLaren *et al.* (2004) observed that the exchangeable Cu percentage was lower than that for Zn, Cd and Ni in soils treated with metal-spiked biosolids. Komarek *et al.* (2008) found in a trial with poplar growing on a metal contaminated soil (Gleyic Cambisols) in the Czech Republic, that the percentage of total Cu in the exchangeable fraction of soil ranged 2.2–2.9%, while for Zn the range was 10–14 %. The lower exchangeable value for Cu is probably due to a higher affinity of Cu to the oxide and specifically adsorbed fraction of solid phase soil than Zn. Copper has stronger adsorption affinity than Zn to oxides (McBride 1994). Several studies have shown that the percentages of exchangeable metal in rhizosphere soils are higher than in bulk soils as a result of root process such as root acid excretion which cause greater acidity in rhizosphere soils. Root excretion of organic anions can potentially change the concentration of micronutrients in the soil solution and possibly increase their availability to plants (Dakora and Phillips 2002; Oburger *et al.* 2009; Ryan *et al.* 2001). In the current study there were no significant differences observed in any of the fractions between bulk and rhizosphere soils. This may be due to a lack of clear demarcation between bulk soil and rhizosphere soil during sampling. The concept of rhizosphere is quite arbitrary. Although it is normally used for describing the zone of microbial activity in soil, which is influenced by the roots of any species, it is very difficult to define the boundary between the rhizosphere and the bulk soil (Russell 1977).

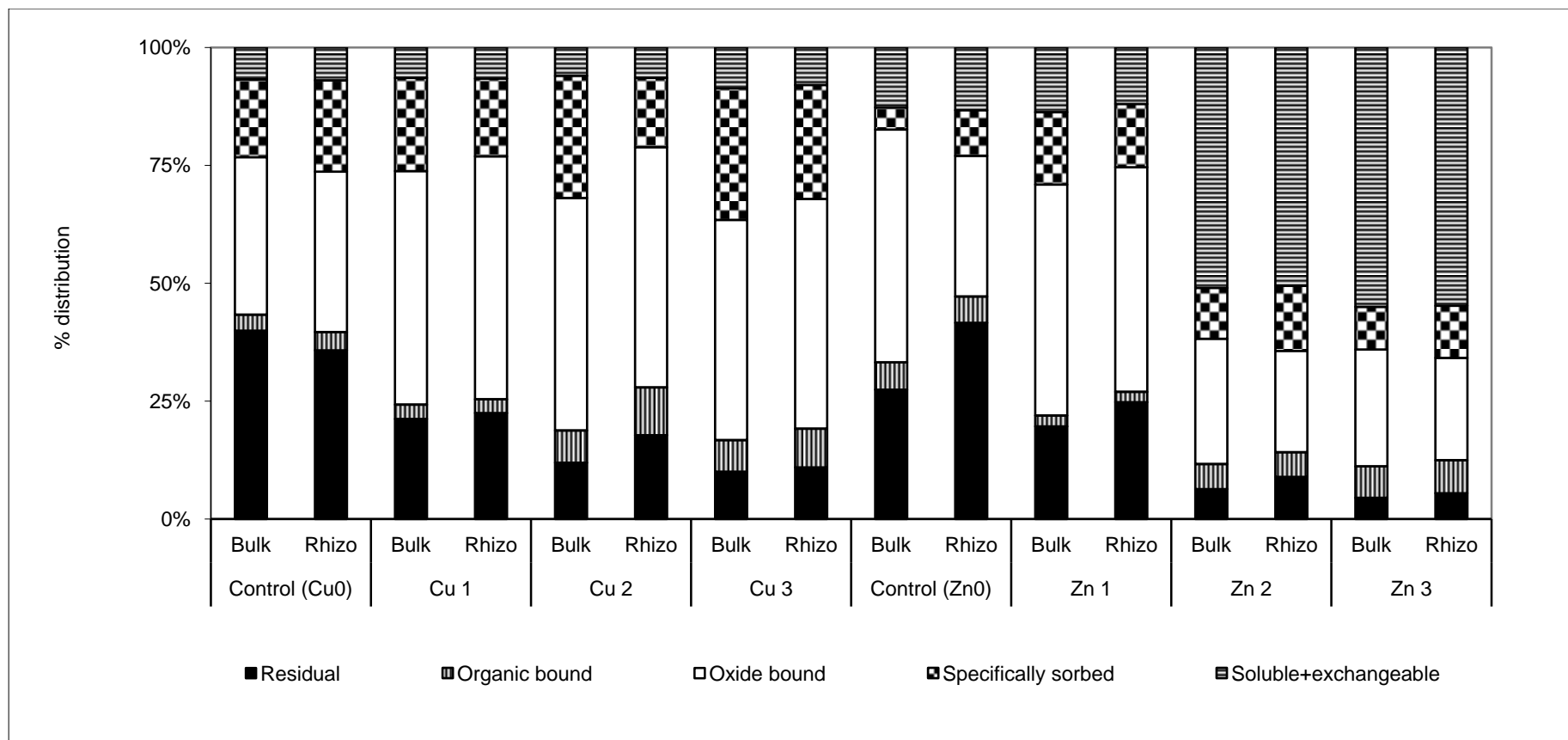


Figure 3.2 Percentage distribution of Cu and Zn fractions in rhizosphere and bulk soils amended with biosolids under poplar

3.3.4 Mycorrhizal colonization

Mycorrhizal colonization of the roots significantly increased with Cu addition up to the Cu2 treatment level and then significantly reduced at the Cu3 level compared to the Cu2 level (Figure 3.3). Even at Cu3, the colonization was greater than for the control.

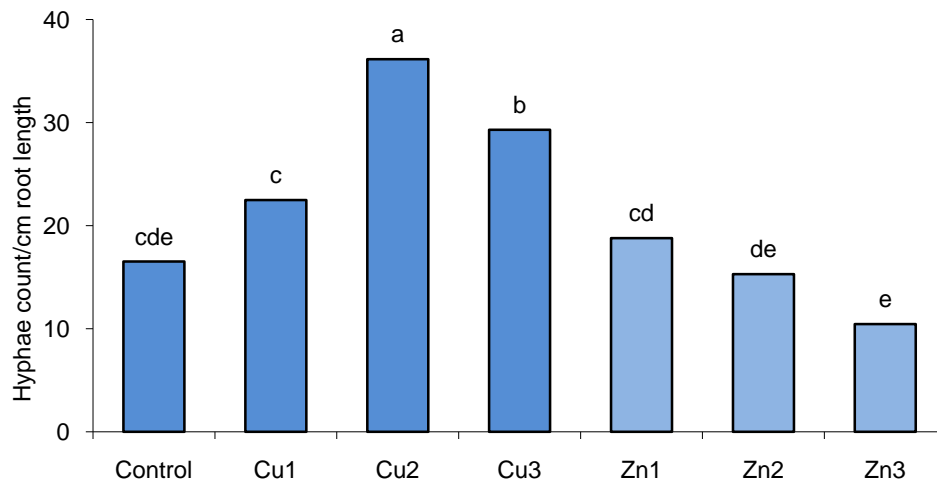


Figure 3.3 Mycorrhiza hyphae counts in poplar roots at different levels of Cu and Zn. Bars with different letters are significantly different ($P \leq 0.05$)

The effect of Cu on mycorrhizal colonization of the roots is interesting; the number of colonies increased with Cu up to the Cu2 treatment, but reduced at the Cu3 level. This might be due to a deficiency of Cu^{2+} in the soil solution for mycorrhizal growth at the control and Cu1 treatment levels, and toxicity at Cu3 level. The Cu^{2+} concentrations in soil solution up to the Cu1 level ($0.009 \mu\text{M}$) are much lower than the value of $0.1\text{--}0.3 \mu\text{M}$ considered adequate for pines grown in solution culture (Gorgé *et al.* 1985). At the Cu^{2+} concentration of $0.27 \mu\text{M}$ found for the Cu3 treatment, the mycorrhiza may be affected by Cu toxicity. Arduini *et al.* (1995) observed that the root elongation was inhibited at $1 \mu\text{M}$. My results indicate that Cu at the highest rate of application is toxic to mycorrhiza but not to poplar because at Cu3 treatment mycorrhiza growth was decreased but not shoot and root growth; root growth instead, increased (Table 3.1). Perhaps mycorrhiza is protecting the plants from the toxic effects of Cu by binding Cu to mycorrhiza

tissues (Denny and Wilkins 1987; Galli *et al.* 1994) and/or by other mechanisms (Dixon and Buschena 1988). The apparent manifestation is that mycorrhiza at the Cu3 level was suffering from Cu toxicity, but not the plants.

The increased levels of Zn addition reduced the level of mycorrhizal colonization, but the differences between treatments were not statistically significant except for the Zn3 treatment which produced mycorrhizal counts significantly lower than the Zn1 treatment (Figure 3.3). Interestingly, the Zn^{2+} concentration for all levels of treatments was much higher than the corresponding value for Cu, but the mycorrhiza growth was significantly affected only at the Zn^{2+} concentration of 4284 μM (Zn3 level). However, plant growth was significantly reduced at the Zn2 level (991 μM Zn^{2+}). This shows that unlike Cu, Zn is more toxic to poplar than to mycorrhiza as reported by Wilkins (1991). Because mycorrhiza counts were statistically similar up to a Zn^{2+} concentrations of 991 μM , this concentration does not seem to be toxic to mycorrhiza. Colpaert *et al.* (2004) showed that the Zn^{2+} concentration necessary to effect a 50% reduction of mycelial biomass production assessed for 235 ectomycorrhizal isolates varied from 3000 to 18000 μM in a media culture. The Zn^{2+} concentration of 4284 μM obtained for toxicity to mycorrhiza in our study falls within the toxicity range reported by Colpaert *et al.* (2004).

3.3.5 Dehydrogenase activity

Copper was found to be toxic to soil microorganisms at all levels of Cu addition, as observed from the significant decrease in soil dehydrogenase activity in both bulk and rhizosphere soils for all Cu treatments relative to the control (Figure 3.4). The difference was not significant between Cu2 and Cu3. Increasing the rate of Zn addition also decreased soil dehydrogenase activity, and the differences were significant at every successive addition of Zn, as was observed for the effect of Zn addition on plant growth reduction (Table 3.1). In a comparison of the two metals, the dehydrogenase activity reduction relative to the control was not as great for Zn as for Cu.

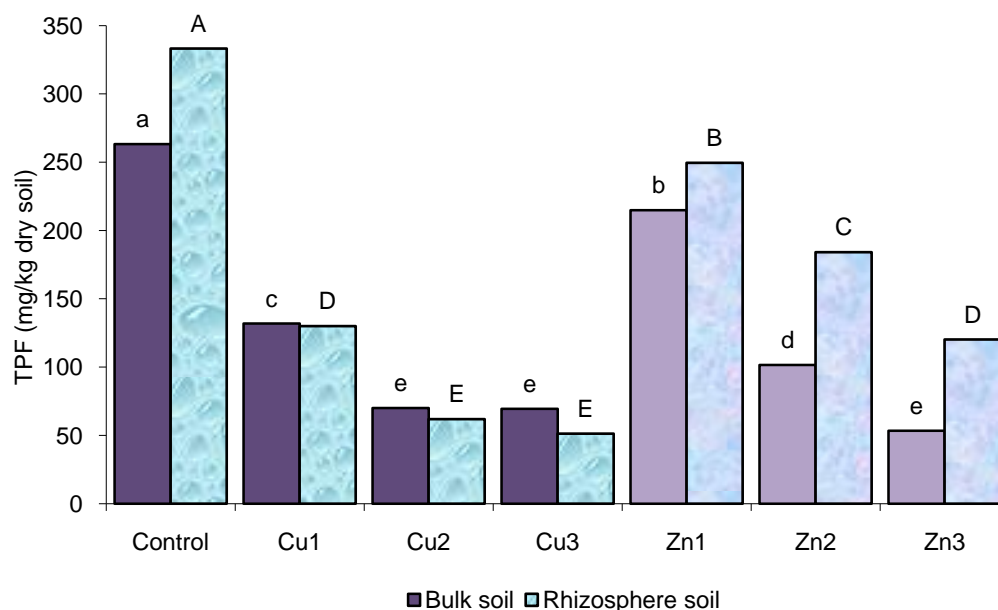


Figure 3.4 Dehydrogenase activity (TPF) in bulk and rhizosphere soils at different levels of Cu and Zn. Bars with different letters are significantly different ($P \leq 0.05$). Simple letters indicate the variance among the bulk soils and capital letters for the rhizosphere soils

Although Cu was not found to be toxic to plants at all levels, and to mycorrhiza at Cu levels up to Cu2, while Zn was toxic to plants and reduced mycorrhiza at low levels of addition, the decrease in dehydrogenase activity with increasing metal addition was greater for Cu than Zn, even with lower rates of Cu addition. This indicates that Cu is a more toxic metal than Zn for soil microorganisms. For example, at approximately the same total metal concentrations of Cu2 (137 mg/kg, pH 5.5) and Zn1 (141 mg/kg, pH 5.4), the dehydrogenase activity was significantly ($P \leq 0.001$) higher for Zn than Cu. This trend was previously reported by Chaperon and Sauve (2007). In their study, forest and agricultural soils were amended with four metals including Cu (50–1000 mg/kg soil) and Zn (200–3000 mg/kg soil). They reported that the total metal concentration to reduce dehydrogenase activity by 50 % was 58 mg/kg for Cu and 752 mg/kg for Zn in forest soils, and 268 and 927 mg/kg respectively for agricultural soils.

In my study the dehydrogenase activity between the rhizosphere and bulk soils did not differ significantly at any Cu or Zn level, except for Zn² where the dehydrogenase activity was higher for rhizosphere soils. Adriano (2001) indicated that rhizosphere soil is very different to bulk soil in terms of pH, redox potential, mineral composition, and organic matter cycling and composition. This is due to the interaction of the activities of soil, plant and microorganisms in the rhizosphere. Many studies have shown that enzyme activity, including dehydrogenase activity, is higher in rhizosphere soil (Joner and Jakobsen 1995; Joner *et al.* 1995; Kang and Freeman 2007).

The dehydrogenase activity data were correlated with various solid- and liquid-phase metal concentrations. The liquid-phase total metal concentration and solid-phase soluble + exchangeable metal concentration showed the highest correlation as was observed for the Cu and Zn spiked biosolids suspensions discussed in Chapter 2. Therefore, the dehydrogenase activity was plotted against these parameters. The concentrations of Cu and Zn in the total soil solution phase and in the soluble+exchangeable solid phase that corresponded to a 50% reduction in dehydrogenase activity (EC₅₀) was determined (calculated by assuming that dehydrogenase activity for the control treatment was 100%). The EC₅₀ values for total solution phase Cu and Zn were 0.1 and 27 mg/L, respectively, and for solid phase exchangeable Cu and Zn were 5 and 169 mg/kg, respectively (Figure 3.5).

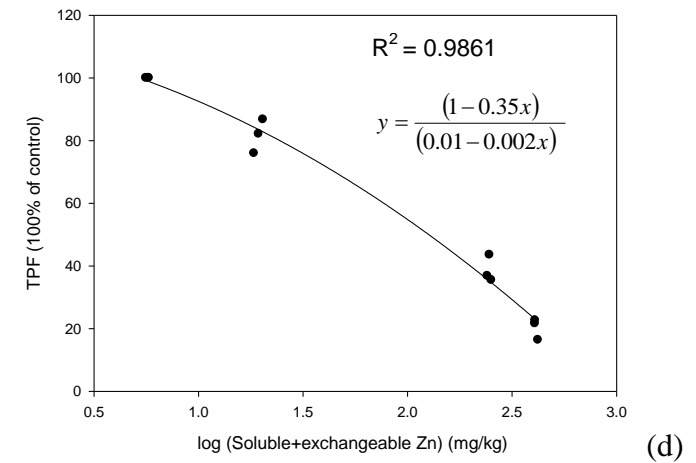
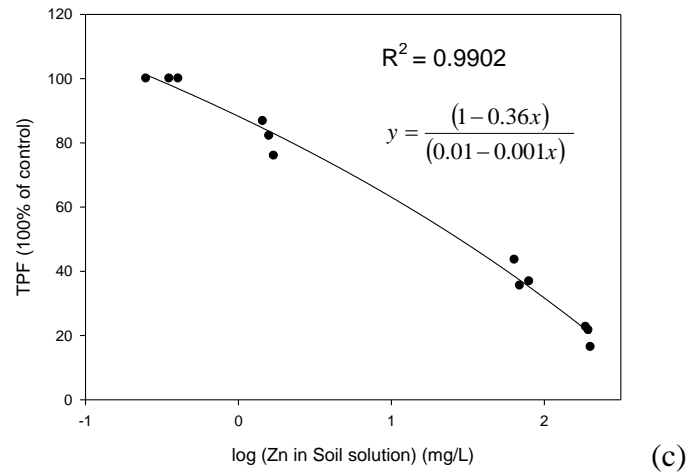
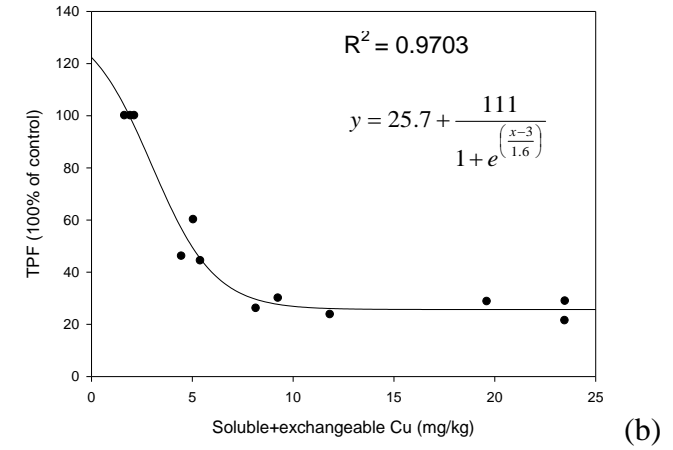
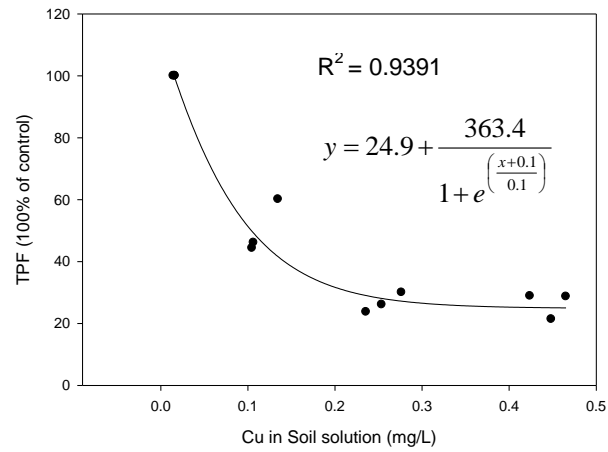


Figure 3.5 Relationship of dehydrogenase activity (TPF) with (a) soil solution Cu, (b) exchangeable Cu, (c) soil solution Zn and (d) exchangeable Zn. Equation 1 in Materials and method section was used for (a) and (b); Equation 2 was used for (c) and (d)

Higher EC₅₀ (dehydrogenase activity) values obtained for total solution metal concentration and soil exchangeable metal concentration for Zn compared with that for Cu obtained in my study are consistent with the results of others. Chaperon and Sauve (2007) reported that the EC₅₀ values for water soluble Cu and Zn were 0.05 and 0.14 mg/L respectively for forest soils, and the corresponding values for agricultural soils were 0.5 and 6.8 mg/L. Horswell *et al.* (2006) used bacterial and fungal bioluminescence-based biosensors as indicators of potential heavy metal toxicity to microorganisms in the needle litter of a mature pine (*Pinus radiata*) forest soil that had been amended with Cu and Zn contaminated sewage sludge. They reported that the effective concentrations that gave a 50% reduction in bioluminescence (EC₅₀ values) for water soluble Cu and Zn were 0.12 mg/L and 1.3 mg/L respectively. Chaudri *et al.* (1999; 2000b) used bacterial biosensors for the calculation of EC₅₀ values with soils that had received heavy metal contaminated sludge for 10 years, and reported that the EC₅₀ values for total soil solution Zn varied from 5.6 to 22.5 mg/L, depending on the bacterial species. In my previous laboratory incubation study (Chapter 2) on sewage sludge spiked with Cu and Zn, I reported that dehydrogenase activity was reduced by 50% (EC₅₀) at total solution phase Cu and Zn concentrations of 0.1 and 20 mg/L respectively, and solid phase exchangeable Cu and Zn concentrations of 410 and 670 mg/kg respectively.

Considering all these studies, it can be concluded that the toxicity thresholds based on dissolved metals seem to be more variable, probably because of the type of assay and soils/biosolids used for toxicity measurement (Hartley *et al.* 1997a). But even with these variations, the EC₅₀ values are relatively larger for Zn relative to Cu, suggesting that Zn is less toxic to soil microorganisms than Cu per unit metal concentration.

3.4 Conclusions

Increased Cu levels in the soil from 12 (control treatment) up to the highest level tested in the trial of 226 mg/kg (Cu3), increased root DM yield but had no effect on leaf DM yield. Free copper ion (Cu^{2+}) concentrations as measured in the soil solution suggest that Cu was deficient for the control, Cu1 and Cu2 treatments ($\leq 0.04 \mu\text{M}$). In contrast to Cu, Zn caused phytotoxicity at total soil Zn concentrations beyond 141 mg/kg (Zn1 level) by reducing leaf and root dry matter yields. The reduction in yields is due to high concentrations of Zn^{2+} . The critical Zn^{2+} concentration that will cause Zn phytotoxicity was calculated to be $88.5 \mu\text{M}$. The lower Cu^{2+} concentrations in soil solution relative to the Zn^{2+} are attributed to a higher percentage of Cu in the soil solution being complexed to dissolved organic matter (95–99% organic Cu) compared with that of Zn (4–24% organic Zn). The Cu concentration in the exchangeable Cu fraction (6–9%) was also significantly lower than that for Zn (12–55%) further supporting the lower availability of Cu to plants.

Copper deficiency in the soil solution of the control treatment resulted in increased mycorrhizal colonization for all Cu amendments as observed for increased root dry matter yield. However, toxicity at the Cu3 level became apparent; mycorrhizal colonization was significantly reduced for Cu3 level relative to Cu2 level. Increased levels of Zn addition reduced the mycorrhizal colony number more than Cu, suggesting Zn to be more toxic to mycorrhiza than Cu.

All rates of Cu and Zn addition decreased soil dehydrogenase activity. Lower rates of Cu addition promoted a higher reduction of dehydrogenase activity relative to Zn, indicating that Cu is a more toxic metal than Zn to soil microorganisms. The EC_{50} values for total solution phase Cu and Zn were 0.1 and 27 mg/L, respectively, and for solid-phase exchangeable Cu and Zn were 5 and 169 mg/kg, respectively.

Overall, the results showed that at the same rate of total soil metal concentration, Zn was more toxic to poplar and mycorrhiza, whereas Cu was more toxic to soil microorganisms. At low rates of addition, Cu seems to have some beneficial effect on poplar root and mycorrhizal growth. Considering the total metal concentration causing toxicity to plants and microorganisms, the current recommended limits in New Zealand of 100 mg/kg for Cu and 300 mg/kg for Zn appears to be high for both metals with respect to soil microbial activity but low for Cu and high for Zn with respect to poplar growth. Further research on adult trees in the field and use of other indices of microbial activity are recommended to confirm these results.

Chapter 4

Response of *Pinus radiata* and soil microbial activity to increasing copper and zinc contamination in a soil treated with metal-amended biosolids *

4.1 Introduction

Pine (*Pinus radiata*) is one of the world's most commercially important conifers, and planting covers a total global area now exceeding 4.3 million hectares. It is grown extensively as an exotic timber species in several countries, such as New Zealand, Australia, Chile, South Africa and Spain (Putoczki *et al.* 2007; Sevillano-Marco *et al.* 2009; Sutton 1999; Toro and Gessel 1999). Pine forestry has a very important place in the economy of New Zealand and constitutes 89.5% of the national plantation forestry land area (approximately 1.6 million ha) (NZFOA 2009/2010). Pine forests have in place intensive management regimes and grow relatively quickly.

Besides being a commercial plantation species, pine finds application as a suitable species for environmental protection on steep and degraded mountain slopes (Bi *et al.* 2010) to control erosion. The species also provides a substantial storage for carbon, and as such has a value in carbon sequestration. As discussed in the earlier chapters, biosolids application to pine forestry soils is often considered to be a relatively safe option for waste disposal. This is because the biosolids have a demonstrated beneficial effect on tree growth (Wang *et al.* 2006), and because there is little risk of contaminants from the biosolids entering the human food chain under this type of land use. However, due to repeat surface applications of biosolids, it has been reported that heavy metal bioavailability may be increased in the litter-rich surface soil, potentially leading to some degree of movement of

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these heavy metals into the underlying mineral soil, and eventually into groundwater (McLaren *et al.* 2007; Su *et al.* 2008). If these lands are converted to crop production in the future, then heavy metals accumulated in the forestry soil may represent an unacceptable risk to the food chain (McLaren *et al.* 2010).

Many studies have investigated the toxicity effect of Cu and Zn on pine using metal contaminated soils or solution culture studies (Arduini *et al.* 1994; Arduini *et al.* 1995; Egiarte *et al.* 2009; Gratton *et al.* 2000; Hartley *et al.* 1999). Metal accumulation and movement in pine forest soils, and the litter layer beneath pine trees, both amended with biosolids, have also been studied in recent trials (Egiarte *et al.* 2009; McLaren *et al.* 2007; Mosquera-Losada *et al.* 2009; Su *et al.* 2008; Toribio and Romanyà 2006). However, quantification of the toxicity effects of Cu and Zn on pine growing in biosolids-amended soil is rare in the literature. Fuentes *et al.* (2007a) showed that soils amended with low levels of Cu- and Zn-contaminated biosolids (44 and 122 mg/kg soil respectively) did not have an adverse affect on pine growth. Interestingly, soil amended with control biosolids (24 and 64 mg/kg soil for Cu and Zn respectively) had a negative effect on photosynthesis during drought. Larchevêque *et al.* (2006) tested the phytotoxic effect of Cu and Zn in soil amended with biosolids-based compost (up to 3.5 and 13 mg/kg EDTA-extractable soil Cu and Zn, respectively) and did not see any toxicity effect on pine seedlings.

Growth of pine plants is often improved when their root systems are infected by mycorrhizal fungi (Theodorou and Bowen 1970). Many studies have reported that the symbiotic ectomycorrhizal (ECM) association can ameliorate metal stress in pines and enhance its growth and vigour under a variety of conditions caused by high concentrations of toxic metals (Adriaensen *et al.* 2006; Adriaensen *et al.* 2005; Ahonen-Jonnarth *et al.* 2000; Rudawska and Leski 2005; Van Tichelen *et al.* 2001). Adriaensen *et al.* (2005) proved that a Cu-adapted ECM fungi isolate (*Suillus luteus*) provided excellent protection against Cu toxicity in pine seedlings exposed to elevated Cu levels up to 60 μM in solution culture. In another experiment, with a Zn dose-response up to 250 μM Zn, Adriaensen *et al.* (2004) showed that the Zn-tolerant ECM fungi (*Suillus bovinus*) was efficient in

protecting pines from Zn stress. Although ECM fungi protect the host plant from metal toxicity, tolerance of ECM to metal seems to depend on their long-term adaptability to the high levels of metal in soil. For example, Colpaert *et al.* (2004) studied the adaptive Zn tolerance (up to 27 000 $\mu\text{M Zn}^{2+}$ in nutrient medium) of four isolates of ECM collected in pioneer pine forests at 14 locations along a Zn pollution gradient and found that the highest tolerance was shown near heavily Zn contaminated sites. These studies indicate that the evolution of metal-specific tolerance capabilities of ECM is strongly triggered by pollution in the local environment. However, literature quantifying the amelioration effect of ECM for Cu and Zn toxicity on pine grown in biosolids-treated soil is lacking.

Chapter 3 has demonstrated the effect of incorporating Cu and Zn spiked biosolids into soils on poplar plant growth, mycorrhiza association with poplar roots, and soil microbial activity. This Chapter presents the results of a similar study that was conducted to determine the bioavailability of Cu and Zn in soils amended with biosolids, their effect on growth and metal uptake by pine, mycorrhiza, and soil microbial activity. A key objective of this work was to quantify critical levels of Cu and Zn in soil that can be considered as phytotoxic to pine. This study also compares the results obtained from the poplar and pine study and presents possible reasons for differences and agreements.

4.2 Materials and methods

A glasshouse trial was conducted using one-year old pine in pots containing soil (13 kg) that was amended with biosolids previously spiked with 3 levels of Cu or Zn. The trial design and treatments are similar to those of the trial on poplar reported in Chapter 3. The control treatment was soil amended with biosolids not spiked with metal. Total metal concentrations (mg/kg soil) in the treatments were for Cu 16 (Cu0–Control), 48 (Cu1), 146 (Cu2), 232 (Cu3); for Zn 36 (Zn0–Control), 141 (Zn1), 430 (Zn2), 668 (Zn3). The seven treatments were replicated three times. One-year old pine clones (clone 96004) were collected from Arborgen plant nursery, New Zealand, and transplanted, one per pot. The pots were arranged in a Randomized Complete Block Design (RCBD) in a glasshouse,

and regularly watered to maintain soil moisture at 80% 'pot field capacity'. The glasshouse temperature was maintained at $8.5 \pm 5^{\circ}\text{C}$ minimum (night) and $18 \pm 6^{\circ}\text{C}$ maximum (day). After 312 days of plant growth the experiment was concluded, and plant shoots, roots and mycorrhiza and soil samples from each pot were progressively collected. The following soil parameters were measured: metal fractions, soil–solution total metal concentration, pH, and dehydrogenase activity in rhizosphere and bulk soils. The following plant parameters were measured: pine needles (separated from stem and branches) and root DM yields, mycorrhizae hyphal density, and root and needle metal concentrations. The sampling procedure, methods of analysis, quality control and data analysis were discussed in Chapter 3.

Mathematical relationships were fitted between dehydrogenase activity and metal concentrations (soluble+exchangeable fraction and solution phase). The equations used for the curve fitting were:

$$y = \frac{a}{1 + e^{-\left(\frac{x-c}{b}\right)}} \quad (1)$$

where y is the dehydrogenase activity; x is the measured metal concentration; and a,b,c are constants

and

$$y = d + \frac{a}{1 + e^{-\left(\frac{x-c}{b}\right)}} \quad (2)$$

where y is the dehydrogenase activity; x is the log to the base 10 of the measured metal concentration ; and a, b, c, d are constants

4.3 Results and discussion

4.3.1 Dry matter yield and metal availability to the plants

Increased Cu levels had no significant effect on either needle or root DM yield (Table 4.1). The concentration of Cu in needles and roots for all Cu treatments was significantly increased with every level of Cu applied. The needle Cu

concentration at all levels of Cu was higher than the critical Cu concentration of 2.1–2.3 mg/kg, generally considered the limit for Cu deficiency, but below the phytotoxic level of 40 mg/kg for juvenile *Pinus radiata* plants (Boardman *et al.* 1997).

Increasing the soil Zn concentration decreased needle and root DM yields beyond the Zn1 treatment (total soil Zn > 141 mg/kg) (Table 4.1). In roots, a much greater DM yield reduction was observed between the Zn1 and Zn2 treatments than was observed for needles. Plant needles at the Zn2 treatment showed severe yellowing and withering. The Zn3 treatment plants died during the experiment (Figure 4.1). The DM yield reductions and severe damage of needles caused by the Zn2 and Zn3 treatments (≥ 430 mg/kg) could be well deduced from the severe yellowing and wilting of the pine needles, and indicate Zn toxicity at moderate levels of Zn addition. Observed serious reductions in the needle chlorophyll concentration (needles turned reddish) may lead to reduced C fixation and thus reduced growth. The data do not allow pinpointing of the exact cause of the yellowing and reddish coloration in pine needles. However, it is likely that the reduction is largely caused by the Zn induced impairment of nutrient transfer to needles, in particular N, Mg, and Fe which play a key role in chlorophyll synthesis and functioning (Adriaensen *et al.* 2006). This was probably due to root damage of the plant through direct contact with a high concentration of bioavailable Zn. The concentration of Zn in the needles and roots for all Zn treatments was significantly increased with every successive level of Zn applied. The needle Zn concentration above the Zn1 treatment was much higher than the phytotoxic level of 200 mg Zn/kg DM defined by Boardman *et al.* (1997). This explains the lower DM yields obtained for the Zn2 and Zn3 treatments. The severe DM reduction above the total soil Zn concentration of 430 mg/kg (Zn2 treatment) tends to support the maximum permissible soil Zn concentration of 300 mg/kg set by NZWWA (2003).

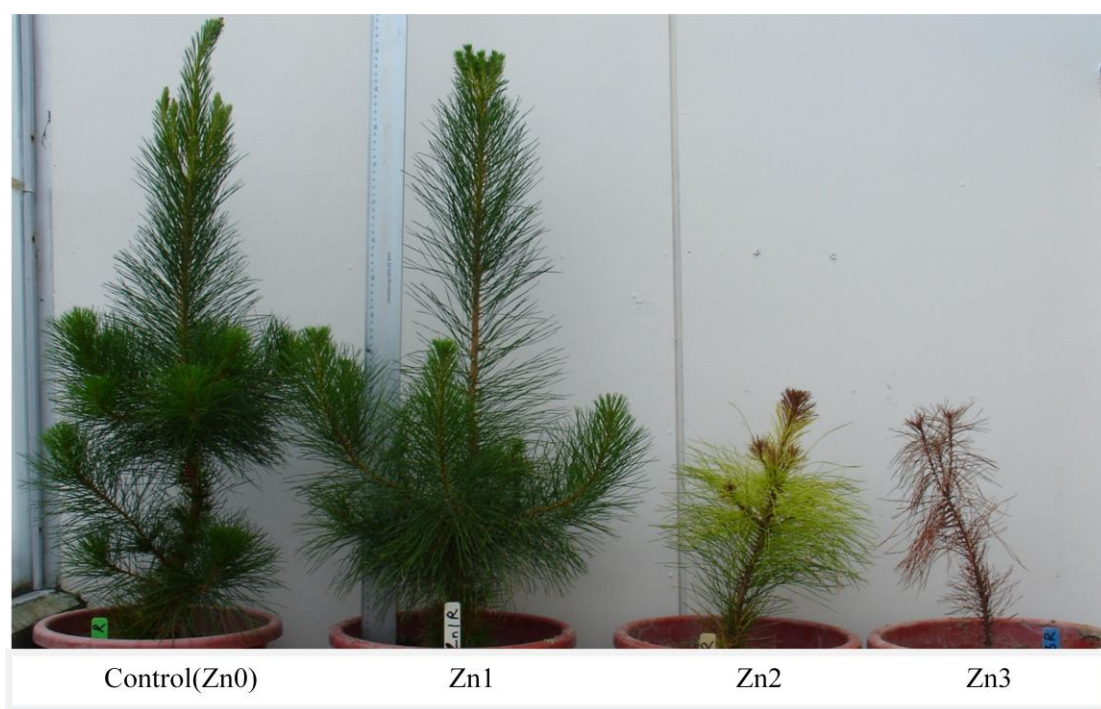
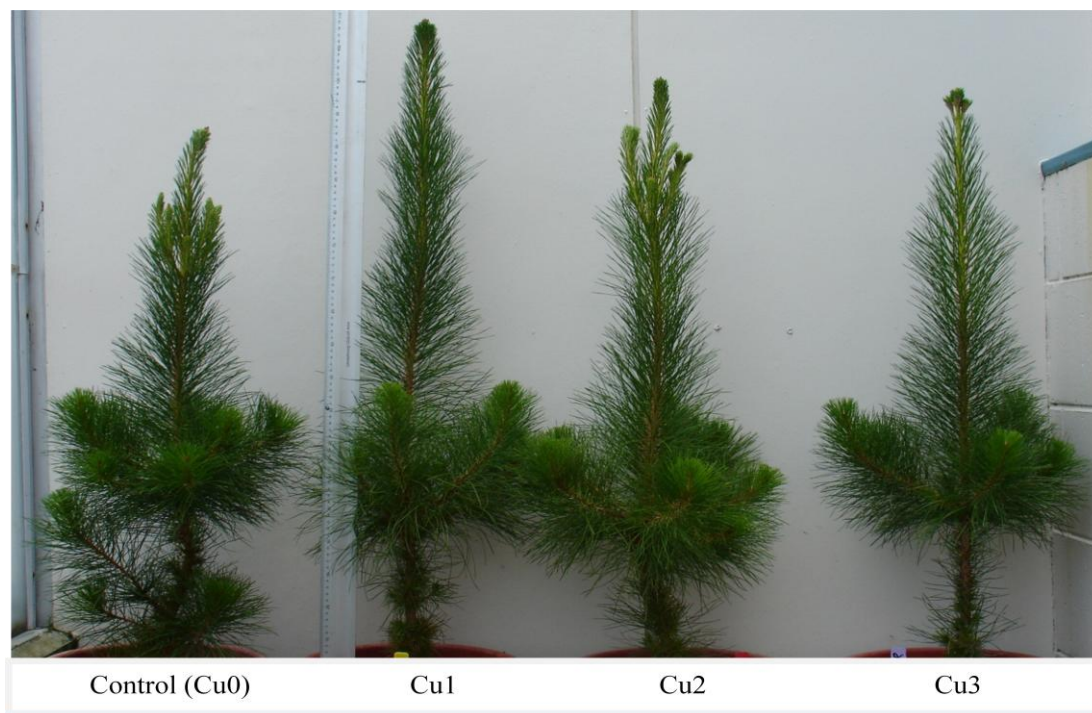


Figure 4.1 Effect of Cu and Zn on growth in pine (312 days after planting): Figure shows no toxic effect of Cu on plant growth, but an increasing phytotoxic effect of Zn with increasing concentration of Zn in soil

The bioconcentration factor (BCF), defined as a ratio of metal concentration in plant shoots to metal concentration in soil (Table 4.1), was much lower for Cu (0.03–0.26) than for Zn (0.8–1.5). The needle:root concentration ratio was also lower for Cu than for Zn suggesting that the translocation of Cu from roots to needles was lower than that for Zn. Copper transport to aboveground plant tissues is generally restricted. Arduini *et al.* (1996) found during an experiment with two-week old seedlings of two pine species, with a range of Cu concentration in solution culture (0.012–5 μM), that a maximum 16.5% of the total root Cu concentration was accumulated in the shoot and concluded that the root was an effective barrier to Cu translocation. They also reported that X-ray microanalysis of root tip sections confirmed the Cu to be strongly accumulated in the cell walls of the cortex, where its concentration sharply reduced from outer to inner cell layers. Hartley *et al.* (1999) reported, for a pot trial with Scots pine (*Pinus sylvestris*) seedlings, that the BCF value for Cu was <1 and for Zn was 2.5 when the soil Cu and Zn values were 10 and 100 mg/kg, respectively.

Table 4.1 Effect of Cu and Zn on total soil metal concentration, soil pH, pine needle metal concentration and DM yield

| Treatment | Total soil | pH | Soil solution | | | | | | | | Metal conc. | | BCF |
|-----------|------------------------|-----|-----------------------|----------------|------|--------|------|---------------------|---|------|-------------|--------|---------|
| | metal conc. (mg/kg) | | metal Conc. (mg/L) | Dry matter (g) | | | | Metal conc. (mg/kg) | | | | Ratio | |
| | | | | | | | | | | | | | |
| | | | | Needle | Root | Needle | Root | Needle: Root | | | | | |
| Cu0 | 16 | 6.1 | 0.03 | 77.6 | a | 34.8 | a | 4.2 | d | 10 | d | 0.42 a | 0.26 a |
| Cu1 | 48 | 5.8 | 0.27 | 67.5 | a | 32.7 | a | 5.3 | c | 65 | c | 0.08 b | 0.11 b |
| Cu2 | 146 | 5.7 | 0.37 | 65.6 | a | 35.6 | a | 6.3 | b | 166 | b | 0.04 b | 0.04 c |
| Cu3 | 232 | 5.7 | 0.54 | 65.0 | a | 39.6 | a | 7.3 | a | 267 | a | 0.03 b | 0.03 c |
| Zn0 | 36 | 6.1 | 0.9 | 72.3 | a | 34.8 | a | 39 | d | 45 | d | 0.85 a | 1.08 ab |
| Zn1 | 141 | 5.5 | 4.4 | 68.1 | a | 33.7 | a | 205 | c | 550 | c | 0.37 b | 1.45 a |
| Zn2 | 430 | 4.8 | 89 | 33.5 | b | 3.1 | b | 341 | b | 2464 | b | 0.14 c | 0.79 b |
| Zn3 | 668 | 4.7 | 362 | 12.6 | c | 1.5 | b | 1000 | a | 5033 | a | 0.20 c | 1.50 a |

Values in columns followed by different letters are significantly different ($P \leq 0.05$). BCF denotes bioconcentration factor

4.3.2 Metal speciation in soil solution phase

The total Cu concentrations (mg/L) in rhizosphere soil solution were 0.03, 0.27, 0.37 and 0.54 for the Cu0, Cu1, Cu2 and Cu3 treatments, respectively. For Zn, the concentrations were 0.9, 4.4, 89 and 362 for the Zn0, Zn1, Zn2 and Zn3 treatments, respectively.

When the soil solution Cu speciation in the rhizosphere soil was predicted using the WHAM speciation model, Cu^{2+} concentrations (μM) in soil solution were 0.001, 0.008, 0.04 and 0.06 for the Cu0, Cu1, Cu2 and Cu3 treatments, respectively (Table 4.2). The low Cu^{2+} concentration for all treatments is attributed to about 99% of Cu in soil solution being complexed to dissolved organic matter. Van Tichelen *et al.* (2001) carried out a trial with ectomycorrhizal (ECM) inoculated Scots pine seedlings grown in hydroponics solution and reported that even at a Cu concentration of 47 μM , shoot Cu concentration was <25 mg/kg. In another study, Fuentes *et al.* (2007b) conducted an experiment with seedlings of several Mediterranean woody species (Aleppo pine, mastic tree, prickly juniper, and evergreen buckthorn) in a hydroponic culture for 12 weeks. They reported that increasing the nutrient solution Cu concentration from 0.047 to 1 μM Cu increased the pine biomass DM, but at 4 μM Cu the shoot and root biomass were decreased by 27% and 33% respectively, as compared to the 1 μM Cu treatment. In the present trial with pine, the maximum Cu^{2+} concentration in soil solution was 0.06 μM showing that even at an elevated treatment level (Cu3), bioavailable Cu was low. This also confirms the reason for the non significant effect of Cu treatment on DM, and lower Cu accumulation in needles relative to roots of pine. Even though the Cu^{2+} concentrations were low, the growth of pine did not increase with increasing Cu^{2+} concentration. However, the needle Cu concentration in the control treatment was higher than the critical Cu concentration for Cu deficiency in radiate pines. The reason for pine not suffering from Cu deficiency despite the low Cu^{2+} concentration in soil solution may be due to ECM helping the plants to take up Cu from the soil solution pool of low Cu concentration more efficiently. The mycorrhiza influence on the Cu uptake of the plant system will be discussed in a later section.

Table 4.2 Percentage of metal species in soil solution

| | M2+ | MHCO ₃ | MSO ₄ | MCl | Total | Organic M |
|-----|-----------------|-------------------|------------------|----------------|------------------|-----------------|
| Cu0 | 0.01 (0.00) | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | 0.01 (0.00) | 99.99 (1.89) |
| Cu1 | 0.20 (0.02) | 0.01 (0.00) | 0.07 (0.01) | 0.00 (0.00) | 0.28 (0.3) | 99.72 (1.34) |
| Cu2 | 0.75 (0.10) | 0.03 (0.01) | 0.22 (0.02) | 0.00 (0.00) | 1.00 (0.12) | 99.00 (2.01) |
| Cu3 | 0.73 (0.10) | 0.02 (0.00) | 0.28 (0.03) | 0.00 (0.00) | 1.04 (0.10) | 98.96 (1.64) |
| Zn0 | 31.65 (1.44) | 0.09 (0.01) | 5.73 (0.24) | 0.02 (0.00) | 37.48 (1.23) | 62.52 (1.08) |
| Zn1 | 61.15 (1.51) | 0.04 (0.01) | 16.79 (0.64) | 0.15 (0.02) | 78.14 (1.51) | 21.86 (0.86) |
| Zn2 | 72.24 (1.47) | 0.01 (0.00) | 27.92 (1.03) | 0.19 (0.02) | 100.36 (2.02) | 0.00 (0.00) |
| Zn3 | 79.83 (1.54) | 0.01 (0.00) | 21.36 (1.12) | 0.20 (0.03) | 101.41 (2.10) | 0.00 (0.00) |

Values are means \pm standard error, n=3 (in parenthesis)

Zn²⁺ concentrations (μ M) for the Zn0, Zn1, Zn2, and Zn3 treatments were 4.5, 41, 983 and 4419 respectively; and contributed 32–80% of the soil solution Zn (Table 4.2). Fuentes *et al.* (2007b) also examined the effect of Zn on Aleppo pine seedlings (*Pinus halepensis*) in hydroponic culture and reported that by increasing the solution Zn concentration from 0.073 to 25 μ M Zn, pine seedling DM was increased, but at 100 μ M Zn the shoot and root biomass were decreased by 35% and 24% respectively, relative to the 25 μ M Zn concentration. Applying these results to the present trial with pines, Zn treatment above the Zn1 level may be phytotoxic to the pine seedlings. This confirms the observed effect on the DM yields and needle Zn concentrations resulted from the increasing Zn concentration in the soil. The critical phytotoxic level of Zn was determined by fitting a curve to relate DM content to the Zn²⁺ concentration in soil solution (Figure 4.2).

Interpretation of the data derived a critical Zn concentration for Zn toxicity of 62 μM . In previously conducted hydroponics experiments, 40 μM Zn caused a reduction in root elongation for grasses which are sensitive to Zn stress (Al-Hiyaly *et al.* 1988), while 76 μM Zn reduced the growth as well as the N and P uptake capacity of Zn-sensitive ECM (*S. bovinus*) associated with pine seedlings (Adriaensen *et al.* 2004).

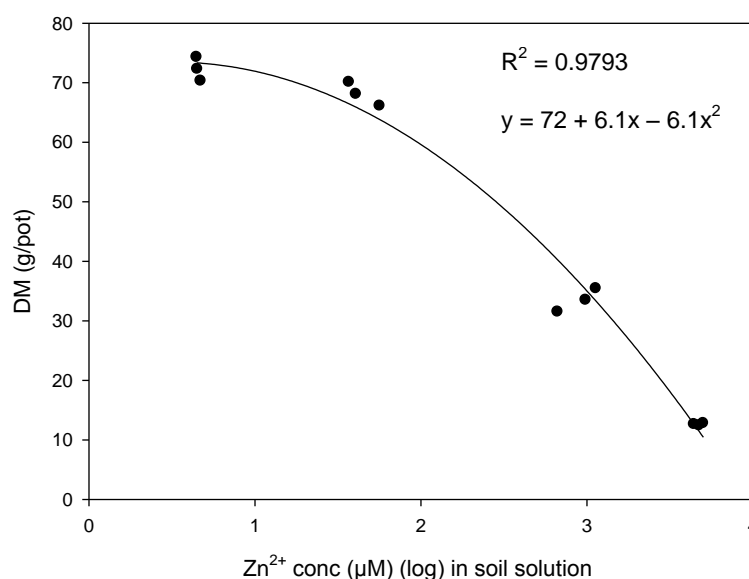


Figure 4.2 Relationship of pine needle DM with the Zn^{2+} concentration (μM) in soil solution

4.3.3 Metal fractions in the soil solid phase

The percentage distribution of Cu and Zn in the various soil solid phase fractions was measured to determine the fate of added metals and to explain the bioavailability and phytotoxicity of Zn and Cu derived from the biosolids (Figure 4.3). When the soil Cu level was low, the residual fraction of Cu was the largest contribution of total soil Cu (43 and 34% for the Cu0 and Cu1 treatments, respectively), followed by the oxide bound Cu fraction (21–42%) and specifically sorbed Cu (19 and 11 for Cu0 and Cu1 treatments, respectively) (Figure 4.3). However, as the Cu level increased, the relative contribution of the various fractions changed. The residual fraction significantly reduced (13–17%), whereas the % of Cu associated with the oxide fraction (52–54%) increased at higher Cu

levels (Cu2 and Cu3). The exchangeable Cu fraction for the control treatment contributed 12% of total soil copper, but in the biosolids-amended soils (from Cu1 to Cu3), was reduced to 5–8% of total Cu concentration. The differences were not significant among the Cu treatments. These results show that increasing the level of Cu changed the relative percentage of Cu among the non-bioavailable Cu fractions, but did not produce much change in the percentage of the bioavailable soluble+exchangeable fraction in the soil. However, the absolute concentration of exchangeable solution Cu increased with an increase in Cu levels (2, 2, 11 and 13 mg/kg soil for the Cu0, Cu1, Cu2 and Cu3 treatments, respectively). The increase in the percentage of Cu in the oxide fractions is probably due to strong sorption of the added Cu to the oxide constituents of the soil. Perhaps with an increase of time in terms of years, the sorbed Cu may move to the residual fraction by diffusion into lattice structures (McBride 1991) and this would lead to increased residual Cu fraction percentage as observed in the native soil (Cu0 treatment). Huang *et al.* (2008) conducted an experiment with ECM inoculated Chinese pine seedlings grown in a brown soil amended with Cu (25–400 mg/kg soil), and reported that the percentage of Cu associated with the non-labile soil Cu fractions increased with increasing total soil Cu concentration (carbonate fraction from 23 to 57%, oxide fraction from 24 to 31% and residual fraction from 11 to 23%), but the percentage of Cu associated with the exchangeable fraction was reduced (from 8 to 1%). However, the absolute exchangeable Cu concentration did not significantly change (3.9–4.1 mg/kg) with the increasing soil Cu concentration. Huang *et al.* (2008)'s study supports the results of the present study, as the increasing amount of Cu added to the soil was mainly associated with non-labile forms and did not change the bioavailable form.

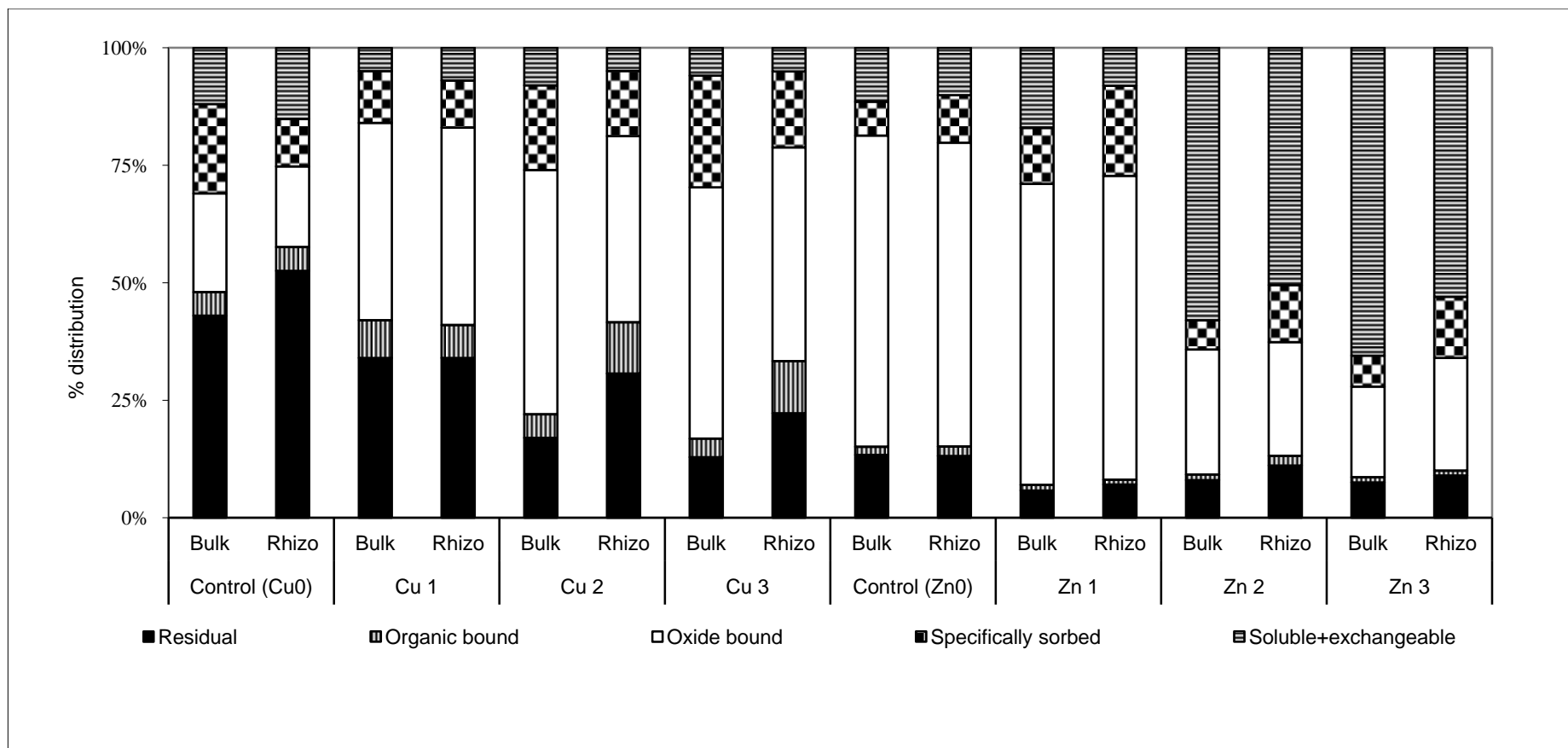


Figure 4.3 Percentage distribution of Cu and Zn fractionation in rhizosphere and bulk soils amended with biosolids under pine

The relative distribution of the Zn fractions was different to that of Cu (Figure 4.3). For low levels of total soil Zn (Zn0 and Zn1) the percentage of oxide bound metal was higher (64–66%) than for the high levels of soil Zn (19–26%), whereas at the higher rates (Zn2 and Zn3) the Zn was mostly present in the exchangeable fraction (58–66%). The bioavailability of Zn as a proportion of the total Zn in soil, up to the Zn1 level, was low, as indicated by the relatively low contribution of the exchangeable fraction to total soil Zn (12–17% up to Zn1). But at higher rates (Zn2 and Zn3) added Zn was highly available (the exchangeable fraction was 58–66%) and toxic to the plant tissues. The absolute concentration of soluble+exchangeable Zn in the Zn0, Zn1, Zn2 and Zn3 treatments was 4, 32, 249 and 437 mg/kg, respectively. The observed effect of the high rates of Zn amendment on plant DM content and needle Zn concentration is consistent with these results. The lower soil pH range (4.4–4.8) for the Zn2 and Zn3 treatment in comparison to the Zn0 and Zn1 treatment (pH 5.6–6.1) could have reduced the sorption of Zn to soil organic matter and minerals, through a reduction in net negative charge on the components of the soil (McBride 1991). This would have led to a higher percentage of Zn in the soluble+exchangeable fractions at higher levels of Zn treatment. The rhizosphere soils did not show any significant difference in Zn fractionation relative to the bulk soil.

4.3.4 Mycorrhiza colonization

Both Cu and Zn at all levels of treatment had no significant effect on mycorrhizal colonization of the roots (Figure 4.4). However, a trend of increasing mycorrhiza colonisation was observed with increasing Cu levels (Figure 4.4) and this may have been due to the adjustment of the ECM population to control the bioavailability of Cu at elevated Cu levels, to protect the host plant from Cu toxicity. The non-significant affect on mycorrhizal colonization with increasing Cu application supports the safe application of Cu to soil up to a total concentration of 226 mg/kg. Adriaensen *et al.* (2005) compared the Cu tolerance of 2 isolates of Cu-tolerant ECM and Cu-sensitive ECM (*Suillus luteus*) inoculated with pine seedling and grown in a solution culture (0.1–60 μ M Cu) for 10 weeks. The shoot weight, nutritional uptake and weight of extraradical

mycelium of ECM were not affected by Cu addition (up to 60 μM) in Cu-tolerant ECM inoculated pine seedlings, whereas in the non-mycorrhizal seedlings, and seedlings colonized by the Cu-sensitive isolate, root growth and nutrient uptake were strongly inhibited under Cu stress conditions. They also reported that the shoot Cu concentration was maintained below 20 mg/kg even at 60 μM for Cu-tolerant ECM seedlings. This was in comparison to the Cu-sensitive and non-ECM inoculated pines, where the shoot concentration exceeded 20 mg Cu/kg DM at the solution concentration of 15 μM Cu. In the present study the Cu^{2+} concentrations in soil solution are below 0.06 μM , which is much lower than the concentrations used in the study of Adriaensen *et al.* (2005). Therefore it is not surprising that Cu was not toxic to pine.

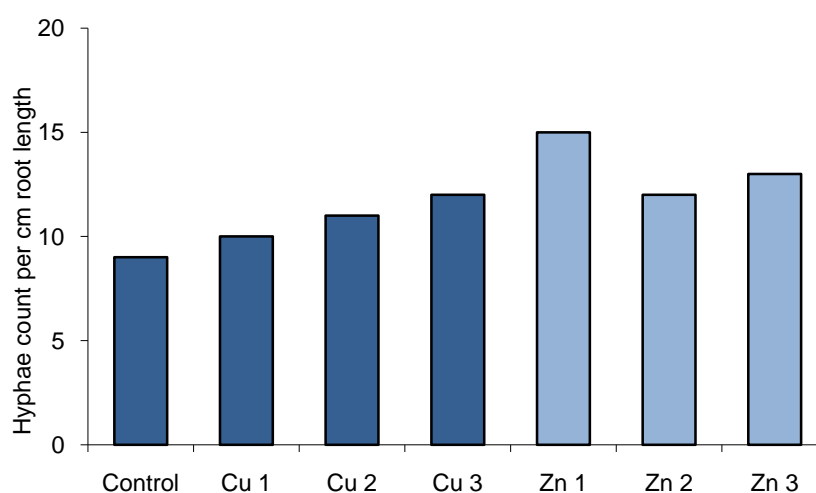


Figure 4.4 Mycorrhiza hyphae counts on pine roots as a function of Cu and Zn treatments of the soil. No significant difference ($P \leq 0.05$) between treatments.

Even though a phytotoxic effect was observed in the plants above the Zn1 treatment (soil solution Zn^{2+} concentrations above 41 μM), the elevated Zn concentration did not affect mycorrhiza colony counts. This indicates that the Zn concentration exceeded the critical toxicity level for the plants, but not for the ECM. However, the ECM was not able to protect the plant from Zn toxicity above this Zn^{2+} concentration. Colpaert *et al.* (2004) showed a high Zn tolerance of 147 ECM isolates (collected in 13 Zn polluted pine forestry sites) in solution culture Zn concentration up to 3000 μM .

4.3.5 Dehydrogenase activity

Even though Cu was not found to be toxic to plants or mycorrhiza, it was found to be toxic to soil microorganisms at all levels of Cu addition, as observed from the decrease in soil dehydrogenase activity with increasing Cu (Figure 4.5).

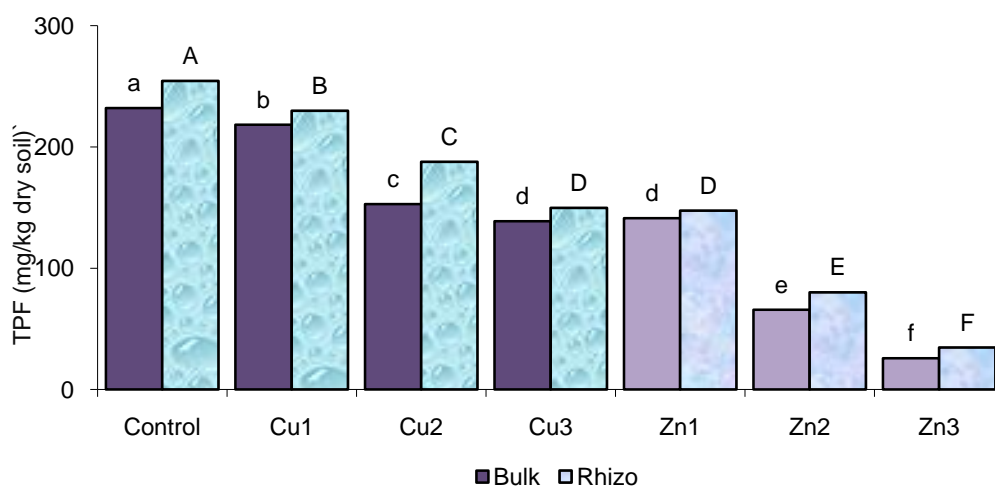


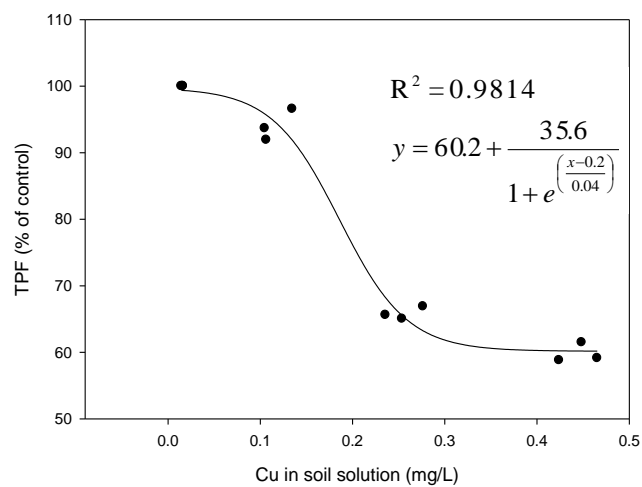
Figure 4.5 Dehydrogenase activity (TPF) in bulk and rhizosphere soils at different levels of Cu and Zn. Bars with different letters are significantly different ($P \leq 0.05$). Simple letters indicate the variance among the bulk soils and capital letters are the rhizosphere soils

The affect of Zn on soil microorganisms was the same; increasing the rate of Zn decreased soil dehydrogenase activity at all levels of Zn addition. However, Zn addition also reduced plant growth (Table 4.1). The decrease in dehydrogenase activity relative to the control was greater for Zn than Cu. When the dehydrogenase activity is compared for the same total soil metal concentration (comparison of TPF for Cu2 and Zn1 levels) activity was higher for Cu than Zn.

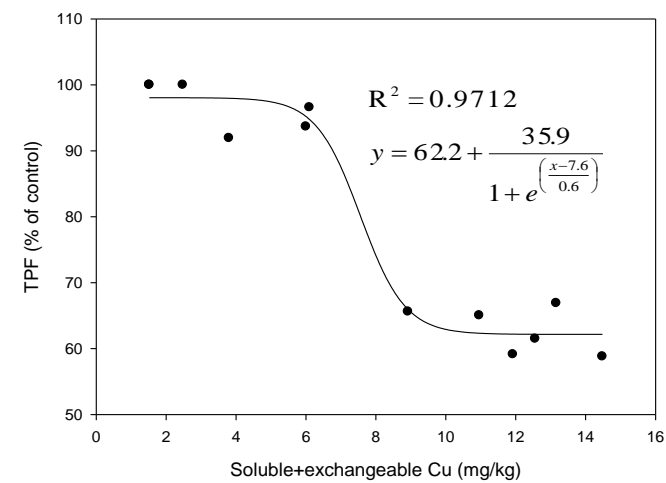
The dehydrogenase activity data were correlated with liquid-phase total metal concentrations and solid-phase soluble+exchangeable metal concentrations. The concentration of metal in the soluble+exchangeable solid phase and in the total solution phase that corresponded to a 50% reduction in dehydrogenase activity (EC_{50}) was determined. For Cu, the EC_{50} value was not however reached, so instead EC_{40} values were calculated. Calculation of EC_{50} for Cu would have

required extrapolation of the curve and likely generated an inaccurate experimental result (Broos *et al.* 2007). The EC₄₀ values for total solution phase and for solid-phase exchangeable Cu were 0.5 mg/L and 14.5 mg/kg, respectively. The EC₅₀ values for total solution phase and for solid-phase exchangeable Zn were 9 mg/L and 55 mg/kg, respectively (Figure 4.6).

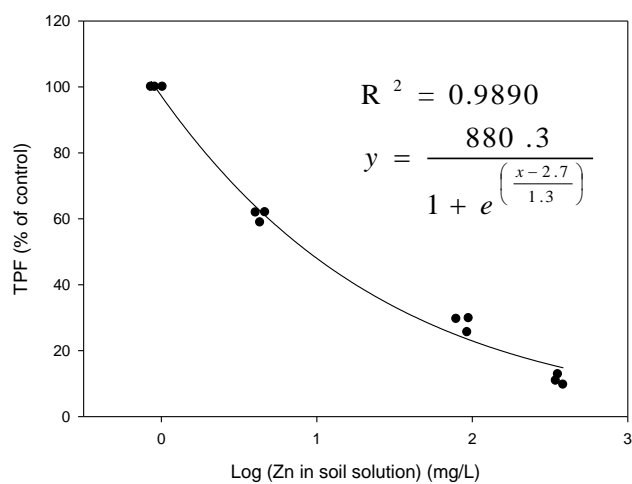
The greater toxicity of Zn over Cu on microbial activity in terms of the total metal concentration (Fig. 4.5), but higher EC₅₀ values (lower toxicity effect) of Zn over Cu (though it was not calculated for Cu in this trial) in terms of the soil solution and exchangeable fractions, gives a good picture of variations in bioavailability between Cu and Zn. Furthermore, this highlights the importance of carefully interpreting results of the toxicity affect of these metals on microbial activity. Broos *et al.* (2007) estimated the EC₅₀ values for Cu and Zn concentration on substrate-induced nitrification and respiration activities for 12 soils (previously amended with Zn (5–9100 mg/kg) and Cu (3–5880 mg/kg) in the field) collected from various parts of Australia. They concluded that when the EC₅₀ values were based on a total metal concentration, there was a strong 1:1 relationship between EC₅₀ values for Cu and Zn across all soils, indicating equal microbial toxicity of these metals in soil. However, when based on the soil solution concentration, Cu generally was more toxic (lower EC₅₀) than Zn, as observed in the current study.



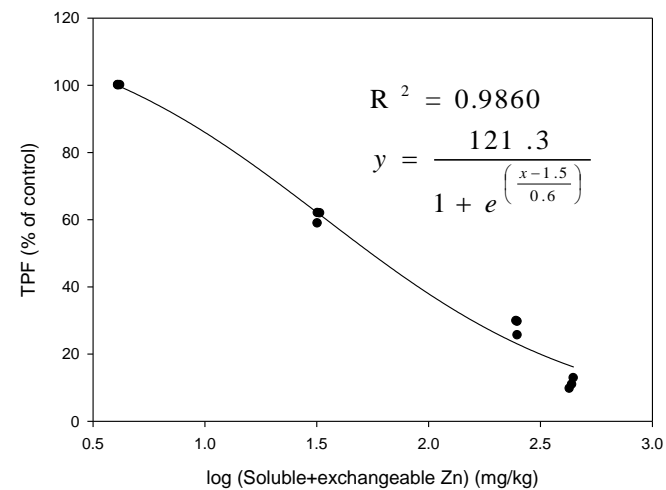
(a)



(b)



(c)



(d)

Figure 4.6 Relationship of dehydrogenase activity (TPF) with (a) soil solution Cu, (b) exchangeable Cu, (c) soil solution Zn and (d) exchangeable Zn. Equation 1 in section 4.2 was used for (a) and (b); Equation 2 was used for (c) and (d)

4.3.6 Comparative effect of biosolids–derived Cu and Zn on poplar and pine

The poplar (Chapter 3) and pine trials were conducted under similar experimental conditions and commenced at the same date. However, poplar was harvested earlier (147 days) than pine (312 days) due to the deciduous nature of the poplar leaves with the start of the winter season. The similarity of experimental design and treatments allows a comparison to be made between poplar and pine on the effects of biosolids–derived Cu and Zn on plant, soil and microbial activities (Table 4.3).

Table 4.3 Comparative effects of Cu and Zn in poplar (Chapter 3) and pine (Chapter 4) trials

| Parameter | | Poplar | Pine |
|---|------|---|------------------|
| Root DM | (Cu) | increased | no effect |
| | (Zn) | Reduced from Zn1 | Reduced from Zn2 |
| Leaf DM | (Cu) | no effect | |
| | (Zn) | Reduced from Zn2 | |
| Leaf concentration | (Cu) | not phytotoxic | |
| | (Zn) | phytotoxic | |
| BCF (Cu and Zn) | | high | low |
| Shoot:root metal conc. Ratio (Cu and Zn) | | high | low |
| Mycorrhiza | (Cu) | Increased up to Cu2 and then reduced at Cu3 | no effect |
| | (Zn) | reduced | no effect |
| Soil microbial activity | | reduced with increasing metal conc. | |
| Soil microbial activity–rate of reduction (Total metal conc.) | | Cu>Zn | Zn>Cu |

The root DM yield was increased with increasing soil Cu concentration in poplar, but was not significantly affected in pine. However, both poplar leaf DM and pine needle DM were not significantly affected by increasing soil Cu concentrations. In both plants, Cu was not phytotoxic and increasing the soil Cu level caused an increase in the leaf Cu concentration. There was no difference in poplar leaf DM and pine needle DM between the Zn0 and Zn1 treatments, but they were reduced beyond the Zn1 treatment due to Zn phytotoxicity. In contrast, root DM in poplar was reduced for an increased soil zinc concentration beyond the control treatment, whereas for pine root, DM was affected only from the Zn1 treatment. However, at higher rates of soil Zn (Zn2 and Zn3), the decrease in root biomass production was similar in both plants. In both plants, the leaf/needle Zn concentration above the Zn1 treatment was much higher than the phytotoxic level. The needle Zn concentration was significantly increased up to the Zn3 level in pine, but in poplar there was no increase in the Zn concentration above the Zn2 level. This may be due to the longer time duration of the pine trial (312 days) compared with the poplar trial (147 days) in addition to plant type differences.

The BCF values (Cu=0.1–0.8 and Zn=5.5–10) and the shoot:root metal concentration ratios (Cu=0.2–1.2 and Zn=0.8–4) for poplar were much higher than the corresponding values for pine (Cu=0.03–0.26 and Zn=0.8–1.5 for BCF; Cu=0.03–0.42 and Zn=0.14–0.85 for needle:root ratio). This is probably due to the higher translocation and accumulation rate of metals in poplar leaves than pine. Poplar is known to be a shoot metal accumulator plant (Castiglione *et al.* 2009; Dos Santos Utmazian and Wenzel 2007).

The total soil solution concentrations of Cu (0.02–0.45 mg/L) and Zn (0.43–334 mg/L) in the poplar trial were similar to the corresponding values for the pine trial (Cu 0.03–0.54 mg/L; Zn 0.9–362 mg/L). The soil solution Cu²⁺ concentration at Cu3 was lower in pine (Cu²⁺ 0.06 µM) than the poplar treatment at Cu3 (Cu²⁺ 0.27 µM). One reason for the lower Cu²⁺ concentration in pine trial could be the longer duration of this trial where more Cu²⁺ would have sorbed to the soil colloids with the increased time. The percentage of total Cu in the soluble+exchangeable soil fractions (6–9 and 5–12% for poplar and pine

respectively) was low, and lower than that of Zn (12–55 and 12–66% for poplar and pine respectively) for both poplar and pine. The lower Cu soil solution and soluble+exchangeable solid phase metal concentrations for both poplar and pine relative to the corresponding Zn concentrations well explains the lack of apparent phytotoxicity of Cu, but obvious phytotoxicity of Zn.

In the poplar trial mycorrhizal colonization was increased in the presence of Cu up to the Cu₂ level and then reduced at Cu₃, whereas colonisation was reduced in the presence of all levels of Zn relative to the control. In pine, both Cu and Zn did not affect the mycorrhizal colonization. The reason for this difference is not clear, but may be due to the species difference of plants and duration of the trials. In the longer duration of the trial with pine, some of the metal-resistant mycorrhiza species may have had time to develop, as has been found for mycorrhiza infection at metal contaminated field sites (Colpaert *et al.* 2004). Both Cu and Zn were found to be toxic to soil microorganisms at all treatments, as observed from the decrease in soil dehydrogenase activity with increasing soil metal concentrations in both plant trials. However, the dehydrogenase activity reduction was greater for Cu than Zn in the poplar trial for all treatments, whereas in pine, activity reduction was higher for Zn than Cu at the same total soil metal concentrations. The EC₅₀ values (50% reduction of dehydrogenase activity of control treatment) for total solution phase and solid-phase exchangeable fraction for Zn only were calculated in both trials.

4.4 Conclusion

An increasing amount of Cu applied to soil in the form of Cu-amended biosolids increased the concentration of Cu in pine needle but had no affect on needle and root DM, even at the highest total soil concentration of 232 mg/kg. However, a severe phytotoxic effect was observed when the total soil Zn concentration exceeded 141 mg/kg (Zn₁). Cu was not phytotoxic because the Cu²⁺ concentration in soil solution was extremely low (0.06μM) due to most of the solution Cu being complexed to dissolved organic matter (approx 99%). The phytotoxic effect of Zn was due to a high Zn²⁺ concentration in soil solution (41–4419 μM, 61–80% of

soil solution Zn). The critical concentration value for phytotoxicity was calculated to be 62 $\mu\text{M Zn}^{2+}$. Soil metal fractionation showed that the total Cu concentration in the exchangeable Cu fraction (2–13 mg Cu/kg soil; 5–12% of total Cu) was significantly lower than that for Zn (4–437 mg Zn/kg; 12–66% of total Zn) suggesting that the solid phase also contributed to Zn phytotoxicity to pine. The lower bioavailability of Cu resulted in a lower BCF value for Cu (0.03–0.26) compared with that for Zn (0.8–1.5). Translocation of Cu from root to needle was also lower than that for Zn as evidenced from the lower needle:root ratio for Cu relative to Zn.

Both Cu and Zn at all levels of addition had no significant affect on mycorrhizal colonization of the roots. However, soil microbial activity was inhibited by both Cu and Zn at all levels of metal addition, but the rate of reduction relative to the control was greater for total Zn than for Cu. The EC_{50} values for the total solution phase and solid–phase exchangeable fraction for Cu was not reached. However, EC_{40} was calculated to be 0.5 mg/L and 14.5 mg/kg. For Zn the corresponding EC_{50} values were 9 mg/L and 55 mg/kg, respectively.

Biosolid–derived Cu and Zn influenced pine plants differently to poplar trees in some aspects such as root dry matter, BCF, leaf:root concentration, and mycorrhizal colonization. However, some similar effects on both plants for these metal contaminants were observed, mainly in terms of metal bioavailability, phytotoxic effects and total microbial activity. The BCF and leaf:root metal concentration ratios were much lower in pine than the corresponding values for poplar indicating that unlike poplar, which is a shoot metal accumulator plant, above ground pine parts and litter do not appear to represent a potential risk to the environment.

Based on these conclusions, it can be summarised that at similar rates of total soil metal concentration, Cu did not show a phytotoxic effect, but Zn was phytotoxic to pine. Nevertheless, both Cu and Zn showed the same trend of effect on mycorrhiza and soil microbial activity. With respect to pine forestry the findings of this study suggest that the current recommended limits for the total Cu and Zn

concentration in New Zealand soil (100 mg/kg Cu; 300 mg/kg for Zn) appear to be toxic to soil microbial activity but do not affect the mycorrhiza activity. However, as far as phytotoxicity is concerned; it is recommended that the total Zn concentration limit in soils be revised as the present limit exceeded the upper critical toxicity level to pine.

Chapter 5

Effect of soil properties on the bioavailability of copper and zinc in eight soils treated with metal-amended biosolids

5.1 Introduction

In Chapters 3 and 4, plants were grown on a biosolids-treated soil and the effects of Cu and Zn on metal bioavailability and soil microbial activity were studied. The fate of Cu and Zn derived from the biosolids was also studied by investigating the metal fractionation in the soil. These studies showed that the bioavailability and the dynamics of the metals added to the soil were different between Cu and Zn. However, these studies were conducted only on one soil type. The potential distribution of Cu and Zn into different chemical fractions in biosolids-amended soil is controlled by the chemical characteristics of each metal, and the chemical and physical characteristics of the soils, especially pH and the content and nature of clay and organic matter. For example, Heemsbergen *et al.* (2010) showed that the bioavailability of biosolids-borne Zn, as determined by CaCl_2 -extraction, was negatively correlated with both pH and clay content in 12 soil types amended with biosolids in Australian agricultural lands. However, they did not find any relationship between soil properties and CaCl_2 -extractable Cu. McBride *et al.* (1997) investigated the effect of soil properties on the availability of metals in several soils including soils previously treated with biosolids. They reported that Cu solubility was negatively correlated with both soil pH and organic matter content, while Zn solubility was negatively correlated only with pH.

It has been observed that metal availability is generally greatest immediately following biosolids applications to soil due to the release of metal ions through the decomposition of soluble/readily degradable organic matter, and the

dissolution of unstable inorganic compounds (Smith 1996). This initial peak is commonly followed by a reduction in metal availability. This may indicate a lessening in importance of biosolids properties and a predominance of soil factors in controlling metal availability, or may be due to the biosolids matrix itself promoting metal retention with increased time after biosolids application (Udom *et al.* 2004). As soil properties influence the availability of metals derived from biosolids, it was of interest to investigate how differential metal availability, as a function of soil properties, influences plant uptake of the metals. However, it is not practical to conduct glasshouse trials similar to the ones described in Chapters 3 and 4 on several soils. Instead, laboratory incubation trials are a viable option to model the relationships between soil properties and the bioavailability of biosolids derived metals.

There are few studies in the literature that assess the chemical forms of different metals in biosolids amended soils after different incubation times. Sánchez-Martín *et al.* (2007) conducted an incubation study with different rates (20 and 200 g biosolids/kg soil) of biosolids were incorporated into soils and incubated for 18 months to determine the distribution of the metals (including Cu and Zn) in different fractions with incubation time. They reported that the exchangeable fraction of Cu and specifically sorbed fractions of Zn were significantly reduced at 18 months after incubation. Zhao *et al.* (1999) conducted a short-term (2 week) incubation study using 72 soils collected from three field experiments where different types and rates of biosolids were used. They reported that the soil solution metal (Cu, Zn, Cd and Ni) concentrations were positively correlated with total soil metal concentrations, soil organic carbon and dissolved organic carbon, and negatively correlated with soil pH. In New Zealand, Gray and McLaren (2006) conducted an experiment on soil factors affecting heavy metal solubility (including Cu and Zn) by using 202 soils sampled from sites that had different types and amounts of heavy metal contamination. They reported that the water soluble Cu concentration was positively correlated only with total Cu, but water soluble Zn was positively correlated with total Zn and negatively correlated with pH and amorphous Fe. Wightwick *et al.* (2010) reported that concentrations of 0.01 M CaCl₂-extractable Cu, measured in surface soils collected from 98 vineyards in 10 different grape-growing regions of Australia, ranged from <0.1 to

0.94 mg Cu/kg and accounted for 0.10–1.03% of the total Cu concentration in the soils. Differences in the CaCl_2 -extractable Cu concentration were positively related to soil properties such as the exchangeable K concentration, %silt, and % CaCO_3 , and negatively related to pH and %clay.

Kabala and Singh (2001) conducted an experiment with four soil profiles (silt, clay-loamy, and sandy soils) located near a Cu smelter in Poland, to investigate the distribution and chemical fractions of Cu, Pb, and Zn and their mobility in relation to soil properties. In the fine-textured soil, the metals were distributed in the order: residual \gg Fe-Mn oxides occluded $>$ organically complexed $>$ exchangeable and specifically adsorbed, while the order of distribution for sandy soils was: residual $>$ organically complexed $>$ Fe-Mn oxides occluded $>$ exchangeable and specifically adsorbed, for both Cu (total soil Cu 97–426 mg/kg) and Zn (total soil Zn 47–51 mg/kg). The difference in distribution order was mainly due to higher Fe/Mn oxide contents in clay soils. They also reported that the ratio of available Cu and Zn to total metal concentration was correlated negatively with the clay content.

The objective of this study was to examine the relationships that exist between soil properties and the chemical fractionations of Cu and Zn in soils amended with biosolids in a laboratory incubation experiment, with eight soils of contrasting properties. Particular attention was paid to the bioavailable exchangeable fraction.

5.2 Materials and methods

A laboratory incubation study was set up with eight soils collected from across the North Island of New Zealand. Selected properties of the soils are given in Table 5.1 and 5.2. The soil samples were collected from the surface horizon (0–10cm), sieved through a 2 mm stainless steel sieve while still field moist, and then air-dried, homogenized and stored for subsequent analysis and the incubation study.

5.2.1 Laboratory incubation setup

Sub-samples of each soil (equivalent to 500 g air dry soil) were amended with biosolids previously spiked with Cu or Zn sulphate to generate a final soil metal concentration equivalent to 150 mg Cu/kg or 450 mg Zn/kg (the Cu₂ and Zn₂ preparations as described in Chapter 2). The control treatment was soil amended with biosolids not spiked with the metals (Cu₀ and Zn₀). Each soil sample was spread in a tray and a calculated volume of spiked biosolids was poured onto the soil and hand mixed to ensure uniform and thorough mixing of biosolids with the soil. The three treatments (control, 150 mg Cu/kg and 450 mg Zn/kg) for each of the eight soils were replicated six times (to allow for duplicate samples to be taken at three sampling times) in plastic bags, (200 mm x 100 mm). Soil moisture was maintained at 60% 'pot field capacity' throughout the incubation. Sufficient micro holes were made in the plastic bags to ensure aerobic conditions were present in the soils. The amended soils were then incubated at 24°C inside an incubator with periodic inspection for moisture content and air circulation. Moisture content was brought to the 60% 'pot field capacity' value if required.

5.2.2 Particle size distribution

The particle size distribution of each soil was determined by using the ultrasonic–dispersion procedure and the pipette method based on the method of Claydon (1989). As a pre-treatment, calcium carbonate was removed by adding 1 M HCl, and then organic matter oxidation was carried out with 30% (v/v) H₂O₂. Dispersion was conducted using an ultrasonic probe to a depth of 1 cm in the soil suspension run for 5 minutes.

5.2.3 Chemical analysis

Acid oxalate–extractable Fe and Al, and dithionite–citrate–extractable Fe and Al concentrations in the control soil were measured using methods described by Blackmore *et al.* (1987). Ammonium acetate extractable Fe and Al in the control soil was measured using method described by McLean *et al.* (1958). For the acid oxalate extraction, 0.02 M ammonium oxalate buffered to pH 3.25 with oxalic

acid was used and extracted for 4 hours (in darkness). Dithionate–citrate extraction was carried out by 1 g sodium dithionate and 22% sodium citrate solution and extracted for 16 hours. Ammonium acetate Fe and Al extractions were carried out at pH 4.8. CEC and exchangeable bases were determined by using the method of ammonium acetate leaching at pH 7 as described by Blackmore *et al.* (1987). Soil organic carbon was measured using a Shimadzu TOC–5000 analyzer (Wu *et al.* 1990).

Subsamples were taken from each bag at 0, 147 and 257 days after incubation, and the soil pH, total soil metal concentration and metal fractionation in soils were measured. The methods of analysis and quality control for the relevant parameters were presented in Chapter 3.

5.2.5 Data analysis

In addition to the data analysis techniques explained in Chapter 2, a set of simple correlation analysis relating the solid–phase soil metal fractions to various soil properties were performed using SAS[®] 9.1.2 (SAS Institute Inc 2004). A stepwise regression procedure was also used to determine the soil properties that were most significantly controlling the bioavailable soluble+exchangeable metal concentrations of the solid–phase soil metal fractions. The final regression equation model contained only those predictors that were found to have significant effects at 5% significant level on modelled outcomes.

Table 5.1 Physical and chemical properties of biosolids amended soils

| Soil site | Soil classification* | pH | CEC (meq/100g) | Organic C (%) | Total N (%) | C:N ratio | Amorphous (%) | | Crystalline (%) | | Sand (%) | Silt (%) | Clay (%) |
|---|----------------------|-----|-------------------|------------------|-------------------|--------------|---------------|------|-----------------|------|-------------|-------------|-------------|
| | | | | | | | Al | Fe | Al | Fe | | | |
| 1 Kaweka, Napier | Allophanic Soil | 5.5 | 15.90 | 5.33 | 0.30 | 17.7 | 2.29 | 0.84 | 1.55 | 0.41 | 47.0 | 38.5 | 14.5 |
| 2 Egmont, Taranaki | Allophanic Soil | 5.3 | 21.66 | 11.20 | 0.79 | 6.8 | 2.79 | 3.02 | 2.38 | 0.03 | 12.0 | 56.5 | 31.5 |
| 3 Palmerston North** | Recent Soil | 5.9 | 11.63 | 1.22 | 0.11 | 50.0 | 0.15 | 0.37 | 0.04 | 0.15 | 74.0 | 15.5 | 10.5 |
| 4 Tuapaka farm, Palmerston North | Brown Soil | 5.3 | 20.53 | 4.79 | 0.42 | 12.6 | 0.43 | 0.76 | 1.03 | 0.40 | 5.0 | 72.5 | 22.5 |
| 5 Kaimatarau, Rongotea | Recent Soil | 5.7 | 19.98 | 4.77 | 0.38 | 13.9 | 0.84 | 0.82 | 0.92 | 0.06 | 38.0 | 49.5 | 12.5 |
| 6 Taupo | Pumice Soil | 5.4 | 13.64 | 2.79 | 0.14 | 39.1 | 2.13 | 0.99 | 0.37 | 0.25 | 34.0 | 45.5 | 20.5 |
| 7 No.4 dairy farm***, Massey University, Palmerston North | Pallic Soil | 5.9 | 22.07 | 3.56 | 0.36 | 15.0 | 0.20 | 0.39 | 0.15 | 0.22 | 59.5 | 28.0 | 12.5 |
| 8 Tangahoe, Northland | Ultic Soil | 4.9 | 15.02 | 0.92 | 0.05 | 115.2 | 0.55 | 1.21 | 4.19 | 5.81 | 3.5 | 44.0 | 52.5 |

*Hewitt (1998)

** Manawatu silt loam

*** Tokomaru silt loam

Table 5.2 Total and exchangeable metal concentrations (mg/kg) of biosolids amended soils (control) at various incubation periods

| | | Total metal | | Exchangeable metal | | | | | |
|-----------|-------------------------|-------------|-----|--------------------|-----|-----|-----|-----|-----|
| | | Cu0 | Zn0 | Cu0 | | | Zn0 | | |
| | | | | Days | | | | | |
| Soil site | | | | 0 | 147 | 257 | 0 | 147 | 257 |
| 1 | Kaweka, Napier | 12 | 31 | 0.6 | 0.6 | 0.6 | 3.5 | 2.9 | 2.5 |
| 2 | Egmont, Taranaki | 21 | 30 | 0.2 | 0.3 | 0.5 | 1.4 | 0.7 | 0.8 |
| 3 | Palmerston North | 10 | 38 | 0.1 | 0.2 | 0.2 | 5.0 | 4.4 | 3.7 |
| 4 | Tuapaka farm* | 10 | 33 | 0.1 | 0.2 | 0.2 | 1.7 | 1.3 | 0.9 |
| 5 | Kaimatarau, Rongotea | 10 | 30 | 0.1 | 0.2 | 0.2 | 2.6 | 1.7 | 2.3 |
| 6 | Taupo | 15 | 33 | 0.1 | 0.2 | 0.3 | 1.5 | 1.6 | 1.7 |
| 7 | No.4 dairy farm, Massey | 12 | 29 | 0.4 | 0.5 | 0.5 | 3.7 | 2.4 | 3.7 |
| | University* | | | | | | | | |
| 8 | Tangahoe, Northland | 22 | 25 | 0.4 | 0.4 | 0.5 | 4.7 | 4.5 | 3.8 |

* Palmerston North

5.3 Results and discussion

5.3.1 Solid phase fractionation

Figure 5.1 shows the distribution of solid-phase Cu for the control treatment soils (a) and for the added-Cu fractions in the soils of the Cu treatment (b). The added-Cu fractions were calculated by subtracting the Cu fractions in the control treatment from the corresponding fractions in the Cu treatment. The exchangeable and specifically adsorbed Cu fractions were lower than the other fractions in all the control soils (0.5–5.7% and 0.8–10% of the total soil Cu for exchangeable and specifically adsorbed fractions, respectively) (Figure 5.1a). The added-Cu generally showed a low distribution in the exchangeable fraction (0.2–3.9% of

total Cu) (Figure 5.1b) except for the Tangahoe soil where it ranged from 7.9 to 9.9% of the total Cu. The percentage of soil Cu associated with the specifically sorbed fraction was significantly higher in the added-Cu pool (3–18.8%) compared to the control soils. The highest proportions of total Cu were found in the organic bound fraction in both the control (14–54.5%) and ‘added Cu’ (28.7–70.5%) soils. The percentage of added-Cu associated with the residual fraction decreased, and that associated with the organic fraction increased with increasing incubation time. This may be due to the progressive breakdown of recalcitrant organic Cu in the residual fraction with time by the soil microbes, with the subsequent binding of Cu to humus. The oxide fraction showed a large variation among the control soils; however the variation was lower in Cu-added soils. The Tangahoe soil showed the greatest association of Cu with the oxide fraction (63.8–67.6%) in the control incubation and this may be due to the high content of Fe and Al oxides in this soil (Table 5.1). The incubation time did not greatly influence the distribution of Cu between soil fractions in the unamended soil, as reported by Sánchez-Martín *et al.* (2007) in their 18 month incubation study in Spain with soils amended with biosolids, but the soil pH was slightly reduced (by 0.3–0.9) with time. In the current study, increased incubation time reduced pH only by 0.2–0.3 units and therefore did not have a significant effect on the control soils. However, in the Cu treatments, increasing the incubation time reduced the pH by 0.9–1.2 and this may be the reason for the observed changes in distribution of Cu fractions throughout the incubation period. i.e. exchangeable Cu decreased with time.

The distribution of Zn between the different solid-phase fractions of the soil was markedly different to the distribution of Cu. Zinc in the control soils was mainly associated with the residual fraction (18.4–31.8 mg Zn/kg) in all soil types, unlike Cu, which was found mainly in the oxide (1.7–16.2 mg Cu/kg) and organic (2.9–7.7 mg Cu/kg) fractions. The percentage of Zn in the residual fraction as a % of the total Zn in the control soils (65.1–89.1%) was much higher than Cu (10.8–32.6%) in this fraction. This is probably because of the lower affinity of Zn relative to Cu for oxides and organic matter present in soil (McBride 1994), thus making it accumulate in the residual fraction. As was the case for Cu, the greatest concentration of Zn associated with the oxide fraction for the control treatment

was observed for the Tangahoe soil (5–6.5 mg Zn/kg), contributing 16.8–19.8% of the total Zn concentration compared with 2–5.2 % for the other soils (Figure 5.2a). This is likely because this soil had the highest content of Fe and Al oxides among all tested soils. Added-Zn distributed mainly in the exchangeable fraction, and the proportion of Zn associated with the exchangeable fraction (13.9–59.1%) was greater than the corresponding proportion for Cu (0.2–9.9%) (Figure 5.2b). This is likely due to the lower affinity of Zn to organic matter and oxides in soils than Cu. Based on this result, biosolids-derived Zn is expected to be much more available to plants and microorganisms than Cu. This is consistent with the findings in Chapter 3 and 4 on the relative toxicity of these two metals to plants and microorganisms. As was the case for Cu, the Tangahoe soil had the highest percentage of added Zn associated with the exchangeable fraction (209.7–242 mg Zn/kg) relative to the other soils, contributing 50–59% of the total added-Zn. Unlike Cu, where the residual fraction in the Cu-added soils decreased with time, for the Zn-added soils the residual fraction increased with time. The increase in residual fraction with time is probably due to Zn in other fractions, especially organic bound Zn, becoming recalcitrant or diffusing into the crystal lattice with increasing time.

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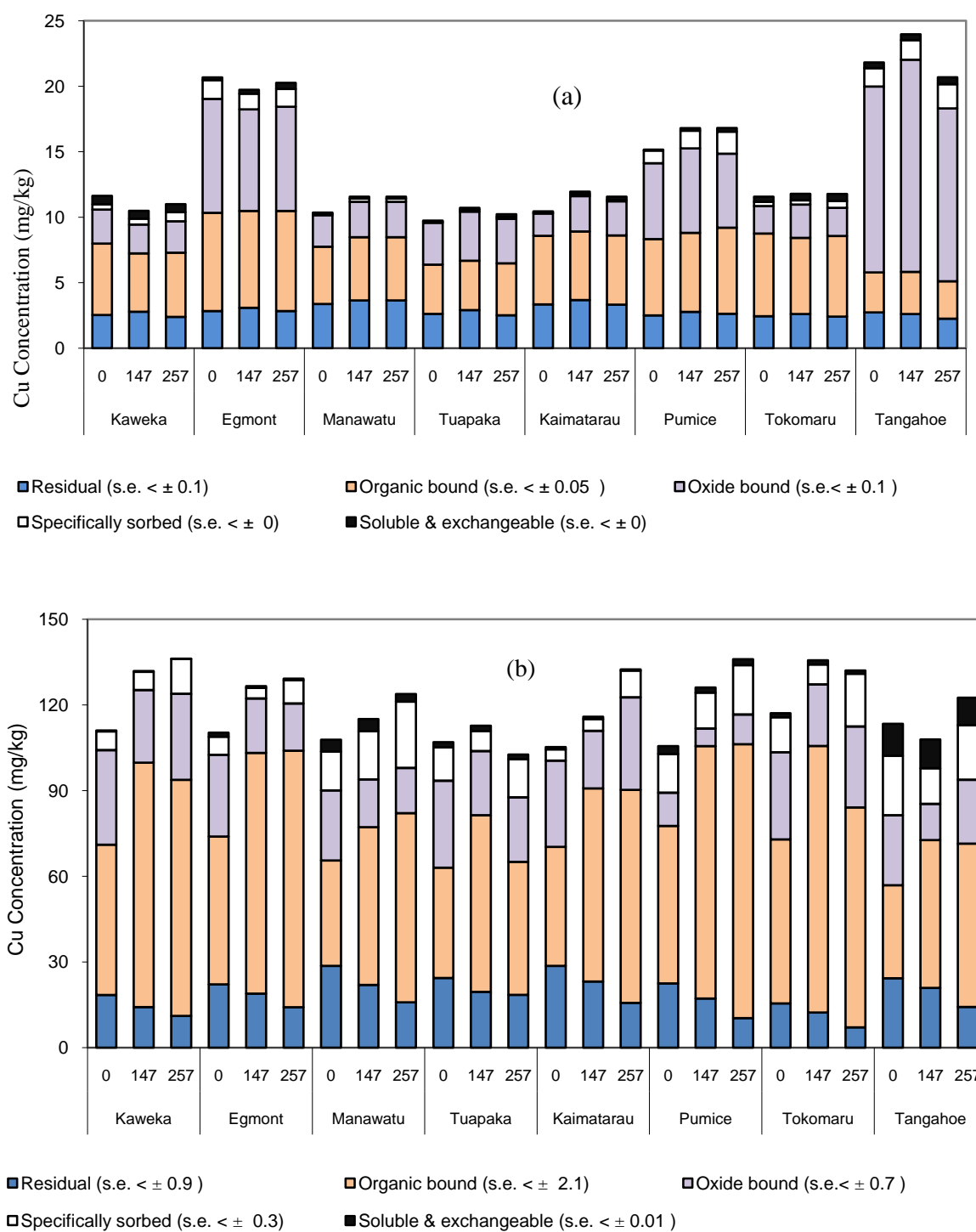


Figure 5.1 Distribution of Cu in various soil fractions in the eight soils amended with biosolids. (a) Control and (b) added Cu after 0, 147 and 257 days of incubation (s.e.=standard error, $n=2$). Added Cu = Cu treatment minus Control

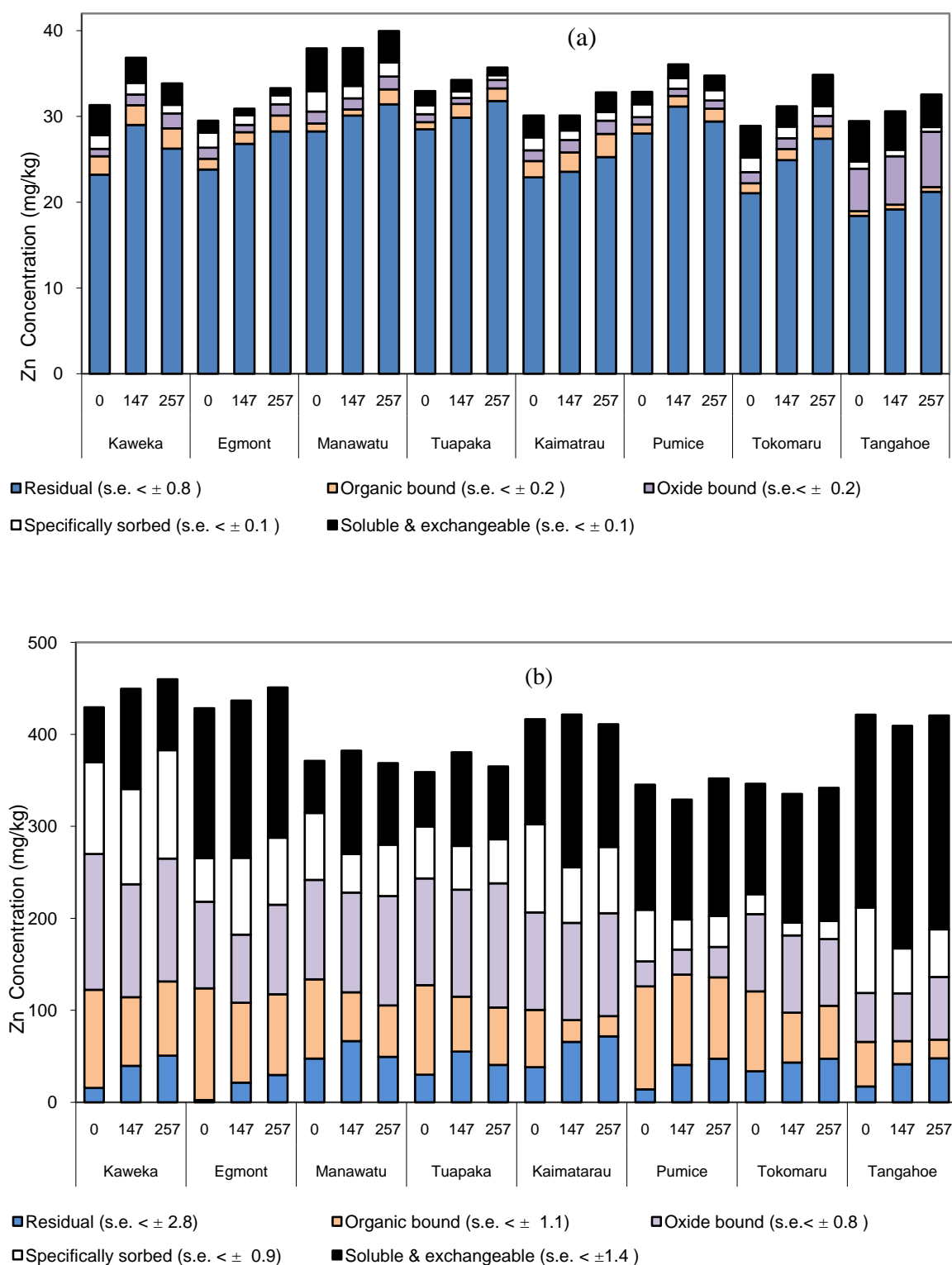


Figure 5.2 Distribution of Zn in various soil fractions in eight soils amended with biosolids. (a) Control and (b) added Zn after 0, 147 and 257 days of incubation (s.e.=standard error, $n=2$). Added Zn = Zn treatment minus Control

5.3.2 Correlation study

As exchangeable Cu and Zn fractions are considered to be the immediately bioavailable Cu and Zn fractions, these fractions were correlated with soil properties. To determine the soil properties that control the concentration of 'added' metals in the exchangeable fraction, Pearson's correlation coefficients were calculated for each property (Table 5.3 and 5.4). The exchangeable Cu did not significantly correlate with any of the soil properties in the control treatments. This may be due to the extremely low exchangeable Cu concentration in the soils (0.1–0.6 mg/kg: 1–5% of total Cu) as well as the influence of the parent materials of the soils, rather than its association with other materials and processes such as anthropogenic contaminants and climate. However, correlations were observed for the Cu-added soil treatment, where the exchangeable Cu range was 0.3–11.2 mg/kg: (0.3–11 % of total Cu). Correlations were positive and highly significant for the exchangeable Cu concentration with crystalline Fe oxide, crystalline Al oxide and clay contents ($r=0.62$ – 0.97); but negative for pH and organic C with moderate to high correlation coefficients ($r=-0.51$ – -0.65) at all incubation periods.

In general, when crystalline Fe and Al oxides concentrations are high, Cu ions are expected to be strongly adsorbed onto the oxide surfaces, mostly in non-exchangeable forms (Khaodhiar *et al.* 2000; Saha *et al.* 1991; Undabeytia *et al.* 2002). This would therefore result in negative correlations between the soil exchangeable Cu concentration and the crystalline Fe and Al oxide contents. However, in the present study, the positive correlations obtained for exchangeable Cu with crystalline Fe and Al oxides do not seem to be consistent with this theory. This may be because the soil concentrations of these oxides were strongly correlated with clay content (Table 5.4, $r=0.86$ for Fe oxide and $r=0.91$ for Al oxide) which provided increased negative charge to cause an increase in the concentration of exchangeable metals. In order to determine which of these soil properties is the main factor controlling exchangeable Cu, a stepwise regression analysis was conducted using these three soil properties. The regression analysis showed that the Fe oxide and Al oxide contents were the controlling soil factors, and that among these two factors, Fe oxide was the main controlling soil factor

contributing to 90% of the variability of exchangeable Cu. The stepwise regression equations are follows;

$$\text{Exchangeable Cu} = 1.7 \text{ FeCr} + 1.6$$

$$r^2=0.895***$$

$$P < 0.0001$$

$$\text{Exchangeable Cu} = 2.2 \text{ FeCr} + 0.8 \text{ AlCr} + 2.3$$

$$r^2=0.929*$$

$$P = 0.026$$

where; FeCr = Crystalline Fe%, AlCr = Crystalline Al%

The reason for the positive correlation of Fe oxide with exchangeable Cu is not clear. It is possible that Cu was strongly adsorbed in a non-exchangeable form, but the 1M $\text{Mg}(\text{NO}_3)_2$ used in the first step of the sequential fractionation procedure (Chapter 2) was able to extract this non-exchangeable form because of the high concentration of the Mg^{2+} in the extraction solution. Another possibility is that Fe oxide forms a coating on the clay minerals thus blocking the negative charges in the clay minerals, and that these oxides were removed by the organic matter in the added biosolids which complexed with the Fe and Al in the oxides, thereby exposing the negative charges in clay particles accessible for exchangeable Cu adsorption.

It may be that the high correlation coefficients obtained for crystalline Fe and Al obtained in this study were due to the extremely high values of crystalline Fe oxide (5.8%) and crystalline Al oxides (4.2%) contents of the Tangahoe soil compared to other soils. In order to test this, the correlation and stepwise regression procedures were repeated by removing the Tangahoe soil from the analysis and using only the other seven soils. The stepwise regression results with the seven soils showed that exchangeable Cu did not correlate with crystalline Fe oxide. The regression analysis here showed that the crystalline Al oxide and CEC were the controlling soil factors, but with a very low coefficient of determination ($r^2=0.54$). Future studies are necessary with a set of much larger number of soils having a range of crystalline Fe oxides to confirm these results.

In the Zn treated soil, as observed for Cu, exchangeable Zn was positively correlated with the crystalline Fe and Al oxides and the clay content of the soils ($r=0.66-0.80$), and negatively correlated with soil pH for all sampling times (Table 5.3). As for Cu, stepwise regression analysis was conducted to determine which of these soil properties is the main factor controlling exchangeable Zn variation, because the soil properties are interrelated (Table 5.4). The regression analysis showed that clay content and pH were the controlling soil factors contributing to explaining 73% of the variability of exchangeable Zn. The stepwise regression equations for these relationships are as follows;

$$\text{Exchangeable Zn} = 1.5 \text{ Clay} + 40$$

$$r^2=0.576***$$

$$P = 0.0006$$

$$\text{Exchangeable Zn} = 2.9 \text{ Clay} - 62.7 \text{ pH} - 336.9$$

$$r^2=0.727*$$

$$P = 0.019$$

where; Clay = Clay%, pH = Soil pH

With increasing clay content and pH the negative charges on organic matter and metal oxides and clay edges increase, thus increasing the exchangeable Cu and Zn concentration in soil.

Table 5.3 Simple linear correlation coefficients (r) for exchangeable metals versus properties of eight soils

| | 0 day | | | | 147 days | | | | 257 days | | | |
|--|-------|---------|----------|---------|----------|---------|----------|---------|----------|---------|----------|---------|
| | Cu0 | Cu1 | Zn0 | Zn1 | Cu0 | Cu1 | Zn0 | Zn1 | Cu0 | Cu1 | Zn0 | Zn1 |
| pH | -0.20 | -0.65** | 0.20 | -0.55* | -0.12 | -0.53* | 0.01 | -0.52* | -0.23 | -0.54* | 0.28 | -0.49 |
| CEC | -0.01 | -0.38 | -0.47 | 0.07 | 0.11 | -0.44 | -0.66** | 0.02 | 0.12 | -0.43 | -0.39 | 0.02 |
| C | -0.04 | -0.51* | -0.65** | 0.02 | 0.03 | -0.61** | -0.74*** | -0.11 | 0.19 | -0.59* | -0.72*** | -0.12 |
| Alam | 0.17 | -0.27 | -0.57* | 0.15 | 0.19 | -0.42 | -0.50* | -0.05 | 0.31 | -0.39 | -0.59* | 0.01 |
| Feam | -0.05 | 0.04 | -0.49* | 0.49* | 0.00 | -0.06 | -0.46 | 0.36 | 0.23 | -0.07 | -0.56* | 0.37 |
| Alcr | 0.40 | 0.73*** | 0.15 | 0.68** | 0.37 | 0.62** | 0.22 | 0.78*** | 0.48 | 0.67** | 0.04 | 0.66** |
| Fecr | 0.38 | 0.97*** | 0.48 | 0.68** | 0.32 | 0.92*** | 0.59* | 0.80*** | 0.33 | 0.95*** | 0.46 | 0.73*** |
| Silt | -0.23 | -0.07 | -0.75*** | 0.10 | -0.26 | -0.18 | -0.67** | 0.03 | -0.17 | -0.17 | -0.81*** | 0.00 |
| Clay | 0.20 | 0.85*** | 0.07 | 0.78*** | 0.18 | 0.75*** | 0.20 | 0.79*** | 0.32 | 0.77*** | 0.02 | 0.76*** |
| * $P < 0.05$ ** $P < 0.01$ *** $P < 0.001$ | | | | | | | | | | | | |

Table 5.4 Simple linear correlation coefficients between the properties of eight soils

| | pH | CEC | C | AlAm | FeAm | AlCr | FeCr | Silt |
|--|----------|--------|---------|--------|-------|---------|---------|------|
| CEC | 0.06 | | | | | | | |
| C | −0.07 | 0.64** | | | | | | |
| AlAm | −0.30 | 0.04 | 0.68** | | | | | |
| FeAm | −0.46 | 0.33 | 0.80*** | 0.70** | | | | |
| AlCr | −0.83*** | 0.02 | 0.12 | 0.19 | 0.52* | | | |
| FeCr | −0.70** | −0.27 | −0.45 | −0.24 | 0.04 | 0.82*** | | |
| Silt | −0.60** | 0.51* | 0.50* | 0.29 | 0.45 | 0.32 | 0.02 | |
| Clay | −0.89*** | −0.04 | −0.01 | 0.10 | 0.49* | 0.91*** | 0.86*** | 0.35 |
| * $P < 0.05$ ** $P < 0.01$ *** $P < 0.001$ | | | | | | | | |

5.4 Conclusions

Cu applied to soil in the form of Cu-amended biosolids generally showed the lowest proportion in the exchangeable fraction (0.3–11.1% of total Cu) among the soil Cu fractions. The highest proportion of total soil Cu was found in the organic bound fraction (21.5–61.4%). The percentage of Cu-added to the soil that was associated with the residual fraction decreased with time, while that associated with the organic bound fraction increased with increasing incubation time, possibly due to the breakdown of recalcitrant organic Cu in the residual fraction by microbes, with the subsequent binding of Cu to humus.

The distribution of Zn to the solid phase fractions of the soil differed to that of the Cu. Added-Zn was associated mainly with the exchangeable fraction (13.9–59.1% of total Zn), and this fraction accounted for a higher proportion of soil Zn than Cu (0.3–11.1% of total Cu). This is likely due to the lower affinity of Zn for organic matter and oxides in soils than Cu, and indicates that biosolids-derived Zn is expected to be much more available to plants and microorganisms than Cu.

Regression analyses showed that the main soil properties controlling exchangeable Cu were the contents of crystalline Fe oxide and Al oxide. Among

these two soil properties, Fe oxide was the dominant factor and contributed to explaining 90% of the variability of exchangeable Cu. Regression analysis showed that clay content and pH were the controlling soil factors that contributing to explaining 73% of the variability of exchangeable Zn. The relationship of exchangeable Cu with soil properties were influenced largely by one soil which had very high Fe and Al oxide contents. Future studies need to use larger number of soils having a wide range of Fe and Al oxide contents to confirm these results.

Chapter 6

Effect of lime and Al waste on poplar yield, and Cu and Zn uptake from a biosolids–amended soil

6.1 Introduction

In previous chapters the toxic effects of biosolids–derived Cu and Zn on the growth of poplar and pine, and on the microbial activity of the soil, including mycorrhizal colony development, were studied. Chapter 5 presented information on the soil properties that influence Cu and Zn availability. All these studies aimed to investigate the toxic effects of Cu and Zn through the use of soil solid and solution phase speciation procedures. The results suggested that different threshold toxic levels of Cu and Zn species exist for plant growth and microbial activity. It was found that many of the biosolids–amended soils have the potential to cause Cu and Zn toxicity to plants and microorganisms. Therefore, it is important to find suitable techniques that can manage the excessive bioavailability of these metals, and thereby mitigate the apparent risk to both the environment and to human health through food chain.

Several techniques have been proposed to manage or remediate metal–contaminated soils. One of these is chemical immobilisation, an *in situ* remediation method in which inexpensive materials are added to contaminated soil to reduce the solubility of heavy metal contaminants. This may then alleviate environmental risk (Brown *et al.* 1996; Pierzynski and Schwab 1993). The technique involves the addition of chemicals to contaminated soils to reduce the solubility of metals through metal sorption and/or precipitation reactions. The application of soil amendments to immobilise heavy metals is considered to be a promising technology to meet requirements for environmentally sound and cost–effective remediation (Friesl *et al.* 2004).

Liming of acidic soils is an ancient agricultural practice to ameliorate soil acidity. Limestone (calcite or dolomite or a variable combination of the two minerals) is the main liming material used, although quicklime, hydrated lime and by-products such as slag and gypsum have also been used (Adriano *et al.* 2002). From a review of the literature, Bolan *et al.* (2008) summarised that liming acid soils affects the biotransformation of metals and their subsequent bioavailability through a number of processes. These include pH-induced changes in heavy metal speciation, pH-induced changes in metal sorption/desorption, the competitive effect of lime-derived Ca^{2+} on metal sorption, pH-induced mobilization of DOC and its subsequent effect on the chelation of metals, changes in redox, and methylation or demethylation reactions.

Some industrial by-products have also been used to ameliorate metal toxicity in soils (Brunori *et al.* 2005; Gheshlaghi *et al.* 2008; Illera *et al.* 2004; Lombi *et al.* 2004). For example, Gheshlaghi *et al.* (2008) conducted a glasshouse study on the effect of treated zeolite, Fe waste (rates 5% and 10% w/w) and lime (0.33 and 0.66% w/w) on the phytoavailability of Cu, Zn and Ni to sunflower grown in long-term biosolids-amended soils in Christchurch, New Zealand. They reported that Cu (total soil Cu 83–177 mg/kg) availability in soils and the Cu concentration in plants was not significantly affected by any amendment. However, for Zn (total soil Zn 323–435 mg/kg) the shoot Zn concentration and soluble soil Zn concentration were reduced by 73–91% and >90%, respectively, and DM was increased by 63% for the combined application of lime (0.33–0.66%) and Fe waste (5–10%) materials.

Aluminium industry waste can potentially be used as a low-cost adsorbent for heavy metal remediation. Aluminium smelters such as that at Tiwai Point in Southland, New Zealand, generate a large quantity of aluminium dross by-product which requires disposal or re-use. Dross contains Al oxides which are known to have a high adsorption capacity for heavy metals, and is highly alkaline (pH 10.1). This material could therefore be expected to immobilise heavy metals and thus reduce the bioavailability of these contaminants in the soil. Despite this

potential, there have been no studies reported on the effects of Al dross on heavy metal solubility, and phytoavailability, when applied to contaminated soils.

There are, however, many studies reported in literature that describe the effect of lime on the response of soil microorganisms to metal inputs to soil (Garau *et al.* 2007; Kostov and Van Cleemput 2001; Lee *et al.* 2008). Garau *et al.* (2007) conducted a laboratory incubation study (6 months) to compare the efficiency of red mud (a bauxite residue–4%), natural zeolite (10%) and lime (0.5%), to immobilize heavy metals present in a polluted acidic soil (Pb 3266, Cd 35.4 and Zn 1495 mg/kg), and to measure the effect of these amendments on soil microbiological properties. The addition of lime or red mud significantly reduced the solubility of Zn (from 244 to 15 mg/kg) by increasing the soil pH from 4.2 to 7.1 (both amendments), but zeolite reduced Zn solubility only to 205 mg/kg because of an associated negligible increase of soil pH (4.8). The reduction of Zn solubility by lime significantly increased the urease and dehydrogenase activities of the soil by 142 and 42%, respectively.

Few studies have investigated the effect of liming on the extent of ectomycorrhizal fungi infection of plants. The effects of several soil amendments (1% Lime, 5% biosolids compost, 7.5% natural zeolite) were studied by Simon *et al.* (2006) with red fescue grown in soil contaminated with mine spoil (pH 3.7, Cu 336 and Zn 3306 mg/kg), that was infected with Zn–tolerant arbuscular mycorrhizal fungi (AMF). They reported that during the 12 weeks after treatment of the soil with amendments, the rate of AMF infection frequency was low for the lime–only treatment (4%), but was high in soil that received a combined treatment of all three amendments (93%). The shoot concentration of Cu was reduced by 9–34% and Zn by 22–44% for the combined treatment relative to the control. The corresponding value for the lime–only treatment was not consistent for shoot Cu concentration, but the shoot Zn concentration was reduced by 9–44% relative to the control.

Studies on the effect of soil amendments on the amelioration of the toxic effect of Cu and Zn in biosolids-amended soils to microorganisms and poplars are rare in the literature. Therefore, a study was conducted with the objective to examine the potential of Al dross to ameliorate Cu and Zn contamination in biosolids-amended soils, both without, and in combination with, a liming material. The ameliorating influence of the lime-only treatment was also studied. Evaluation of the various treatments was carried out by examining the effects on Cu and Zn bioavailability to poplar plants, ectomycorrhizal fungus, and soil microbial activity.

6.2 Materials and methods

6.2.1 Soils

Soil for this experiment was obtained from the soils amended with biosolids spiked with three levels of Cu or Zn used in the previous trials with poplar and pine (Chapter 2 and 3). The Cu1, Cu2 and Cu3 and Zn1, Zn2 and Zn3 treatment levels were mixed together for each metal in a cleaned concrete mixer to make a bulk Cu or Zn soil, and then sieved to pass through a <5 mm stainless steel sieve. The samples were then air-dried at 28⁰C in the glass house. Subsamples were taken and ground to pass through a 2 mm stainless steel sieve prior to laboratory analysis. The bulk soils were stored in polyethylene bags until being used for the pot experiment constituting this chapter. The total Cu and Zn concentrations of the soil mixtures (for Cu 144 and Zn 417 mg/kg soil) were both above the current guideline limits (100 mg/kg for Cu and 300 mg/kg for Zn, (NZWWA 2003) for biosolids-amended soils.

6.2.2 Amendments

Two soil amendments were used in this study: Al dross, and lime material. The lime material was obtained from Ravensdown Fertiliser Cooperative and contained 72% of CaCO₃. The Al dross was a by-product from New Zealand

Aluminium Smelters Limited (NZAS) in Tiwai Peninsula, Southland, New Zealand. The particle size range of this material was 5 mm–100 mm. The average percentage of Al was 0.21%. The concentrations of major elements present in the Al dross as determined by 65% HNO₃ digestion (60°C) followed by ICP/MS analysis, are presented in Table 6.1. As expected, the Al concentration was the highest, but much lower than the total Al content of 53% reported by Manfredi *et al.* (1997). The low recovery of Al obtained in the current study is probably due to the weak acid used for the digestion. The pH was measured as 10.1.

Table 6.1 The average conc. (mg/kg) of chemical elements in the Al dross sample

| Ca | K | Na | Mg | Fe | Al | B | Cu | Ni | Zn |
|----|----|-----|-----|----|------|-----|-----|-----|------|
| 30 | 11 | 143 | 1.2 | 21 | 2133 | 1.3 | 3.3 | 0.4 | 0.05 |

6.2.3 Glasshouse trial

A glasshouse trial was conducted using poplar (*Populus deltoides* x *yunnanensis*) in pots containing various levels of lime and Al dross added to the soil (12 kg air-dried soil) that was previously amended with biosolids spiked with Cu or Zn. The control treatment was the Cu or Zn spiked soil, but with no lime or Al dross added. Poplar cuttings for the pot experiment were obtained from the ‘Tree line nursery’ plantation, a division of RST Environmental Solutions, Palmerston North, New Zealand.

Two weeks prior to the trial, the soil (as explained in 6.2.1) for each pot was spread into a large tray. A calculated weight of lime or Al dross and essential nutrients (equivalent to 50 kg N, 30 kg P, 30 kg K and 30 kg Mg/ha) was spread onto the soil and hand mixed to ensure uniform and thorough mixing of the applied treatments. The trial treatments were; Control (C—not treated with amendments), 0.1% lime (L1), 0.5% lime (L2), 2% Al dross (A1), 6% Al dross (A2), and 0.1% lime+2% Al dross (L1A1) for both Cu (6 treatments) and Zn (6 treatments). These rates were selected based on a 2 weeks laboratory incubation study (Appendix) that was conducted prior to the planning of this experiment. The

rates which increased soil pH from approximately 6.5 to 7.0 were selected as the higher rates and half or one-third of these rates were selected as the lower rates for the trial. The 6 treatments were replicated three times and arranged in a Randomized Complete Block Design (RCBD) in a glasshouse for each metal.

Poplar cuttings (20 cm length) were previously grown to form roots in black plastic bags (200 mm x 100 mm) containing a mixture of soil obtained from the same poplar plantation (to provide a source of mycorrhizae hyphae) and potting mixture in the ratio of 1:1. The cuttings with roots were transplanted, one per pot, and regularly watered to maintain soil moisture at 80% 'pot field capacity'. The glasshouse temperature was maintained at $13\pm 2^{\circ}\text{C}$ minimum (night) and $24\pm 2^{\circ}\text{C}$ maximum (day) during the experiment. After 142 days of plant growth the experiment was concluded, and plant shoots, roots and mycorrhiza, and soil samples from each pot, were progressively collected. The following parameters were measured: total metal concentration, soil metal fractions (soluble and exchangeable, specifically adsorbed fractions only), pH, microbial biomass carbon (MBC) and dehydrogenase activity in rhizosphere soils, as well as poplar foliar and root DM yields, mycorrhizae hyphal density, and shoot and root metal concentrations. The sampling procedure, methods of analysis, quality control and data analysis were as discussed in Chapter 3 except for MBC. In addition, exchangeable Al of the post-harvest rhizosphere soil was determined by 1M KCl extraction (soil:KCl=1:5, overnight stand) using the method described by Blackmore *et al.* (1987). Plant available Al was determined through extraction of the rhizosphere soil with 0.1M CaCl_2 using the method described by Houba *et al.* (2000) (Soil: CaCl_2 =1:10, extracted for 2 hrs). The Al in the extract was analyzed by flame atomic absorption spectrometry with N_2O /air gas burner.

6.2.4 Microbial biomass carbon (MBC)

Microorganisms are generally considered as the driving force or catalyst behind the organic matter decomposition process (Smith and Paul 1995). In this sense, the magnitude of MBC may indicate the potential rate of C flux (Franzluebbers *et*

al. 1999a; 1999b). Several techniques are now available to measure MBC, including chloroform fumigation–incubation (CFI), chloroform fumigation–extraction (CFE) and substrate–induced respiration (SIR) methods (Martens 1995).

The CFE method was used in the current study. In this method, the magnitude of soil microbial biomass is estimated by measuring organic C in extracts following the disruption of cell membranes with chloroform. This fumigation direct extraction method is recommended for measuring soil MBC in acid soils, freshly sampled soils and soils recently amended with substrates (Tate *et al.* 1988). Gregorich *et al.* (1990) have calibrated and modified the extraction method of Tate *et al.* (1988). The method of Gregorich *et al.* (1990) was adopted in this experiment. The soil samples were divided into 2 batches and the first batch was treated as a control (no CHCl₃ added), equivalent to 25 g oven–dry soil extracted with 50 ml 0.5 M K₂SO₄. The second batch was extracted after the addition of 1 ml of ethanol–free CHCl₃ pipetted directly onto the soil. Extractions were conducted by shaking soil suspensions for 30 min on a mechanical shaker and then bubbling CO₂ free air through the samples to remove CHCl₃. The soil organic C in the extractant was quantified using a Shimadzu TOC–5000 analyser (Kyoto, Japan) (Wu *et al.* 1990).

6.3 Results and discussion

6.3.1 Poplar yield and metal accumulations

Table 6.2 shows the poplar root and leaf dry matter yield and metal concentration (Cu or Zn) for the various treatments.

In Cu contaminated soil, application of Al dross at all levels significantly decreased root DM yield, but the yield was not significantly influenced by lime application. The lowest root DM was observed for the CuA2 treatment. However, the amendments did not have any readily desirable significant effect on leaf DM

yield with the exception of CuL1A1 which promoted a 17% increase in leaf DM relative to the control.

Amendment with lime and Al dross at all levels of treatment had a major effect on the root and leaf Cu concentration for the contaminated soils, significantly decreasing the Cu concentration by up to 58% in roots and 56% in leaves relative to the control treatment. The leaf Cu concentration for all plants was above the critical Cu concentration of 1–5 mg/kg generally considered the limit for Cu deficiency in plants (Robson and Reuter 1981) and below the phytotoxic range of 25–40 mg/kg as defined by Chaney (1989). Therefore the plants were neither deficient nor toxic to Cu for all treatments level. However, the results indicate that Cu uptake was reduced by the addition of both the ameliorants, suggesting that future Cu toxicity due to continuous application of Cu in biosolids can be prevented by these ameliorants. But despite Cu not exceeding a toxicity threshold, a burning of leaf edges were observed in the lower leaves of poplar for the CuA2 treatment. This observation, in combination with reduced root DM for both the Al dross treatments, suggests a non-Cu related toxicity of the treatments, possibly Al toxicity arising from high levels of Al addition in Al dross.

Root DM yield was increased with lime application to the Zn contaminated soil, but the increase was only significant for ZnL2 (40% increase) relative to the control. However, the effect of Al dross application on root DM for the Zn contaminated soil was similar to that for the Cu contaminated soil (reduction in root DM). However, this effect was only significant for ZnA2 (79% decrease) relative to the control. The trend of the effect of lime and Al dross application on leaf DM production was more apparent for the Zn contaminated soil than for the Cu contaminated soil. The addition of lime increased the leaf DM as it did for root DM, but the effects were small and not significant. However, Al dross significantly decreased leaf DM (from 18 to 57% decrease) in the Zn contaminated soil at both rates of application. Even though ZnA1 decreased leaf DM, ZnL1 had no effect, and the combination of these two ameliorants (ZnL1A1) significantly increased leaf DM content relative to the control.

Table 6.2 Effect of lime (L) and Al dross (A) applications on poplar DM yield and metal concentration

| Treatments | Dry matter (g/pot) | | | | Metal conc. (mg/kg) | | | | Metal conc. Ratio (Leaf:Root) | | BCF | |
|------------|--------------------|----|------|----|---------------------|---|--------|----|----------------------------------|----|------|----|
| | Leaf | | Root | | Leaf | | Root | | | | | |
| Cu C | 56.3 | bc | 24.1 | a | 17.2 | a | 129.2 | a | 0.1 | a | 0.12 | a |
| Cu L1 | 55.6 | c | 20.6 | a | 13.0 | b | 98.9 | b | 0.1 | a | 0.09 | b |
| Cu L2 | 61.9 | ab | 22.6 | a | 8.9 | c | 82.5 | bc | 0.1 | a | 0.05 | c |
| Cu A1 | 56.6 | bc | 14.1 | b | 12.7 | b | 86.5 | bc | 0.1 | a | 0.09 | b |
| Cu A2 | 51.9 | c | 10.0 | b | 7.6 | c | 54.7 | d | 0.2 | a | 0.06 | c |
| Cu L1A1 | 66.0 | a | 13.7 | b | 11.4 | b | 75.9 | c | 0.2 | a | 0.08 | b |
| Zn C | 60.1 | b | 14.0 | bc | 3147.9 | a | 1108.9 | a | 2.9 | a | 7.5 | a |
| Zn L1 | 61.6 | b | 16.8 | ab | 1963.5 | b | 1064.5 | a | 1.9 | b | 4.7 | b |
| Zn L2 | 63.9 | b | 19.6 | a | 667.6 | d | 504.2 | c | 1.3 | bc | 1.6 | d |
| Zn A1 | 49.2 | c | 11.2 | c | 1019.7 | c | 703.3 | b | 1.5 | bc | 2.4 | c |
| Zn A2 | 25.9 | d | 2.9 | d | 176.8 | e | 219.4 | d | 0.9 | c | 0.5 | e |
| Zn L1A1 | 77.3 | a | 12.7 | c | 491.0 | d | 385.9 | cd | 1.4 | bc | 1.3 | de |

Values in columns followed by different letters are significantly different ($P \leq 0.05$)

The root Zn concentration was significantly decreased for all treatments (up to 95% relative to control) except for ZnL1 which showed no statistical difference to the control. The apparent trend was for a decreasing Zn concentration with increasing rate of soil amendments. The Zn concentration of the control plants was above the phytotoxicity threshold range (500–1500 mg Zn/kg DM) defined by Chaney (1989). But the ZnA2 and ZnL1A1 treatments reduced the leaf concentration eliminating Zn toxicity (leaf concentration 176.8 and 491 mg/kg respectively). However, severe burning effects on the leaf edges were observed in the poplar leaves (Figure 6.1) for the ZnA2 treatment as observed for CuA2 treatment. This observation, in combination with reduced root and leaf DM for the Al dross treatments, also suggests probable Al toxicity arising from high levels of Al addition in Al dross, as discussed for the Cu contaminated soil. However, the burning effect on leaves was more severe for Zn than for the Cu contaminated soil.



Figur 6.1. Leaf chlorosis and browning in poplar treated with ZnA2

6.3.2 Al phytotoxicity in Al dross treatments

To confirm the apparent Al toxicity at the higher Al dross level treatments (CuA2 and ZnA2), possibly caused by the excessive application of Al to these soils, the 0.1M CaCl₂ extractable Al and exchangeable Al (1M KCl extraction) concentrations of the postharvest rhizosphere soil were measured. The CaCl₂ extractable Al and exchangeable Al levels in the Al dross treatments were extremely high (Table 6.3) and close to reported critical Al toxicity levels (3–5 and 40–270 mg/kg, respectively) for most plants (Edmeades *et al.* 1983; Hume *et al.* 1988; Sumner 1979). The values are much higher for the Zn contaminated soil than the Cu contaminated soil, explaining the severity of the Al toxicity problem in the Zn contaminated soil, and providing a possible explanation for the higher yield reduction in the Zn soil.

Table 6.3 CaCl₂ (0.01M) extractable and exchangeable (1M KCl) Aluminium (mg/kg) in soils

| Treatments | CaCl ₂ | | KCl | |
|------------|-------------------|---|-----------------|---|
| | extractable Al | | exchangeable Al | |
| Cu control | 0.1 | d | 6.6 | d |
| CuL1 | 0.1 | d | 5.6 | e |
| CuL2 | 0.1 | d | 6.8 | d |
| CuA1 | 1.3 | b | 19.3 | b |
| CuA2 | 3.3 | a | 42.2 | a |
| CuL1A1 | 1.0 | c | 18.2 | c |
| Zn control | 0.1 | d | 4.0 | d |
| ZnL1 | 0.02 | d | 3.9 | d |
| ZnL2 | 0.1 | d | 2.3 | e |
| ZnA1 | 1.4 | b | 23.3 | b |
| ZnA2 | 6.2 | a | 52.1 | a |
| ZnL1A1 | 1.1 | c | 17.0 | c |

Values in columns followed by different letters are significantly different ($P \leq 0.05$)

At neutral or weakly acidic pH, Al is reported to exist in the form of insoluble aluminosilicate, hydroxide or oxide (Bartlett and Riego 1972; Bojarczuk 2004; Matsumoto 2000). Despite the fact that Al is insoluble under these pH conditions, there are indications that it can still be taken up and become toxic to plants. For example, Farina *et al.* (1980) conducted an experiment with corn plants grown in the greenhouse on three highly weathered soils to measure availability of exchangeable Al at pH values near or above neutral (pH > 6.5). They reported that on two of the soils tested, yields decreased significantly as the pH approached neutrality (pH > 6.5). They found that the leaf Al concentration increased as the pH increased from 5 to 7, and suggested that the yield decrease was due to Al toxicity.

6.3.3 Beneficial effect of combined application of lime and Al dross

The higher leaf DM content and lower Cu and Zn concentration in leaves for both Cu and Zn contaminated soils amended with the combined application of lime and Al dross (CuL1A1 or ZnL1A1), relative to soil amended with these treatments applied separately at the same or a higher rate, indicates that there are some interactive effects of these treatments on the amelioration of Cu and Zn phytotoxicity in Cu and Zn contaminated soils. The increased DM yield for these treatments may be due to the addition of Ca through lime incorporation, which reduced the Al:Ca ratio on soil exchange sites. A number of studies have shown that liming or the presence of high levels of Ca in soil can reduce Al toxicity in an acidic soil environment by reducing the Al:Ca ratio (Álvarez *et al.* 2005; Cronan and Grigal 1995; Manoharan *et al.* 1996; Yang and Chen 2001). Based on a review of many laboratory and field studies with multiple forest species, Cronan and Grigal (1995) reported that with a soil solution Al/Ca molar ratio of 1 there was 50% risk of Al toxicity, if the ratio was 2 there was 75% risk, and if the ratio was 5 the risk was between 95 and 100%.

The increase in DM effected by the combined application of lime+Al dross (CuL1A1 and ZnL1A1) may also be due to the increase in soil pH caused by these treatments (Table 6.4). Even though Al dross increased soil pH, it did not increase the DM yield. This is likely because of the Al toxicity as discussed in the previous section. The DM decrease Al dross treatments might also be due to formation of Al_{13} and consequent Al toxicity as reported by Kinraide (1990). Lime also increased the soil pH but did not increase leaf DM yield, or reduce Zn phytotoxicity, unlike the lime+Al dross combination. Perhaps, the pH increase caused by the low rate of lime application was not high enough to increase DM yield. The higher rate of lime (L2) increased pH to the same level as the lime+Al dross combination, but the leaf DM yield for the Zn contaminated soil was significantly lower relative to ZnL1A1. The reason for this result is not clear. However, it is possible that high rates of Ca addition through the lime application might have reduced Mg uptake by the plants, subsequently causing a yield reduction as reported by Famina (1980). There may also be some beneficial effect of Al dross itself at low rates, when combined with the lime.

Table 6.4 Effect of lime and Al dross applications on soil pH

| Treatments | Cu | | | | | | Zn | | | | | |
|------------|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|-----|------|
| | C | L1 | L2 | A1 | A2 | L1A1 | C | L1 | L2 | A1 | A2 | L1A1 |
| Soil pH | 5.4 | 5.8 | 6.6 | 6.1 | 6.6 | 6.3 | 4.9 | 5.3 | 6.3 | 5.7 | 6.3 | 6.0 |

6.3.4 Bioconcentration factor (BCF)

The BCF was higher for both control treatments (CuC and ZnC; 0.12 and 7.5, respectively) compared to the lime– and Al dross–amended soils (Table 6.2). Increasing the treatment levels of lime or Al dross significantly reduced the BCF relative to a lesser application rate. For example, the BCF for the CuA1 treatment was 0.09 and for the CuA2 treatment was 0.06. This negative relationship of BCF with increasing rates of amendments suggests that the addition of increasing rates of lime or Al dross reduced metal accumulation in the plant shoot. A similar effect was observed in the leaf to root concentration ratio for the Zn contaminated soils,

but the effect was not significant. For Cu no difference in the leaf to root concentration ratio was observed.

6.3.5 Available soil metal concentrations

The treatment effects on the soluble+exchangeable and specifically adsorbed metal concentrations in soils are shown in Table 6.5. For both the Cu and Zn contaminated soils, all amendments significantly reduced the soluble+exchangeable metal concentration relative to the control soil. The relative concentration of the specifically adsorbed metal fraction was significantly reduced by the Al dross treatments, and when lime and dross were added to the soil together (L1A1), relative to the controls for both the Cu and Zn contaminated soils (Table 6.5). The lowest soluble+exchangeable (0.9 mg/kg) and specifically adsorbed (14.6 mg/kg) Cu concentrations in the Cu contaminated soil were observed for the treatment of CuA2. Similarly, for the Zn contaminated soil, the application of ZnA2 reduced the soluble+exchangeable Zn concentration to the lowest value recorded for the trial (8.2 mg/kg). However, the lowest specifically adsorbed Zn concentration was recorded for the Zn L1A1 treatment (4.9 mg/kg).

The decreasing trend in the concentration of bioavailable Cu and Zn (defined as the sum of soluble+exchangeable and specifically adsorbed metal) is consistent with the decreasing concentration of Cu and Zn in the leaves (Table 6.2). The higher soil pH (6.6 and 6.3 for Cu and Zn respectively) resulting from the addition of a high rate of Al dross or lime, likely caused a decrease in Cu and Zn solubility and therefore bioavailability. However, in general, bioavailable Cu and Zn concentrations were significantly lower for the Al dross (A2) application than for the lime (L2) application, despite the same pH for these two treatments for each metal (Table 6.4). This suggests an additional non-pH effect that is apparent only for Al dross to ameliorate Cu and Zn phytotoxicity. The greater efficiency of dross to reduce the bioavailable Cu and Zn concentration, relative to lime (at the same pH), could be due to Al derived from Al dross exchanging with Cu and Zn in the exchange complex. This is confirmed by the higher exchangeable Al in Al

dross treatments (Table 6.3). The reduction in the specifically adsorbed Cu and Zn fractions by Al dross application is also due to Al added in Al dross competing with Cu and Zn for the specifically adsorbing sites.

The lime+Al dross treatment (CuL1A1 or ZnL1A1) showed similar values for soluble+exchangeable metal concentrations and lower values for specifically adsorbed metal concentrations, relative to CuL2 or ZnL2. However, both the soluble+exchangeable metal concentration and specifically adsorbed fractionation were lower for CuL1A1 than CuL1, and lower for ZnL1A1 than ZnL1 and ZnA1. This may be due to the additive effects of lime and Al dross – the Ca in lime and Al in Al dross both competing with Cu and Zn for the exchangeable and specifically adsorbing sites.

Table 6.5 Effect of lime and Al dross applications on soil metal fractions (mg/kg)

| Treatments | Soluble+Exchangeable Cu/Zn | Specifically adsorbed Cu/Zn |
|------------|----------------------------|-----------------------------|
| Cu C | 6.7 a | 25 a |
| Cu L1 | 3.8 b | 23 a |
| Cu L2 | 1.7 cd | 22.2 a |
| Cu A1 | 1.9 c | 18.9 b |
| Cu A2 | 0.9 d | 14.6 c |
| Cu L1A1 | 1.6 cd | 17 bc |
| Zn C | 175.7 a | 52.2 a |
| Zn L1 | 126.6 b | 45.4 b |
| Zn L2 | 43.3 d | 50.1 a |
| Zn A1 | 54.6 c | 37.4 c |
| Zn A2 | 8.2 e | 12.6 d |
| Zn L1A1 | 42.7 d | 4.9 e |

The soluble+exchangeable Cu concentrations were at least 10 times lower than the corresponding Zn concentration for the all treatments. This may be due to lower level of total Cu (144 mg/kg soil) than total Zn (414 mg/kg soil) in the

contaminated soils. Furthermore, a larger proportion of Cu was probably bound to soil organic matter (see Chapter 2–4 on soil Cu fractionation) than Zn. The much higher concentration of Zn in the soluble+exchangeable fraction than Cu may be the reason for the higher root concentration of Zn relative to Cu. The high concentration of Zn in the roots would have exchanged with Al in the root metal binding sites, and the displaced Al could have been translocated to the shoots, especially in the Al dross treatments where the roots were probably loaded with Al because of the high concentration of soluble Al in the soil (Table 6.3). The Al exchanged by Zn in the roots would have translocated to the shoots to cause Al toxicity and reduction in shoot DM (Table 6.2).

6.3.6 Mycorrhizal colonization

In the, Cu contaminated soil, mycorrhizal colonization of the roots significantly increased only with the CuL1A1 treatment; the other treatments did not influence the colony numbers relative to the control (Figure 6.2). In the Zn contaminated soil also, the ZnL1A1 treatment increased mycorrhizal colonization, but a significant decrease with ZnA2 was observed relative to the control. The higher extent of mycorrhiza colonization for the treatments CuL1A1 and ZnL1A1 may be due to the soils having a pH close to neutral and a bioavailable concentration of Cu and Zn that is below a toxic level, both conditions which are considered to be favourable for mycorrhizal hyphae development. Yamanaka (2003) conducted an experiment on the growth of 15 strains of both saprotrophic and ectomycorrhizal ammonia fungi in a culture media initially adjusted to pH 3, 4, 5, 6, 7, 8 or 9, and reported that the ectomycorrhizal species showed maximum growth at pH 6. Despite both the higher rates of lime (L2) and Al dross (A2) increasing soil pH to near neutral, these treatments did not significantly increase the mycorrhiza colonization for both the Cu and Zn contaminated soils. The higher rate of lime increased mycorrhizal colonization as expected, but the result was not significant, whereas the higher rate of Al dross significantly decreased mycorrhizal colonization in the Zn contaminated soil. The significantly lower mycorrhiza

colony development for ZnA2 relative to the control in this soil is attributed to the adverse effects of excessive Al derived from Al dross (Table 6.3) on mycorrhiza.

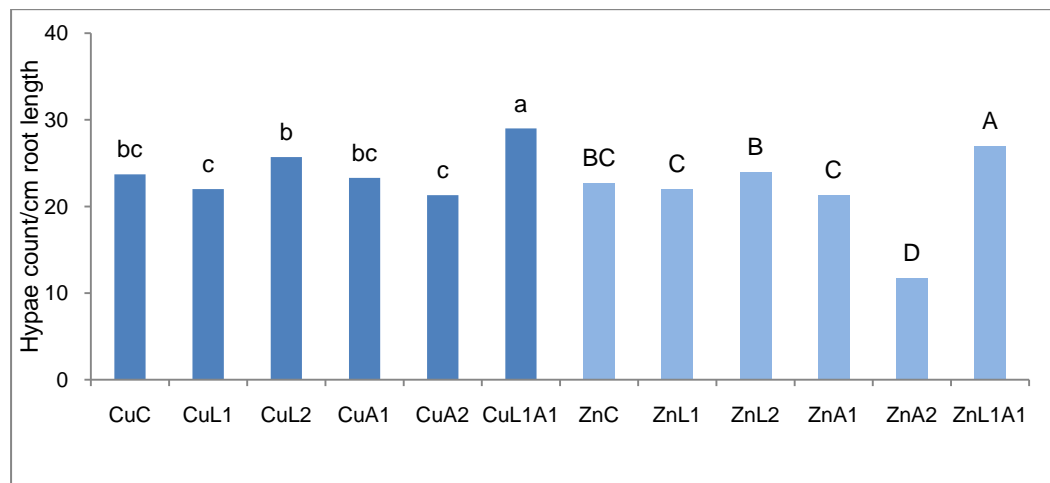


Figure 6.2 Ectomycorrhiza hyphae count in roots of poplar grown in lime and Al dross treated soils contaminated with Cu and Zn derived from biosolids. Bars with different letters are significantly different ($P \leq 0.05$). Simple letters indicate the variance among the Cu contaminated soils and capital letters for the Zn contaminated soils

6.3.7 Dehydrogenase activity and microbial biomass carbon

A significant increase in dehydrogenase activity was observed with lime and Al dross treatments of the Cu contaminated soil (Figure 6.3). Furthermore, increasing the rate of lime and Al dross application significantly increased the dehydrogenase activity. Lime and Al dross treatment significantly increased MBC relative to the control soil for the Cu contaminated soil, except for the treatment CuA1 where MBC was the same as the Cu control. Treatments CuA2 and CuL1A1 promoted the highest MBC (Figure 6.3) values recorded for the Cu contaminated soil.

In the Zn contaminated soil, dehydrogenase activity was also significantly increased relative to the control treatment for all treatments, except for ZnL1. Increasing the rate of lime application increased activity, however there was no significant increase in activity with an increasing rate of dross application. Application of lime and Al dross significantly increased MBC of the Zn soil

relative to the control, but the concentration of MBC did not significantly differ among the lime and Al dross treatments.

The higher microbial activity (dehydrogenase activity and MBC) observed for the higher levels of lime and Al dross application may be due to the increase in pH resulting from lime and Al dross application providing a more favorable environment for soil microbial activity, as was observed for mycorrhiza colony development. A simple linear correlation analysis indicated that soil dehydrogenase activity had a strong positive correlation ($r=0.7$, $P<0.0001$) with soil pH, while MBC had moderate positive correlation ($r=0.4$, $P=0.02$). Many studies in the literature have reported that soil pH has a significant positive correlation with soil microbial activity (Dalal 1998; Dee *et al.* 2003; Filep and Szili-Kovács 2010; Lee *et al.* 2008). For example, Lee *et al.* (2008) conducted a field trial to determine the effect of crushed oyster-shell meal on improving soil chemical and biological properties and crop plant productivity (Chinese cabbage) grown in sandy and silt loam soils (pH 5.8–6.2). They reported that application of crushed oyster-shell (16 Mg/ha) increased soil pH to 6.9–7.4 and significantly increased the MBC ($r=0.8$ – 0.9 , $P<0.01$) and microbial biomass nitrogen concentrations ($r=0.5$ – 0.8 , $P<0.01$), and stimulated soil enzyme activities ($r=0.6$ – 0.8 , $P<0.01$).

It is interestingly note that the higher level of Al dross treatment (CuA2 or ZnA2) promoted higher values for both soil dehydrogenase activity and MBC, but lower mycorrhizal colony number and prominent chlorosis in poplar relative to the control. This indicates that high soil Al concentrations caused by the Al dross (Table 6.3) affects poplar plant growth (section 6.3.1) and mycorrhiza colony development (section 6.3.3), but has no effect on soil microbial activity.

Both dehydrogenase activity and MBC generally responded in a similar way to the treatments in both the Cu and Zn contaminated soil. A simple linear correlation analysis indicated that dehydrogenase activity had a strong positive correlation ($r=0.68$, $P<0.0001$) with MBC. These results are in agreement with

those of Kunito *et al.*(2001) who found a high correlation of dehydrogenase activity with MBC ($r=0.938$, $P<0.01$) in an investigation of Cu (140–144 mg/kg) and Zn (216–292 mg/kg) influence on microorganisms in agricultural soils amended with biosolids or compost.

In addition to the direct effect of soil pH changes caused by lime and Al dross on microbial activity, addition of these amendments also reduced the plant-available Cu and Zn concentrations to levels well below the toxic threshold concentrations, as assessed by quantification of the soluble+exchangeable and specifically adsorbed metal fractions (Table 6.5). The latter effect may have also increased the microbial activity and MBC. As the control soils had soluble+exchangeable Cu and Zn concentrations close to or above the EC_{50} values (Cu 27 mg/kg; Zn 55–169 mg/kg) determined in the earlier chapters (Chapter 3 and 4), the microbial activity may have been affected by Cu and Zn in the control soils. Addition of amendments, lime and Al dross has decreased the soluble+exchangeable Cu and Zn to levels well below the EC_{50} values, and this could also be a reason for the increase in microbial activity effected by the ameliorants.

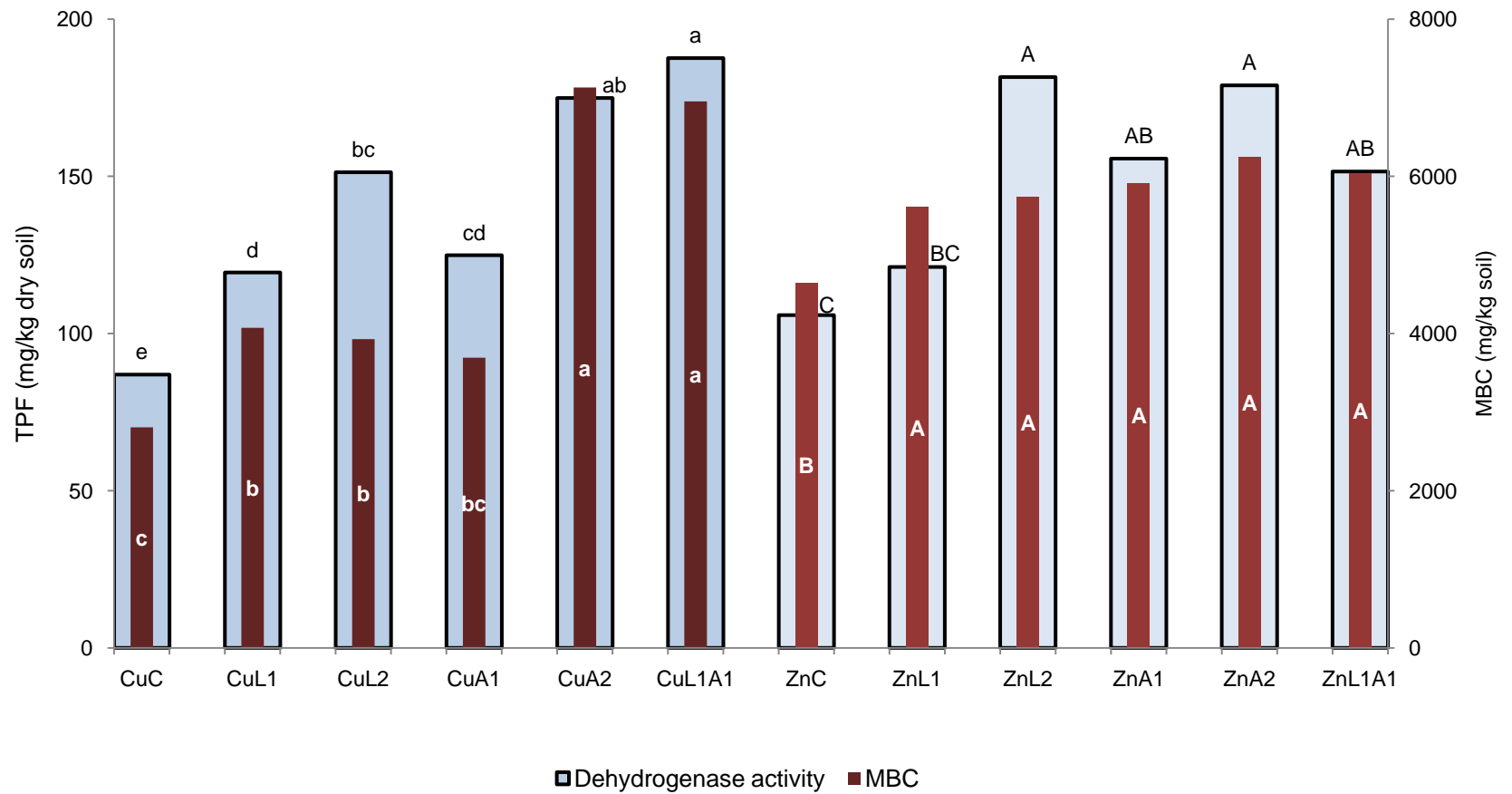


Figure 6.3 Dehydrogenase activity (TPF) and MBC for lime and Al dross treated rhizosphere soils in Cu and Zn contaminated soils. Bars with different letters are significantly different ($P \leq 0.05$). Simple letters indicate the variance among the Cu contaminated soils and capital letters for the Zn contaminated soils.

6.4 Conclusions

The application of lime and Al dross as a mixture (0.1% lime and 2% Al dross) was able to reduce Cu and Zn uptake and also significantly increase DM yield of poplar in both the Cu and Zn contaminated soils. The reduction in Zn uptake by the plants alleviated Zn phytotoxicity. The mycorrhizal population and microbial activity were also improved for the combined lime+Al dross treatment indicating that this is a good strategy to ameliorate Cu and Zn toxicity. Single applications of lime (0.1 and 0.5%) and a low level of Al dross amendment (2%) did not significantly reduce Zn toxicity. Even though a higher level of Al dross (6%) reduced Cu and Zn bioavailability and improved microbial activity, it reduced poplar yield and mycorrhiza colonization, probably due to the presence of toxic quantities of Al derived from the Al dross in the root zone. The overall results indicate that combined application of low rates of lime and Al dross (w/w) may be a better choice for ameliorating Cu and Zn toxicity to poplar plants and may also represent a possible way of disposing of Al waste. The rate of Al dross in the combined treatment must however be kept low to prevent possible secondary problems of Al toxicity.

Chapter 7

Overall conclusions and recommendations for future work

7.1 Need for the study

Application of biosolids to forest and agricultural lands provides a low-cost disposal option for biosolids with many potential benefits to soil. However, the practice may result in accumulations of potentially toxic heavy metals, and thus regulations are implemented to limit the amount of metals applied through biosolids application. Current guidelines in New Zealand and many other countries are not ideal because they are based on total metal concentrations in soils and biosolids, rather than bioavailable metal fractions which are better indicators of the potential for metals to interact with plants and microorganisms. Therefore, more environmentally appropriate regulations, based on the available metal fraction, need to be developed. The bioavailability of metals is dependent on the chemical nature of the metals, and the chemical, physical and biological properties of biosolids and soil. Moreover the subsequent release of the bioavailable metal fraction in soil depends on the interactions of biosolids with soil, plants and soil microorganisms. Hence, knowledge concerning the dynamics of biosolids-derived heavy metals in soils is a subject of paramount importance for developing specific strategies to modulate biosolids application guidelines. The overall aim of this thesis was to assess the bioavailability of biosolids-derived Cu and Zn in soil, to investigate the effect of biosolids-derived Cu and Zn on plant and soil microbial activity with special attention on mycorrhiza, and to investigate the effect of application of two ameliorants to soil on the reduction of the bioavailability of these two metals. Copper and Zn are the metals of greatest concern in biosolids because of the high concentration of these two metals in most biosolids (internationally), and because of their relatively high toxicity to microbes and plants.

7.2 Research outline

New Zealand biosolids generally have low heavy metal concentrations due to low levels of industrial activity in this country. Therefore, for research purposes, these biosolids need to be amended with heavy metals to study the long-term effects on soils, plants and microbes of incorporating contaminated biosolids that may present an environmental risk, into soil. A review of literature in Chapter 1 highlighted the importance of using a correct protocol to spike heavy metals into the biosolids prior to soil application for research purposes (Gibbs *et al.* 2006), and the equilibrium time period necessary for the metals to completely incorporate into the biosolids matrix. Use of an appropriate protocol simulates ‘real-life’ metal-contaminated biosolids. Chapter 2 of this thesis focussed on this subject, with special emphasis on the spiking of Cu and Zn to biosolids. As biosolids in New Zealand are currently applied to forest lands, two important forest plants, poplar and pine, were selected for study. As these two trees have ectomycorrhiza, the research was designed to study the effect of Cu and Zn not only on plants, but also on ectomycorrhiza, which to my knowledge has not been reported previously (Chapter 3 and 4). The hypothesis that total soil metal concentrations above the currently recommended maximum permissible levels of 100 and 300 mg/kg for Cu and Zn respectively in New Zealand (NZWWA 2003), can cause toxicity to poplar, pine and soil microorganisms, was also tested in Chapter 3 and 4. The review of literature also indicated that there is very limited information available on the comparative toxicities of heavy metals to plants, soil and mycorrhizae. Chapter 4 therefore examined the comparative effects of biosolids-derived Cu and Zn on poplar and pine, and also soil microbial activity.

As soil properties influence the bioavailability of metals derived from biosolids, a laboratory incubation trial was setup with eight soils collected from across the North Island of New Zealand, to model the relationships between soil properties and the bioavailability of biosolids-derived metals (Chapter 5). The studies described in Chapter 2 to Chapter 5 aimed to investigate the toxic effects of Cu and Zn through soil solid and solution phase speciation procedures. The results suggested that different threshold toxic levels of Cu and Zn chemical species exist

for plant growth and microbial activity. It was found that many of the biosolids–amended soils have the potential to cause Cu and Zn toxicity to plants and microorganisms. Therefore, it is important to find suitable amelioration methods to reduce excessive bioavailability of these metals in soil, to control risk to both the environment and human health through the food chain. Chapter 6 examined the potential of Al dross produced in New Zealand and a lime material to ameliorate Cu and Zn bioavailability in biosolids–amended soils. The effect of combination of these two ameliorates was also examined. Evaluation of the various treatments was carried out by examining the effect of these amendments on Cu and Zn bioavailability to poplar plants, and ectomycorrhizal and soil microbial activity.

7.3 Important findings of this study

In the section, the key points of the doctoral research outcome are summarised.

7.3.1 A shorter period of equilibration (2 months) than the previously used 6–9 months may be sufficient for Cu and Zn to be fully incorporated into the biosolids matrix (Chapter 2)

In Chapter 2 fractionation of Cu and Zn in biosolids amended separately with three levels of Cu and Zn sulphate, and anaerobically incubated for 4 months, showed that metal incorporation into the biosolids matrix appeared to be complete after two months. Therefore, in research practice where biosolids are spiked with metals to simulate biosolids that are naturally contaminated with metals, a shorter period of equilibration (2 months) than previously used (6–9 months) may be sufficient to ensure a high degree of incorporation of metals into the biosolids matrix. The majority of Cu in the biosolids solid phase was found in the organic and residual geochemical fractions, but Zn was associated mostly with the oxide, specifically bound, and exchangeable fractions. In the liquid phase, almost all Cu was organically bound, whereas Zn was mainly present as free Zn ions (Zn^{2+}) and ZnSO_4 species. The microbial activity measured as dehydrogenase activity was reduced by 50% (EC_{50} defined as the effective concentration for 50% activity

reduction) at a total solution–phase concentration of 0.1 and 20 mg/L for Cu and Zn respectively, and a solid–phase exchangeable Cu and Zn concentrations of 410 and 670 mg/kg, respectively.

7.3.2 Biosolids–derived Cu was more toxic to microorganisms than Zn, but Zn was more toxic to poplar and mycorrhiza (Chapter 3)

The results of a 147 day pot trial on poplar illustrated that at the same total soil metal concentration, biosolids–derived Cu was more toxic to soil microorganisms than Zn, whereas Zn was more toxic to poplar and ECM fungi. Considering the total metal concentration causing toxicity to plants and microorganisms, the currently recommended maximum metal concentration limits in New Zealand of 100 mg/kg for Cu and 300 mg/kg for Zn appear to be high for both metals with respect to soil microbial activity, but low for Cu and high for Zn with respect to poplar growth.

7.3.3 Ectomycorrhiza fungi symbiosis with pine was well developed and not affected by Cu and Zn. As for poplar, both Cu and Zn were toxic to microorganisms, but Zn was more toxic than Cu. Only Zn reduced pine growth (Chapter 4)

Biosolids–derived Cu and Zn influenced pine plants differently to poplar trees in some aspects such as root DM, BCF, leaf:root concentration and mycorrhizal colonization. However, some similar effects of these metal contaminants on both pine and poplar were observed, mainly in terms of metal bioavailability, phytotoxic effects and total microbial activity. As was seen for poplar plants, at similar rates of total soil metal concentration, Cu did not show a phytotoxic effect, but Zn was phytotoxic to pine. Both metals did not influence ECM fungi colony development, but decreased microbial activity. With respect to pine forestry, the findings of this study suggest that the current recommended limits for the total Cu and Zn concentration in New Zealand soils (100 mg/kg Cu; 300 mg/kg for Zn) the metals appear to be toxic to soil microbial activity but do not affect the

mycorrhiza activity. However, with respect to phytotoxicity in plants, the total Zn concentration limit for soil is too high.

7.3.4 The total solution-phase (mg/L) and solid-phase exchangeable (mg/kg) Cu and Zn concentrations for 50% microbial activity (dehydrogenase activity) reduction (EC₅₀) were as follows; (Chapter 2, 3 and 4)

The EC₅₀ values (50% reduction of dehydrogenase activity of control treatment) for total solution phase and solid-phase exchangeable fraction for Cu and Zn were performed in the biosolids incubation and glasshouse trials.

| | Solid phase exchangeable | | Total solution phase | |
|----------------------------|--------------------------|-----|----------------------|------|
| | metal | | metal | |
| | mg/kg | | mg/L | |
| | Cu | Zn | Cu | Zn |
| Biosolids incubation trial | 410 | 670 | 0.1 | 20 |
| Poplar I trial | 27 | 169 | 0.1 | 27 |
| Pine trial | 14.5 (EC ₄₀) | 55 | 0.5 | 14.5 |

7.3.5 Crystalline Fe oxide was found to be the main soil property controlling the exchangeable soil Cu concentration. Whereas for exchangeable Zn, the dominant soil properties were soil clay content and pH (Chapter 5)

Laboratory incubation trials with eight soils collected from across the North Island of New Zealand showed that biosolids-derived Zn is expected to be much more available to plants and microorganisms than Cu due to the lower affinity of Zn for organic matter and Fe and Al oxides in soil than Cu. The functional relationships of biosolids-derived Cu and Zn with soil properties showed that the main soil properties controlling exchangeable Cu were the contents of crystalline Fe oxide and Al oxide. Among these two soil properties, crystalline Fe oxide was the dominant factor and contributed to explaining 90% of the variability of

exchangeable Cu. The concentration of exchangeable Cu increases with the soil content of crystalline Fe oxide. For Zn, clay content and pH were the controlling soil factors that contributing to explaining 73% of the variability of exchangeable Zn. The concentration of exchangeable Zn increases with an increasing soil clay content in soil, but decreases with increasing pH.

7.3.6 Application of 0.1% lime and 2% alkaline Al dross as a mixture ameliorated the toxic effect of biosolids–derived Cu and Zn in poplar, improved microbial activity and mycorrhiza colony development (Chapter 6)

Studies on the effect of soil amendments on the amelioration of the toxic effect of biosolids–derived Cu and Zn to microorganisms and poplar revealed that application of lime and Al dross (pH 10) as a mixture (0.1% lime and 2% Al dross) was able to mitigate Cu and Zn phytotoxicity and also significantly increase DM yield of poplar for both Cu and Zn contaminated soils. The ECM fungi population and microbial activity were improved for a combined lime+Al dross treatment. The overall results indicate that combined application of low rates of lime and Al dross (w/w) may be a better choice for ameliorating Cu and Zn toxicity in poplar plants than that lime or dross treatment on their own, and a possible way of disposing of Al waste. The rate of Al dross in the combined treatment needs to be kept low to prevent a possible secondary effect of Al toxicity.

7.4 Recommendations for future work

- The study reported in this thesis showed that a significant increase in poplar root growth was associated with progressively higher concentration of Cu in soil. In this study, the toxic Cu concentration could not be assessed because no poplar plants showed any signs of Cu toxicity. Pine also did not show any toxic effect at higher Cu treatment. Furthermore, EC₅₀ values for solid and solution phase Cu concentrations needed to reduce microbial activity by 50% in pine soil were not reached. Therefore, future trials on poplar and pine need

to test higher rates of Cu addition to soil (as biosolids) to determine the optimum rates of Cu and Cu^{2+} concentration in soil solution for poplar and pine growth and, to investigate the critical toxic threshold concentrations.

- The study reported in this thesis demonstrated that there are influences of biosolids-derived Cu and Zn on poplar and pine plants and ECM fungi development. In order to establish better functional relationships of bioavailable Cu and Zn and plants with ECM, trials need to be conducted on plants inoculated with different ECM fungi isolates.
- A limitation of the study described in this thesis, and of similar studies, is the current lack of techniques suited to measure the narrow range of biochemical changes that take place in the rhizosphere of plants. Small changes may remarkably influence the uptake of metals. For example, in the poplar trial, plant root growth and ECM fungal colony numbers increased with increasing Cu levels. It was suggested that ECM controls the toxicity of Cu by accumulating excess Cu in their cell walls. But experimental investigation of this theory was not possible. Sensitive techniques are required to quantify the role of ECM hyphae in regulating metal uptake by plants, and such sensitive techniques need to be developed and utilised in future studies.
- In the laboratory incubation study, the relationship observed between exchangeable Cu and soil properties was influenced largely by one of the eight soils used which had a very high Fe and Al oxide content. Future studies need to use a larger number of soils having a wide range of Fe and Al oxide contents to confirm the observed results.
- Application of a wide range of Al dross concentrations in combination with different rates of lime is needed to study their interactive effect of these two amendments in ameliorating Cu and Zn bioavailability in soils.
- The findings of the glasshouse trials in this thesis, especially the critical toxicity soil Cu and Zn concentrations, need to be confirmed and further

investigated through conducting long-term trials on adult plants in the field on contrasting soils.

The findings of the studies in this thesis are applicable to environmental regulations with respect to heavy metal limits that seek to protect agricultural and forest land, human and animal health, and soil and drinking water quality, in scenarios where biosolids are applied to soil. The findings will contribute to better sustainable reuse of biosolids, a ubiquitous material that is not waste, but a resource; an organic material rich in the essential nutrients.

References

ADEME (1995) Les différents procédés de stockage des boues d'épuration avant valorisation en agriculture. (Agences De L'eau: Angers Cedex 01).

Adriaensen K, Van Der Lelie D, Van Laere A, Vangronsveld J, Colpaert JV (2004) A zinc-adapted fungus protects pines from zinc stress. *New Phytologist* **161**, 549-555.

Adriaensen K, Vangronsveld J, Colpaert J (2006) Zinc-tolerant *Suillus bovinus* improves growth of Zn-exposed *Pinus sylvestris* seedlings. *Mycorrhiza* **16**, 553-558.

Adriaensen K, Vralstad T, Noben JP, Vangronsveld J, Colpaert JV (2005) Copper-adapted *suillus luteus*, a symbiotic solution for pines colonizing Cu mine spoils. *Applied and Environmental Microbiology* **71**, 7279-7284.

Adriano DC (2001) 'Trace elements in terrestrial Environments: Biogeochemistry, bioavailability and risks of metals.' (Springer: New York).

Adriano PC, Bolan NS, Koo Bon Jun., Naidu R, Lelie D.van der., Vangronsveld J, Wenzel WW (2002) Natural remediation processes : Bioavailability interactions in contaminated soils. In '17th World Congress of Soil Science'. (World Congress of Soil Science publishers: Thailand).

Ahonen-Jonnarth U, Van Hees PAW, Lundstrom US, Finlay RD (2000) Organic acids produced by mycorrhizal *Pinus sylvestris* exposed to elevated aluminium and heavy metal concentrations. *New Phytologist* **146**, 557-567.

Alcacio TE, Hesterberg D, Chou JW, Martin JD, Beauchemin S, Sayers DE (2001) Molecular scale characteristics of Cu(II) bonding in goethite-humate complexes. *Geochimica et Cosmochimica Acta* **65**, 1355-1366.

Al-Hiyaly SA, McNeilly T, Bradshaw AD (1988) The effects of zinc contamination from electricity pylons-evolution in a replicated situation. *New Phytologist* **110**, 571-580.

Allen HE, Cho SH, Neubecker TA (1983) Ion exchange and hydrolysis of Type A zeolite in natural waters. *Water Research* **17**, 1871-1879.

Alloway B (2009) Soil factors associated with zinc deficiency in crops and humans. *Environmental Geochemistry and Health* **31**, 537-548.

Alloway BJ (1995) 'Heavy metals in soils.' (Chapman and Hall: Glasgow).

Alloway BJ (2005) 'Copper-deficient soils in Europe.' (International Copper Association: New York).

Alloway BJ (2008a) Copper and zinc in soils: Too little or too much ? In 'NZ Trace Elements Group Conference' Waikato University, Hamilton).

Alloway BJ (2008b) 'Zinc in soils and crop nutrition.' (International Zinc Association and International Fertilizer Industry Association: Brussels and Paris).

Álvarez E, Fernández-Marcos ML, Monterroso C, Fernández-Sanjurjo MJ (2005) Application of aluminium toxicity indices to soils under various forest species. *Forest Ecology and Management* **211**, 227-239.

Alvarez JM, Lopez-Valdivia LM, Novillo J, Obrador A, Rico MI (2006) Comparison of EDTA and sequential extraction tests for phytoavailability prediction of manganese and zinc in agricultural alkaline soils. *Geoderma* **132**, 450-463.

Álvarez-Ayuso E, García-Sánchez A (2003) Palygorskite as a feasible amendment to stabilize heavy metal polluted soils. *Environmental Pollution* **125**, 337-344.

Anderson E (1993) 'Plants of central Queensland their identification and uses.' (Department of Primary Industries: Brisbane).

Arduini I, Godbold DL, Onnis A (1994) Cadmium and copper change root growth and morphology of *Pinus pinea* and *Pinus pinaster* seedlings. *Physiologia Plantarum* **92**, 675-680.

Arduini I, Godbold DL, Onnis A (1995) Influence of copper on root growth and morphology of *Pinus pinea* L. and *Pinus pinaster* Ait. seedlings. *Tree Physiology* **15**, 411-415.

Arduini I, Godbold DL, Onnis A (1996) Cadmium and copper uptake and distribution in Mediterranean tree seedlings. *Physiologia Plantarum* **97**, 111-117.

Arisi ACM, Mocquot B, Lagriffoul A, Mench M, Foyer CH, Jouanin L (2000) Responses to cadmium in leaves of transformed poplars overexpressing Γ -glutamylcysteine synthetase. *Physiologia Plantarum* **109**, 143-149.

Ashworth DJ, Alloway BJ (2008) Influence of dissolved organic matter on the solubility of heavy metals in sewage-sludge-amended Soils. *Communications in Soil Science and Plant Analysis* **39**, 538 - 550.

AWA (2009) Australasian biosolids partnership. Australian Water Association Limited, NSW.

Baath E, Frostegard A, Diaz-Ravina M, Tunlid A (1998) Microbial community-based measurements to estimate heavy metal effects in soil: The use of phospholipid fatty acid patterns and bacterial community tolerance. *Ambio* **27**, 58-61.

Bais HP, Weir TL, Perry LG, Gilroy S, Vivanco JM (2006) The role of root exudates in rhizosphere interactions with plants and other organisms. *Annual Review of Plant Biology* **57**, 233-266.

Barea J-M, Pozo MJ, Azcon R, Azcon-Aguilar C (2005) Microbial co-operation in the rhizosphere. *J. Exp. Bot.* **56**, 1761-1778.

Barry GA (1997) Total heavy metal status of horticultural soils in Queensland. Final Report of Project VG 404. Horticultural Research and Development Corporation, Gordon, NSW.

Bartlett RJ, Riego DC (1972) Toxicity of hydroxy aluminum in relation to pH and phosphorus. *Soil Science* **114**, 194-200.

Basta NT, McGowen SL (2004) Evaluation of chemical immobilization treatments for reducing heavy metal transport in a smelter-contaminated soil. *Environmental Pollution* **127**, 73-82.

Beckett PHT, Davis RD (1978) The additivity of the toxic effects of Cu, Ni and Zn in young barley. *New Phytologist* **81**, 155-173.

Bell RW, Dell B, Huang L (2004) Importance of micronutrients for crop nutrition. In 'International Fertilizer Industry Association Symposium on Micronutrients' p. 17. (IFIA: New Delhi, India).

Benedetti MF (2006) Metal ion binding to colloids from database to field systems. *Journal of Geochemical Exploration* **88**, 81-85.

Bertsch PM, Seaman JC (1999) Characterization of complex mineral assemblages: Implications for contaminant transport and environmental remediation. *Proceedings of the National Academy of Sciences of the United States of America* **96**, 3350-3357.

Bezdicsek DF, Beaver T, Granatstein D (2003) Subsoil ridge tillage and lime effects on soil microbial activity, soil pH, erosion, and wheat and pea yield in the Pacific Northwest, USA. *Soil and Tillage Research* **74**, 55-63.

Bi H, Long Y, Turner J, Lei Y, Snowdon P, Li Y, Harper R, Zerihun A, Ximenes F (2010) Additive prediction of aboveground biomass for *Pinus radiata* (D. Don) plantations. *Forest Ecology and Management* **259**, 2301-2314.

Billett MF, Fitzpatrick EA, Cresser MS (1991) Long-term changes in the Cu, Pb, and Zn content of forest soil organic horizons from North-East Scotland. *Water, Air, & Soil Pollution* **59**, 179-191.

Bissonnette L, St-Arnaud M, Labrecque M (2010) Phytoextraction of heavy metals by two Salicaceae clones in symbiosis with arbuscular mycorrhizal fungi during the second year of a field trial. *Plant and Soil*.

Blackmore LL, Searle PL, Daly BK (1987) New Zealand Soil Bureau Scientific Report 80. New Zealand.

Boardman R, Cromer RN, Lambert MJ, Webb MJ (1997) Forest plantations. In 'Plant analysis: An interpretation manual'. (Eds DJ Reuter, JB Robinson) pp. 505–566. (CSIRO Publishing: Melbourne).

BODTrakTM (1997) 'Instrument manual.' (Hach Company: USA).

Bojarczuk K (2004) Effect of aluminium on the development of poplar (*Populus tremula* L. × *P. alba* L.) *in vitro* and *in vivo*. *Polish Journal of Environmental Studies* **13**, 261-266.

Bolan NS, Rowarth J, de la Luz Mora M, Adriano D, Curtin D (2008) Chapter 17 Biological transformation and bioavailability of nutrient elements in acid soils as affected by liming. In 'Developments in Soil Science'. (Ed. R Naidu) pp. 413-446. (Elsevier).

Borghi M, Tognetti R, Monteforti G, Sebastiani L (2007) Responses of *Populus* × *euramericana* (*P. deltoides* × *P. nigra*) clone Adda to increasing copper concentrations. *Environmental and Experimental Botany* **61**, 66-73.

Borghi M, Tognetti R, Monteforti G, Sebastiani L (2008) Responses of two poplar species (*Populus alba* and *Populus x canadensis*) to high copper concentrations. *Environmental and Experimental Botany* **62**, 290-299.

Brennan RF, Armour JD, Reuter DJ (1993) Diagnosis of zinc deficiency. In 'Zinc in soils and plants'. (Ed. AD Robson). (Kluwer Academic Publishers: Dordrecht).

Bright DA, Healey N (2003) Contaminant risks from biosolids land application: Contemporary organic contaminant levels in digested sewage sludge from five treatment plants in Greater Vancouver, British Columbia. *Environmental Pollution* **126**, 39-49.

Brookes PC, McGrath SP (1984) Effects of metal toxicity on the size of the soil microbial biomass. *Journal of Soil Science* **35**, 341-346.

Broos K, Warne MSJ, Heemsbergen DJ, Stevens D, Barnes MB, Correll RL, McLaughlin MJ (2007) Soil factors controlling the toxicity of copper and zinc to microbial processes in Australian soils. *Environmental Toxicology and Chemistry* **26**, 583-590.

Brown S, Chaney RL, Hallfrisch JG, Xue Q (2003) Effect of biosolids processing on lead bioavailability in an urban soil. *Journal of Environmental Quality* **32**, 100-108.

Brown SL, Chaney RL, Lloyd CA, Angle JS, Ryan JA (1996) Relative uptake of cadmium by garden vegetables and fruits grown on long-term biosolid-amended soils. *Environmental Science & Technology* **30**, 3508-3511.

Brundrett MC, Abbott LK (1994) Mycorrhizal fungus propagules in the jarrah forest: I. Seasonal study of inoculum levels. *New Phytologist* **127**, 539-546.

Brunner AM, Busov VB, Strauss SH (2004) Poplar genome sequence: functional genomics in an ecologically dominant plant species. *Trends in Plant Science* **9**, 49-56.

Brunori C, Cremisini C, Massanisso P, Pinto V, Torricelli L (2005) Reuse of a treated red mud bauxite waste: studies on environmental compatibility. *Journal of Hazardous Materials* **117**, 55-63.

Burton ED, Hawker DW, Redding MR (2003) Sludge-derived Cu and Zn in a humic-gley soil: effect of dissolved metal-organic matter complexes on sorption and partitioning. *Soil and Sediment Contamination: An International Journal* **12**, 23 - 46.

Cairney JWG, Meharg AA (1999) Influences of anthropogenic pollution on mycorrhizal fungal communities. *Environmental Pollution* **106**, 169-182.

Campbell JA (1998) The London convention, evolving to more robust impact assessment. In 'SPE International Conference on Health, Safety, and Environment in Oil and Gas Exploration and Production'. Caracas, Venezuela. (Society of Petroleum Engineers Inc.).

Cancès B, Ponthieu M, Castrec-Rouelle M, Aubry E, Benedetti MF (2003) Metal ions speciation in a soil and its solution: experimental data and model results. *Geoderma* **113**, 341-355.

Caravaca F, Garcia C, Hernández MT, Roldán A (2002) Aggregate stability changes after organic amendment and mycorrhizal inoculation in the afforestation of a semiarid site with *Pinus halepensis*. *Applied Soil Ecology* **19**, 199-208.

Carrasco L, Caravaca F, Álvarez-Rogel J, Roldán A (2006) Microbial processes in the rhizosphere soil of a heavy metals-contaminated Mediterranean salt marsh: A facilitating role of AM fungi. *Chemosphere* **64**, 104-111.

Castaldi P, Santona L, Melis P (2005) Heavy metal immobilization by chemical amendments in a polluted soil and influence on white lupin growth. *Chemosphere* **60**, 365-371.

Castiglione S, Franchin C, Fossati T, Lingua G, Torrigiani P, Biondi S (2007) High zinc concentrations reduce rooting capacity and alter metallothionein gene expression in white poplar (*Populus alba* L. cv. Villafranca). *Chemosphere* **67**, 1117-1126.

Castiglione S, Todeschini V, Franchin C, Torrigiani P, Gastaldi D, Cicatelli A, Rinaudo C, Berta G, Biondi S, Lingua G (2009) Clonal differences in survival capacity, copper and zinc accumulation, and correlation with leaf polyamine levels in poplar: A large-scale field trial on heavily polluted soil. *Environmental Pollution* **157**, 2108-2117.

Cavallaro N, McBride MB (1984) Zinc and copper sorption and fixation by an acid soil clay: effect of selective dissolutions. *Soil Science Society of America Journal* **48**, 1050-1054.

Centre for Ecology and Hydrology (2002) 'Windermere humic aqueous model (WHAM): Equilibrium chemical speciation for natural waters.' (Natural Environmental Research Council: Wallingford, UK).

Chander K, Brookes PC (1991) Is the dehydrogenase assay invalid as a method to estimate microbial activity in copper-contaminated soils? *Soil Biology and Biochemistry* **23**, 909-915.

Chander K, Brookes PC (1993) Residual effects of zinc, copper and nickel in sewage sludge on microbial biomass in a sandy loam. *Soil Biology and Biochemistry* **25**, 1231-1239.

Chander K, Joergensen RG (2002) Decomposition of ¹⁴C labelled glucose in a Pb-contaminated soil remediated with synthetic zeolite and other amendments. *Soil Biology and Biochemistry* **34**, 643-649.

Chaney RL (1989) Toxic element accumulation in soils and crops: Protecting soil fertility and agricultural food chains. In 'Inorganic contaminants in the vadose zone'. (Ed. NB B Bar-Yosef, J Goldshmid) pp. 140-158. (Springer-Verlag: Berlin).

Chaperon S, Sauvé S (2007) Toxicity interaction of metals (Ag, Cu, Hg, Zn) to urease and dehydrogenase activities in soils. *Soil Biology and Biochemistry* **39**, 2329-2338.

Chaudhuri D, Tripathy S, Veeresh H, Powell MA, Hart BR (2003) Relationship of chemical fractions of heavy metals with microbial and enzyme activities in sludge and ash-amended acid lateritic soil from India. *Environmental Geology* **45**, 115-123.

Chaudri A, Allain C, Barbosa-Jefferson V, Nicholson F, Chambers B, McGrath S (2000a) A study of the impacts of Zn and Cu on two rhizobial species in soils of a long-term field experiment. *Plant and Soil* **221**, 167-179.

Chaudri AM, Knight BP, Barbosa-Jefferson VL, Preston S, Paton GI, Killham K, Coad N, Nicholson FA, Chambers BJ, McGrath SP (1999) Determination of acute Zn toxicity in pore water from soils previously treated with sewage sludge using bioluminescence assays. *Environmental Science & Technology* **33**, 1880-1885.

Chaudri AM, Lawlor K, Preston S, Paton GI, Killham K, McGrath SP (2000b) Response of a *Rhizobium*-based luminescence biosensor to Zn and Cu in soil solutions from sewage sludge treated soils. *Soil Biology and Biochemistry* **32**, 383-388.

Chlopecka A, Adriano DC (1996) Mimicked In-Situ Stabilization of Metals in a Cropped Soil: Bioavailability and Chemical Form of Zinc. *Environmental Science & Technology* **30**, 3294-3303.

Christie P, Li X, Chen B (2004) Arbuscular mycorrhiza can depress translocation of zinc to shoots of host plants in soils moderately polluted with zinc. *Plant and Soil* **261**, 209-217.

Christie T, Brathwait B, Thompson B (2002) Zeolites: Mineral commodity report V. New Zealand Mining.

Chukwuma MC, Eshett ET, Onweremadu EU, Okon MA (2010) Zinc availability in relation to selected soil properties in a crude oil polluted eutric tropofluvent. *International Journal of Environmental Science and Technology* **7**, 261-270.

Ciccu R, Ghiani M, Serici A, Fadda S, Peretti R, Zucca A (2003) Heavy metal immobilization in the mining-contaminated soils using various industrial wastes. *Minerals Engineering* **16**, 187-192.

Clarke C (2006) Biosolids. In 'appeared in Water and Wastewater Treatment'. (Environmental Data Interactive Exchange: Surrey).

Claydon JJ (1989) Determination of particle size distribution of fine grained soils - pipette method: DSIR Division of Land & Soil Sciences technical record LH5. Division of Scientific and Industrial Research, Wellington, New Zealand.

Colpaert JV, Muller LAH, Lambaerts M, Adriaensen K, Vangronsveld J (2004) Evolutionary adaptation to Zn toxicity in populations of *Suilloid* fungi. *New Phytologist* **162**, 549-559.

Cronan CS, Grigal DF (1995) Use of calcium/aluminum ratios as indicators of stress in forest ecosystems. *Journal of Environmental Quality* **24**, 209-226.

Dai J, Xu M, Chen J, Yang X, Ke Z (2007) PCDD/F, PAH and heavy metals in the sewage sludge from six wastewater treatment plants in Beijing, China. *Chemosphere* **66**, 353-361.

Dakora FD, Phillips DA (2002) Root exudates as mediators of mineral acquisition in low-nutrient environments. *Plant and Soil* **245**, 35-47.

Dalal RC (1998) Soil microbial biomass—what do the numbers really mean? *Australian Journal of Experimental Agriculture* **38**, 649-665.

Davis RD, Carlton-Smith CH (1984) An investigation into the phytotoxicity of zinc, copper and nickel using sewage sludge of controlled metal content. *Environmental Pollution Series B, Chemical and Physical* **8**, 163-185.

Dee BM, Haynes RJ, Graham MH (2003) Changes in soil acidity and the size and activity of the microbial biomass in response to the addition of sugar mill wastes. *Biology and Fertility of Soils* **37**, 47-54.

Degryse F, Buekers J, Smolders E (2004) Radio-labile cadmium and zinc in soils as affected by pH and source of contamination. *European Journal of Soil Science* **55**, 113-122.

Degryse F, Smolders E, Parker DR (2009) Partitioning of metals (Cd, Co, Cu, Ni, Pb, Zn) in soils: concepts, methodologies, prediction and applications –a review. *European Journal of Soil Science* **60**, 590-612.

Delas J (1984) Les toxicités métalliques dans les sols acides. *Le Progrès Agricole et Viticole* **101**, 96-102.

Denny HJ, Wilkins DA (1987) Zinc tolerance in *Betula* spp. IV. The mechanism of ectomycorrhizal amelioration of zinc toxicity. *New Phytologist* **106**, 545-553.

Di Baccio D, Kopriva S, Sebastiani L, Rennenberg H (2005) Does glutathione metabolism have a role in the defence of poplar against zinc excess? *New Phytologist* **167**, 73-80.

Di Baccio D, Tognetti R, Sebastiani L, Vitagliano L (2003) Responses of *Populus deltoides* × *Populus nigra* (*Populus* × *euramericana*) clone I-214 to high zinc concentrations. *New Phytologist* **159**, 443-452.

Dietrich DM, Lamar RT (1990) Selective medium for isolating *Phanerochaete chrysosporium* from soil. *Applied and Environmental Microbiology* **56**, 3088-3092.

Dixon R, Buschena C (1988) Response of ectomycorrhizal *Pinus banksiana* and *Picea glauca* to heavy metals in soil. *Plant and Soil* **105**, 265-271.

Doelman P (1986) Resistance of soil microbial communities to heavy metals. In 'Microbial Communities in Soil: FEMS Symposium'. Copenhagen. (Ed. J.V. Kjoller and L.H. Sorensen) pp. 369–384. (Elsevier, Essex).

Doelman P, Haanstra L (1989) Short- and long-term effects of heavy metals on phosphatase activity in soils: An ecological dose-response model approach. *Biology and Fertility of Soils* **8**, 235-241.

Dos Santos Utmazian MN, Wenzel WW (2007) Cadmium and zinc accumulation in willow and poplar species grown on polluted soils. *Journal of Plant Nutrition and Soil Science* **170**, 265-272.

Echevarria G, Morel JL, Fardeau JC, Leclerc-Cessac E (1998) Assessment of Phytoavailability of Nickel in Soils. *Journal of Environmental Quality* **27**, 1064-1070.

Edmeades DC, Smart CE, Wheeler DM (1983) Aluminium toxicity in New Zealand soils. Preliminary results on the development of diagnostic criteria. *New Zealand Journal of Agricultural Research* **26**, 493-501.

Egiarte G, Pinto M, Ruiz-Romera E, Arbestain MC (2009) Changes in heavy metal concentrations in acid soils under pine stands subjected to repeated applications of biosolids. *Soil Science* **174**, 372-379.

Elliott HA, Dempsey BA, Maille PJ (1990) Content and Fractionation of Heavy Metals in Water Treatment Sludges. *Journal of Environmental Quality* **19**, 330-334.

Entry JA, Rygielwicz PT, Watrud LS, Donnelly PK (2002) Influence of adverse soil conditions on the formation and function of Arbuscular mycorrhizas. *Advances in Environmental Research* **7**, 123-138.

Environment-Protection-Authority (1996) 'South Australian biosolids guidelines for the safe handling, reuse or disposal of biosolids.' (Department of Environment and Natural Resources: Adelaide).

Farfel MR, Orlova AO, Chaney RL, Lees PSJ, Rohde C, Ashley PJ (2005) Biosolids compost amendment for reducing soil lead hazards: a pilot study of Orgro® amendment and grass seeding in urban yards. *Science of The Total Environment* **340**, 81-95.

Farina MPW, Sumner ME, Plank CO, Letsch WS (1980) Aluminum toxicity in corn at near neutral soil pH levels. *Journal of Plant Nutrition* **2**, 683 - 697.

Fernandes SAP, Bettiol W, Cerri CC (2005a) Effect of sewage sludge on microbial biomass, basal respiration, metabolic quotient and soil enzymatic activity. *Applied Soil Ecology* **30**, 65-77.

Fernandes SAP, Bettiol W, Cerri CC, Camargo P (2005b) Sewage sludge effects on gas fluxes at the soil-atmosphere interface, on soil $\delta^{13}\text{C}$ and on total soil carbon and nitrogen. *Geoderma* **125**, 49-57.

Ferrol N, González-Guerrero M, Valderas A, Benabdellah K, Azcón-Aguilar C (2009) Survival strategies of arbuscular mycorrhizal fungi in Cu-polluted environments. *Phytochemistry Reviews* **8**, 551-559.

Filep T, Szili-Kovács T (2010) Effect of liming on microbial biomass carbon of acidic arenosols in pot experiments. *Plant, soil and environment* **56**, 268–273.

Fomina MA, Alexander IJ, Colpaert JV, Gadd GM (2005) Solubilization of toxic metal minerals and metal tolerance of mycorrhizal fungi. *Soil Biology and Biochemistry* **37**, 851-866.

Fornes F, García-de-la-Fuente R, Belda RM, Abad M (2009) 'Alperujo' compost amendment of contaminated calcareous and acidic soils: Effects on growth and trace element uptake by five Brassica species. *Bioresource Technology* **100**, 3982-3990.

Fotovát A, Naidu R (1998) Changes in composition of soil aqueous phase influence chemistry of indigenous heavy metals in alkaline sodic and acidic soils. *Geoderma* **84**, 213-234.

Franchin C, Fossati T, Pasquini E, Lingua G, Castiglione S, Torrigiani P, Biondi S (2007) High concentrations of zinc and copper induce differential polyamine responses in micropropagated white poplar (*Populus alba*). *Physiologia Plantarum* **130**, 77-90.

Franzluebbers AJ, Haney RL, Hons FM (1999a) Relationships of chloroform fumigation-incubation to soil organic matter pools. *Soil Biology and Biochemistry* **31**, 395-405.

Franzluebbers AJ, Haney RL, Hons FM, Zuberer DA (1999b) Assessing biological soil quality with chloroform fumigation-incubation: Why subtract a control? *Canadian Journal of Soil Science* **79**, 521-528.

French CJ, Dickinson NM, Putwain PD (2006) Woody biomass phytoremediation of contaminated brownfield land. *Environmental Pollution* **141**, 387-395.

Friesl W, Horak O, Wenzel. WW (2004) Immobilization of heavy metals in soils by the application of bauxite residues: Pot experiments under field conditions. *Journal of Plant Nutrition and Soil Science* **167**, 54-59.

Fuentes A, Lloréns M, Sáez J, Soler A, Aguilar MI, Ortuño JF, Meseguer VF (2004) Simple and sequential extractions of heavy metals from different sewage sludges. *Chemosphere* **54**, 1039-1047.

Fuentes D, Disante KB, Valdecantos A, Cortina J, Ramón Vallejo V (2007a) Response of *Pinus halepensis* Mill. seedlings to biosolids enriched with Cu, Ni and Zn in three Mediterranean forest soils. *Environmental Pollution* **145**, 316-323.

Fuentes D, Disante KB, Valdecantos A, Cortina J, Vallejo VR (2007b) Sensitivity of Mediterranean woody seedlings to copper, nickel and zinc. *Chemosphere* **66**, 412-420.

Fytili D, Zabaniotou A (2008) Utilization of sewage sludge in EU application of old and new methods—A review. *Renewable & Sustainable Energy Reviews* **12**, 116-140.

Gadd GM (1993) Interactions of fungi with toxic metals. *New Phytology* **124**, 25-60.

Gadd GM (2010) Metals, minerals and microbes: geomicrobiology and bioremediation. *Microbiology* **156**, 609-643.

Galli U, Schüepp H, Brunold C (1994) Heavy metal binding by mycorrhizal fungi. *Physiologia Plantarum* **92**, 364-368.

Garau G, Castaldi P, Santona L, Deiana P, Melis P (2007) Influence of red mud, zeolite and lime on heavy metal immobilization, culturable heterotrophic microbial populations and enzyme activities in a contaminated soil. *Geoderma* **142**, 47-57.

García-Delgado M, Rodríguez-Cruz MS, Lorenzo LF, Arienzo M, Sánchez-Martín MJ (2007) Seasonal and time variability of heavy metal content and of its chemical forms in sewage sludges from different wastewater treatment plants. *Science of The Total Environment* **382**, 82-92.

Gasco G, Lobo MC, Guerrero F (2005) Land application of sewage sludge: A soil columns study. *Water SA* **31**, 309-318.

Gheshlaghi ZT, McLaren RG, Adams JA (2008) Effect of treated zeolite, iron waste, and liming on phytoavailability of Zn, Cu, and Ni in long-term biosolids-amended soils. *Australian Journal of Soil Research* **46**, 509-516.

Gibbs PA, Chambers BJ, Chaudri AM, McGrath SP, Carlton-Smith CH (2006) Initial results from long-term field studies at three sites on the effects of heavy metal-amended liquid sludges on soil microbial activity. *Soil Use and Management* **22**, 180-187.

Giller KE, Witter E, McGrath SP (1998) Toxicity of heavy metals to microorganisms and microbial processes in agricultural soils: A review. *Soil Biology & Biochemistry* **30**, 1389-1414.

Giovannetti M, Mosse B (1980) An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. *New Phytologist* **84**, 489-500.

Göhre V, Paszkowski U (2006) Contribution of the arbuscular mycorrhizal symbiosis to heavy metal phytoremediation. *Planta* **223**, 1115-1122.

González-Chávez MC, Carrillo-González R, Wright SF, Nichols KA (2004) The role of glomalin, a protein produced by arbuscular mycorrhizal fungi, in sequestering potentially toxic elements. *Environmental Pollution* **130**, 317-323.

Gorgé JL, Lastra O, Chueca A, Lachica M (1985) Use of photosynthetic parameters for the diagnosis of copper deficiency in *Pinus radiata* seedlings. *Physiologia Plantarum* **65**, 508-512.

Gove L, Cooke CM, Nicholson FA, Beck AJ (2001) Movement of water and heavy metals (Zn, Cu, Pb and Ni) through sand and sandy loam amended with biosolids under steady-state hydrological conditions. *Bioresource Technology* **78**, 171-179.

Gratton WS, Nkongolo KK, Spiers GA (2000) Heavy metal accumulation in soil and jack pine (*Pinus banksiana*) needles in Sudbury, Ontario, Canada. *Bulletin of Environmental Contamination and Toxicology* **64**, 550-557.

Gray CW, McLaren RG (2006) Soil Factors Affecting Heavy Metal Solubility in Some New Zealand Soils. *Water, Air, & Soil Pollution* **175**, 3-14.

Gregorich EG, Wen G, Voroney RP, Kachanoski RG (1990) Calibration of a rapid direct chloroform extraction method for measuring soil microbial biomass C. *Soil Biology and Biochemistry* **22**, 1009-1011.

Gwenzi W, Munondo R (2008) Long-term impacts of pasture irrigation with treated sewage effluent on shallow groundwater quality. *Water Science & Technology* **58**, 2443-2452.

Hammer D, Keller C (2002) Changes in the Rhizosphere of Metal-Accumulating Plants Evidenced by Chemical Extractants. *J Environ Qual* **31**, 1561-1569.

Hartley J, Cairney JWG, Freestone P, Woods C, Meharg AA (1999) The effects of multiple metal contamination on ectomycorrhizal Scots pine (*Pinus sylvestris*) seedlings. *Environmental Pollution* **106**, 413-424.

Hartley J, Cairney JWG, Meharg AA (1997a) Do ectomycorrhizal fungi exhibit adaptive tolerance to potentially toxic metals in the environment ? *Plant and Soil* **189**, 303-319.

Hartley J, Cairney JWG, Sanders FE, Meharg AA (1997b) Toxic interactions of metal ions (Cd^{2+} , Pb^{2+} , Zn^{2+} and Sb^{3-}) on in vitro biomass production of ectomycorrhizal fungi. *New Phytologist* **137**, 551-562.

Hartley W, Edwards R, Lepp NW (2004) Arsenic and heavy metal mobility in iron oxide-amended contaminated soils as evaluated by short- and long-term leaching tests. *Environmental Pollution* **131**, 495-504.

Hassinen V, Vallinkoski V-M, Issakainen S, Tervahauta A, Kärenlampi S, Servomaa K (2009) Correlation of foliar *MT2b* expression with Cd and Zn concentrations in hybrid aspen (*Populus tremula* \times *tremuloides*) grown in contaminated soil. *Environmental Pollution* **157**, 922-930.

Hedley MJ, Nye PH, White RE (1983) Plant-induced changes in the rhizosphere of rape (*Brassica napus* var. Emerald) seedlings. IV. The effect of rhizosphere phosphorus status on the pH, phosphatase activity and depletion of soil phosphorus fractions in the rhizosphere and on the cation-anion balance in the plants. *New Phytologist* **95**, 69-82.

Heemsbergen DA, McLaughlin MJ, Whatmuff M, Warne MSJ, Broos K, Bell M, Nash D, Barry G, Pritchard D, Penney N (2010) Bioavailability of zinc and copper in biosolids compared to their soluble salts. *Environmental Pollution* **158**, 1907-1915.

Hewitt AE (1998) 'New Zealand soil classification: Landcare Research Science Series No.1.' (Manaaki Whenua Press: Lincoln, NZ).

Horswell J, Weitz HJ, Percival HJ, Speir TW (2006) Impact of heavy metal amended sewage sludge on forest soils as assessed by bacterial and fungal biosensors. *Biology and Fertility Soils* **42**, 569-576.

Houba VJG, Temminghoff EJM, Gaikhorst GA, van Vark W (2000) Soil analysis procedures using 0.01 M calcium chloride as extraction reagent. *Communications in Soil Science and Plant Analysis* **31**, 1299-1396.

Huang Y, Li T, Huang Z-J, Fei Y-H (2008) Ectomycorrhizal fungus-induced changes of Cu and Cd speciation in the rhizosphere of chinese pine seedlings. *Pedosphere* **18**, 758-765.

Huang Y, Tao S, Chen YJ (2005) The role of arbuscular mycorrhiza on change of heavy metal speciation in rhizosphere of maize in wastewater irrigated agriculture soil. *Journal of Environmental Sciences (China)* **17**, 276-280.

Hume L, Ofsoski N, Reynolds J (1988) Influence of pH, exchangeable aluminium and 0.02M CaCl₂-extractable aluminium on the growth and nitrogen-fixing activity of white clover (*Trifolium repens*) in some New Zealand soils. *Plant and Soil* **111**, 111-119.

Ikhsan J, Johnson BB, Wells JD (1999) A comparative study on the adsorption of transition metals on kaolinite. *Journal of Colloid and Interface Science* **217**, 403-410.

Illera V, Garrido F, Serrano S, García-González MT (2004) Immobilization of the heavy metals Cd, Cu and Pb in an acid soil amended with gypsum- and lime-rich industrial by-products. *European Journal of Soil Science* **55**, 135-145.

Iskandar IK, Adriano DC (1997) Remediation of soils contaminated with metals: a review of current practices in the USA. In 'Remediation of soils contaminated with metals'. (Eds IK Iskandar, DC Adriano). (Science Reviews: Northwood, UK).

Jacquot-Plumey E, Caussanel J-P, Gianinazzi S, Van Tuinen D, Gianinazzi-Pearson V (2003) Heavy metals in sewage sludges contribute to their adverse effects on the arbuscular mycorrhizal fungus *Glomus mosseae*. *Folia Geobotanica* **38**, 167-176.

Jassogne L, Hettiarachchi G, Chittleborough D, McNeill A (2009) Distribution and speciation of nutrient elements around micropores. *Soil Science Society of America Journal* **73**, 1319-1326.

Jentschke G, Winter S, Godbold DL (1999) Ectomycorrhizas and cadmium toxicity in Norway spruce seedlings. *Tree Physiology* **19**, 23-23.

João R, Michael WA (2000) Microporous titanosilicates and other novel mixed octahedral-tetrahedral framework oxides. *European Journal of Inorganic Chemistry* **2000**, 801-818.

Joner EJ, Jakobsen I (1995) Growth and extracellular phosphatase activity of arbuscular mycorrhizal hyphae as influenced by soil organic matter. *Soil Biology and Biochemistry* **27**, 1153-1159.

Joner EJ, Magid J, Gahoonia TS, Jakobsen I (1995) P depletion and activity of phosphatases in the rhizosphere of mycorrhizal and non-mycorrhizal cucumber (*Cucumis sativus* L.). *Soil Biology and Biochemistry* **27**, 1145-1151.

Kabala C, Singh BR (2001) Fractionation and Mobility of Copper, Lead, and Zinc in Soil Profiles in the Vicinity of a Copper Smelter. *Journal of Environmental Quality* **30**, 485-492.

Kabata-Pendias A (1993) Behavioural properties of trace metals in soils. *Applied Geochemistry* **8**, 3-9.

Kabata-Pendias A (2004) Soil-plant transfer of trace elements--an environmental issue. *Geoderma* **122**, 143-149.

Kabata-Pendias A, Pendias H (2001) 'Trace elements in soils and plants.' (CRC Press: Boca Raton, Florida).

Kaminsky W, Kummer AB (1989) Fluidized bed pyrolysis of digested sewage sludge. *Journal of Analytical and Applied Pyrolysis* **16**, 27-35.

Kamphake LJ, Hannah SA, Cohen JM (1967) Automated analysis for nitrate by hydrazine reduction. *Water Research* **1**, 205-216.

Kandeler E, Tscherko D, Bruce KD, Stemmer M, Hobbs PJ, Bardgett RD, Amelung W (2000) Structure and function of the soil microbial community in microhabitats of a heavy metal polluted soil. *Biology and Fertility of Soils* **32**, 390-400.

Kang H, Freeman C (2007) Interactions of marsh orchid (*Dactylorhiza* spp.) and soil microorganisms in relation to extracellular enzyme activities in a peat soil. *Pedosphere* **17**, 681-687.

Kao P-H, Huang C-C, Hseu Z-Y (2006) Response of microbial activities to heavy metals in a neutral loamy soil treated with biosolid. *Chemosphere* **64**, 63-70.

Keller C, McGrath SP, Dunham SJ (2002) Trace metal leaching through a soil-grassland system after sewage sludge application. *Journal of Environmental Quality* **31**, 1550-1560.

Kelly JJ, Häggblom M, Tate III RL (1999) Changes in soil microbial communities over time resulting from one time application of zinc: a laboratory microcosm study. *Soil Biology and Biochemistry* **31**, 1455-1465.

Khan MJ, Jones DL (2009) Effect of composts, lime and diammonium phosphate on the phytoavailability of heavy metals in a copper mine tailing soil. *Pedosphere* **19**, 631-641.

Khaodhiar S, Azizian MF, Osathaphan K, Nelson PO (2000) Copper, Chromium, and Arsenic Adsorption and Equilibrium Modeling in An Iron-Oxide-Coated Sand, Background Electrolyte System. *Water, Air, & Soil Pollution* **119**, 105-120.

Khiari B, Marias F, Zagrouba F, Vaxelaire J (2004) Analytical study of the pyrolysis process in a wastewater treatment pilot station. *Desalination* **167**, 39-47.

Khoshgoftarmanesh AH, Shariatmadari H, Karimian N, Kalbasi M, van der Zee SEATM (2006) Cadmium and Zinc in Saline Soil Solutions and their Concentrations in Wheat. *Soil Science Society of America Journal* **70**, 582-589.

Kiekens L (1995) Zinc. In 'Heavy Metals in Soils'. (Ed. BJ Alloway). (Blackie Academic & Professional: Glasgow, UK).

Kinraide TB (1990) Assessing the rhizotoxicity of the aluminate ion, $\text{Al}(\text{OH})_4^-$. *Plant Physiology* **94**, 1620-1625.

Komárek M, Tlustos P, Száková J, Chrastný V (2008) The use of poplar during a two-year induced phytoextraction of metals from contaminated agricultural soils. *Environmental Pollution* **151**, 27-38.

Koomen I, McGram SP, Giller KE (1990) Mycorrhizal infection of clover is delayed in soils contaminated with heavy metals from past sewage sludge applications. *Soil Biology and Biochemistry* **22**, 871-873.

Koprivova A, Kopriva S, Jager D, Will B, Jouanin L, Rennenberg H (2002) Evaluation of transgenic poplars over-expressing enzymes of glutathione synthesis for phytoremediation of cadmium. *Plant Biology* **4**, 664-670.

Kostov O, Van Cleemput O (2001) Microbial activity of Cu contaminated soils and effect of lime and compost on soil resiliency. *Compost Science & Utilization* **9**, 336.

Kovács B, Prokisch J, Györi Z, Kovács AB, Palencsár AJ (2000) Analytical methods and quality assurance - Studies on soil sample preparation for inductively coupled plasma atomic emission spectrometry analysis. *Communications in Soil Science and Plant Analysis* **31**, 1949 - 1963.

Kozdrój J (1995) Microbial responses to single or successive soil contamination with Cd or Cu. *Soil Biology and Biochemistry* **27**, 1459-1465.

Krpata D, Peintner U, Langer I, Fitz WJ, Schweiger P (2008) Ectomycorrhizal communities associated with *Populus tremula* growing on a heavy metal contaminated site. *Mycological Research* **112**, 1069-1079.

Krupa P, Kozdroj J (2004) Accumulation of heavy metals by ectomycorrhizal fungi colonizing birch trees growing in an industrial desert soil. *World Journal of Microbiology and Biotechnology* **20**, 427-430.

Kunito T, Saeki K, Goto S, Hayashi H, Oyaizu H, Matsumoto S (2001) Copper and zinc fractions affecting microorganisms in long-term sludge-amended soils. *Bioresource Technology* **79**, 135-146.

Larchevêque M, Ballini C, Korboulewsky N, Montès N (2006) The use of compost in afforestation of Mediterranean areas: Effects on soil properties and young tree seedlings. *Science of The Total Environment* **369**, 220-230.

Laureysens I, De Temmerman L, Hastir T, Van Gysel M, Ceulemans R (2005) Clonal variation in heavy metal accumulation and biomass production in a poplar coppice culture. II. Vertical distribution and phytoextraction potential. *Environmental Pollution* **133**, 541-551.

Lee CH, Lee DK, Ali MA, Kim PJ (2008) Effects of oyster shell on soil chemical and biological properties and cabbage productivity as a liming materials. *Waste Management* **28**, 2702-2708.

Lee IH, Kuan Y-C, Chern J-M (2006) Factorial experimental design for recovering heavy metals from sludge with ion-exchange resin. *Journal of Hazardous Materials* **138**, 549-559.

Liangpeng Y, Jian M, Yan L (2007) Soil salt and nutrient concentration in the rhizosphere of desert halophytes. *Acta Ecologica Sinica* **27**, 3565-3571.

Liu Q, Loganathan P, Hedley MJ, Grace LJ (2008) Effect of Mycorrhizal Inoculation on Rhizosphere Properties, Phosphorus Uptake and Growth of Pine Seedlings Treated With and Without a Phosphate Rock Fertilizer. *Journal of Plant Nutrition* **31**, 137 - 156.

Logan TJ, Chaney RL (1983) Utilization of municipal wastewater and sludge on land metals,. In 'Utilization of municipal wastewater and sludge on land'. (Eds AL Page, TL Gleasen, JE Smith, Jr., IK Iskander, LE Sommers) pp. 235-326. (University of California: Riverside, CA).

Loganathan P, Hedley MJ, Grace ND (2008) Pasture soils contaminated with fertilizer-derived cadmium and fluorine: livestock effects. *Reviews of Environmental Contamination and Toxicology* **192**, 29-66.

Lombi E, Hamon RE, Wieshammer G, McLaughlin MJ, McGrath SP (2004) Assessment of the use of Industrial by-products to remediate a copper- and arsenic-contaminated soil. *Journal of Environmental Quality* **33**, 902-910.

Ma LQ, Rao GN (1997) Chemical Fractionation of Cadmium, Copper, Nickel, and Zinc in Contaminated Soils. *J Environ Qual* **26**, 259-264.

Macnicol R, Beckett P (1985) Critical tissue concentrations of potentially toxic elements. *Plant and Soil* **85**, 107-129.

Magesan GN, Wang HL (2003) Application of municipal and industrial residuals in New Zealand forests: an overview. *Australian Journal of Soil Research* **41**, 557-569.

Malerius O, Werther J (2003) Modeling the adsorption of mercury in the flue gas of sewage sludge incineration. *Chemical Engineering Journal* **96**, 197-205.

Manceau A, Marcus MA, Tamura N, Proux O, Geoffroy N, Lanson B (2004) Natural speciation of Zn at the micrometer scale in a clayey soil using X-ray fluorescence, absorption, and diffraction. *Geochimica et Cosmochimica Acta* **68**, 2467-2483.

Manfredi O, Wuth W, Bohlinger I (1997) Characterizing the physical and chemical properties of aluminum dross. *JOM Journal of the Minerals, Metals and Materials Society* **49**, 48-51.

Manoharan V, Loganathan P, Tillman RW, Parfitt RL (1996) Assessing aluminum phytotoxicity in long-term phosphate fertilized pasture soils. *Communications in Soil Science and Plant Analysis* **27**, 1731 - 1748.

Mapanda F, Mangwayana EN, Nyamangara J, Giller KE (2007) Uptake of heavy metals by vegetables irrigated using wastewater and the subsequent risks in Harare, Zimbabwe. *Physics and Chemistry of the Earth, Parts A/B/C* **32**, 1399-1405.

Martens R (1995) Current methods for measuring microbial biomass C in soil: Potentials and limitations. *Biology and Fertility of Soils* **19**, 87-99.

Martin F, Tuskan GA, DiFazio SP, Lammers P, Newcombe G, Podila GK (2004) Symbiotic sequencing for the *Populus* mesocosm. *New Phytologist* **161**, 330-335.

Martinez CE, McBride MB, Kandianis MT, Duxbury JM, Yoon S, Bleam WF (2002) Zinc-sulfur and cadmium-sulfur association in metalliferous peats: Evidence from spectroscopy, distribution coefficients, and phytoavailability. *Environmental Science & Technology* **36**, 3683-3689.

Martinez F, Cuevas G, Calvo R, Walter I (2003) Biowaste effects on soil and native plants in a semiarid ecosystem. *Journal of Environmental Quality* **32**, 472-479.

Mathur SP, Levesque MP (1983) The effects of using copper for mitigating histosol subsidence on: 2. the distribution of copper, manganese, zinc, and iron in an organic soil, mineral sublayers, and their mixtures in the context of setting a threshold of phytotoxic soil-copper. *Soil Science* **135**, 166-176.

Matsumoto H (2000) Cell biology of aluminum toxicity and tolerance in higher plants. In 'International Review of Cytology' pp. 1-46. (Academic Press. New York)

Mattigod SV, Sposito G (1979) Chemical modeling in aqueous systems. In 'American Chemical Society Symposium Series 93'. (Ed. EA Jenne) pp. 837-856. (American Chemical Society: Washington, DC).

McBride M, Sauve S, Hendershot W (1997) Solubility control of Cu, Zn, Cd and Pb in contaminated soils. *European Journal of Soil Science* **48**, 337-346.

McBride MB (1981) 'Forms and distribution of copper in solid and solution phases of soil.' (Academic Press: New York).

McBride MB (1991) Processes of heavy and transition metal sorption by soil minerals. In 'Interactions at the soil colloid-soil solution interface'. (Eds GH Bolt, MF De Boodt, MHB Hayes, MB McBride) pp. 149-175. (Kluwer Academic Publishers: Dordrecht).

McBride MB (1994) 'Environmental Chemistry of Soils.' (Oxford University Press: New York).

McBride MB, Blasiak JJ (1979) Zinc and copper solubility as a function of pH in an acid soil. *Soil Science Society of America Journal* **43**, 866-870.

McBride MB, Bouldin DR (1984) Long-Term Reactions of Copper(II) in a Contaminated Calcareous Soil. *Soil Science Society of America Journal* **48**, 56-59.

McBride MB, Martinez CE (2000) Copper phytotoxicity in a contaminated Soil: Remediation rests with adsorptive materials. *Environmental Science & Technology* **34**, 4386-4391.

McBride MB, Martinez CE, Topp E, Evans L (2000) Trace Metal Solubility and Speciation in A Calcareous Soil 18 Years After No-Till Sludge Application. *Soil Science* **165**, 646-656.

McBride MB, Richards BK, Steenhuis T (2004) Bioavailability and crop uptake of trace elements in soil columns amended with sewage sludge products. *Plant and Soil* **262**, 71-84.

McBride MB, Richards BK, Steenhuis T, Spiers G (1999) Long-Term Leaching of Trace Elements in A Heavily Sludge-Amended Silty Clay Loam Soil. *Soil Science* **164**, 613-623.

McGrath SP, Brookes PC, Giller KE (1988) Effects of potentially toxic metals in soil derived from past applications of sewage sludge on nitrogen fixation by trifolium repens L. *Soil Biology and Biochemistry* **20**, 415-424.

McLaren RG, Black A, Clucas LM (2010) Changes in Cu, Ni, and Zn availability following simulated conversion of biosolids-amended forest soils back to agricultural use. *Australian Journal of Soil Research* **48**, 286-293.

McLaren RG, Clucas LM (2001) Fractionation of copper, nickel, and zinc in metal-spiked sewage sludge. *Journal of Environmental Quality* **30**, 1968-1975.

McLaren RG, Clucas LM, Speir TW, van Schaik AP (2007) Distribution and movement of nutrients and metals in a *Pinus radiata* forest soil following applications of biosolids. *Environmental Pollution* **147**, 32-40.

McLaren RG, Clucas LM, Taylor MD (2005) Leaching of macronutrients and metals from undisturbed soils treated with metal-spiked sewage sludge. 3. Distribution of residual metals. *Australian Journal of Soil Research* **43**, 159-170.

McLaren RG, Clucas LM, Taylor MD, Hendry T (2003) Leaching of macronutrients and metals from undisturbed soils treated with metal-spiked sewage sludge. 1. Leaching of macronutrients. *Australian Journal of Soil Research* **41**, 571-588.

McLaren RG, Clucas LM, Taylor MD, Hendry T (2004) Leaching of macronutrients and metals from undisturbed soils treated with metal-spiked sewage sludge. 2. Leaching of metals. *Australian Journal of Soil Research* **42**, 459-471.

McLaughlin MJ, Hamon RE, McLaren RG, Speir TW, Rogers SL (2000) Review: A bioavailability-based rationale for controlling metal and metalloid contamination of agricultural land in Australia and New Zealand. *Australian Journal of Soil Research* **38**, 1037-1086.

McLaughlin MJ, Smolders E (2001) Background zinc concentrations in soil affect the zinc sensitivity of soil microbial processes - a rationale for a metalloregion

approach to risk assessments. *Environmental Toxicology and Chemistry* **20**, 2639-2643.

McLean EO, Heddleson MR, Hallowaychuk RL (1958) Aluminium in soils. *Soil Science Society of America Proceedings* **22**, 382-387.

Meagher RB, Rugh CL, Kandasamy MK, Wang NJ (1997) Engineered phytoremediation of mercury pollution in soil and water using bacterial genes. . In 'Fourth International Conference on the Biogeochemistry of Trace Elements'. I.K.

Meharg AA (2003) The mechanistic basis of interactions between mycorrhizal associations and toxic metal cations. *Mycological Research* **107**, 1253-1265.

Meharg AA, Cairney JWG (2000) Ectomycorrhizas -- extending the capabilities of rhizosphere remediation? *Soil Biology and Biochemistry* **32**, 1475-1484.

Melo WJ, Junior EFF, Mouta ER, Guedes ACTP, Oliveira LR, Melo GMP, Melo VP (2007) Dehydrogenase activity in sewage sludge treated with different sources of cadmium. In 'Biochemistry of trace elements: Environmental protection, remediation and human health'. (Eds Y. Zhu, N. Lepp, R Naidu) pp. 95-98. (Tsinghua University: China).

Mench M, Vangronsveld J, Lepp NW, Edwards R (1998) Physico-chemical aspects and efficiency of trace element immobilization by soil amendments. In 'Metal contaminated soils: In situ inactivation and phytoremediation'. (Eds J Vangronsveld, SD Cunningham) pp. 151–182. (Springer: New York).

Mesquita ME, Carranca C (2005) Effect of dissolved organic matter on copper - zinc competitive adsorption by a sandy soil at different pH values. *Environmental Technology* **26**, 1065 - 1072.

Mills T, Arnold B, Sivakumaran S, Northcott G, Vogeler I, Robinson B, Norling C, Leonil D (2006) Phytoremediation and long-term site management of soil

contaminated with pentachlorophenol (PCP) and heavy metals. *Journal of Environmental Management* **79**, 232-241.

Moattar F, Hayeripour S (2004) Application of chitin and zeolite adsorbents for treatment of low level radioactive liquid wastes. *International Journal of Environmental Science and Technology* **1**, 45-50.

Mosquera-Losada MR, López-Díaz ML, Rigueiro-Rodríguez A (2009) Zinc and copper availability in herbage and soil of a *Pinus radiata* silvopastoral system in Northwest Spain after sewage-sludge and lime application. *Journal of Plant Nutrition and Soil Science* **172**, 843-850.

Naidu R, Kim KR (2008) Contaminant fate, dynamics and bioavailability: Biochemical and molecular mechanism at the soil: Root interface. *Journal of Soil Science and Plant Nutrition* **8**, 56-63.

Nissen LR, Lepp NW, Edwards R (2000) Synthetic zeolites as amendments for sewage sludge-based compost. *Chemosphere* **41**, 265-269.

Nolan AL, Lombi E, McLaughlin MJ (2003a) Metal bioaccumulation and toxicity in soils-Why bother with speciation? *Australian Journal of Chemistry* **56**, 77-91.

Nolan AL, McLaughlin MJ, Mason SD (2003b) Chemical Speciation of Zn, Cd, Cu, and Pb in pore waters of agricultural and contaminated soils using donnan dialysis. *Environmental Science & Technology* **37**, 90-98.

Nolan AL, Zhang H, McLaughlin MJ (2005) Prediction of zinc, cadmium, lead, and copper availability to wheat in contaminated soils using chemical speciation, diffusive gradients in thin films, extraction, and isotopic dilution techniques. *J Environ Qual* **34**, 496-507.

NRC (2002) 'Biosolids applied to land: Advancing standards and practices.' (The National Academics Press: Washington, D.C.).

NZFOA (2009/2010) 'New Zealand plantation forest industry: Facts and figures 2009/2010.' (New Zealand Forest Owners Association: Wellington, NZ).

NZWWA (2003) 'Guidelines for the safe application of biosolids to land in New Zealand.' (New Zealand Water and Wastes Association: Wellington).

Obbard JP (2001) Ecotoxicological assessment of heavy metals in sewage sludge amended soils. *Applied Geochemistry* **16**, 1405-1411.

Oburger E, Kirk GJD, Wenzel WW, Puschenreiter M, Jones DL (2009) Interactive effects of organic acids in the rhizosphere. *Soil Biology and Biochemistry* **41**, 449-457.

Odegaard H, Paulsrud B, Karlsson I (2002) Wastewater sludge as a resource: Sludge disposal strategies and corresponding treatment technologies aimed at sustainable handling of wastewater sludge. *Water, Science and Technology* **46**, 295-303.

Ogut M, Coyne M, Thom WO (2009) Weathered fly ash does not affect soil and biosolid carbon mineralization. *Communications in Soil Science and Plant Analysis* **40**, 1790 - 1802.

Oliver IW, Merrington G, McLaughlin MJ (2004) Australian biosolids: Characterization and determination of available copper. *Environmental Chemistry* **1**, 116-124.

Oliver IW, Merrington G, McLaughlin MJ (2006) Copper partitioning among mineral and organic fractions in biosolids. *Environmental Chemistry* **3**, 48-52.

Oste LA, Lexmond TM, Van Riemsdijk WH (2002) Metal immobilization in soils using synthetic zeolites. *J Environ Qual* **31**, 813-821.

Parker DR, Pedler JF (1997) Reevaluating the free-ion activity model of trace metal availability to higher plants. *Plant and Soil* **196**, 223-228.

Parkinson D, Gray JRG, Williams ST (1971) 'Methods for studying the ecology of soil microorganisms.' (Oxford Blackwell Scientific Publication: UK).

Parkpain P, Sirisukhodom S, Carbonell-Barrachina AA (1998) Heavy metals and nutrients chemistry in sewage sludge amended Thai soils. *Journal of Environmental Science and Health, Part A: Toxic/Hazardous Substances and Environmental Engineering* **33**, 573-597.

Pastor J, Aparicio AM, Gutierrez-Maroto A, Hernández AJ (2007) Effects of two chelating agents (EDTA and DTPA) on the autochthonous vegetation of a soil polluted with Cu, Zn and Cd. *Science of The Total Environment* **378**, 114-118.

Pathak A, Dastidar MG, Sreekrishnan TR (2008) Bioleaching of heavy metals from anaerobically digested sewage sludge. *Journal of Environmental Science and Health, Part A: Toxic/Hazardous Substances and Environmental Engineering* **43**, 402 - 411.

Paya-perez A, Sala J, Mousty F (1993) Comparison of ICP-AES and ICP-MS for the Analysis of Trace Elements in Soil Extracts. *International Journal of Environmental Analytical Chemistry* **51**, 223 - 230.

Peacock S, Rimmer DL (2000) The Suitability of an Iron Oxide-Rich Gypsum By-Product as a Soil Amendment. *Journal of Environmental Quality* **29**, 1969-1975.

Pepper IL, Gerba C. P., Brendecke J. W. (1995) 'Environmental Microbiology-A Laboratory Manual.' (Academic Press: San Diego).

Peuke AD, Rennenberg H (2005) Phytoremediation with transgenic trees. *Zeitschrift fur Naturforschung - Section C Journal of Biosciences* **60**, 199-207.

Pierzynski GM, Schwab AP (1993) Bioavailability of Zinc, Cadmium, and Lead in a Metal-Contaminated Alluvial Soil. *Journal of Environmental Quality* **22**, 247-254.

Putoczki TL, Pettolino F, Griffin MDW, Moller R, Gerrard JA, Bacic A, Jackson SL (2007) Characterization of the structure, expression and function of *Pinus radiata* D. Don arabinogalactan-proteins. *Planta* **226**, 1131-1142.

Querol X, Alastuey A, Moreno N, Alvarez-Ayuso E, Garcia-Sánchez A, Cama J, Ayora C, Simón M (2006) Immobilization of heavy metals in polluted soils by the addition of zeolitic material synthesized from coal fly ash. *Chemosphere* **62**, 171-180.

Quoreshi AM, Khasa DP (2008) Effectiveness of mycorrhizal inoculation in the nursery on root colonization, growth, and nutrient uptake of aspen and balsam poplar. *Biomass and Bioenergy* **32**, 381-391.

Ramulu USS (2001) 'Reuse of municipal sewage and sludge in agriculture.' (Scientific Publishers: Jodhpur).

Rangel-Castro JI, Ken K, Nick O, Graeme WN, Ian CA, Charlie MS, Phil I, Andy M, Jim IP (2005) Stable isotope probing analysis of the influence of liming on root exudate utilization by soil microorganisms. *Environmental Microbiology* **7**, 828-838.

Reichenberg F, Mayer P (2006) Two complementary sides of bioavailability: Accessibility and chemical activity of organic contaminants in sediments and soils. *Environmental Toxicology and Chemistry* **25**, 1239-1245.

Richards BK, Steenhuis TS, Peverly JH, McBride MB (2000) Effect of sludge-processing mode, soil texture and soil pH on metal mobility in undisturbed soil columns under accelerated loading. *Environmental Pollution* **109**, 327-346.

Robinson B, Mills T, Petit D, Fung L, Green S, Clothier B (2000) Natural and induced cadmium-accumulation in poplar and willow: Implications for phytoremediation. *Plant and Soil* **227**, 301-306.

Robson AD, Reuter DJ (1981) Diagnosis of copper deficiency and toxicity. In 'Copper in soils and plants'. (Eds JF Loneragan, AD Robson, RD Graham) pp. 287-312. (Academic Press: London).

Romkens PFAM, Salomons W (1998) Cd, Cu and Zn solubility in arable and forest soils: Consequences of land use changes for metal mobility and risk assessment. *Soil Science* **163**, 859-871.

Rosa AH, Rocha JC, Burba P (2002) Extraction and exchange behavior of metal species in therapeutically applied peat. *Talanta* **58**, 969-978.

Rudawska M, Leski T (2005) Trace elements in fruiting bodies of ectomycorrhizal fungi growing in Scots pine (*Pinus sylvestris* L.) stands in Poland. *Science of The Total Environment* **339**, 103-115.

Rusk JA, Hamon RE, Stevens DP, McLaughlin MJ (2004) Adaptation of soil biological nitrification to heavy metals. *Environmental Science & Technology* **38**, 3092-3097.

Russell RS (1977) 'Plant root systems: Their function and interaction with the soil.' (McGraw-Hill Book Company (UK) Limited: London).

Ryan P, Delhaize E, Jones D (2001) Function and mechanism of organic anion exudation from plant roots. *Annual Review of Plant Physiology and Plant Molecular Biology* **52**, 527-560.

Saha JK, Mondal AK, Hazra GC, Mandal B (1991) Depth wise distribution of copper fractions in some ultisols. *Soil Science* **151**, 452-458.

Saifullah, Meers E, Qadir M, de Caritat P, Tack FMG, Du Laing G, Zia MH (2009) EDTA-assisted Pb phytoextraction. *Chemosphere* **74**, 1279-1291.

Sakal R (2000) Long-term effects of nitrogen, phosphorous, potassium, and zinc management on crop yields and micronutrients availability in rice-wheat cropping system on calcareous soils. In 'Long-term soil fertility experiments in rice-wheat cropping systems in rice-wheat consortium paper series 6'. (Eds IP Abrol, KF Bronson, JM Duxbury, RK Gupta) pp. 73-82. (Rice-Wheat Consortium for the Indo-Gangetic Plains: New Delhi, India).

Salt DE, Prince RC, Pickering IJ, Raskin I (1995) Mechanisms of cadmium mobility and accumulation in Indian mustard *Plant Physiology* . **109**, 1427–1433.

Sánchez-Martín MJ, García-Delgado M, Lorenzo LF, Rodríguez-Cruz MS, Arienzo M (2007) Heavy metals in sewage sludge amended soils determined by sequential extractions as a function of incubation time of soils. *Geoderma* **142**, 262-273.

SAS Institute Inc (2004) 'SAS® 9.1.2 Users' Guide.' (SAS Institute Inc.: Cary, NC).

Schroder TJ, Hiemstra T, Vink JPM, van der Zee SEATM (2005) Modeling of the SolidâSolution Partitioning of Heavy Metals and Arsenic in Embanked Flood Plain Soils of the Rivers Rhine and Meuse. *Environmental Science & Technology* **39**, 7176-7184.

Schwertmann U, Taylor RM (1989) Iron oxides. In 'Minerals in soil environments'. (Eds JB Dixon, SB Weed) pp. 379-438. (ASA and SSSA: Madison, WI).

Seames WS, Fernandez A, Wendt JOL (2002) A study of fine particulate emissions from combustion of treated pulverized municipal sewage sludge. *Environmental Science & Technology* **36**, 2772-2776.

Sebastiani L, Scebba F, Tognetti R (2004) Heavy metal accumulation and growth responses in poplar clones Eridano (*Populus deltoides* x *maximowiczii*) and I-214 (*P. x euramericana*) exposed to industrial waste. *Environmental and Experimental Botany* **52**, 79-88.

Sell J, Kayser A, Schulin R, Brunner I (2005) Contribution of Ectomycorrhizal Fungi to Cadmium Uptake of Poplars and Willows from a Heavily Polluted Soil. *Plant and Soil* **277**, 245-253.

Sevillano-Marco E, Fernandez-Manso A, Castedo-Dorado F (2009) Development and applications of a growth model for *Pinus radiata* D. Don plantations in El Bierzo (Spain). *Investigacion Agraria: Sistemas y Recursos Forestales* **18**, 64-80.

Shober AL, Stehouwer RC, Macneal KE (2003) On-farm assessment of biosolids effects on soil and crop tissue quality. *Journal of Environmental Quality* **32**, 1873-1880.

Shrivastava SK, Banerjee DK (1998) Operationally determined speciation of copper and zinc in sewage sludge. *Chemical Speciation and Bioavailability* **10**, 137-143.

Shrivastava SK, Banerjee DK (2004) Speciation of Metals in Sewage Sludge and Sludge-amended Soils. *Water, Air, & Soil Pollution* **152**, 219-232.

SigmaPlot 10 (2006) 'A scientific graphing and data analysis software package.' (Systat Software Inc.: USA).

Simon L, Tamás J, Kovács E, Kovács B, Biró B (2006) Stabilisation of metals in mine spoil with amendments and growth of red fescue in symbiosis with mycorrhizal fungi. *Plant, soil and environment* **52**, 385-391.

Sims JT (1986) Soil pH Effects on the Distribution and Plant Availability of Manganese, Copper, and Zinc. *Soil Sci Soc Am J* **50**, 367-373.

Sinclair AH, Edwards AC (2008) Micronutrient deficiency problems in agricultural crops in europe. In 'Micronutrient deficiencies in global crop production'. (Ed. BJ Alloway) pp. 225-244. (Springer: Netherlands).

Singh B, Natesan SA, Singh BK, Usha K (2005) Improving zinc efficiency of cereals under zinc deficiency. *Current Science* **88**, 36-44.

Singh KP, Mohan D, Sinha S, Dalwani R (2004) Impact assessment of treated/untreated wastewater toxicants discharged by sewage treatment plants on health, agricultural, and environmental quality in the wastewater disposal area. *Chemosphere* **55**, 227-255.

Singh MV (2008) Micronutrient deficiencies in crops and soils in India. In 'Micronutrient deficiencies in global crop production'. (Ed. BJ Alloway) pp. 93–126. (Springer: Dordrecht).

Singh RP, Agrawal M (2008) Potential benefits and risks of land application of sewage sludge. *Waste Management* **28**, 347-358.

Smith JL, Paul EA (1995) The significance of soil microbial biomass estimations. In 'Soil Organic Matter Management for Sustainable Agriculture'. (Eds RDB Lefroy, GJ Blair, ET Craswell) pp. 357-396. (Australian Centre for International Agricultural Research: Canberra).

Smith S, Read D (1997) 'Mycorrhizal symbiosis.' (Academic Press: London).

Smith SR (1996) 'Agricultural recycling of sewage sludge and the environment.' (CAB International: Wallingford).

Sommers LE, Van Volk V., Giordano PM, Sopper WE, Bastian R (1987) Effects of soil properties on accumulation of trace elements by crops. In 'Land application of sludge'. (Eds AL Page, TJ Logan, J Ryan). (Food chain implications: Lewis, Chelsea).

Speir TW, van Schaik AP, Hunter LC, Ryburn JL, Percival HJ (2007) Attempts to derive EC50 values for heavy metals from land-applied Cu-, Ni-, and Zn-spiked sewage sludge. *Soil Biology and Biochemistry* **39**, 539-549.

Srivastava PC, Gupta UC (1996) 'Trace elements in crop production.' (Science Publishers: Lebanon, NH).

Stobrawa K, Lorenc-Plucinska G (2008) Thresholds of heavy-metal toxicity in cuttings of European black poplar (*Populus nigra* L.) determined according to antioxidant status of fine roots and morphometrical disorders. *Science of The Total Environment* **390**, 86-96.

Stover RC, Sommers LE, Silviera DJ (1976) Evaluation of metals in waste water sludge. *Journal of Water Pollution Control Federation* **48**, 2165-2175.

Stuczynski T, Siebielec G, Daniels WL, McCarty G, Chaney RL (2007) Biological aspects of metal waste reclamation with biosolids. *J Environ Qual* **36**, 1154-1162.

Su DC, Wong JWC (2004) Chemical speciation and phytoavailability of Zn, Cu, Ni and Cd in soil amended with fly ash-stabilized sewage sludge. *Environment International* **29**, 895-900.

Su J, Wang H, Kimberley MO, Beecroft K, Magesan GN, Hu C (2008) Distribution of heavy metals in a sandy forest soil repeatedly amended with biosolids. *Australian Journal of Soil Research* **46**, 502-508.

Sumner ME (1979) Response of alfalfa and sorghum to lime and P on highly weathered soils. *Agronomy Journal* **71**, 763-766.

Sumner ME (1979) Response of alfalfa and sorghum to lime and P on highly weathered soils. *Agronomy Journal* **71**, 763-766.

Sun L, Chen S, Chao L, Sun T (2007) Effects of Flooding on Changes in Eh, pH and Speciation of Cadmium and Lead in Contaminated Soil. *Bulletin of Environmental Contamination and Toxicology* **79**, 514-518.

Sutton WRJ (1999) The need for planted forests and the example of radiata pine. *New Forests* **17**, 95-109.

Takacs T, Voros I (2003) Effect of metal non-adapted arbuscular mycorrhizal fungi on Cd, Ni and Zn uptake by ryegrass. *Acta Agronomica Hungarica* **51**, 347-354.

Tandy S, Healey JR, Nason MA, Williamson JC, Jones DL (2009a) Heavy metal fractionation during the co-composting of biosolids, deinking paper fibre and green waste. *Bioresource Technology* **100**, 4220-4226.

Tandy S, Healey JR, Nason MA, Williamson JC, Jones DL (2009b) Remediation of metal polluted mine soil with compost: Co-composting versus incorporation. *Environmental Pollution* **157**, 690-697.

Tao S, Chen YJ, Xu FL, Cao J, Li BG (2003) Changes of copper speciation in maize rhizosphere soil. *Environmental Pollution* **122**, 447-454.

Tate KR, Ross DJ, Feltham CW (1988) A direct extraction method to estimate soil microbial c: effects of experimental variables and some different calibration procedures. *Soil Biology and Biochemistry* **20**, 329-335.

Taylor JP, Wilson B, Mills MS, Burns RG (2002) Comparison of microbial numbers and enzymatic activities in surface soils and subsoils using various techniques. *Soil Biology and Biochemistry* **34**, 387-401.

Tecator (1983) 'Application note (AN62/83): Determination of the sum of nitrate and nitrite in water by flow injection analysis.' (Foss North America Technology: Eden Prairie, MN).

Tessier A, Campbell PGC, Bisson M (1979) Sequential extraction procedure for the speciation of particulate trace metals. *Analytical Chemistry* **51**, 844-851.

Theodorou C, Bowen GD (1970) Mycorrhizal responses of Radiata pine in experiments with different fungi. *Australian Forestry Journal* **34**, 183-191.

Thiele JH (2009) 'Municipal sludge digester upgrade for biofuel production.' (Bioenergy Association: Wellington).

Tipping E, Rieuwerts J, Pan G, Ashmore MR, Lofts S, Hill MTR, Farago ME, Thornton I (2003) The solid-solution partitioning of heavy metals (Cu, Zn, Cd, Pb) in upland soils of England and Wales. *Environmental Pollution* **125**, 213-225.

Todeschini V, Franchin C, Castiglione S, Burlando B, Biondi S, Torrigiani P, Berta G, Lingua G (2007) Responses of two registered poplar clones to copper, after inoculation, or not, with arbuscular mycorrhizal fungi. *Caryologia* **60**, 146-155.

Tognetti R, Sebastiani L, Minnocci A (2004) Gas exchange and foliage characteristics of two poplar clones grown in soil amended with industrial waste. *Tree Physiology* **24**, 75-82.

- Tokalioglu S, Kartal S (2002) Atomic absorption spectrometric determination of heavy metal contents of soils around different highways in Turkey and statistical interpretation of the data. *Journal of Trace and Microprobe Techniques* **20**, 127 - 140.
- Toribio M, Romanyà J (2006) Leaching of heavy metals (Cu, Ni and Zn) and organic matter after sewage sludge application to Mediterranean forest soils. *Science of The Total Environment* **363**, 11-21.
- Toro J, Gessel S (1999) Radiata pine plantations in Chile. *New Forests* **18**, 33-44.
- Tullio M, Pierandrei F, Salerno A, Rea E (2003) Tolerance to cadmium of vesicular arbuscular mycorrhizae spores isolated from a cadmium-polluted and unpolluted soil. *Biology and Fertility of Soils* **37**, 211-214.
- Uchimiya M, Lima IM, Thomas Klasson K, Chang S, Wartelle LH, Rodgers JE (2010) Immobilization of heavy metal ions (CuII, CdII, NiII, and PbII) by broiler litter-derived biochars in water and soil. *Journal of Agricultural and Food Chemistry*.
- Udom BE, Mbagwu JSC, Adesodun JK, Agbim NN (2004) Distributions of zinc, copper, cadmium and lead in a tropical ultisol after long-term disposal of sewage sludge. *Environment International* **30**, 467-470.
- Undabeytia T, Morillo E, Ramos AB, Maqueda C (2002) Mutual Influence of Cu and a Cationic Herbicide on Their Adsorption-Desorption Processes on Two Selected Soils. *Water, Air, & Soil Pollution* **137**, 81-94.
- Unterbrunner R, Puschenreiter M, Sommer P, Wieshammer G, Tlustos P, Zupan M, Wenzel WW (2007) Heavy metal accumulation in trees growing on contaminated sites in Central Europe. *Environmental Pollution* **148**, 107-114.

USEPA (1988) Ocean Dumping Ban Act of 1988. (United States Environmental Protection Agency: Washington, DC).

USEPA (1995) Land application of sewage sludge and domestic septage. United States Environmental Protection Agency EPA/625/R-95/001, Washington, DC.

USEPA (2005) Biosolids. (United States Environmental Protection Agency: Washington, DC).

Van Tichelen KK, Colpaert JV, Vangronsveld J (2001) Ectomycorrhizal protection of *Pinus sylvestris* against copper toxicity. *New Phytologist* **150**, 203-213.

Vangronsveld J, Ruttens A, Mench M, Boisson J, Lepp NW, Edwards R, Penny C, Van der Lelie D (2000) In Situ Inactivation and Phytoremediation of Metal-and Metalloid-contaminated Soils: Field Experiments. In 'Bioremediation of Contaminated Soils'. (Eds J Wise, D Trantolo, E Cichon, H Yang, U Stotmeister) pp. 859-884. (Marcel Dekker Inc.: New York).

Vervaeke P, Luyssaert S, Mertens J, Meers E, Tack FMG, Lust N (2003) Phytoremediation prospects of willow stands on contaminated sediment: A field trial. *Environmental Pollution* **126**, 275-282.

Vesilind AP, Ramsey TB (1996) Effect of drying temperature on the fuel value of wastewater sludge *Waste management & Research* **14**, 189-196.

Virkutyte J, van Hullebusch E, Sillanpää M, Lens P (2005) Copper and trace element fractionation in electrokinetically treated methanogenic anaerobic granular sludge. *Environmental Pollution* **138**, 517-528.

Walsh LM, Erhardt WH, Seibel HD (1972) Copper Toxicity in Snapbeans (*Phaseolus vulgaris* L.). *J Environ Qual* **1**, 197-200.

Wang H, Kimberley MO, Magesan GN, McKinley RB, Lee JR, Lavery JM, Hodgkiss PDF, Payn TW, Wilks PJ, Fisher CR, McConchie DL (2006) Midrotation effects of biosolids application on tree growth and wood properties in a *Pinus radiata* plantation. *Canadian Journal of Forest Research* **36**, 1921-1930.

Weissenhorn I, Glashoff A, Leyval C, Berthelin J (1994) Differential tolerance to Cd and Zn of arbuscular mycorrhizal (AM) fungal spores isolated from heavy metal-polluted and unpolluted soils. *Plant and Soil* **167**, 189-196.

Weng L, Temminghoff EJM, Lofts S, Tipping E, Van Riemsdijk WH (2002) Complexation with dissolved organic matter and solubility control of heavy metals in a sandy soil. *Environmental Science & Technology* **36**, 4804-4810.

Weng L, Temminghoff EJM, Van Riemsdijk WH (2001) Contribution of individual sorbents to the control of heavy metal activity in sandy soil. *Environmental Science & Technology* **35**, 4436-4443.

Wightwick AM, Salzman SA, Reichman SM, Allinson G, Menzies NW (2010) Inter-Regional Variability in Environmental Availability of Fungicide Derived Copper in Vineyard Soils: An Australian Case Study. *Journal of Agricultural and Food Chemistry* **58**, 449-457.

Wilkins DA (1991) The influence of sheathing (ecto-)mycorrhizas of trees on the uptake and toxicity of metals. *Agriculture, Ecosystems & Environment* **35**, 245-260.

Wu J, Joergensen RG, Pommerening B, Chaussod R, Brookes PC (1990) Measurement of soil microbial biomass C by fumigation-extraction--an automated procedure. *Soil Biology and Biochemistry* **22**, 1167-1169.

Wu Q, Hendershot WH, Marshall WD, Ge Y (2000) Speciation of cadmium, copper, lead, and zinc in contaminated soils. *Communications in Soil Science and Plant Analysis* **31**, 1129 - 1144.

Wu X, Aasen I (1994) Models for predicting soil zinc availability for barley. *Plant and Soil* **163**, 279-285.

Yamanaka T (2003) The effect of pH on the growth of saprotrophic and ectomycorrhizal ammonia fungi in vitro. *Mycologia* **95**, 584-589.

Yang YH, Chen SM (2001) Physiological effects of aluminium/calcium ratios on aluminium toxicity of mungbean seedling growth. *Journal of Plant Nutrition* **24**, 585-597.

Yin Y, Impellitteri CA, You S-J, Allen HE (2002) The importance of organic matter distribution and extract soil:solution ratio on the desorption of heavy metals from soils. *The Science of The Total Environment* **287**, 107-119.

Zhang H, Sun L, Sun T (2008a) Solubility of ion and trace metals from stabilized sewage sludge by fly ash and alkaline mine tailing. *Journal of Environmental Sciences* **20**, 710-716.

Zhang H, Young SD (2005) Characterizing the availability of metals in contaminated soils. II. The soil solution. *Soil Use and Management* **21**, 459-467.

Zhang H, Zhao F-J, Sun B, Davison W, McGrath SP (2001) A new method to measure effective soil solution concentration predicts copper availability to plants. *Environmental Science & Technology* **35**, 2602-2607.

Zhang L, Masui M, Mizukoshi H, Ninomiya Y, Kanaoka C (2007) Formation of submicron particulates (PM₁) from the oxygen-enriched combustion of dried sewage sludge and their properties. *Energy & Fuels* **21**, 88-98.

Zhang L, Masui M, Mizukoshi H, Ninomiya Y, Koketsu J, Kanaoka C (2008) Properties of water-soluble and insoluble particulate matter emitted from dewatered sewage sludge incineration in a pilot-scale ash melting furnace. *Fuel* **87**, 964-973.

Zhao F, McGrath SP, Dunham SJ (1999) Factors affecting the solubility of zinc, cadmium, copper and nickel in sewage sludge amended soils. In 'Proceedings of Fifth International Conference on the Biogeochemistry of Trace Metals'. Vienna. (Ed. WW Wenzel, D.C. Adriano, B. Alloway, H.E. Doner, C. Keller, N.W. Lepp, M. Mench, R. Naidu and G.M. Pierzynski) pp. 270-271. (Austria Eds).

Zhao K, Selim HM (2010) Adsorption-desorption kinetics of Zn in soils: Influence of phosphate. *Soil Science* **175**, 145-153.

Zhou LX, Wong JWC (2001) Effect of dissolved organic matter from sludge and sludge compost on soil copper sorption. *Journal of Environmental Quality* **30**, 878-883.

Zorpas AA, Constantinides T, Vlyssides AG, Haralambous I, Loizidou M (2000) Heavy metal uptake by natural zeolite and metals partitioning in sewage sludge compost. *Bioresource Technology* **72**, 113-119.

Zou C, Gao X, Shi R, Fan X, Zhang F (2008) Micronutrient deficiencies in crop production in China. In 'Micronutrient deficiencies in global crop production'. (Ed. BJ Alloway) pp. 127–148. (Springer: Dordrecht).

Zublena JP (1991) Soil facts: Nutrients removed by crops in North Carolina. (North Carolina Cooperative Extension Service: North Carolina).

Appendix

Chapter 6: Effect of lime and Al waste on poplar yield, and Cu and Zn uptake from a biosolids-amended soil

Preliminary study (2weeks incubation study with lime and Al waste)

Objective

To determine the levels of lime (L) and Al waste (A) needed as amendments to ameliorate Cu and Zn toxicity to poplar plants

Treatments

The soils were mixed with following rates of lime and Al wastes.

| | | | | | | | | | |
|----|---------|--------|--------|--------|--------|--------|--------|------|-------|
| Cu | Control | 0.1% L | 0.3% L | 0.6% L | 0.9% L | 1.2% L | 1.5% L | 2% A | 10% A |
| Zn | Control | 0.1% L | 0.3% L | 0.6% L | 0.9% L | 1.2% L | 1.5% L | 2% A | 10% A |

- The samples were duplicated and incubated at 80% field capacity and at 28⁰C for 2 weeks
- Samplings were done twice, one and two weeks after incubation and then pH and soluble & exchangeable fraction were measured.

Results

pH and soluble & exchangeable fraction of metals (a. Cu and b. Zn) in 1 week and 2 weeks after incubation of biosolids amended soils incorporated with lime (L) and Al wastes (A).

a.

| | | 1 wk after incubation | | 2 wks after incubation | |
|---------|---------|-----------------------|--------------------------------------|------------------------|--------------------------------------|
| | | pH | Soluble & Exchangeable (mg/kg) | pH | Soluble & Exchangeable (mg/kg) |
| Cu Soil | Control | 6.1 | 3.16 | 5.8 | 5.70 |
| | 0.1% L | 6.5 | 1.26 | 6.6 | 3.54 |
| | 0.3% L | 6.8 | 0.98 | 6.9 | 3.50 |
| | 0.6% L | 7.4 | 0.81 | 7.5 | 2.88 |
| | 0.9% L | 7.6 | 0.86 | 7.6 | 2.90 |
| | 1.2% L | 7.6 | 0.75 | 7.7 | 2.45 |
| | 1.5% L | 7.6 | 0.79 | 7.7 | 2.55 |
| | 2% A | 6.7 | 1.52 | 6.8 | 2.93 |
| | 10% A | 7.5 | 0.79 | 7.5 | 2.89 |

b.

| | | 1 wk after incubation | | 2 wks after incubation | |
|---------|---------|-----------------------|--------------------------------------|------------------------|--------------------------------------|
| | | pH | Soluble & Exchangeable (mg/kg) | pH | Soluble & Exchangeable (mg/kg) |
| Zn Soil | Control | 5.7 | 166.58 | 5.5 | 220.56 |
| | 0.1% L | 6.1 | 115.63 | 5.8 | 123.24 |
| | 0.3% L | 6.5 | 54.83 | 6.4 | 60.50 |
| | 0.6% L | 6.9 | 28.16 | 6.8 | 17.17 |
| | 0.9% L | 7.1 | 13.87 | 7.0 | 9.60 |
| | 1.2% L | 7.2 | 5.65 | 7.3 | 3.39 |
| | 1.5% L | 7.3 | 5.52 | 7.5 | 1.57 |
| | 2% A | 6.2 | 24.63 | 6.2 | 59.45 |
| | 10% A | 7.2 | 21.28 | 7.6 | 10.18 |

The treatments which produced the optimum pH values for plant growth (pH 6.5–7.0) were selected for the glasshouse trial. These were 0.1 and 0.5% lime and 2 and 6% Al dross.