Copper Availability in Biosolids

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In fulfilment of the requirements for the degree of

Doctor of Philosophy

A thesis submitted to

Soil and Land Systems

School of Earth and Environmental Sciences

The University of Adelaide

Australia

June, 2004

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Abstract

Biosolids from sewage treatment plants present both problems and opportunities for society. They are rich in organic matter, nutrients and trace elements and so can be effective soil conditioners, potentially improving both soil structure and fertility. However, they commonly contain high concentrations of heavy metals, which can accumulate to toxic levels in soils receiving frequent or high dose applications. Copper (Cu) is one of the metals of chief concern because it often has high concentrations in biosolids and is capable of exerting a toxic effect on soil microbes. Limits are placed on the amounts of biosolids that can be applied to land to prevent soil accumulation of metals, but these regulatory limits are based on the total metal concentrations in soils and biosolids rather than on the portion that is ecologically active. Therefore, current regulations do not take into account the fact that much of the metal content is bound up in a way that renders it non-active, and thus poses no threat to the environment. A more environmentally relevant regulatory system would set its limits using the available portion of metals. Therefore it is important to quantify this available fraction, and to establish a method by which it can be consistently measured. To do this the nature of biosolids needs to be better understood, and the factors controlling the available fraction need to be identified. Also, it is important to determine how the available fraction may change with time.

This PhD project surveyed 24 biosolids from around Australia and characterised them in terms of chemical and physical properties. Available Cu was measured using radio isotopic techniques (^{64}Cu), a Cu^{2+} ion selective electrode, solution extraction, and other methods. A model for predicting available Cu was produced, using the total Cu concentration and the Cu^{2+} ion activity in solution extracts:

$$\text{Available Cu (mg/kg)} = 281.5 \text{ Log Total Cu} - 14.9\ pCu^{2+} - 459 \qquad (R^2 = 0.806)$$

where 'logTotal Cu' is $\log_{10}$ total biosolid Cu concentration (mg/kg).

A 21-month incubation experiment was conducted to monitor Cu availability over time, with the conclusion that it will remain constant if pH is maintained. Biosolid/soil pH had a strong effect on available Cu, hence a regulatory system based on the available Cu fraction that incorporates a pH protection index is proposed. Mineralisation of organic matter did not lead to increases in available Cu, thus no evidence for the time bomb hypothesis was found. However, organic matter was found to be important for Cu sorption in some biosolids, indicating that over a longer term effects of organic mineralisation on Cu availability may be seen, and therefore longer trials (*i.e.* > 10 years) are needed to determine the long-term fate of biosolid Cu.

Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person except where due reference has been made in the text.

I give consent for this thesis, when deposited in the University Library, to be available for loan and photocopying.

13/10/2004

Ian William Oliver **Date**

Acknowledgements

I would like to thank the South Australian Water Corporation (SA Water) for providing supplemental funds in the first year of this research.

A huge thank you goes to my supervisors, Dr Mike McLaughlin and Dr Graham Merrington, whose advice, direction, encouragement and good humour throughout my candidature were invaluable. Quite simply, they are dead set legends.

Many others provided valuable support during this epic production, with Colin Rivers, laboratory manager extraordinaire, at the top of the list. Dr. Ron Smernick (Dr NMR), also merits special mention. To all the staff and post grads within the University of Adelaide's Department of Soil Science (in all its incarnations; Soil Science, Soil and Water, Soil and Land Systems), I thank you for your suggestions, help and camaraderie. In particular, I thank Ryan Farquharson for tending my samples while I was in Europe. To the members of Mike's team upstairs at CSIRO I say thanks, for many analyses, critical comment and support.

I'd also like to thank my friends and colleagues at the Laboratory for Soil and Water Management, K.U. Leuven, with whom I spent a golden year during my Belgian hiatus. Although not strictly part of my PhD, the experience allowed me to return a better scientist, keen to finish my own research and tackle anything else thrown at me (Erik Smolders and crew, dank u wel).

Last but not least, I'd like to thank my family and friends for their support and encouragement, and for their often asked question of what I'm going to do when I stop playing in the dirt and get a real job. We will see …

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1. General Introduction and Literature Review

1.1 Generation and Disposal of Sewage Sludge (Biosolids)

The treatment and processing of waste water by sewage treatment plants generates a residual material known as sewage sludge, or biosolids. Waste water treatment (and so biosolid production) is a multi stage process, beginning with raw sewage undergoing primary treatment. Primary treatment involves the physical processes of screening to remove larger fragments of debris, aeration to promote precipitation and settling, and sedimentation of solids in primary sedimentation tanks (EWS 1992). Secondary treatment involves biological processes, whereby organisms (*i.e.* bacteria, fungi, protozoa and algae) are utilised for their ability to break down organic matter, and to convert suspended particles and dissolved nutrients into more chemically stable forms. The sediments (or sludges) produced during primary and secondary treatment then undergo stabilisation, commonly via anaerobic digestion. This treatment sees the sludge transferred to sealed digestion tanks, where it is subjected to anaerobic decomposition. Bacterial activity in the digesters reduces the organic matter content of the sludge, reduces the level of pathogens present, and also reduces sludge mass by approximately 40% (Ross *et al.* 1991). The digested sludge is then pumped to evaporation lagoons or to mechanical presses for drying, producing a soil-like material (*i.e.* biosolids).

It is estimated that 220 000 tons of dry biosolids are generated in Australia annually, all of which require some form of disposal or re-use. Due to increasing pressure to discontinue the practice of ocean disposal, much of the biosolids produced in this country are simply stockpiled, disposed of as land-fill, incinerated, or used to cap municipal waste dumps or mine sites (Beavers 1993). However, in line with the community's developing recycling ethos, and the ever tightening budgets of waste water treatment authorities, a growing trend away from these seemingly

wasteful, non cost-effective disposal methods has emerged. This trend has been strongest in NSW, where it is estimated that up to 99% of the biosolids produced by Sydney Water are disposed of via land application (Beavers 1993). Other waste treatment authorities (including those in SA, Vic and Qld) are implementing similar programs, or are conducting feasibility trials (Beavers 1993). In the UK the application of biosolids to agricultural land is a long established practice, with approximately 40-60% of that produced disposed of in this way (McGrath 1987; DETR 1998). In the USA the figure is approximately 40% overall (Lue-Hing *et al.* 1994), with some individual states (*e.g.* Maryland) applying up to 90% (Walker 1994). This recycling of biosolids, via land application, can provide many potential benefits to agricultural systems.

1.2 Benefits of Biosolid Land Application

Biosolids are rich in organic matter, commonly up to 60% dry weight (Ross *et al.* 1991), and in macro- and micronutrients. Thus biosolids can have a range of beneficial effects when applied to agricultural land. Due largely to the organic matter content of the material, improvements in soil physical properties such as increases in aggregate stability, porosity, infiltration rate and available water content have been repeatedly documented after biosolid application (Hall and Coker 1981; Morel and Guckert 1981; Oberle and Keeney 1994). Biosolid phosphorus (P) and nitrogen (N) can significantly increase the fertility and productivity of soils receiving biosolid applications, and thus can reduce the need for inorganic P and N fertilizer additions on treated soils. This is attested to by the vast array of studies reported in the literature which detail increased plant yields following biosolid application (Chang *et al.* 1982; Cimino and Toscano 1993; Dowdy *et al.* 1978; Gardiner *et al.* 1995; Narwal *et al.* 1983; Peverly and Gates 1994; Reddy and Dunn 1986; Sanders *et al.* 1987). The trace element (*i.e.* Cu, Zn, and Ni, etc.) content of biosolids may also be partially responsible for the reported increases in soil fertility following biosolid addition. This is

because of the demonstrated capability of biosolids to supply these micronutrients at rates equivalent to or greater than that commonly supplied by applications of commercial fertilizers (Weggler-Beaton 1996). Application of biosolids to agricultural land can therefore be seen as a particularly beneficial option in Australia, due to Australian soils being generally low in organic matter, and low in N and P minerals (Hubble *et al.* 1983; Spain *et al.* 1983). Further, many Australian soils are also deficient in trace elements.

1.3 Land Application of Biosolids: Potential Detrimental Effects

Although many benefits may be gained through land application of biosolids, the practice does have the potential to create detrimental effects. The biosolid components of major concern in this regard are: pathogenic organisms, nutrients, organic contaminants, and heavy metals.

1.3.1. Pathogens, Nutrients and Organic Contaminants

Pathogens present in faecal material and other wastes have the potential to be transferred into biosolids. Such pathogenic organisms may include viruses, bacteria, fungi, protozoa and nematodes, all of which can pose risks to human and/or animal health (Ross *et al.* 1991). The high fertilizer value of biosolids has already been discussed, but biosolid nutrients (principally P and N) can pose a threat to environmental quality if applied inappropriately or at rates in excess of plant requirements. Losses from application sites (through leaching of nitrates, and through surface run-off containing both phosphates and nitrates) can lead to significant pollution of ground and surface waters (Smith 1996). This pollution can, in turn, result in accelerated rates of eutrophication in receiving waters, and to the associated outbreaks of algal blooms. However, the threats posed by pathogenic organisms and by biosolid nutrients are reduced through a number of routine operations, and by application guidelines. In the case of pathogens, biosolids are either

heat-treated, stockpiled for a certain time, or there is a withholding period imposed between application and land use (DETR 1998; SAEPA 1996). In the case of nutrients, guidelines are set for the types of sites where application is deemed suitable. These guidelines are commonly based on the degree of slope and proximity to surface and ground water, etc. (*i.e.* DETR 1998; Ross *et al.* 1991; SAEPA 1996).

A range of organic pollutants (man-made chemicals including pesticides, industrial solvents and plasticisers) can occur in biosolids. These chemicals are widely considered as risk factors due to their often high levels of toxicity, carcinogenic properties, and environmental persistence. Further, they are perceived as particularly harmful because of their entirely artificial nature (Smith 1996). However, despite their notoriety and perceived threat, there is general consensus within the scientific community (based on current knowledge) that organic contaminants present in biosolids are unlikely to cause significant environmental problems (O'Connor 1994; Wild and Jones 1991). This is because their concentrations are usually very low (mg/kg-µg/kg range) in biosolids, and these are further diluted at least 100 fold in typical agricultural land application scenarios (O'Connor 1994). These chemicals are expected to become an even lesser threat in the future, due to their frequency of use declining and the enforcement of stricter controls on their use and disposal (Smith 1996).

1.3.2. Heavy Metals

Due to the considerations raised in the previous section, it is the heavy metal content of biosolids that is perceived as posing the greatest risk to environmental health in connection with biosolid application (Kabata-Pendias and Pendias 1992; Smith 1996)). Thus restriction of biosolid heavy metal contents forms the basis of many reuse regulations (*i.e.* DETR 1998; SAEPA 1996). Heavy

metals can enter the sewer system through road run-off, atmospheric deposition, pipe corrosion, and through domestic and industrial waste inputs. Some of the metals commonly found in biosolids include cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), manganese (Mn), mercury (Hg), nickel (Ni) and zinc (Zn). Some of these metals are essential trace elements involved in plant and animal nutrition, however all of them can be toxic at elevated concentrations. Land application of biosolids can lead to accumulations of these heavy metals in soil (King and Hajjar 1990; MacLean *et al.* 1987; McGrath 1987; Nyamangara 1998). This can adversely affect soil microbial systems (Dahlin and Witter 1993), and may also lead to increased plant up-take of metals (Chang *et al.* 1987; Gardiner *et al.* 1995; Pichtel and Anderson 1997; Zwarich and Mills 1982). Increased plant uptake has many potential implications, including direct phytotoxic symptoms, reduced yields, and possible flow-on effects through food chains (affecting both animals and humans). Excess metal concentrations in agricultural soils therefore have the potential to cause problems in terms of soil fertility, agricultural productivity, and food safety and marketability. It is for these reasons that metal concentration based limits are imposed on the agricultural use of biosolids. However, in Australia (as in many other countries), the limits are based on total soil and biosolid metal concentrations (*i.e.* SAEPA 1996). Such limits are inadequate because they do not take into account the many soil-metal interactions which effectively render much of the added metals unavailable (Kiekens and Cottenie 1981; McLaren and Crawford 1973b). Similarly, the chemistry of the biosolids themselves (*i.e.* the interactions between metals and other biosolid components), which also limits metal-organism interactions (Heckman *et al.* 1987; Jing and Logan 1992; John and Van Laerhoven 1976), are not considered. Thus, current regulations do not distinguish between the various forms of metals present, so that insoluble, non-reactive and non-available forms are deemed to be as hazardous as forms that are highly soluble, reactive and toxic. Many studies have shown that the non-available forms of

metals in biosolids, and in biosolid amended soils, far outweigh the available (*i.e.* potentially useful/toxic) fractions (McGrath 1987; Tsadilas *et al.* 1995). Thus it is important to determine the potentially available proportions of various metals present in biosolids, so that more accurate assessments can be made as to the likely risks of metal contamination occurring following biosolid application (and also so that more appropriate application regulations can be devised). The metals of primary concern, *i.e.* those whose total concentrations most commonly restrict biosolid re-use (and so warrant investigation in terms of their available fractions), are Cu and Zn (Nyamangara 1998; Smith 1996).

1.4 Biosolid Copper

1.4.1 Sources of Biosolid Copper

1.4.1.1 Copper Plumbing Systems

The corrosion/abrasion of Cu from plumbing systems is, in the majority of cases, the principal source of Cu in biosolids (Smith 1996). Exceptions occur in areas where industrial discharges containing high levels of Cu (*i.e.* from electroplating plants) are released directly into sewers, in which case these sources may dominate Cu input. However, even in such areas Cu plumbing systems are still a major source of biosolid Cu – commonly contributing around 30-40% of total Cu input (German Copper Institute 1995, as cited by Landner *et al.* 2000).

The amount of Cu released through pipe corrosion and abrasion depends on the age of the plumbing and on the physico-chemical properties of the water supply. The rate of corrosion generally decreases with time, due to the formation of a coating layer that restricts contact between the active components (the electrochemical pair). Therefore Cu dissolution occurs at a faster rate in newer pipes than in older ones (Comber and Gunn 1996), as shown in Figure 1.4.1.

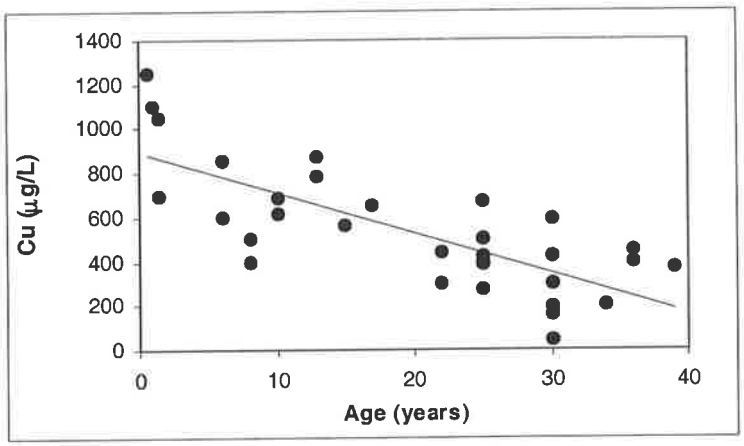


Figure 1.4.1: Relationship between hot water Cu concentration and age of household pipes (Comber & Gunn 1996).

Therefore, it may be expected that sewage works servicing newer estates (residential, industrial or commercial) will produce biosolids of higher Cu content than those servicing older estates (assuming other factors are equal).

The corrosivity of the water supply, determined mostly by its pH but also by its calcium (Ca) and carbonate concentrations (or 'hardness') and dissolved CO_2 (Broo *et al.* 1997), is a major factor determining the Cu input to sewers from plumbing systems. In general, water with pH above 8 is considered non-corrosive, while water having a pH less than 6.5 is considered corrosive. Cruse *et al.* (Cruse *et al.* 1985) produced a simplified diagram (Figure 1.4.2) indicating the relationship between pH of mains water and the dissolution of Cu from plumbing.

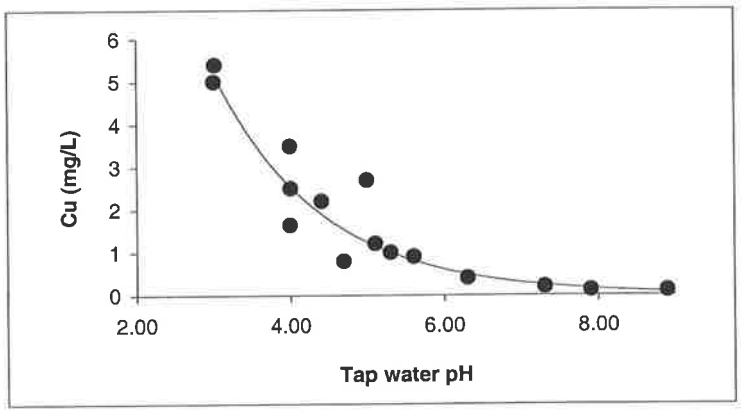


Figure 1.4.2: Mains water Cu concentration as affected by pH. Redrawn from Cruse *et al.* (1988).

1.4.1.2 Other Sources

Other inputs of Cu to sewer systems, and thus contributions to biosolid Cu, can come from industrial and commercial wastes, atmospheric deposition and road run-off, and Cu containing additives (flocculents) used in sewage treatment. Industrial/commercial wastes (mainly from brass polishing, electroplating and metal finishing industries, but also to a lesser extent from vehicle mechanics, hospitals and laboratories) can be a significant source of Cu. In some cases, these industrial/commercial inputs have been found to be the dominant sources – representing up to 60% of total Cu input to sewers (German Copper Institute 1995, as cited in Landner *et al.* 2000). However the amount derived from industry is generally much less, and is dependent upon the type of industrial activities conducted in the region, the level of waste pre-treatment prior to sewer discharge, and whether industrial wastes are discharged to the municipal sewer. In the UK, the contribution of industrial/commercial wastes to total sewer Cu input was estimated to be around 3% (Smith 1996 and references therein).

The importance of atmospheric deposition and run-off as a source of biosolid Cu depends on the existence of separate or combined sewer and storm water systems. With combined systems, run-off from roads, railways and houses (containing Cu from corrosion of vehicle components and roof cladding, etc.) can add to the sewer Cu load. The amount contributed depends on a number of factors, including the extent of road networks in the area, the volume and type of traffic, and factory emission standards. Data on actual percentages of total sewer Cu input contributed by run-off are sparse, but a survey of the literature by Landner *et al.* (2000) identified some studies where broad estimates were given for Sweden, Switzerland and the UK. The values quoted were 20% (Sweden), 10% (Switzerland) and <4% (UK).

1.4.2 Copper Concentrations in Biosolids

The amount and concentration of Cu in biosolids can vary greatly, both between different sewage treatment plants and over time within a single plant, due to the variations in input discussed above. Also, the treatment processes employed at a given treatment plant will affect the final Cu concentration in the biosolids produced. The variability of Cu concentration was highlighted by a survey of the biosolids applied to land in the Sydney region (Ross *et al.* 1991). From 72 samples, the survey revealed a concentration range of 705 – 2783 mg Cu/kg (dry solids), with a mean concentration of 1427 mg Cu/kg. Similarly, in the USA, a study found that eight sewage treatment plants within a single state (Indiana) produced biosolids with mean Cu concentrations ranging from 662 to 8381 mg Cu/kg dry solids (Sommers *et al.* 1976).

1.5 Background Levels and Regulations

Background Cu concentrations are important because regulations covering the re-use of biosolids (on agricultural land) often take into account existing soil levels (*e.g.* SAEPA 1996). Such regulations acknowledge the role played by existing Cu contents in the accumulation of Cu to potentially toxic levels in soils. It is the weathering and break down of primary minerals, such as bornite (Cu_5FeS_4) and chalcopyrite ($CuFeS_2$), that is the initial source of Cu in unpolluted soils (Parker 1981). Table 1.5.1 lists Cu concentration ranges determined for a number of natural (*i.e.* unpolluted), Australian surface soils (McKenzie 1959; Tiller 1963).

Table 1.5.1: Typical Copper Concentrations For Australian Soils

Soil	Cu Concentration (mg / kg dw)
Sandy soils & podsols	22-52
Gleysols	38-61
Rendzinas	7-43
Kastanozems & brown soils	83-140
Ferralsols	2-96

The maximum permissible concentration of Cu in agricultural soils varies between (and within) countries (Table 1.5.2, and see McLaughlin *et al.* 2000 for further comparisons and discussion). Applications of biosolids are therefore restricted so as not to exceed these levels. Further, in many areas, biosolid application is restricted in compliance with annual limits set down for the maximum amount of metals that can be applied to soils (Table 1.5.2). Additional regulations, covering aspects such as proximity to surface and ground waters, and degree of slope, etc., are also commonly in place (*i.e.* DPIWE 1999; SAEPA 1996). In South Australia, biosolid application is also restricted to sites with soil pH > 5.5.

Table 1.5.2: Soil Cu Regulations for Various Regions in Australia and the World

Country/Region	Max. Permissible Soil Cu Concentration (mg / kg dw)	Annual Load Limit (kg / ha)
Australia		
- New South Wales*	100	-
- South Australia#	200	12
- Tasmania++	42	-
- Victoria $	100	-
Denmark	40	-
France	100	-
United Kingdom	80, 100, 135, 200 **	7.5
United States	775	75

** Values for UK are for soils with pH 5.0<5.5, 5.5<6.0, 6-7, & >7 respectively.
* (EPA NSW 1997), # (SAEPA 1996), ++(DPIWE 1999), $ (VBTWG 2000).
Other information compiled by Smith (1996).

1.6 Copper Associations in Biosolids and Soils

1.6.1 Inorganic Forms

1.6.1.1 Solution Inorganic Copper

Although soluble nitrate, chloride, sulphate and phosphate complexes of Cu can occur in soil solutions, the most significant inorganic species are hydroxy and carbonate complexes and free

Cu^{2+} ions (McBride 1981; Ritchie and Sposito 1995). The solubilities of inorganic Cu^{2+} species in solution are shown as a function of pH in Figure 1.6.1.

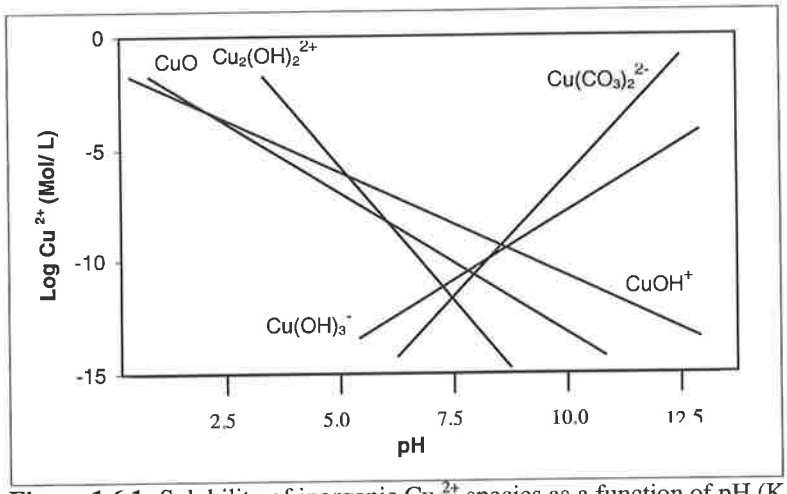


Figure 1.6.1: Solubility of inorganic Cu^{2+} species as a function of pH (Kabata-Pendias and Pendias, 1992).

Overall solubility of Cu^{2+} species reaches a minimum at about pH 7 – 8 (Figure 1.6.1). The species $CuOH^+$ and $Cu_2(OH)_2^{2+}$ are most prevalent below pH 7, while above pH 8 anionic species become important. However, in alkaline conditions Cu tends to flocculate or precipitate out of solution, so that concentration is greater at lower pH (*i.e.* pH < 4, Kiekens and Cottenie 1981). As is the case for most metal cations, the activity of Cu^{2+} also increases as pH declines (Cavallaro and McBride 1980; Sauve *et al.* 1997). The solubility of inorganic metal compounds is, however, generally not the dominant factor determining soil solution metal concentrations (Tiller 1996). This is particularly the case for Cu, as stability constants indicate that Cu minerals are too soluble to account for the typically low concentrations of total Cu found in soil solutions (McBride 1981). Indeed, soil solution Cu concentrations are commonly less than $6x10^{-7}$ mol/L in non biosolids-amended soils (Bradford *et al.* 1971; Lindsay 1991; Yamasaki *et al.* 1975), while values of $2.5x10^{-6}$ mol/L may be expected in heavily amended soils (*i.e.*McBride *et al.* 1999). Thus it has been suggested that precipitation-dissolution reactions do not primarily control Cu concentration, but rather adsorption-desorption processes.

Inorganic species, however, form only a small fraction of solution Cu in biosolid-amended soils (and soils generally). Many studies have demonstrated that organic complexes dominate solution Cu (*i.e.* Hodgson *et al.* 1965; Hodgson *et al.* 1966), leaving inorganic species representing from 10% to less than 1% of total solution Cu (Dudley *et al.* 1987; Emmerich *et al.* 1982a; Sauve *et al.* 1997). Furthermore (and importantly for plant uptake), free Cu^{2+} ions have been shown to comprise only between 0.2% and 3.0% of solution Cu in biosolid-amended soils (Emmerich *et al.* 1982a; Minnich and McBride 1987). It should also be noted here that solution Cu (including both 'free' and complexed forms) represents a minute portion of total biosolid/soil Cu. From the data of Sauve *et al.* (1997) it can be seen that the amount of soluble Cu (as a percentage of total Cu) ranged from 0.03% to 0.19% in 15 samples of biosolid-treated soil (with a mean of 0.11%). Thus the vast majority of Cu in biosolids and in soils is associated with the solid phase.

1.6.1.2 Solid Phase Inorganic Forms

Copper may be present as an absorbate on inorganic surfaces such as silicate clays, metal oxides and hydroxides, and carbonates. Two mechanisms of adsorption have been loosely defined, they are 'non-specific' and 'specific' adsorption. The former involves simple ion exchange, whereby Cu is electrostatically bound to sites of permanent negative charge (McBride 1981). This non-specifically adsorbed Cu can exchange freely with ions in solution, and is easily displaced when excess Ca^{2+} (or other muliti-valent cations) are present in solution (McLaren and Crawford 1973a). Copper adsorbed in this way represents a very small fraction of the total adsorbed metal, and in some cases has been considered negligible when compared to total surface adsorbed Cu (*i.e.* McLaren and Crawford 1973b). The latter mechanism, 'specific' adsorption, results in Cu being held by surfaces even in the presence of excess solution Ca^{2+} which would prevent Cu

adsorption by standard cation exchange. According to McBride (1981), Cu^{2+} (with the possible exception of Pb^{2+}) is the most strongly specifically adsorbed divalent transition / heavy metal on Fe and Al oxy-hydroxides. Adsorption to these surfaces may involve the formation of direct surface Cu-O-Fe or Cu-O-Al bonds (McBride 1978a). McLaren and Crawford (1973b) showed that specific adsorption of Cu also occurs on Mn oxides, and that the adsorption capacity of these is greater than that of Fe and Al oxides.

However, according to solid phase fractionation schemes (*cf.* sections 1.8 and 1.9.2.2) adsorbed forms do not form the bulk of the inorganic Cu in biosolid-amended soils. The largest inorganic fractions are the occluded forms (occluded by silicate clays) and that held inside mineral structures (termed residual Cu) (Nyamangara 1998). This Cu is non-diffusible, and so does not exchange with solution Cu (McLaren and Crawford 1974). Much of the 'native' Cu in soils is likely to be associated with the occluded and residual fractions.

1.6.2 Organic Forms

Copper has an extremely high affinity for organic matter, much more so than most other metals (McBride 1981). Thus in pure biosolids, Cu can be expected to be largely associated with the organic matter fraction. Copper (and other metals) can be strongly complexed by organic matter due to the presence of multidentate sites on organic ligands, which bind ions like a claw in a process termed chelation (Stevenson 1994). The binding (electron donor) sites are most often on O, S and N atoms (Figure 1.6.2) (Parker 1981).

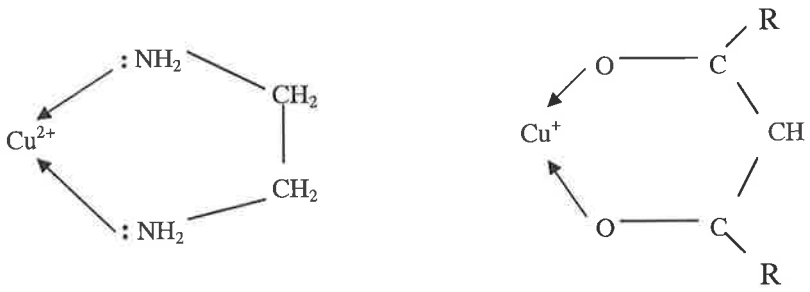


Figure 1.6.2: Two chemical structures showing bidentate Cu^{2+} binding (Parker 1981).

Organic materials derived from biosolids and soils are extremely heterogeneous, making it difficult to identify and classify compounds. However a large amount of research has focused on Cu binding by a complex group of compounds referred to as fulvic and humic acids. Complexation of Cu by these humic substances is believed to primarily involve carboxylate, phenolic hydroxyl, carbonyl and amine functional groups (McBride 1978b; Stevenson 1994; Stevenson and Fitch 1981). At low Cu concentrations (or high organic matter contents), complexation with humic substances will principally occur through the chelation process outlined above, thus forming strong covalent Cu-organic matter bonds (Stevenson 1994). As the contamination level increases, sites capable of covalent bond formation become saturated, hence further binding occurs through weaker electrostatic forces (Stevenson 1994). Therefore Cu-organic matter complexation in biosolids and soils may involve both covalent and ionic bonding, with the relative importance of each process depending on the level of organic matter Cu saturation. Figure1.6.3 shows Cu as a 2:1 chelate complex holding two humic acid molecules in a chain, as a 1:1 complex at the end of a chain, and in a salt-type linkage with an isolated carboxylate group (Stevenson and Fitch 1981):

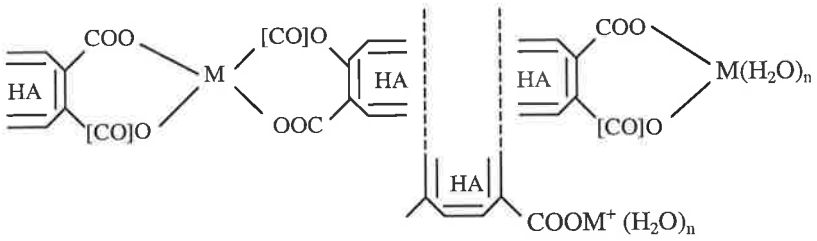


Figure 1.6.3: Humic acid (HA) – metal (M) chelate complexes (2:1 and 1:1), and a metal – carboxylate group (COOH) salt-type linkage (Stevenson and Fitch, 1981).

1.6.2.1 Solid and Solution Phase Organic Forms

It has already been mentioned that organic forms of Cu predominate in soil solution for both amended and non biosolids-amended soils. Organic forms commonly comprise 98-99% of that held in solution (Emmerich *et al.* 1982a; Hodgson *et al.* 1965; Sauve *et al.* 1997), which in turn generally represents <1% of total Cu (Sauve *et al.* 1997). The solubility of Cu-humic complexes depends on a range of factors, including the molecular weight of the complex, and on the pH and ionic strength of the soil solution.

Fulvic acids tend to be more soluble than humic acids due to their relatively low molecular weights. Also, fulvic complexes are soluble over a wider pH range, being soluble in both acid and alkaline conditions (Kiekens and Cottenie 1981). These points, together with the observation that Cu^{2+} is preferentially complexed by fulvates (Smith 1996), provide an explanation as to why fulvic acid complexes tend to dominate soil solution Cu. This was illustrated by Verloo (1979, as cited by Kiekens and Cottenie 1981), who found that 93% of the Cu in a soil leachate was present as fulvate complexes. Other organic ligands that may form soluble Cu complexes include simple

compounds such as organic acids (i.*e.* citric and oxalic), amino acids, phenols and phenolic acids, sugar acids (*i.e.* gluconic and glucoronic), and organic phosphates (Stevenson 1994).

Humic acid complexes tend to form insoluble precipitates below pH 5, and only become appreciably soluble when pH rises above 6 (Kiekens and Cottenie 1981). However, the presence of Ca^{2+} (or other multivalent ions) in solution causes flocculation and precipitation of humates, thus removing them from solution. Hence humic acids are often thought of as organic 'sinks' for Cu in soils (Stevenson and Chen 1991). Solid-phase humic-Cu complexes thus form a substantial component of biosolid and soil Cu. This was shown by Nyamangara (1998), who found 67% of the Cu in biosolid-amended soils to be present in the insoluble organic fraction.

1.7 Copper in Plants & Ecosystems

1.7.1 Copper Essentiality in Plants

It has been known since the 1930's that Cu is an essential plant micronutrient. Molecules containing Cu (particularly proteins and enzymes) have vital roles in such processes as photosynthesis, respiration, carbohydrate distribution, lignification, N metabolism, free radical detoxification, and desaturation of long-chain fatty acids (Kabata-Pendias and Pendias 1992; Romheld and Marschner 1991). Copper proteins are also believed to be involved in the maintenance of thylakoid membrane stability (Bussler 1981). The names, principal locations, and apparent main functions of several of the better-known Cu-containing enzymes are listed in Table 1.7.1 (Walker and Webb 1981).

Table 1.7.1: Plant Copper Enzymes

Enzyme / Protein	Sub-cellular Localisation	*Main Function (s)*
Cytochrome oxidase	mitochondria	Terminal oxidase in mitochondrial electron transport chain (*i.e.* respiration).
Plastocyanin	chloroplast	Intermediate in photosynthetic electron transport chain.
Phenolase and Laccase	chloroplast	Oxidation of plant phenols and aromatic amines (with products subsequently used in photosynthetic and respiratory electron transport chains).
Superoxide dismutase	chloroplast	Regulates superoxide radicals ($O_2^{-}\bullet$) generated during photosynthesis.

Copper deficiency (induced by low soil-Cu availability and/or by imbalances with other nutrients) depresses the activities of Cu-containing enzymes (Walker and Webb 1981), which in turn impacts on the over-all metabolism of plants. Thus Cu deficiency can lead to reduced rates of photosynthesis and respiration, resulting in diminished growth and lower yields. Chlorosis and structural malformations of leaves may also occur as a consequence of an inadequate Cu supply (Bussler 1981). Copper deficiencies have also been known to cause pollen sterility, resulting in reduced reproductive rates (Romheld and Marschner 1991). In addition, decreased lignification (due to reductions in the activities of the enzymes phenolase and laccase) may reduce plant structural strength and resistance to disease (Bussler 1981; Robson and Reuter 1981; Romheld and Marschner 1991). The tissue concentrations below which these effects of deficiency occur are highly species, cultivar, and even plant-part specific, thus it is not possible to definitively state a minimal plant requirement for Cu. However, some generalised ranges based on tissue concentrations can be given for crop plants, pastures and vegetables (units in μg Cu/g dry matter): deficient 0-5; sufficient 5-25; toxic 20-100 (Huett *et al.* 1997; Pinkerton *et al.* 1997; Reuter *et al.* 1997).

1.7.2 Plant Uptake

Copper, due to its extremely high affinity for soil colloids, is one of the most tightly bound metals in soils and sediments (Baker and Senft 1995; Kabata-Pendias and Pendias 1992). Thus it has been suggested that the majority of Cu taken up by plants is accessed via root interception of soil Cu deposits (Gilkes and Sadleir 1979; Jarvis 1981; Jarvis and Whitehead 1981; Oliver and Barber 1966). However, solubilisation of soil Cu by dissolved organic matter in soil solution may also play a role in delivering Cu to root surfaces (Checkai *et al.* 1987, and see *section 1.8.2*). Thus Cu is in contrast to other micronutruents such as Fe, Mn, and Zn, which are primarily supplied to roots by diffusion, and B, being mainly supplied through mass flow (Jarvis 1981). However, although contrasting mechanisms may be responsible for bringing different metals to root surfaces, plant metal uptake from the root surface can be generally described in terms of the following sequence of events: Penetration of root cell walls, absorption into epidermal/cortical cells[1] (across the cell membrane), symplasmic movement (via plasmodesmata) to the root's interior, and finally release into mature xylem vessels (*i.e.* the conducting tissue) through which translocation to aerial plant parts occurs (Kochian 1991) (Figure 1.7.1).

The first plant-based barrier to metal uptake is therefore the cell wall of root cells. Plant cell walls are complex, consisting of cellulose microfibrils embedded in a matrix of pectins, lignins, and glycoproteins (Clarkson 1974). Pores in the cell wall create a tortuous pathway for water and solute movement, with the pore surfaces being lined with carboxyl, amino and other organic functional groups capable of tightly binding metals (Kochian 1991).

[1] Water and solutes may move radially through the root via the apoplasm (*i.e.* the free space external to cellular cytoplasm) to the endodermal layer (where further penetration is prevented by the Casparian strip), so that inner cortical or endodermal cells may be the site for absorption into the symplasm. However, evidence suggests that this is a minor transport pathway for heavy metals, thus the majority of absorption occurs in the outer cells (Brams and Fiskell 1971; Kochian 1991).

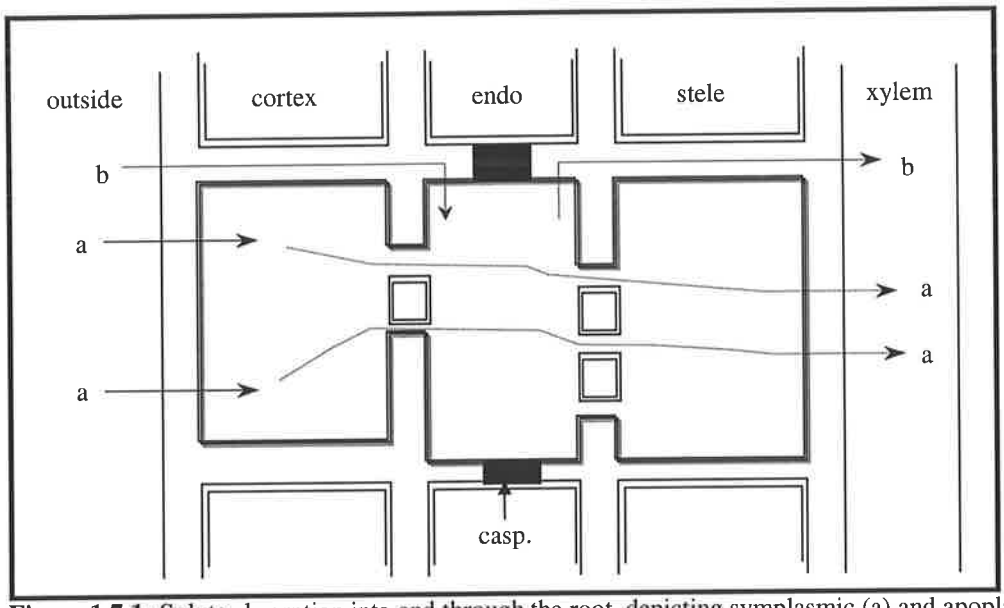


Figure 1.7.1: Solute absorption into and through the root, depicting symplasmic (a) and apoplasmic (b) pathways (outside = external solution; endo = endodermal cells; and casp. = casparian strip - after Kochian, 1991).

This has particular implications for Cu, due to this metal's high affinity for organic functional groups. Indeed, it has been reported that cell walls contain many binding sites that are highly Cu specific, with Cu being adsorbed to multidentate organic ligands involving N, S, and O atoms (Graham 1981). Therefore much of the Cu removed from soil solution by plant roots does not progress beyond root cell walls (Brams and Fiskell 1971; Loneragan 1981), which explains the accumulation of Cu often observed at the surface, and around peripheral cells, of roots (Brams and Fiskell 1971). Adsorption to cell walls is therefore a major factor contributing to the low absorption rate of Cu in plants, viz 0.1-7.0 nmol Cu/h/g root fresh weight compared to 5-200 nmol Zn (Graham 1981; Kausar *et al.* 1976; Loneragan 1975).

Absorption into the symplasm (crossing the cell plasma membrane) is the next barrier to uptake. It is generally regarded that the free ion, Cu^{2+}, is the form of Cu absorbed by the cell (Graham 1981; Kochian 1991; Robson and Reuter 1981), but, due to results of numerous studies (*i.e.* Taylor and Foy 1985), the possibility of complexed species also being absorbed cannot be totally

discounted. Evidence that Cu^{2+} is the form absorbed was provided by Brams and Fiskel (1971) and by Sauve *et al.* (1996), as both groups of investigators found Cu uptake to be dependent upon the concentration of Cu^{2+} in solution, and not on other solution components. However, the data produced by the study of Sauve *et al.* (1996) has been severely criticized for being highly influenced by the results of one soil, to the point where if removed the ability of Cu^{2+} activity in solution to predict plant Cu uptake was no greater than that of total soil Cu (McLaughlin 2002). Nevertheless, Dragun *et al.* (1976) provided support that Cu^{2+} is the form taken up by plants by observing that Cu uptake rates are much faster from solutions containing free Cu^{2+} than from solutions with complexed species. Their results showed that, for a four-week growth period, shoots and roots of corn plants both accumulated more Cu from a $1.6x10^{-7}$ mol/L Cu solution (with no chelating agents) than from a $9.8x10^{-5}$ mol/L Cu solution containing $4x10^{-4}$ M DTPA (Dragun *et al.* 1976). Further, other studies, such as the electron paramagnetic resonance studies of Goodman and Linehan (1979), have produced evidence indicating that although the vast majority of soil solution Cu is organically complexed, Cu appears to dissociate from organic ligands prior to absorption. However, in view of the conflicting evidence of Taylor and Foy (1985) and others, it may be the case that Cu is preferentially absorbed as the free ion (in terms of rate and relative amount), with complexed forms being taken up to a far lesser degree. At least, the role of organic complexes in bringing Cu to the site of uptake needs to be recognized.

The absorption mechanism is not fully understood, however, based on evidence that metabolic inhibitors decrease Cu absorption (Bowen 1969), it is thought to involve an active transport process. One theory proposes that a membrane bound protein (or carrier), powered by cellular metabolism, facilitates the movement of Cu^{2+} across the plasmalemma, and subsequently releases the ion into cytoplasm (Graham 1981). However as yet no protein has been identified as being

responsible for this mechanism, hence the exact transport process remains unknown (Kochian 1991; Walker and Webb 1981). To date evidence for active transport is not conclusive, thus the possibility of passive processes also being involved in Cu absorption (particularly at elevated solution concentrations), as theorised by a number of workers (Giordano *et al.* 1974; Graham 1981), cannot be dismissed.

Once inside the cytoplasm Cu^{2+} is believed to be re-bound into metal-organic chelates, with most evidence pointing to amino acids as the ligands (Goodman and Linehan 1979; Loneragan 1981). The Cu-chelates may then move via the symplasm to the interior of the root, eventually reaching the living xylem parenchema cells from where the Cu is 'unloaded' into the mature (dead) xylem vessels (Figure 1.7.1). Mechanisms controlling the release of Cu (and other metals) into xylem vessels are yet to be identified, and may involve both active and passive processes (Kochian 1991; Loneragan 1981). Mass flow of solution through the vascular tissue then transports Cu (once again largely as an organic chelate) to other plant parts (Loneragan 1981). However, as at the root cell wall, the strong affinity of Cu for organic ligands results in much of the metal binding to sites along the symplasmic pathway, and also to sites on xylem walls (Brams and Fiskell 1971). This binding tendency accounts for Cu having one of the lowest absorption and translocation rates of all the essential metals, and also partly explains the far greater accumulation of Cu in roots relative to other metals (Mitchell *et al.* 1978). The potential extent of this root accumulation/retention has been highlighted by observations that Cu may be retained even when aerial plant parts are experiencing severe Cu deficiency (Loneragan 1981). Thus the low rate of absorption of Cu, its resistance to translocation within plants, and its strong affinity for soil particles, all conspire to make the metal's transfer rate from soil to plant tops comparatively low (Sauerbeck 1991). This can be seen by comparing transfer coefficients for different metals, where

the term 'transfer coefficient' is defined as the metal concentration in the plant divided by the concentration in soil. For both Zn and Cd, the range of transfer coefficient values has been shown to be from 1-10, whereas the range for Cu has been found to be from 0.1 –1.0 (Sauerbeck 1991).

1.7.3 Copper Toxicity

Copper, like other trace elements, is potentially toxic to plants and other organisms at excessive concentrations (Figure 1.7.2).

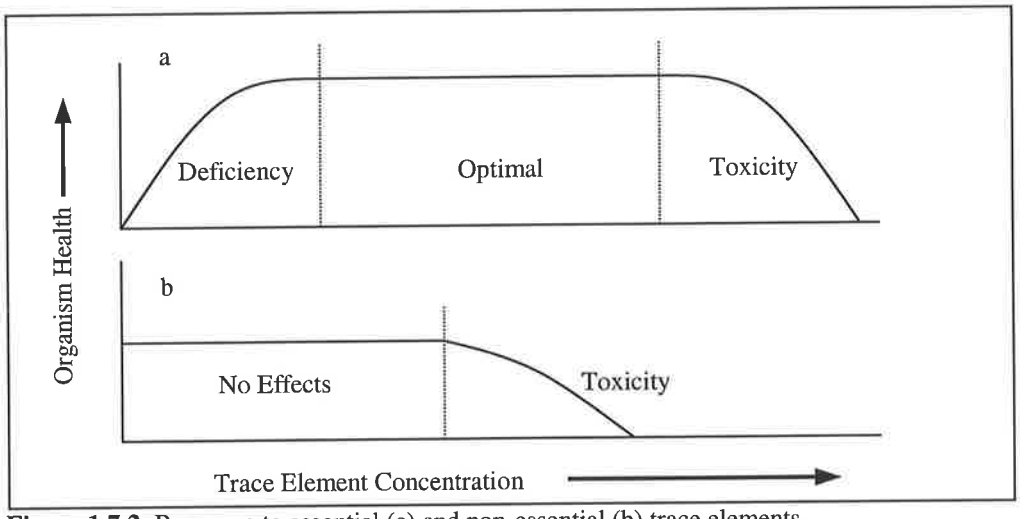


Figure 1.7.2: Response to essential (a) and non-essential (b) trace elements.

When in excess Cu can exert a range of direct toxic effects on plants, including alterations to cell membrane permeability (causing leakage of K^+, PO_4^{3-}, and other ions), peroxidation of chloroplast membrane lipids (resulting in inhibition of photosynthetic pathways), and weakening of inner cell wall tissues (Brams and Fiskell 1971; Woolhouse and Walker 1981). Elevated concentrations may also inhibit root elongation, resulting in affected plants exhibiting thick, stunted roots characteristic of metal toxicity (Brams and Fiskell 1971; Dragun *et al.* 1976). In addition, Cu excess may induce deficiencies in other elements such as Mo, Zn, and P (Bussler 1981; Kabata-Pendias and Pendias 1992). Thus plant yield, growth rate, nutritional status and

survivorship can all be adversely affected by excessive amounts of Cu. The reported critical concentrations at which these effects occur are highly varied, as they depend on species (and possibly on cultivar), plant part examined, plant age, and measure of toxic effect (*i.e.* percentage yield reduction - 10, 20 or 50%, growth rate decline, or visible symptoms, etc.). For example, some plants (*i.e.* wheat) have reportedly shown yield reductions at leaf tissue concentrations as low as 10 µg/g, while others (such as cotton and soybean) have not shown reductions in yield at tissue concentrations of 100 µg/g (Macnicol and Beckett 1985). However, as a general benchmark, the critical leaf/shoot concentration (or toxic threshold concentration) is often cited as approximately 20 µg/g (Beckett and Davis 1977; Kabata-Pendias and Pendias 1992; Robson and Reuter 1981). The soil concentrations corresponding to this plant tissue threshold concentration will vary depending on soil factors such as pH, organic matter type and content, texture, and cation exchange capacity. One study (Davis and Carlton-Smith 1984), conducted on a biosolid-amended soil (a sandy loam of pH 7.0), found the critical tissue concentration at which perennial ryegrass yield declined (22 µg/g) corresponded to a total soil-biosolid Cu concentration of 105 mg / kg. In terms of EDTA extractable Cu, this value corresponded to a soil-biosolid concentration of 56 mg / kg (Davis and Carlton-Smith 1984).

Although toxicity to plants is a primary concern in relation to biosolid use, soil microbes and microbial activity may be far more sensitive to metal inputs. Increased soil metal contents from biosolid applications have, in some instances, led to decreased microbial populations, lower microbial enzyme activity, reduced biodiversity within the microbial community, and to reductions in soil organic matter/nutrient turn-over rate (Chander and Brookes 1991; Chander and Brookes 1993; Dahlin and Witter 1993; Dahlin *et al.* 1997; Giller *et al.* 1998; McGrath *et al.* 1988a). In terms of microbial toxicity, Cu has been identified as being one of the most potent

metals present in biosolids (Chander and Brookes 1993). Hattori (1992) found microbial toxicities of various heavy metals to be in the order Cd = Cu > Ni > Zn > Pb, when rates of both respiration and colony formation were studied. Copper has also been found to be particularly toxic to certain groups of microbes, with many species of fungi and N-fixing bacteria being especially sensitive (Chaudri *et al.* 1992; Dahlin *et al.* 1997; McGrath *et al.* 1988a). Thus the content and availability of biosolid Cu has important implications for N cycling within amended soils, as well as for general microbial function and for soil fertility and productivity.

A growing body of evidence indicates that for Cu, the most toxic form of the element is the free ion (Cu^{2+}), rather than complexed species. Much of this evidence comes from studies involving aquatic organisms (*i.e.* Allen and Hansen 1996; Kim *et al.* 1999; Ma *et al.* 1999), where the toxicity of Cu in solution was reduced through chelation with organic ligands. For example, Kim *et al.* (1999) observed that addition of dissolved organic matter (as both purified humic acids and 'natural' dissolved organic matter from river water) substantially raised the Cu LC_{50} for the zooplankton species *Ceriodaphnia dubia*. The Cu LC_{50} was raised from 20 µg/L in the absence of chelating agents, to 119 µg/L with ligands present (at 2.5 mg DOM/L) (Kim *et al.* 1999). Some evidence has also come from studies involving higher plants, such as the work by Dragun *et al.* (1976). These authors found that corn plants grown in solution culture suffered toxic symptoms at a Cu concentration of 2.5×10^{-6} mol/L when chelating agents were absent, whereas when DTPA was added to solution (at 4×10^{-4}M) no toxicity was observed even when the Cu concentration exceeded 9.4×10^{-5} mol/L (due to the majority of Cu being in a complexed, non-toxic form) (Dragun *et al.* 1976). The authors inferred from this that as long as the activity of Cu^{2+} in solution is maintained at or above a pCu^{2+} of 5.78 (*i.e.* an effective Cu^{2+} concentration < 2.5×10^{-6} mol/L), toxicity will not occur in these test plants regardless of the total Cu concentration (Dragun *et al.*

1976). Investigations such as these (*i.e.* those showing Cu^{2+} to be the toxic form) provide further support for the notion that the free ion is the biologically active species in Cu absorption (*cf. section 1.7.2*). However, Taylor and Foy (1985) found that complexed Cu (as CuEDTA) could induce toxicity in wheat at high concentrations in solution culture (800 μM CuEDTA), and that toxic effects differed to those observed when Cu was supplied as $CuSO_4$.

1.7.4 Food Chain Effects

Accumulation and movement of metals through the food chain is a major concern in biosolid re-use programs, particularly in relation to potential human health effects. Indeed limits (*i.e.* DETR 1998; SAEPA 1996) are commonly placed on the amounts of biosolids that can be applied to agricultural land in order to ensure that food stuffs produced on treated sites do not cause maximal daily intake levels (MDI's) to be exceeded when consumed. However, the major metal of concern in regard to food chain contamination is Cd (Gardiner *et al.* 1995; Whatmuff 1996), whereas Cu is not considered as a risk for the soil-plant-human pathway. The minimal risk of Cu toxicity to humans via the food chain is due to a number of factors, including: 1) phytotoxicity occurs at plant Cu concentrations below levels dangerous to humans (Zwarich and Mills 1982); 2) copper is largely retained by roots, and does not readily accumulate in edible plant parts (Brams and Fiskell 1971; Cimino and Toscano 1993; Dowdy and Larson 1975; Mitchell *et al.* 1978), and 3) uptake and distribution rates of Cu (*i.e.* transfer coefficients - *cf. section 1.7.2*) are generally very low due to the metal's high affinity for soil colloids (Baker and Senft 1995; Kiekens and Cottenie 1981; McBride 1981; McLaren and Crawford 1973b). Thus, in regard to biosolid application, the risk of Cu toxicity to humans is essentially limited to direct ingestion of biosolid/amended soil materials (with children being the main concern).

Although humans may not be at risk from Cu in the food chain, certain groups of animals may be. Ruminants, particularly sheep, are sensitive to the Cu concentration of pasture plants. Symptoms of Cu toxicity have been observed in sheep feeding on pasture with Cu concentrations less than 10 mg/kg (Scheinberg 1991), which is approximately half of the concentration associated with phytotoxicity. These effects are exacerbated by low Mo concentrations (<0.5 mg/kg) in pasture plants, due to the reduced Mo-Cu antagonism effects that regulate Cu uptake in the gut (Scheinberg 1991). Animals may also receive elevated doses of Cu through directly ingesting particles of amended soil when grazing. Thus toxicity to grazing animals, phytotoxicity, and microbial toxicity (including effects on microbial diversity and community functionality), are all areas of concern in regard to biosolid Cu.

1.7.5 Mobility in the Environment

The mobility of biosolid borne metals in the environment is generally low, as attested to by the wealth of reports documenting metal accumulations in biosolid-amended soils (Aitken and Cummins 1997; Brallier *et al.* 1996; Dam Kofoed 1981; MacLean *et al.* 1987; McBride *et al.* 1997; McGrath and Cegarra 1992; O'Riordan and McGrath 1997; Samaras and Kallianou 2000). Many investigations have found metals to be largely retained within the zone of biosolid-soil incorporation, and not to leach or otherwise migrate to lower layers to any significant degree (Chang *et al.* 1982; deVries and Merry 1980; Emmerich *et al.* 1982a; McGrath 1987; McLaren *et al.* 1999). This is particularly so for Cu, as its affinity for soil colloids (especially organic materials) makes it one of the least mobile heavy metals in soils (Kabata-Pendias and Pendias 1992). For example, Dowdy *et al.* (1991) reported that despite 14 years of massive, annual biosolid application (totaling 1070 kg Cu / ha), the Cu concentration below the zone of

incorporation was not significantly different to that of untreated soils. The study did reveal some migration of Cd and Zn to lower layers, but the amounts detected only represented around 4-5% of the applied metals (Dowdy *et al.* 1991). Others (Chang *et al.* 1982; Holtzclaw *et al.* 1978; McLaren *et al.* 1999; Tackett *et al.* 1986) concur that Cd, Ni and Zn tend to be the more mobile metals in biosolids, rather than Cu.

However, despite the low amounts of biosolid metals identified in subsoil layers (suggesting leaching is minimal), losses from treated soils do occur. McGrath (1987) found that soil movement (via tillage and erosion, etc.) could account for some of the apparent losses of metals from application sites (those being losses identified by deficits in measured metal contents relative to calculated amounts of metals applied). Other studies (Chang *et al.* 1982; McBride *et al.* 1997; McBride *et al.* 1999) identified selective deficits in concentrations of metals in treated soils, indicating that bulk soil movement was not solely responsible for apparent losses. The conclusion drawn from these latter studies was that some metal leaching must occur, but that which does occurs via preferential flow paths, with metals transported as relatively non-adsorptive organic chelates (thus explaining the lack of adsorption/accumulation in subsoils) (Chang *et al.* 1982; McBride *et al.* 1997). Additional routes of metal loss may include plant off-take (uptake and subsequent removal at harvest), microbial mediated movement, and uptake by soil fauna and subsequent removal via the food chain, but these are generally considered to be minor (Kabata-Pendias and Pendias 1992; McBride *et al.* 1997).

However, as outlined above, biosolid borne metals (and Cu in particular) are generally immobile, and are mostly confined to surface layers when applied to soil. Thus accumulation tends to occur, which creates the risk of phytotoxicity, threatens the viability and functionality of soil microbial

populations, and also presents a risk to animals and humans (principally children) via direct ingestion of soil materials.

1.8 Copper Bioavailability & Controlling Factors

Bioavailability can be defined as the capacity for a substance to be transferred from a growth medium (*i.e.* biosolids or soil) to a living organism (*i.e.* a plant root or microbe). In regards to Cu, (and other metals) not all forms present in soils and biosolids are directly accessible to organisms, and much of the metal is not bioavailable. This has been consistently reported in metal uptake studies (*i.e.* Sims and Kline 1991), in which plant tissue concentrations have not correlated with the total Cu concentrations of the growth medium. It has been shown that Cu in solution, together with the solution-exchangeable Cu adsorbed on solids, comprises the 'pool' of Cu that is plant accessible (Brun *et al.* 1998; Gerritse *et al.* 1983). In this context a number of 'Cu pools' can be distinguished (in soils and/or biosolids), with the total number and specifications of pools dependent upon which of the many published fractionation schemes is considered (however, caution is required when interpreting results from such schemes - *cf. section 1.9.2.2*). In the scheme devised by McLaren and Crawford (1973a), the following fractions were identified:

1. Solution + Exchangeable
2. Weakly adsorbed (to organic and inorganic surfaces)
3. Organically bound
4. Occluded
5. Residual

Because it has been suggested that 'solution + exchangeable' Cu is the fraction that plants can access (as plants access Cu and other micronutrients from solution), factors that control the movement of Cu into and out from this pool may also control Cu bioavailability. Thus, viewed from this perspective, bioavailability is a function of the availability of dissolved species in solution, and the ability of the soil/biosolid to buffer metal concentrations in solution

(McLaughlin *et al.* 2000). These two components have been described respectively as the Intensity (*I*) and Capacity (*C*) factors, with the total amount of metal present in equilibrium with solution Cu referred to as the Quantity (*Q*) factor (Khasawneh 1971; Tiller *et al.* 1972b). The variables having most influence on these factors are pH, organic matter (type and content), biological activity, Cu content (level of contamination), and time. However, these factors are not independent, they interact strongly and have confounding effects which together influence Cu bioavailability in a very complex way (which is not yet fully understood).

1.8.1 pH

Soil (or biosolid) pH is widely recognised as the dominant factor influencing metal bioavailability. The pH affects Cu bioavailability directly by its influence on Cu solubility, and on Cu^{2+} adsorption and speciation (Fig 1.6.1). By gradually lowering the pH of soil suspensions (a sandy loam with soil pH 5.5, and a heavy clay with pH 6.65), Kiekens and Cottenie (1981) found the percentage of added Cu remaining in inorganic soluble forms was approximately 25% at pH 3, 5% at pH 4, and <1% at pH 4.5. Thus higher pH led to lower inorganic Cu concentrations in solution, and hence to potentially lower rates of plant uptake. McLaren and Crawford (1973a; 1973b) similarly found pH to affect the partitioning of soil Cu between solid and solution phases. They concluded that reductions in the quantity of solution inorganic Cu were due to increased levels of specific adsorption by soil constituents at higher pH. In addition to influencing metal partitioning (between solid and solution phases), it is also well known that pH strongly influences Cu speciation in solution (which also affects bioavailability). It has been shown that lower pH values promote an increased activity of Cu^{2+} ions (Cavallaro and McBride 1980; Sauve *et al.* 1997), which may in turn lead to greater rates of Cu absorption by roots (Minnich *et al.* 1987; Sauve *et al.* 1996).

Solution pH also indirectly effects Cu bioavailability by its influence on organic matter. The pH of a soil or biosolid medium will influence the extent of ionisation of acidic functional groups on organic molecules, which influences the polarity (and so solubility) of organic compounds. This partially explains why humic acids generally form insoluble Cu complexes at low pH, while they become more soluble as pH increases (Kiekens and Cottenie 1981). Increased ionisation of acidic functional groups at higher pH values also allows a greater degree of Cu compelxation, as Cu may occupy a higher proportion of exchange sites on organic compounds due to decreased competition from H^+ (Dudley *et al.* 1986). The solubility of the organic complex (as influenced by molecular weight, solution ionic strength, and other factors) then determines whether the chelate remains in solution. Therefore pH increase often results in an increase in the proportion of organically complexed Cu in solution, at the expense of inorganic forms, with organic forms becoming overwhelmingly dominant above pH 4 (Kiekens and Cottenie 1981). This effect of pH on Cu binding by organic matter has a complicated flow-on effect on bioavailabilty; being that although more Cu may be brought into solution by increased complexation with soluble organics, the complexed forms may be less readily available to plants.

However, the effect of pH on Cu bioavailability to plants is not as profound within the pH range usually associated with biosolid application (*i.e.* pH >5.5). Although changes in pH strongly affect bioavailability below pH 5, changes to pH above this value have been shown to have only a limited effect. For example; King and Hajjar (1990) found that while increasing the pH of biosolid amended soil from 4 to 6 decreased Cu uptake by tobacco plants from 2000 µg/pot to 800 µg/pot, further pH increases did not reduce Cu uptake. Sauerbeck (1991) also demonstrated this effect by growing a wide array of plants (including both mono- and dicots) in a range of

biosolid-amended soils that had been pH adjusted to varying degrees. He found that altering pH within the slightly acidic to slightly alkaline range did not affect Cu uptake (Sauerbeck 1991). Similar findings were reported by Brallier *et al.* (1996), who found uptake of Cu by maize and selected vegetable plants was not generally affected by liming biosolid-amended soils (whereas Cd, Ni, and Zn uptake were consistently reduced). The same outcome has been noted for ryegrass grown on biosolid amended soils (Dijkshoorn *et al.* 1981; Sanders *et al.* 1986), where Cu uptake was independent of pH above a threshold value of 5.5 (while Zn and Ni uptake remained pH dependent throughout the range 4 – 7.5). Similar observations prompted Davies *et al.* (1987) to go so far as to say that there was no role for pH in affecting plant Cu uptake (pH range 6 – 7.5). This insensitivity to pH change, in terms of plant Cu uptake, is not restricted to biosolids or biosolid-amended soils. The same trend has been observed in soils contaminated with Cu from poultry manure (Sims 1986) and from fungicides (Brun *et al.* 1998). However, it may be that the effects of pH change on Cu availability are masked to some degree in plant uptake studies due to the strong homeostasis that plants exhibit in terms of shoot Cu concentrations (Jarvis 1981). Still, it may be that pH is not as dominant a factor in controlling bioavailability of Cu as it is for other metals found in biosolids (at least when considering the pH range within which biosolids may be applied under current regulations). Other factors, such as type and content of organic matter, may therefore be more important. However, the interaction effects between pH and these other factors may still be very significant.

1.8.2 Organic Matter

Organic matter type and content in soils and biosolids generally have a strong effect on metal bioavailability, with this being particularly so for Cu due to the strong affinity of organic matter for this metal. From stability constants it is evident that Cu minerals are too soluble to account for

the low concentrations of Cu found in soil solutions (McBride 1981), thus it is not precipitation-dissolution reactions that primarily control Cu concentration, but rather adsorption-desorption processes. While the exchangeability of Cu adsorbed by organic matter was found to be lower than that adsorbed by clay and oxide surfaces (20% compared to 60 and 75% respectively, determined by isotopic exchange techniques (McLaren and Crawford 1974), see *section 1.9.4*), it has been suggested nevertheless that organic matter ultimately controls the release of Cu to solution (due to the large proportion of Cu held in the organic fraction) (McLaren and Crawford 1973a; 1973b). Thus organic solids hold the reserves of available Cu, and hence, in regards to Cu, determine the buffer capacity (*C*). The equilibria between Cu pools (controlling the release of Cu to solution) was postulated by McLaren and Crawford (1973a) as follows:

Solution + Exchangeable ↔ Specifically adsorbed ↔ Organically bound

Therefore organic solids serve the function of holding Cu in a kinetically available but thermodynamically stable (insoluble) form (McBride 1981), although some Cu may be held by organic matter irreversibly (McLaren and Crawford 1974; Petruzzelli *et al.* 1978).

The extent of organic matter's dominance over soil Cu buffer power is increased as organic matter content increases (both in absolute terms and relative to Mn oxide content) (McLaren and Crawford 1973a). Thus in biosolids, which have a far greater organic matter content than most Australian soils, Cu buffering capacity would be expected to be completely dominated by organic matter. Therefore Cu bioavailability in biosolids may be largely determined by organic matter concentration and type.

Organic matter type and content determine the number of sites available for metal binding, and thus determine how much can be bound before a saturation level is reached. The Cu binding

capacity of organic matter is approximately equal to the content of acidic functional groups it contains (Stevenson and Fitch 1981), which (considering humic acids as the principal organic solid) may be in the range of 48-160 mg Cu/g humic acid (Stevenson and Fitch 1981). As binding sites become saturated, increases in binding through weaker unidentate bonds or through simple electrostatic forces will occur. This was shown by Keefer *et al.* (1984), who found evidence for two mechanisms of Cu binding which they simply described as 'weak' and 'strong' bonds respectively. The weakly held Cu is more able to exchange with ions in solution, and so is more bioavailable. Therefore the nature and amount of organic matter, together with the total Cu content, will determine the relative amounts of Cu held by weak or strong forces, and so ultimately influence the amount of free ion in solution (which in turn determines *I*). This has been shown in numerous studies where increases in total Cu content have led to higher solution Cu^{2+} activities (McGrath *et al.* 1988b; Sauve *et al.* 1997), while conversely increases in soil organic matter contents have resulted in decreased activities (Minnich and McBride 1987). The importance of organic matter, in regards to Cu bioavailability, is recognised by the suggested method of reporting soil Cu status, that being in terms of g Cu/kg organic C (Lexmond 1981).

Although solid humic substances may act as a sink for Cu (Holtzclaw *et al.* 1978; Stevenson and Chen 1991), complexation by soluble organics (i.*e.* fulvic acids) can increase the quantity of Cu held in solution (Checkai *et al.* 1987). This may occur through chelation and subsequent solubilisation of Cu held to solid surfaces, and through prevention (inhibition) of adsorption to mineral surfaces (McBride 1981). Therefore soluble, organic chelating agents can increase the mobility of Cu, and so may be expected to promote increased plant uptake through bringing greater amounts of dissolved Cu into close proximity to roots. This effect has been noted on numerous occasions (Checkai *et al.* 1987; Minnich *et al.* 1987), where, for a given level of Cu^{2+}

activity, increases in the amounts of soluble, organically complexed Cu have led to increased plant uptake. However, chelation with soluble organics may have a double-edged effect, as although more Cu may be brought into solution, the complexed forms may not be plant available. It has been shown that increases in soluble organic Cu (associated with organic matter from biosolids) can decrease the proportion and total amount of free Cu^{2+} in solution (Minnich and McBride 1987), and so reduces bioavailability (through reducing *I*). Thus the relationship between soluble organic Cu concentration, Cu^{2+} activity, and Cu bioavailability is not straightforward.

1.8.3 Biological Activity

As discussed in the previous section (1.8.2), soluble organic molecules can influence Cu bioavailability by solubilising and mobilising previously bound Cu, and by affecting Cu speciation. The activities of soil/biosolid organisms can result in the release of such organic molecules (Nielsen 1976), hence biological activity may have a direct influence on Cu availability (Jarvis 1981). Root exudates, microbial exudates, and organic degradation products are all capable of Cu complexation and solubilisation, thus the level of biological activity will affect the rate of Cu mineralisation, complexation, and the amount present in solution in organic forms.

1.8.4 Time

The passage of time has three possible outcomes with respect to metal bioavailability, those being that availability may either increase, decrease, or remain unchanged. The first of these potential outcomes (bioavailability increasing) has become widely known as the 'Time-bomb'

effect (Chaney and Ryan 1993), while the second (bioavailability decreasing) has been termed 'Reversion' (Figure 1.8).

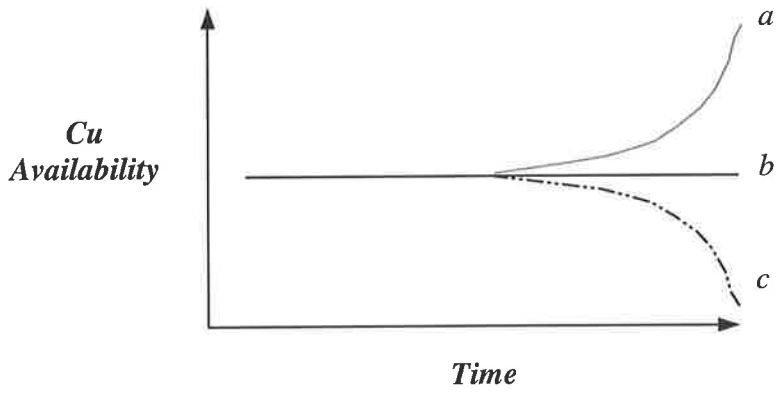


Figure 1.8: Theoretical effects of time on metal availability: a) Time bomb effect; b) No change; and c) Reversion.

The concept of the time-bomb is based on the fact that biosolid metals are largely associated with the organic matter fraction (Emmerich *et al.* 1982b; McLaren and Crawford 1973a). According to the theory, once biosolid applications cease, decomposition processes reduce the total content of organic matter, and then, once the content falls below a certain threshold level, metals previously bound to the organic matter become readily available (McBride 1995). Thus, if allowed to decompose to low levels in biosolid-amended soils, the protective effect of organic matter (that of binding metals in non-readily available forms) may be lost, and phytotoxicities may occur (Nyamangara 1998). However, to date very few studies have shown any evidence for the time-bomb effect. Khan *et al.* (1998) did observe that metal availability in sludge cake from a steelworks site (measured by DTPA extraction) increased with time, however a pH decrease was also noted. More convincingly, Hooda and Alloway (1994) found Cd and Pb retention capacity in sludged soils from England and India decreased significantly over a period of 450 days. This reduction was attributed to loss of organic matter via decomposition, and was apparent even when the pH of the treated soils was strictly maintained.

By contrast, evidence supporting the concept of reversion has steadily accumulated. For example, Emmerich *et al.* (1982b) found Ni and Zn bioavailability decreased with time, due to metals 'reverting' to less available forms (as determined by sequential extraction). The authors observed that the 'available fractions' decreased after a 25 month incubation period, while the 'residual fractions' increased (Emmerich *et al.* 1982b). A number of mechanisms have been proposed as contributors to the reversion process, including that of metals released from organic matter (via mineralisation) being rendered unavailable through occlusion by Fe and Al oxides or other inorganic components (Sadovnikova *et al.* 1996). Other processes of reversion may include diffusion into soil pores not accessible to plants or bulk solution, adsorption by recalcitrant organic components, increased binding strength of metal-organic matter bonds with ageing, solid state diffusion, or other slow soil-metal fixation reactions that reduce metal availability (Cook *et al.* 1999; Smith 1996). However, evidence for reversion is not conclusive, as shown by a number of studies in which conflicting evidence was found. For example, Beckett *et al.* (1983) reported on a number of separate experiments that showed metal availability to (respectively) increase, decrease and stay the same with time. Furthermore, many of the published findings supporting the theory of reversion are from solid phase fractionation studies (using sequential or single solution extraction schemes), which have inherent flaws and many limitations (*cf. section 1.9.2*). Also, there is some contradictory evidence available that suggests metal bioavailability, and particularly that of Cu, may remain constant with time (Bidwell and Dowdy 1987; Chang *et al.* 1997; Dowdy *et al.* 1978; Dowdy *et al.* 1991; McGrath 1987). Although long-term studies in this area are rare, one which has been reported (McGrath and Cegarra 1992) found no change in the bioavailability of metals 20 years after cessation of biosolid application. Similarly, Brown *et al.* (1998) found no statistically significant differences in lettuce Cd concentrations between crops

grown periodically for a period of 15 years after biosolid application, even though up to 80% of the added organic carbon in the biosolids had been oxidised.

Another point to consider is that there may be an additional process involved in apparent reductions in metal availability with time. A slow down in the microbial decomposition rate in amended soils, which commonly follows the initial surge of microbial activity triggered by biosolid application (Chaney 1994), may decrease the mobilisation rate of metals, potentially giving a false impression of reduced availability. The rate of mineralisation and metal solubilisation by microbes is greatest during the first few years following biosolid addition (Dudley *et al.* 1986), thus availability and mobility is often greatest during this time (Chaney 1994; Davis 1981). Once the readily decomposable organic matter becomes depleted, apparent availability may decrease in line with decreased microbial activity. Thus, if not considered, this biological process may cause estimates of reversion to be over stated. The severe shortage of long-term studies means questions about the effect of time remain, and so none of the theories can yet be dismissed. This includes the time-bomb concept, as it may be that the time-bomb effect has simply had insufficient time to occur. Further, the results of the few long-term studies that have been conducted (Brown *et al.* 1998; McBride *et al.* 1997; McGrath and Cegarra 1992) cannot be universally accepted as the norm because of the extreme spatial and temporal variations observed in biosolid contents/properties (*cf. section 1.4*), and because of regional climatic variations. Therefore, due to the sparsity of research and the conflicting nature of that which does exist, investigations are needed into time-dependent changes in metal bioavailability, particularly in regard to Cu.

1.8.5 Other metals

The presence of other metals in biosolids and soils can affect the bioavailability of Cu, with the reverse also being true. Organic matter binds Cu more strongly than most other metals, hence Cu is not normally displaced from binding sites on organic compounds (Petruzzelli *et al.* 1978). However, the uptake of Cu ions already in solution will be affected by other metal ions, through both the depression of Cu^{2+} activity due to increases in solution ionic strength, and through direct competition for membrane absorption. It has been shown that, in the case of plant uptake, Zn and Cu are antagonistic (Giordano *et al.* 1974; Kausar *et al.* 1976), as increases in the concentration of one decrease uptake of the other. This is believed to be due to Cu and Zn being taken up by plants via the same mechanism (Graham 1981), thus the ions are in direct competition with each other. However, in contradiction to this well recognised effect, Davis & Carlton-Smith (Davis and Carlton-Smith 1984) found that Zn (at elevated concentrations) enhanced Cu toxicity to perennial ryegrass grown on biosolid-amended soils. The same authors also found that Ni depressed Cu uptake, whereas elevated Cu concentrations increased Ni absorption (Davis and Carlton-Smith 1984). Interactions such as these may have an important influence on the bioavailability/toxicity of biosolid metals, because Cu, Ni and Zn are often all at highly elevated concentrations in biosolids.

1.9 Copper Bioavailability Measurement

In recognition of the shortcomings of using total metal concentrations for soil/biosolid assessment, a great deal of research has been directed towards determining the bioavailable portion of metals. In the course of this research a wide array of measurement techniques have been employed, ranging from short term, abiotic laboratory tests, through to long-term field trials involving multiple plant species. However, due to all of the techniques having limitations of one

form or another (with some suffering more than others), as yet no universal measure has been adopted, thus in any investigation the technique(s) chosen depends on the particular circumstance of the research. Some of the more recognised bioavailability assessment methods are outlined and discussed below.

1.9.1 Plant Uptake Studies

Plant uptake is the obvious measure of bioavailable (or more specifically phytoavailable) metals in biosolids and soils. Clearly, plants will only assimilate metals that are in plant accessible fractions (or pools) of the growth medium. Not surprisingly then, studies focusing on plant uptake abound in the literature (Chang *et al.* 1987; Davis and Carlton-Smith 1984; deVries and Tiller 1978; Gardiner *et al.* 1995; MacLean *et al.* 1987; Sauerbeck 1991; Zwarich and Mills 1982). However, plants can vary in their ability to assimilate soil Cu, with variations in the nature of root exudates, root morphology, genetic predispositions, root-shoot translocation rates, hyperaccumulating ability, and other plant attributes all contributing to plant uptake assays being quite often species (or even variety/cultivar) specific (Cimino and Toscano 1993; deVries and Merry 1980; Graham 1981; Graham *et al.* 1981; Sauerbeck 1991). Futhermore, in addition to different plant species having varying abilities to assimilate the soil Cu that is accessible to them, it has even been shown that some plant species may be capable of accessing additional soil metal pools that other species cannot. An example of this was provided by Hamon *et al.* (1997) who, using isotopic tracer techniques, found that the pool of soil Cd accessible to canola (*Brassica napus*) was much reduced compared to that of other plant species. This further adds to the potential species specificity of plant uptake assays.

An additional problem with plant uptake studies that is particularly important in the case of Cu is that, although different plant species may assimilate different amounts of Cu, within a single test species the range of shoot tissue Cu concentrations observed is likely to be small even if soil Cu concentrations vary widely, due to strong Cu homeostasis in the plant (Jarvis 1981). Therefore, as suggested by Jarvis and Whitehead (1981), plant assays involving analysis of root Cu concentrations may offer a better alternative, but this is not always possible.

Another more general limitation of plant-based assays is that once phytotoxic concentrations are reached in plant tissues, further uptake may be impaired, so that potentially available metals remaining in the growth medium are not absorbed (and therefore not measured) due to reduced plant function (McBride and Martinez 2000). Also, differences in uptake are likely to be observed in studies conducted in pots compared to those conducted in the field, due to reduced nutrient movement in pots and other factors (Chaney *et al.* 2000; Davis 1981). In addition, seasonal variations can affect uptake rates in field trials, again resulting in divergent bioavailability measurements (Chang *et al.* 1987). Therefore plant-based bioavailability measurements can be highly case specific, requiring careful consideration when interpretations or inferences are made. Other detractions from plant uptake studies are the length of time required to run them (growth periods), their labour intensive nature (*i.e.* preparation, sowing, watering, maintenance and harvesting, etc.), and the inherent dangers of utilising living organisms as test subjects (*i.e.* unexpected or unaccountable mortality).

In regard to the current project, the problem of species specificity is exacerbated by the fact that there are very few (if any) published works reporting plant uptake from pure biosolids. The majority of research has focused on biosolid/soil mixtures (Jing and Logan 1992; Pichtel and

Anderson 1997; Zwarich and Mills 1982), which produce confounded bioavailability results reflecting properties of both soil and biosolids together. Some studies have measured metal uptake by plants grown in biosolids mixed with relatively inert materials such as pine bark (Handreck 1994) and heat-treated, acid washed sand (deVries 1983a), which may more closely reflect biosolid metal bioavailability. However, such investigations are few, with those considering Cu uptake particularly scarce.

1.9.2 Chemical/Solution Extraction of Metals

Chemical extraction methods avoid many of the problems associated with plant-based techniques, and, significantly, they are generally less time, capital and labour intensive. Chemical techniques also require less space than biotic measurements, making them much more convenient for a laboratory setting. Chemical extraction of metals involves the addition of a solution to the soil or biosolid (ratios of solid: liquid vary), an equilibration period (again, times used vary widely, from minutes to days), followed by the recovery and subsequent analysis of the solution for metal contents, speciation and/or activity. Centrifugation and/or filtration are probably the most common methods of solution recovery, however other methods, including purpose designed moisture samplers (Knight *et al.* 1998), are also employed.

1.9.2.1 Single Extractants

As the name suggests, single extractant techniques involve a single solution being added to the growth medium being analysed (*i.e.* soil, biosolid, or a mixture). Neutral salts such as $CaCl_2$, $Ca(NO_3)_2$, and KNO_3 are often used (deVries 1983b; Sauve *et al.* 1996; Silviera and Sommers 1977), with the intent that the extracting solution will exchange with the most labile (supposedly readily plant available) metal fraction. Thus such extractants may give an indication of the

intensity factor – *I* (*cf.* section 1.8) in solution (McLaughlin *et al.* 2000). These neutral salt extractants are comprised of an extracting ligand (an anion) that forms weak bonds with biosolid/soil metals, and a cation for exchange (*e.g.* Ca^{2+} or NH_4^+) (Jing and Logan 1992). The concentrations used vary between studies, but a common range is from 0.01M (*i.e.* deVries 1983b; Sauve *et al.* 1996) to 1.0M (Silviera and Sommers 1977).

Organic chelating agents such as EDTA (ethylenediaminetetraacetic acid) and DTPA (diethylenetriaminepentaacetic acid) are also commonly used for single solution extractions. The intent is that the organic ligand will desorb metals from sorption sites on the biosolid (or soil), without greatly perturbing chemical equilibrium or altering the nature of surface metal species (Jing and Logan 1992). The metals extracted are considered to represent the bioavailable portion, or at least the potentially plant available portion including the 'bioavailable reserves' (deVries 1983b; Sims and Kline 1991). Thus at higher concentrations these extractants may give an indication of the effective (in terms of plant available metal) quantity factor – *Q*, in the sample (McLaughlin *et al.* 2000). These reagents are commonly added in a salt solution such as $CaCl_2$ or $Ca(NO_3)_2$ (Fujii and Corey 1986; Jing and Logan 1992). However, the concentrations used vary from study to study, which may be a factor contributing to the inconsistent results reported in the literature. The potential for inconsistency was shown by de Vries (1983b), who demonstrated how changes to the concentration of the added chelating agent can alter the amount of metal extracted, and thus alter the amount identified as 'available'. It was found that 0.01M DTPA extracted 27% more Cu from biosolids than did a concentration of 0.005M (deVries 1983b). The discrepancy was most likely due to saturation of the chelating agent at the lower concentration. Similarly, variations in the solid: solution ratio used in such assessments may also contribute to inconsistencies in bioavailability measurements. Fujii and Corey (1986) found that changing the

ratio of soil: extract solution from 1:5 to 1:2.5 significantly changed the amount of exchangeable Zn determined in a biosolid-amended soil. Evidence such as this suggests that bioavailability measurements using these extractants may be quite method specific.

In terms of which is the more useful of the common single solution extractants (neutral salt, organic chelate, or water) for estimating bioavailability, it depends on the metal being investigated, the properties of the biosolid or soil examined, and on the length of time considered (*i.e.* current or long-term availability – the attempted quantification of I or Q). For example, Mitchell *et al.* (1978) found that water extracts were more closely correlated with plant uptake of Cu and Ni in lettuce and wheat crops, while DTPA extraction was a better predictor for Zn. Conflicting results were obtained by Minnich *et al* (1987), who found water (saturation) extraction to be a poor predictor of Cu uptake. Handreck (1994), meanwhile, found that DTPA extracts were better correlated with plant Cu uptake from biosolid/pine-bark mixtures than were $CaCl_2$ extracts. However, Sauve *et al.* (1996; 1997) argue that 0.01M $CaCl_2$ more closely reflects soil solution than other extractants, and so suggest that this extractant gives a better indication of the metal fraction in equilibrium with the soil solution. Various other authors (Minnich *et al.* 1987; Sanders *et al.* 1987) also view $CaCl_2$ as the best single solution extractant for predicting available Cu.

However, despite such findings and opinions, other research suggests that single solution extractants may only be of limited use when assessing the availability of biosolid Cu. Haq *et al.* (1980) tested nine extractants (including H_2O, $AlCl_3$ + HCl, EDTA, DTPA, acetic acid and *aqua regia*) on soil contaminated with biosolids, then correlated the results with uptake by plants (Swiss chard, *Beta vulgaris*) grown in the same medium. They found that while Zn uptake could

be adequately predicted by extraction with CH_3COONH_4, and Cd and Ni uptake by extraction with acetic acid, none of the extractants trialled were successful as surrogate measures of Cu uptake (Haq *et al.* 1980). Similarly, Beckett *et al.* (1983) observed that the amount of EDTA extractable Cu in biosolid-amended soils varied independently of changes in plant Cu uptake. They therefore concluded that "extractable and available are not the same", and that it would be unwise when forming guidelines to equate extractable with available forms of Cu (Beckett *et al.* 1983). However, this common failure to correlate extractable Cu with plant shoot Cu concentrations in such experiments is again likely to be due to the strong Cu homeostasis expressed by plant species, as discussed above (*section 1.9.1*).

1.9.2.2 Sequential Extraction Schemes

Sequential extraction schemes involve a biosolid (or soil) sample being treated with a series of extracting reagents in a set sequence, where each successive reagent extracts metals from a more resistant fraction. Each extractant, once recovered, is analysed to determine the amount, species or activity of metal it removed from the sample. The extraction scheme thus attempts to separate metals present in the sample into distinct fractions (or 'pools'). Depending on the nature of the scheme employed, a number of the identified fractions may be classified as 'available', hence the extraction scheme can be used to determine the amount and proportion of metals that are bioavailable.

A vast array of sequential extraction/fractionation schemes have been reported in the literature, ranging from 3-step to 7-step models covering an enormous variety of extractants, concentrations, and solid: liquid ratios (*i.e.* Brallier *et al.* 1996; Emmerich *et al.* 1982b; Scancar *et al.* 2000; Silviera and Sommers 1977; Sims and Kline 1991; Sloan *et al.* 1997). An example of

a sequential extraction scheme used in a soil/biosolid study, showing the principle forms (fractions) of metals extracted by each reagent, is given below (McGrath and Cegarra 1992):

Step	Reagent	Primary Fraction Extracted
1	0.1 M $CaCl_2$	Water-soluble & Exchangeable
2	0.5 M NaOH	Organically bound
3	0.05 M Na_2EDTA	Carbonate forms
4	Aqua Regia (4:1 HCl:HNO_3)	Residual

In such schemes the water soluble and exchangeable pool is considered to reflect the readily available metal fraction, with the organically bound and carbonate forms representing the potentially (long-term) available portions (Brallier *et al.* 1996; McGrath and Cegarra 1992; Sims and Kline 1991). Caution is required, however, when interpreting results of such fractionation studies, due to the many flaws and limitations of the technique and the lack of validation with any biological end point. One of the main limitations is that the solid phase fraction targeted by each of the reagents is not extracted exclusively by that reagent, but rather there is considerable overlap in the forms of metals removed by the various extractants. Therefore the fractions identified in the scheme are described as being operationally defined (by the extractant used), containing chemically similar forms of metals with some overlap between pools (Emmerich *et al.* 1982b; Sims and Kline 1991). In addition to the problem of less than desirable selectivity of individual extractants, the reagents may remove varying amounts of metals depending on preceding steps in the fractionation scheme. For example, McGrath and Cegarra (1992) reported that while EDTA extracted 50% of total Pb in biosolid samples when used in isolation, it extracted 80% of the total when following NaOH in a sequential scheme. Stover *et al.* (Stover *et al.* 1976) also cautioned against the effects of sequencing on a reagent's extracting capacity. The various reagents may thus remove more or less of the total metals in each of the targeted pools, so giving an estimation of metal fractionation that is subject to wide variation. Consequently, the bioavailable fractions identified also suffer from a lack of precision. Not surprisingly then, the

identified metal fractions often correlate poorly with plant uptake (Pichtel and Anderson 1997; Sims and Kline 1991; Tsadilas *et al.* 1995), thus they cannot be expected to relate directly to the bioavailable proportion of metals (McGrath and Cegarra 1992).

Due to these problems many researchers view sequential extraction schemes as qualitative, or at best semi-quantitative, measures of metal partitioning (Emmerich *et al.* 1982b; McGrath and Cegarra 1992; Sims and Kline 1991). Alternatively, fractionation by sequential extraction could be seen as indicating the relative solubility of metals, rather than their availability (McLaughlin 2004, *pers comm.*). The many variations and modifications of sequential extraction schemes utilised also makes it difficult to compare separate investigations, resulting (once again) in measurements that are often highly case specific. Therefore the usefulness of sequential extraction schemes may be limited to defining broad differences between samples within a single research project. That is, for determining such things as changes in the proportion of metals in various fractions after a given treatment, changes over specified time periods, or for identifying differences between the fractionation of metals in biosolids/soils of differing origin.

1.9.3 Solution Ion Concentration/Activity

The dominant form of Cu absorbed by organisms is believed to be the cationic Cu species Cu^{2+} (Brams and Fiskell 1971; Graham 1981; Kochian 1991; Robson and Reuter 1981, and *c.f.* section 1.8.2), thus Cu bioavailability/toxicity may be directly related to the concentration (or activity) of Cu^{2+} in solution (Kim *et al.* 1999; Ma *et al.* 1999; Sauve *et al.* 1996). Therefore a range of methods, including Ion Selective Electrodes (ISEs), exchange resins, membrane dialysis, and computer speciation models, are often used to determine Cu^{2+} ion activity, and thus give an indication of the amount of available metal. These devices may be used to analyse displaced soil

solutions, single solution extractions (section 1.9.2.1), or extractions from sequential extraction schemes (section 1.9.2.2).

Recently the Cu^{2+} ISE has received much attention (Dumestre *et al.* 1999; Ma *et al.* 1999; Minnich and McBride 1987; Sauve *et al.* 1996; Sauve *et al.* 1997), largely because this device is relatively easy to use and provides rapid results (Amacher 1984). Also, and importantly in the context of this study, the Cu^{2+} ISE has proven to be particularly suited to the analysis of contaminated soils, including those amended with biosolids (Sauve *et al.* 1997). This is because the elevated metal concentrations of these soils result in solutions/extracts of higher ion activity (compared to non-contaminated soils), which are more within the range that can be confidently quantified by the Cu^{2+} ISE (*i.e.* $pCu^{2+} < 13$) (Sauve *et al.* 1995; Sauve *et al.* 1997). Numerous studies have shown ion activities measured by the Cu^{2+} ISE to be effective predictors of plant Cu uptake. For example, Minnich *et al* (1987) consistently observed a high regression coefficient ($r^2 > 0.9$) when analysing Cu^{2+} activity and root Cu uptake data (however the experiment was limited to a small range of soils). Similarly, Sauve *et al* (1996) found Cu^{2+} activity (measured using an ISE) to be a better predictor of plant Cu accumulation from contaminated soils than either $CaCl_2$ extracts or total Cu measurements. However, as discussed previously (*section 1.7.2*) the study by Sauve *et al* (1996) has been severely criticized for being highly influenced by the results of one soil. McLaughlin (2002) pointed out that when the values for that soil were removed from the dataset the ability of Cu^{2+} activity in solution to predict plant Cu uptake was no greater than that of total soil Cu. The lack of robustness in the results from the Sauve *et al* (1996) study was due to the Cu homeostasis expressed by the test plants in regard to shoot Cu concentration. For example, with the values from the highly influential soil removed from the dataset the ranges of shoot tissue Cu concentrations were very small; 8.1 – 12.6 mg/kg for lettuce

(*Lactuca sativa*), 15.3 – 40.0 mg/kg for radish (*Raphanus sativa*), and 17.0 – 32.3 mg/kg for ryegrass (*Lolium perenne*). Compared to this the range of Cu concentrations in root tissues observed by Minnich *et al* (1987) was very large (approximately 50 – 360 mg/kg), and therefore conclusions drawn from their data are more robust.

However, the Cu^{2+} ISE does have limitations. For example Amacher (1984) demonstrated that it is not suitable for use in soil suspensions or pastes, due to junction potentials being different for different physical phases (*i.e.* between solution, and solids in suspension). Thus the method may only be accurate in soil solutions/extracts. Others point out that although the ISE relies less on calculated values of Cu complexes, it (like all electro-chemical methods) still uses calculations and approximations to convert *emf* values to ion activities (Amacher 1984; Minnich and McBride 1987).

In regard to determining metal availability, there is a general limitation common to all ion activity measurement techniques; that being that because ion activity indicates the instantaneously available metal, it is solely a measure of the intensity factor I (*cf.* section 1.8). That is, ion activity indicates the available metal at the exact point in time when the measurement is taken, and does not take into account the Cu buffering capacity of the soil (C), nor the total potentially available metal present (*effective* Q). Thus in isolation, single ion activity measurements cannot determine bioavailability (McBride and Martinez 2000; Minnich *et al.* 1987), as measurements of both I and Q are necessary (McLaughlin *et al.* 2000; Minnich and McBride 1987; Minnich *et al.* 1987). Therefore ISE measurements (or other measures of ion activity) should be combined with techniques that also give indications of the parameters C and Q, in order to gain a more complete measure of bioavailability.

1.9.4 Isotopic Tracer Techniques

Isotopic techniques are based on the addition of a radioactive isotope to a sample, which then comes to equilibrium with native, non-radioactive isotopes of the same element in the solid and liquid phases. The degree to which the added tracer exchanges with the native metal on the solid phase determines the "exchangeable" or "labile" fraction of that element in the sample, and it is this fraction (along with that in solution) that is considered to be plant available (Echevarria *et al.* 1998; Lopez and Graham 1970; Tiller *et al.* 1972a). Isotopic techniques of this kind were first applied in soil research to measure the availability of soil P (Larsen 1952; McAuliffe *et al.* 1947; Russell *et al.* 1954), and have since been adapted and utilised to measure the availability of various trace metals (Delas *et al.* 1960; Hamon *et al.* 1997; Nakhone and Young 1993; Pandeya *et al.* 1998; Tiller *et al.* 1972a). Work with Cu has been limited due to the short half-life of the most suitable Cu radioisotope, ^{64}Cu ($t_{1/2}$ = 12.8 hours (Parker 1981)). However, Delas *et al.* (1960) used ^{64}Cu to determine the Cu exchange kinetics in 10 French soils with a history of $CuSO_4$ treatment dating back 70 years. Similarly, McLaren and Crawford (1974) used ^{64}Cu to determine the exchangeable Cu content of 24 British soils, while Lopez and Graham (1970; 1972) conducted related tests on soils from the USA. However, it appears that no work has been reported concerning the application of radioisotopic techniques to biosolid Cu.

There are two main variations of isotopic exchange investigations, with the results of each being termed E and L values respectively. Investigations producing E values involve direct sampling of a soil equilibrium solution, with the ratio of radioactive to non-radioactive isotopes in the solution then being determined. The process usually involves replicates of soil samples (2 – 5 g, depending on the study) being equilibrated with water or a dilute neutral salt solution such as

0.01M $CaNO_3$ (at soil: solution ratios commonly varying between 1: 2.5 and 1: 25, *e.g.* Fujii and Corey 1986; McLaren and Crawford 1974; Young *et al.* 2000) for periods of 24 hours or longer. A small quantity (usually an insignificant amount in terms of total mass) of the radioactive isotope of the element being examined is then added to the suspension and allowed to equilibrate for a further period (from hours to days, depending on the element and the aim of the study). The equilibrated solution is then usually centrifuged and filtered, and finally analysed to determine the ratio of radioactive to non-radioactive isotopes. This ratio is used to determine the amount of the element in the sample that is isotopically exchangeable, with the result reported in units of mg exchangeable element / kg sample (or equivalent) (equation 1.9.1). Experiments generating L values differ in that they determine the ratio of radioactive to non-radioactive isotopes in tissues of plants grown in soils spiked with the radioactive isotope (*i.e.* Hamon *et al.* 1997; Tiller *et al.* 1972a) (equation 1.9.2).

$$CuE = \frac{Cu(sol) * TA}{CR * M} \qquad \textbf{Eq 1.9.1}$$

where:
CuE = Isotopically exchangeable Cu (mg/kg)
Cu(sol)= Concentration of Cu in extract solution (mg/L)
TA = Total activity of ^{64}Cu added (Bq)
CR = Count rate in solution (Bq/L)
M = Mass of biosolid sample (kg)

$$L = \frac{Cu^{*}added}{SA_{Cu}} \qquad \textbf{Eq 1.9.2}$$

where: L = labile Cu (μg/g)
Cu*added = ^{64}Cu activity added (Bq/g)
SA_{Cu} = Cu specific activity in plant (Bq/μg Cu)

and, specific activity is calculated:

$$SA_{Cu} = \frac{^{64}Cu \text{ activity in plant (Bq)}}{\text{Cu taken up (μg)}}$$

Because the L value method utilises plants themselves to determine what is phytoavailable, some workers consider L values to be the best index of nutrient/contaminant availability (*i.e.* Pandeya *et al.* 1998). However, L value measurements are not always practical, or even possible, with some metals, with Cu being a prime example. The short half-life of the ^{64}Cu isotope, combined with the extremely slow rate of plant Cu uptake, make L value assessments of Cu from soil or soil-like media a practical impossibility using the radioisotope. Therefore radioisotopic

investigations of soil (or biosolid) Cu availability are essentially restricted to E values. Although E value methods do not use plants, their results have been shown to correlate highly with plant element uptake (*i.e.* Frossard *et al.* 1994; Pandeya *et al.* 1998). Similarly, it has been shown that the isotopically exchangeable fraction of soil metal (as determined by E values) is essentially the pool accessed by plants (Echevarria *et al.* 1998; Tiller *et al.* 1972a). Therefore E value investigations have many of the benefits of L value studies, but without the inconvenience and time and space requirements associated with plant-based assays. However, evidence suggests that E values may not always approximate L values, and may over-estimate metal availability in some conditions. In the case of Zn, E values have been shown to fail in alkaline soils, due to fixation of the added Zn tracer at high pH (Tiller *et al.* 1972a), or to problems with non-isotopically exchangeable colloidal metal (< 0.2 μm) in solution (Lombi *et al.* 2003). Both of these problems violate the assumptions of the E value calculation (equation 1.9.1), which relies on the added tracer remaining fully exchangeable with the metal in the labile pool (*i.e.* the fraction that is exchangeable between solid and solution phases). However, for Cu, the colloidal metal issue was found to be of little, if any, significance (Lombi *et al.* 2003), so does not pose a problem for investigations into isotopically exchangeable Cu. Similarly, the potential problem of fixation of the added tracer, as identified for Zn above, may not be an important issue for Cu (because Cu is less affected than Zn by fixation to inorganic components at elevated pH), but it still needs to be considered if assessments are to be made in alkaline conditions.

Nevertheless, with due consideration to their possible limitations, radioisotopic methods can be considered as effective measures of bioavailability. In fact numerous authors (Barber 1995; Fujii and Corey 1986; Lopez and Graham 1970), have concluded that isotopically determined labile

pools represent the soil's active and potentially active metals (*i.e.* effective Q), and thus are very effective measures of availability.

1.10 Aims & Objectives

The current state of knowledge regarding biosolid Cu, as outlined above, can be summarized as follows; a) biosolids contain substantial mineral and organic components, b) Cu bound in mineral forms may be permanently held, while those in organic forms much less so, c) Cu preferentially binds to organic components, d) upon decomposition of organic matter the previously bound Cu may be released to the environment or re-sorbed by the residual mineral fraction, thus Cu is a good "test case" for the time bomb v protection debate. Therefore, the specific aims and objectives of this study are to; 1) determine the availability of Cu in various Australian biosolids, 2) identify any time-dependent changes in Cu availability and determine the role of organic matter decomposition in any such changes, 3) compare and evaluate different availability measurement techniques for their applicability to biosolid Cu, and 4) establish an empirical model for predicting Cu availability based on measured biosolid properties and characteristics.

2. Biosolids Characterisation and Cu Availability

2.1 Introduction

The physical and chemical properties of Australian biosolids, and how they vary, are not well known, nor is it known how this variability may affect metal availability. This chapter therefore describes a body of work in which biosolids from various regions of Australia were characterised in terms of pH, electrical conductivity (EC), total element concentrations, and other properties. Chemical assays of biosolid metal availability were conducted. Due to the importance of Cu in the context of this thesis (*c.f.* Chapter 1), procedures specifically designed to determine aspects of Cu availability were performed, including isotopic dilution (using ^{64}Cu) and ion activity measurements. The aims of the work were to characterise Australian biosolids in terms of general properties, to determine biosolid metal availability (particularly that of Cu), to identify the key factors controlling Cu availability, and to develop empirical models that aim to predict the availability of biosolid Cu from easily measured biosolid properties.

2.2 Methods

Biosolids were collected from 18 sewage treatment plants located at various sites across Australia. The selected sites covered both domestic and industrial catchments. At sites where stockpiles were kept, biosolids of different ages were sampled. Thus, taking age into account, 24 individual biosolids were characterised. Table 2.2.1 shows the source treatment plant, age at time of collection, production details, and other information for each of the biosolids examined.

Table 2.2.1: Biosolid Source and Production Data

Biosolid	Origin*	Age (at testing)	Major Waste Input	Digestion type or other treatment	Drying system
Bolivar 95	SA	5 years	Industrial + domestic	Anaerobic[#]	Evaporation
Bolivar 97	SA	3 years	Industrial + domestic	Anaerobic	Evaporation
Chelsea 77	Vic	23 years	Domestic	Anaerobic	Evaporation
Chelsea 87	Vic	13 years	Domestic	Anaerobic	Evaporation
Chelsea 96	Vic	4 years	Domestic	Anaerobic	Evaporation
Chelsea 98	Vic	2 years	Domestic	Anaerobic	Evaporation
Cronulla	NSW	< 5 years	Domestic	Anaerobic	Centrifuge
Fairfield	Qld	< 2 years	Domestic	Anaerobic	Belt Press
Gumeracha	SA	2 months	Domestic	Imhoff Tank[+]	Evaporation
Hahndorf	SA	2 months	Domestic	Oxidation Ditch	Evaporation
Heathfield	SA	2 months	Domestic	Extended Aeration	Evaporation
Luggage Point	Qld	1 year	Domestic	Anaerobic	
Luggage Point 95	Qld	5 years	Domestic	Anaerobic	
Myponga	SA	2 months	Domestic	Imhoff Tank	Evaporation
Oxley	Qld	1 year	Industrial + domestic	Anaerobic	Centrifuge
Port Adelaide	SA	5 years	Industrial + domestic	Anaerobic	Evaporation
Port Kembla	NSW	< 5 years	Heavy industry + domestic	Anaerobic	Belt Press
Sandgate	Qld	1 year	Domestic	Anaerobic	Belt Press
St Marys	NSW	< 5 years	Industrial + domestic	Aerobic	Belt Press
Victor Harbor	SA	2 months	Domestic	Imhoff Tank	Evaporation
Wacol	Qld	1 year	Domestic	Extended Aeration	Belt Press
Werribee 83	Vic	17 years	Industrial + domestic	Land filtration	Evaporation
Werribee 97	Vic	3 years	Industrial + domestic	Land filtration	Evaporation
West Hornsby	NSW	< 5 years	Domestic	Anaerobic	Centrifuge

* Australian state: SA= South Australia, Vic = Victoria, NSW = New South Wales, Qld = Queensland.
[#] see Chapter 1 (section 1.1) for description of general sewage treatment and digestion processes.
[+] Imhoff tanks are phase separation tanks where sedimentation allows settling out of solids. Settled solids undergo 'cold digestion' at base of tank. Solids are later transferred to drying beds.

2.2.1 Biosolid Preparation

Because many samples were very moist upon collection, biosolids were oven-dried at 35-40°C for up to 5 days. After this they were considered air-dry, and were crushed and ground to pass a 2 mm sieve. Moisture contents of subsamples were determined (two days drying at 105°C) to enable all reported results to be stated in terms of oven-dry mass.

2.2.2 General Properties

Biosolid pH was measured in duplicate (n=2) 1:5 solid: 0.01M $CaCl_2$ solution extracts, using an Orion pH electrode and an Orion meter (Expandable Ion Analyzer EA940). Electrical conductivity (EC) was measured in 1:5 solid: water (deionised) extracts (n=2), using an Orion

conductivity meter (170). Organic carbon concentration was determined by dichromate oxidation (Allison 1965) (n=2). Total carbon was measured by LECO combustion (Nelson and Sommers 1982) (n=3). Hot water-extractable carbohydrate (HWC), a measure of the readily degradable C present, was determined via the method of Lu *et al.* (1998) (n=3). Total element concentrations were determined in *aqua regia* (1:3 HNO_3: HCl) digests by Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) (Zarcinas *et al.* 1987) (n=3 or 4).

2.2.3 $CaCl_2$-Extractable Elements

Biosolid samples (in triplicate) were equilibrated with 0.01M $CaCl_2$ (1:5 solid: solution) for 24 hours on an end-over-end shaker. After equilibration samples were first centrifuged at 1200 *g* for 10 minutes, with the supernatants then transferred into new vessels that were centrifuged again at 32600 *g* for 15 minutes. The resulting supernatant solutions were then filtered (0.45 μm) and analysed by ICP-AES to determine the concentration of extractable metals. This two-step centrifugation procedure was adopted due to the extreme difficulty incurred when filtering many of the samples in preliminary tests.

2.2.4 Isotopically Exchangeable Cu (E-values)

Isotopically exchangeable Cu (CuE) was determined following the method of McLaren and Crawford (1974). Triplicate biosolid samples (4 g) were equilibrated with 40 mL 0.01M $CaCl_2$ for 40 hours on an end-over-end shaker, after which the radioisotope ^{64}Cu was added (in 0.1 mL solution containing approximately 4 MBq activity). After a further 24-hour equilibration period, samples were centrifuged for 10 minutes at 1200 *g*, the supernatant solutions filtered through 0.45 μm filters, and then measured for radioactivity (gamma counter) and total solution Cu (ICP-AES). Isotopically exchangeable Cu (the E-value, or CuE) was determined as follows:

$$CuE = \frac{Cu(sol) * TA}{CR * M} \qquad \textbf{Eq 2.2.1}$$

where: CuE = Isotopically exchangeable Cu (mg/kg); Cu(sol)= Concentration of Cu in 0.01M $CaCl_2$ solution (mg/L); TA= Total activity of ^{64}Cu added (Bq); CR= Count rate in solution (Bq/L); M= Mass of biosolid sample (kg).

2.2.5 Effect of pH adjustment on Isotopically Exchangeable Cu

To determine whether varying the pH of an individual biosolid would cause changes to the measured E value, three biosolids were treated with varying concentrations of acid during a separate E value investigation. Bolivar 95, Chelsea 98 and Port Kembla biosolids underwent CuE determination as per the method described in section 2.2.4 above, but were equilibrated with 0.01M $CaCl_2$ solutions that had been dosed to seven levels of acid concentration (n=2). A 1M HNO_3 solution, prepared in 0.01M $CaCl_2$, was used to prepare the various solutions (Table 2.2.2). The amounts of acid to be added were determined in a preliminary titration procedure, the resulting titration curves of which are shown in Figure 2.2.1. Subsamples of supernatants were taken from each sample prior to E value determination, and these were used to measure pH and Cu^{2+} activity (pCu^{2+}) using the techniques described in the next section (2.2.6). The E value and pCu^{2+} were used to determine the partition coefficient, or Kd, in the pH adjusted samples (here Kd = CuE/Cu^{2+}activity). The CuE equation was slightly modified for the Kd calculation, so that only the isotopically exchangeable Cu on the solid was used to determine Kd, and not the exchangeable fraction in the solid plus that in the solution phase (Nakhone and Young 1993).

$$CuE_{(solid)} = \frac{Cu(sol) * [TA-\{CR*vol\}]}{CR * M} \qquad \textbf{Eq. 2.2.2}$$

where: $CuE_{(solid)}$ = Isotopically exchangeable Cu in the solid (mg/kg); Cu(sol)= Concentration of Cu in 0.01M $CaCl_2$ solution (mg/L); TA= Total activity of ^{64}Cu added (Bq); CR= Count rate in solution (Bq/L); M= Mass of biosolid sample (kg); and vol = volume of extract solution (L).

Table 2.2.2: Biosolid pH Adjustment

B95	Ch98	PK
-----mmols H^+ / g sample-----		
0.00	0.00	0.00
0.10	0.06	0.10
0.30	0.13	0.20
0.50	0.19	0.30
0.75	0.25	0.40
0.95	0.31	0.50
1.13	0.35	0.60

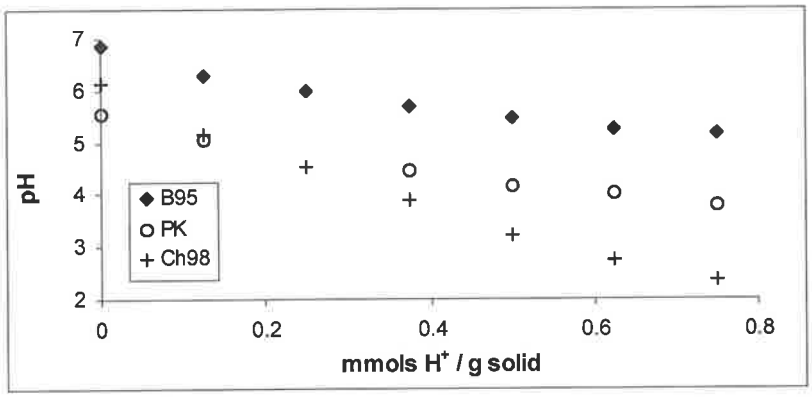


Figure 2.2.1: Titration curves for Bolivar 95, Chelsea 98, and Port Kembla biosolids

2.2.6 Copper Ion Activity (pCu^{2+})

Because Cu^{2+} is believed to be the form of Cu most readily assimilated by organisms (Graham 1981; Kochian 1991; Robson and Reuter 1981), and because Cu^{2+} activity has been successfully related to Cu uptake and/or toxicity (Dumestre *et al.* 1999; McBride 2001; Vulkan *et al.* 2000), the negative log molar cupric ion activity (pCu^{2+}) was measured in biosolid solution extracts using a Cu^{2+} ion selective electrode (Orion 9429), a silver-silver chloride double junction reference electrode (Orion 900200), and a mV meter (Orion 720). Duplicate biosolid samples (4 g) were equilibrated with 40 mL 0.01M $CaCl_2$ solution for 24 hours on an end-over-end

shaker. Samples were then centrifuged for 10 minutes at 1200 *g*, the supernatant decanted, and the pCu^{2+} determined.

Calibration standards were prepared using ethylenediamine dihydrochloride (EN) (Ma *et al.* 1999), $Cu(NO_3)_2$, KNO_3, NaOH, and deionised H_2O. All standards had 40 mL 1.57 x 10^{-6}M $Cu(NO_3)_2$ (prepared in 0.1M KNO_3, to match ionic strength of samples), and 5 mL 0.1M EN. The pH was adjusted to achieve the desired Cu^{2+} activity in the standards by adding varying volumes of 0.01M NaOH (Minnich and McBride 1987). Deionised water was used to maintain a consistent volume across standards (60 mL). The activity of Cu^{2+} in the standards was calculated using GEOCHEM-PC (V2), and a calibration curve of pCu^{2+} and mV produced (Figure 2.2.2).

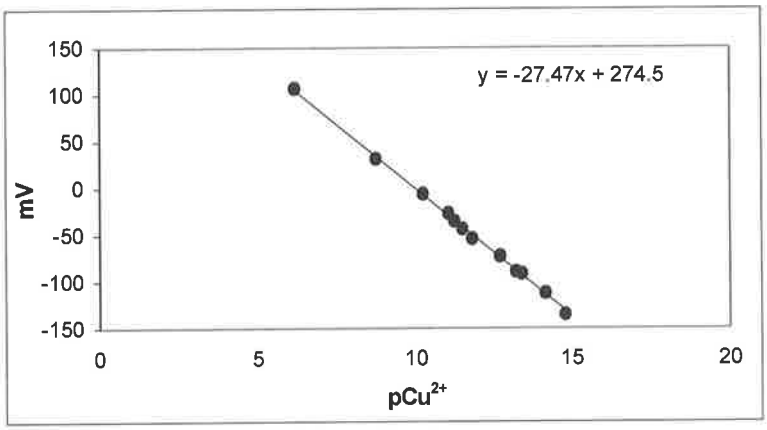


Figure 2.2.2: An example of the calibration curves constructed to determine cupric ion activity (pCu^{2+}) from mV readings. The curve deviates slightly from the ideal Nernstian response, which has a slope of -29.5mV.

2.2.7 Statistical Analysis

Statistical analyses (ANOVA and multiple linear regression) were performed using Genstat 5 for Windows (release 4.1) (Anon. 1998) and Microsoft Excel.

2.3 Results and Discussion

2.3.1 General Biosolid Properties

Table 2.3.1: General Biosolid Properties (± s.e., n=2 or 3)

Biosolid	pH	±	EC (mS/cm)	±	Org. C (%)	±	Total C (%)	±	HWC^ (µg/g)	±	Total N (%)
Bolivar 95	7.23	0.01	6.81	0.02	12.1	0.08	22.3	0.41	4229	24	2.5
Bolivar 97	6.46	0.01	3.68	0.03	15.8	0.77	25.7	0.24	6104	272	2.9
Chelsea 77	6.21	0.05	1.82	0.03	3.1	1.12	5.8	0.16	997	106	1.2
Chelsea 87	5.73	0.03	2.12	0.02	4.3	0.19	6.5	0.06	838	155	0.7
Chelsea 96	5.17	0.01	2.05	0.01	2.8	0.77	5.7	0.21	1728	230	0.8
Chelsea 98	6.19	0.00	1.97	0.00	6.9	0.33	9.3	0.12	1767	225	0.7
Cronulla	6.15	0.01	3.86	0.01	20.3	0.45	27.9	0.17	4678	205	2.9
Fairfield	6.55	0.01	4.68	0.02	16.0	1.10	32.7	0.03	6347	250	2.5
Gumeracha	5.21	0.02	0.61	0.06	7.2	0.07	15.8	0.22	3585	170	1.7
Hahndorf	5.62	0.01	1.13	0.02	15.6	0.92	25.0	0.02	3793	262	2.3
Heathfield	5.50	0.01	3.27	0.05	20.1	0.86	29.0	0.10	4721	458	3.2
Luggage Point	6.67	0.00	6.90	0.09	19.1	3.12	35.9	0.11	8229	528	2.3
Luggage Point 95	4.96	0.01	5.01	0.01	12.0	0.35	10.9	0.26	4196	270	2.9
Myponga	5.62	0.00	1.37	0.03	18.0	0.52	24.8	0.71	5718	1435	2.5
Oxley	6.47	0.00	5.85	0.12	15.6	1.75	33.4	0.13	11065	148	4.0
Port Adelaide	6.80	0.01	11.35	0.01	5.9	0.53	9.3	0.18	4932	1364	2.7
Port Kembla	5.63	0.01	4.46	0.02	19.8	0.95	25.1	0.18	6631	349	3.0
Sandgate	6.27	0.01	5.59	0.02	19.1	1.24	36.7	0.32	13413	214	5.5
St Marys	5.76	0.01	5.41	0.02	17.7	0.55	24.7	0.01	14982	116	3.8
Victor Harbor	5.93	0.01	2.12	0.04	16.2	2.14	21.1	0.32	8288	309	2.2
Wacol	6.41	0.01	3.61	0.06	15.0	2.28	38.5	0.34	11189	718	6.2
Werribee 83	4.11	0.02	5.21	0.01	20.6	0.20	26.2	0.06	6142	129	5.3
Werribee 97	5.06	0.02	1.79	0.01	18.6	1.27	28.7	0.08	3225	251	4.9
West Hornsby	6.71	0.00	1.04	0.02	19.3	0.71	24.1	0.11	10995	169	7.4

^ HWC – Hot water-extractable carbohydrate, in µg glucose equivalents /g soil, (Lu *et al.* 1998).

Results for the general characterisation procedures reveal the widely varying physical and chemical properties of the biosolids surveyed, such as pH values ranging from 4.1 to 7.2, and EC values between 0.61 and 11.35 mS/cm (Table 2.3.1). This reflects the varying inputs to sewers in different regions, and the differing treatment processes and drying systems used at the source treatment plants. The organic carbon (OC) contents of these biosolids were much lower than many values reported for biosolids from overseas. For example, the highest amount observed in this study was 20.6% OC (oven dry value), with a median of 15.9%. In a study of 11 biosolids from Indiana (USA), Stover *et al.* (1976) found a minimum value of 19.8% OC, a median of 23.6% and a maximum of 28.3%. Similarly, other overseas studies where biosolid OC has been

recorded (and was assessed using comparable methods) routinely report much higher percentages of OC (Table 2.3.2).

Table 2.3.2: Organic Carbon Contents of Overseas Biosolids

Study	Country	Biosolid OC %	Moisture*
Parkpain *et al.* 2000	Thailand	20	air dry
Zwarich and Mills 1982	Canada	26	air dry
Carlson-Ekvall and Morrison 1997	Sweden	33	oven dry
Pandeya *et al.* 1998	India	46	not stated
Zhou and Wong 2001	China (Hong Kong)	48	not stated

*Moisture content when tested.

Upon examination of biosolid total metal concentrations (Table 2.3.3), a significant difference was found between the Pb concentrations of biosolids derived predominantly from domestic sources and those with industrial waste inputs, with the mean of the industry influenced biosolids (185 mg/kg) being more than double the mean of the domestic ones (89 mg/kg - see ANOVA output below). Significant differences were not found for any other elements.

ANOVA	*Count*	*Sum*	*Average*	*Variance*
Domestic sludge Pb	16	1426	89	779
Industrial sludge Pb	8	1484	185	29792

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	49501	1	49501	4.94	0.0368	4.30
Within Groups	220237	22	10011			
Total	269738	23				

When total element concentrations were examined in relation to type of drying procedure employed in biosolids production, significant differences were found, for five elements, between biosolids that underwent evaporative drying and those de-watered by other means such as centrifugation (Table 2.3.4). It was expected that evaporatively dried products would have higher concentrations of highly soluble elements, however, somewhat surprisingly, Na concentration showed no significant difference between drying methods. The higher concentrations of Ca, S and P in the non-evaporatively dried biosolids may be due to chemical reagents added to facilitate the dewatering process, which may either add additional amounts (in the case of Ca), or increase

retention in the solids (in the case of P and S). However, the difference in Al concentrations between evaporatively dried and non-evaporatively dried biosolids may simply be an artefact of other treatment processes used in the plants employing evaporative drying systems (namely, the incorporation of alum sludge from drinking water clarification).

Table 2.3.4: Total Element Concentrations with Significant Differences ($p \leq 0.05$) Between Evaporatively and Non-evaporatively Dried Biosolids

	Mean conc. (mg/kg) Evaporativley dried	*Mean conc. (mg/kg) Non-Evaporativley dried*	*F*	*P-value*	*F crit*
Al	25731	10821	15.8	0.001	4.35
Fe	15298	46599	6.6	0.018	4.35
Ca	10192	29435	14.4	0.001	4.35
P	9283	32163	32.3	<0.001	4.35
S	5794	12588	29.7	<0.001	4.35

Table 2.3.3: Biosolid Total Element[‡] Concentrations (mg/kg ± s.e., n=3)

Biosolid	Al	±	Fe	±	Cu	±	Cd	±	Pb	±	Zn	±	Ca	±	Na	±	K	±	P	±	S	±
Bolivar 95	29025	596	15300	152	701	20	2.4	0.04	158	4.9	728	21	38837	1050	5557	106	6895	112	12816	342	8962	140
Bolivar 97	28815	506	13691	231	942	11	3.5	0.16	166	0.7	1012	7	28863	237	1832	49	4851	122	16283	198	8594	74
Chelsea 77	32981	863	16981	368	258	7	4.3	0.21	103	5.9	403	10	6463	195	1193	46	1935	53	7038	158	2733	80
Chelsea 87	34609	734	17735	268	291	4	4.7	0.24	89	0.7	439	4	7128	72	1181	32	2112	77	7606	82	3330	68
Chelsea 96	41251	1299	20253	1690	203	4	3.9	0.16	81	0.8	323	8	6654	73	596	21	1887	62	6277	140	2314	65
Chelsea 98	33384	543	19368	305	291	4	5.4	0.16	107	1.5	458	4	7920	94	1681	24	2325	67	8909	105	3411	45
Cronulla	9018	46	37269	341	775	9	3.2	0.07	79	0.9	807	5	48563	576	1200	7	1186	7	29914	281	13330	214
Fairfield	11794	100	7378	79	398	4	1.9	0.04	64	0.9	790	7	23924	208	2343	25	2831	25	16054	129	10478	107
Gumeracha	34318	529	28750	435	1818	106	2.3	0.14	57	3.4	750	11	928	68	262	5	6467	94	4624	126	3209	76
Hahndorf	21592	1078	11027	182	1916	29	3.3	0.06	114	3.6	1795	16	1891	50	253	16	2157	40	10789	471	6439	98
Heathfield	14594	523	14038	50	2190	29	4.5	0.06	129	2.5	2185	32	2679	101	376	4	2056	49	10566	129	11281	88
Lug Point	7317	17	14315	54	828	4	4.3	0.09	105	0.9	1001	6	21259	59	2872	7	2264	9	24622	359	15649	46
Lug Point 95	18233	291	24420	268	506	13	12.3	0.36	128	2.8	708	23	17692	316	1294	28	3168	20	13665	447	10109	141
Myponga	11379	44	10533	119	1852	93	3.6	0.06	125	6.4	2335	84	1528	22	385	7	1433	12	6352	151	5831	188
Oxley	11669	77	10568	57	696	4	4.5	0.04	122	1.3	1131	5	25995	216	1198	8	2367	19	18889	140	11782	52
Port Adelaide	17874	234	14556	123	325	30	3.0	0.23	77	5.2	488	40	20433	1100	9292	599	6219	70	6239	514	6137	379
Port Kembla	10949	98	82515	1238	964	12	20.7	0.25	80	1.2	1530	23	42814	1315	661	9	575	7	42924	602	18505	458
Sandgate	9131	70	13135	251	522	5	2.6	0.04	67	1.3	1084	9	32722	805	1587	19	1337	8	17638	274	15784	305
St Marys	11234	291	110512	670	597	3	3.2	0.04	57	0.3	687	5	15502	116	937	4	1836	49	52568	214	9587	44
Victor Harbor	5490	298	5638	207	2095	143	2.3	0.13	65	4.0	1479	64	2410	59	401	16	1361	50	7898	220	7184	443
Wacol	5374	213	5067	218	394	6	1.9	0.06	35	0.2	488	7	17289	181	1637	44	7410	131	29478	442	10223	194
Werribee 83	28929	541	12621	67	800	4	13.7	0.07	587	4.7	912	10	6578	143	379	18	3035	42	13622	53	5666	76
Werribee 97	25988	432	13684	58	909	4	9.9	0.21	238	1.0	1560	9	10371	55	831	15	2666	37	10947	76	6019	23
West Hornsby	17397	888	106348	1085	851	10	3.1	0.08	79	0.8	690	5	28676	473	621	15	3659	276	49837	444	11015	93
IRS 1[*] (ms)	15500	200	27500	200	12.6	0.1	0.4	0.10	20.9	0.5	34	0.1	1300	20	88	1.1	2535	49	487	11	230	2
IRS 1 (doc)	14680	400	28100	800	12.8	0.2	0.2	0.02	18.1	0.4	34	1.5	1400	50	90	3.5	2553	74	500	0	230	0
IRS 4 (ms)	11000	200	7900	100	5.6	0.1	0.3	0.04	8.6	0.1	13	0.3	39600	4100	136	4.4	3261	37	304	9	205	1
IRS 4 (doc)	10680	300	7150	200	5.4	0.1	0.2		6.7	0.2	13	0.5	41300	3200	135	4.5	3158	59	300	0	190	10

[‡] *Aqua Regia* digest

[*] IRS = Internal Reference Standard (CSIRO), ms = measured value, doc = documented value.

2.3.2 $CaCl_2$-Extractable Elements

Table 2.3.5 shows $CaCl_2$-extractable element contents as percentages of total element concentrations, while Table 2.3.6 presents the values in absolute terms (mg/kg).

Table 2.3.5: Mean $CaCl_2$ Extractable Element Concentrations as % of Totals (± s.e., n=3)

Biosolid	Cu	±	Zn	±	Mg	±	Mn	±	Ni	±	K	±	P	±	S	±
Bolivar 95	8.0	0.11	0.2	0.01	13	0.1	0.9	0.02	26.0	0.34	16	0.18	0.28	0.00	44	0.71
Bolivar 97	4.9	0.02	0.2	0.01	16	0.2	1.7	0.08	7.0	0.14	11	0.12	0.34	0.01	33	1.62
Chelsea 77	0.4	0.00	0.4	0.06	22	0.4	2.3	0.33	1.7	0.15	12	0.43	0.49	0.02	43	3.55
Chelsea 87	0.4	0.00	0.8	0.06	25	0.6	3.5	0.42	1.8	0.20	9	0.18	0.63	0.03	35	5.17
Chelsea 96	0.6	0.01	2.0	0.13	36	1.1	7.7	0.10	2.8	0.11	10	0.05	1.38	0.02	59	1.25
Chelsea 98	0.5	0.00	0.3	0.01	21	0.2	1.8	0.02	1.9	0.06	15	0.23	0.70	0.01	39	0.98
Cronulla	0.9	0.03	0.2	0.01	27	0.1	1.7	0.01	3.1	0.29	17	0.09	0.18	0.00	29	0.42
Fairfield	6.4	0.05	0.3	0.01	26	0.3	1.1	0.03	19.2	0.99	16	0.72	5.77	0.02	38	0.88
Gumeracha	0.3	0.00	5.7	0.05	3	0.0	9.4	0.08	1.9	0.15	3	0.02	0.85	0.04	13	0.57
Hahndorf	0.3	0.00	2.6	0.10	7	0.0	4.9	0.21	2.2	0.18	8	0.07	0.27	0.01	13	0.44
Heathfield	0.9	0.00	5.4	0.02	24	0.2	10.1	0.05	5.2	0.23	13	0.05	0.65	0.00	26	0.49
Lug Point	4.1	0.05	0.2	0.01	24	0.1	1.2	0.02	8.8	0.10	47	0.61	5.28	0.09	40	0.94
Lug Point 95	0.4	0.01	5.0	0.03	27	0.0	15.5	0.09	4.3	0.10	5	0.14	0.20	0.03	29	0.26
Myponga	0.3	0.00	4.9	0.06	13	0.1	8.9	0.28	1.9	0.27	10	0.08	0.23	0.00	21	0.72
Oxley	3.7	0.24	0.2	0.01	26	0.2	1.5	0.06	9.2	0.50	21	0.23	2.64	0.08	47	0.94
Port Adelaide	1.1	0.01	0.3	0.01	28	0.5	1.9	0.04	2.2	0.16	13	0.16	0.76	0.01	59	1.75
Port Kembla	0.8	0.01	0.9	0.01	38	0.1	3.8	0.02	3.3	0.33	10	0.46	0.07	0.00	29	0.49
Sandgate	1.1	0.05	0.4	0.01	28	0.2	3.1	0.03	7.6	0.10	33	0.46	0.84	0.02	31	0.51
St Marys	0.8	0.02	0.5	0.00	30	0.6	2.5	0.11	2.5	0.33	15	0.17	0.10	0.00	45	1.91
Victor Harbor	0.3	0.01	0.8	0.06	13	0.2	3.6	0.10	1.5	0.14	13	0.11	0.96	0.03	23	0.24
Wacol	9.6	0.13	0.9	0.05	7	0.2	0.7	0.02	6.7	0.44	49	0.69	17.92	0.21	18	0.16
Werribee 83	0.6	0.00	16.6	0.16	8	0.0	13.9	0.48	7.2	0.11	18	0.30	0.71	0.04	19	0.43
Werribee 97	0.4	0.00	3.4	0.09	17	0.1	7.4	0.04	3.4	0.07	13	0.31	0.41	0.01	7	0.31
West Hornsby	1.1	0.01	0.2	0.01	21	0.2	1.0	0.02	2.5	0.11	4	0.12	0.09	0.00	31	0.42

Table 2.3.6: Biosolid $CaCl_2$ Extractable Element Concentrations (mg/kg ± s.e., n=3)

	Cu	±	Zn	±	Mg	±	Mn	±	Ni	±	Cd	±	K	±	P	±	S	±
Bolivar 95	56	0.76	2	0.0	1181	8	2	0.02	12.9	0.10	0.03	0.01	1083	7.3	36	0.3	3900	37
Bolivar 97	43	1.28	2	0.2	805	11	3	0.09	4.3	0.05	0.01	0.01	546	3.5	56	0.8	2804	81
Chelsea 77	1	0.01	2	0.1	769	15	2	0.17	0.8	0.04	0.04	0.02	226	4.8	35	1.0	1175	56
Chelsea 87	1	0.01	3	0.2	940	21	4	0.27	1.0	0.07	0.02	0.01	187	2.2	48	1.3	1172	99
Chelsea 96	1	0.01	6	0.2	1116	34	7	0.05	1.2	0.03	0.09	0.03	185	0.6	87	0.6	1362	17
Chelsea 98	1	0.01	1	0.0	876	9	3	0.02	1.1	0.02	0.01	0.01	346	3.0	63	0.6	1343	19
Cronulla	7	0.22	2	0.1	1204	4	4	0.02	0.8	0.04	0.03	0.01	205	0.7	54	0.7	3885	33
Fairfield	26	0.21	2	0.0	934	9	3	0.06	3.3	0.10	0.00	0.01	462	11.7	927	1.8	3989	53
Gumeracha	5	0.05	43	0.2	232	1	19	0.10	0.6	0.03	0.03	0.03	204	0.6	39	1.1	426	11
Hahndorf	5	0.07	46	1.0	174	1	6	0.16	0.5	0.03	0.03	0.01	181	0.8	29	0.6	807	16
Heathfield	19	0.03	117	0.3	777	6	23	0.06	1.5	0.04	0.07	0.01	269	0.6	69	0.3	2890	32
Lug Point	34	0.38	2	0.1	1926	5	4	0.04	9.0	0.06	0.01	0.03	1053	7.9	1301	13.4	6289	85
Lug Point 95	2	0.06	35	0.1	1282	2	101	0.34	2.6	0.03	0.52	0.04	164	2.5	28	2.1	2883	15
Myponga	5	0.08	115	0.9	267	3	10	0.18	0.6	0.05	0.10	0.00	148	0.7	15	0.1	1243	24
Oxley	26	1.65	2	0.0	1168	11	6	0.12	19.4	0.61	0.03	0.02	492	3.1	499	9.2	5565	64
Port Adelaide	4	0.03	2	0.0	1838	36	3	0.03	0.8	0.03	0.00	0.01	796	5.7	48	0.3	3642	62
Port Kembla	8	0.07	14	0.1	1768	3	23	0.06	1.0	0.06	0.09	0.02	57	1.5	31	0.0	5304	53
Sandgate	6	0.26	4	0.1	973	7	17	0.08	2.4	0.02	b/d*		438	3.5	149	2.2	4968	46
St Marys	5	0.10	3	0.0	1271	25	8	0.19	0.7	0.05	b/d		282	1.8	53	1.2	4272	106
Victor Harbor	6	0.30	12	0.5	280	5	4	0.06	0.4	0.02	0.02	0.00	171	0.8	76	1.5	1642	10
Wacol	38	0.50	4	0.1	504	13	1	0.03	1.4	0.05	b/d		3613	29.3	5284	35.0	1801	9
Werribee 83	5	0.02	151	0.8	151	1	8	0.17	5.6	0.05	1.37	0.01	547	5.2	97	3.4	1083	14
Werribee 97	3	0.03	48	0.7	411	2	10	0.03	2.9	0.04	0.18	0.01	334	4.7	45	0.4	423	11
West Hornsby	9	0.12	1	0.1	731	7	5	0.05	0.8	0.02	b/d		159	2.5	45	0.1	3381	27

*b/d below detection

There was a wide variation (1 – 56 mg/kg) in $CaCl_2$-extractable Cu concentrations in the biosolids (Table 2.3.6). When viewed in relation to total concentrations (Table 2.3.3) it is clear no relationship existed between total and extractable Cu. Regression analysis confirmed this, returning an R^2 value of 0.001. This lack of relationship is consistent with the results of Bhogal *et al.* (2003), who found no relation between NH_4NO_3-extractable Cu and total Cu in biosolid-amended soils. Similarly, Brun *et al.* (1998) found no relation between $CaCl_2$-extractable Cu and total Cu in vineyard soils contaminated with Cu-based fungicides.

Correlation analysis of the results revealed significant relationships ($p \leq 0.05$) existed between $CaCl_2$-extractable Cu and biosolid pH ($r = 0.59$), and between $CaCl_2$-extractable Cu and total C% ($r = 0.51$). This is in contrast to results found by Smith (1997) and Sauve *et al.* (1997), who determined that soluble Cu in the soils they examined was not related to pH (however, these researchers were examining mineral soils, not biosolids). The relationship found here was positive, suggesting that at higher pH more Cu was available for extraction. The relationship was best described by a polynomial rather than a linear model (Figure 2.3.1a). The positive relationship is likely to be due to increased dissolved organic matter concentrations at higher pH (Yin *et al.* 2000; You *et al.* 1999), which would retain a higher concentration of Cu in solution through the formation of soluble Cu-organic complexes, and this is despite the high Ca concentration in the extract solution which is known to minimise organic matter dispersion (Oste *et al.* 2002). Stepwise multiple linear regressions of the data confirmed pH and total C% to be important variables influencing extractable Cu ($R^2 = 0.46$, Eq. 2.3.1), and that other variables (*i.e.* EC and total Cu) were not significant. When total C% (measured by LECO) was replaced by OC% (wet oxidation) in the model, the R^2 fell to 0.36, thus total C% was a greater explanatory

variable. The implications of this may be that either carbonates play a role in controlling a portion of the neutral salt-extractable Cu in some of the biosolids, or that the resistant organic C not identified by the wet oxidation method, which is known to suffer from incomplete combustion of some organic forms (Navarro *et al.* 1991; Nelson and Sommers 1982), may be important.

Ext Cu (mg/kg) = 10.97 (3.42) pH + 0.625 (0.241) TC% - 66.2 (20.1) (R^2 = 0.455) **Eq. 2.3.1**

where Ext Cu = $CaCl_2$ extractable Cu, TC% = total C%, and figures in parentheses indicate standard error estimates.

ANOVA	*d.f.*	*s.s.*	*m.s.*	*v.r.*	*F pr.*	R^2
Regression	2	2796	1398	10.62	<0.001	0.455
Residual	21	2765	131.7			
Total	23	5561	241.8			

ANOVA - Accumulated

Variable	*d.f.*	*s.s.*	*m.s.*	*v.r.*	*F pr.*	*Cumulative* R^2
pH	1	1909	1909	14.5	0.001	0.313
TotC%	1	887	887	6.74	0.017	0.455
Residual	21	2765	132			
Total	23	5561	242			

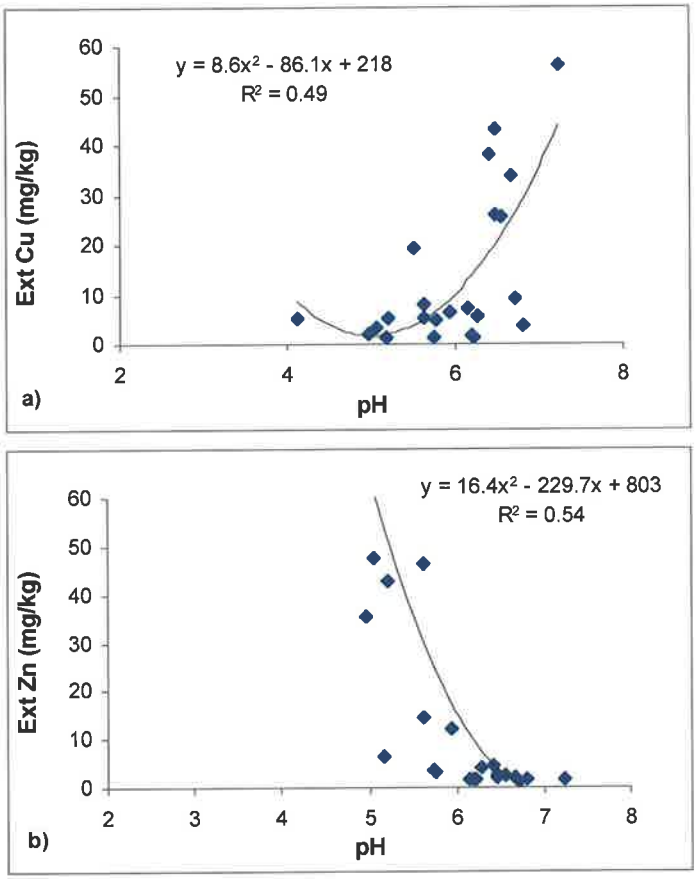


Figure 2.3.1: $CaCl_2$-extractable Cu (a) and Zn (b) as a function of biosolid pH.

There was a significant relationship between $CaCl_2$-extractable Zn and total Zn (r = 0.61), and between extractable Zn and pH (r = -0.69) (Figure 2.3.1b, and Table 2.3.7). When the extreme value of Werribee 83 (151 mg extractable Zn/kg, more than 7 times the mean of all other biosolids) was removed from the dataset, the relationship between extractable Zn and total Zn became even stronger (r = 0.80). Multiple linear regression produced a model explaining 65% of the variation in extractable Zn (complete dataset, Eq. 2.3.2):

Ext Zn (mg/kg) = 0.355 (0.01) TotZn – 34.35 (7.62) pH + 194.5 (48.8) (R^2 = 0.644) **Eq. 2.3.2**

where; Ext Zn = $CaCl_2$ extractable Zn, TotZn = total Zn (mg/kg), and parentheses indicate error estimates.

ANOVA	*d.f.*	*s.s.*	*m.s.*	*v.r.*	*F pr.*	R^2
Regression	2	28099	14050	21.82	<0.001	0.644
Residual	21	13522	644			
Total	23	41621	1810			

ANOVA – Accumulated						
	d.f.	*s.s.*	*m.s.*	*v.r.*	*F pr.*	*Cumulative* R^2
Total Zn	1	15030	15030	23.34	<0.001	0.322
pH	1	13069	13069	20.3	<0.001	0.644
Residual	21	13522	644			
Total	23	41621	1810			

Table 2.3.7: Correlation Coefficients (r) for $CaCl_2$-Extractable and Total Element Concentrations, and for Extractable Element Concentrations and pH

Element	Extractable & Total^	Extractable & pH
Cu	-0.031	0.586*
Zn	0.609*	-0.687*
Cd	0.559*	-0.545*
Mg	-0.147	0.525*
Mn	0.575*	0.159
Ni	0.827*	0.025
K	0.628*	0.306
P	0.198	0.213
S	0.846*	0.312

^ Extractable and Total concentrations in mg/kg.
* significant at the 95% level, double sided T-test.

Unlike the results for Cu, the relationship between extractable Zn and pH was negative, as was the relationship between extractable Cd and pH (Table 2.3.7). This reflects the lower affinity that

Zn and Cd have for organic matter compared to Cu (McBride 1981), because higher dissolved organic matter levels associated with increased pH did not raise Cd and Zn extract solution concentrations as they did for Cu (believed to be through solubilisation of Cu-organic complexes, as discussed above). Rather, the concentrations of Zn and Cd in extract solutions were lower at higher pH levels, due to greater sorption of inorganic Zn^{2+} and Cd^{2+} ions onto solid surfaces.

Although pH, total concentrations, and C contents explain some of the variation observed in extractable element concentrations, large proportions of the variances remain unexplained. This suggests there are other unidentified factors influencing the solubility of biosolid metals, which will likely affect bioavailability.

2.3.3 Isotopically Exchangeable Copper (CuE)

Isotopically exchangeable Cu (CuE) ranged from 28 mg/kg in Wacol biosolids to over 400 mg/kg in those from Heathfield (Table 2.3.8). In terms of the percentage of total Cu that was isotopically exchangeable (%CuE), the spread was from 7% in Wacol biosolids to 38% for Port Kembla (Table 2.3.8). This range of values extends higher than the range observed by McLaren and Crawford (1974) in 24 British soils. They found the %CuE ranged from 2 to 21% in the soils they examined, and that the majority of the isotopically exchangeable Cu was associated with the organic fraction. The authors inferred from their results that the greater the amount of Cu in the organic fraction relative to the total amount of organic matter, the greater the amount of CuE (McLaren and Crawford 1974). This inference followed the concept of increased strength of binding at low adsorption site coverage (McLaren and Crawford 1973b).

Table 2.3.8: Isotopically Exchangeable Cu (CuE, mean (n=3) ± s.e.) and $CaCl_2$ Extractable Cu as % CuE

Biosolid	CuE (mg/kg)	±	CuE (%)*	±	$CaCl_2$ Ext. Cu as % CuE
Bolivar 95	222	4.6	32	0.8	25.3
Bolivar 97	190	2.5	20	0.3	22.6
Chelsea 77	65	6.9	25	2.7	1.8
Chelsea 87	51	0.7	18	0.2	2.0
Chelsea 96	64	1.2	32	0.6	1.8
Chelsea 98	126	3.9	43	1.3	1.0
Cronulla	65	0.7	8	0.1	10.7
Fairfield	76	7.2	19	1.8	33.5
Gumeracha	385	18.2	21	0.8	1.3
Hahndorf	279	8.5	15	0.4	1.8
Heathfield	416	13.1	19	0.5	4.6
Luggage Pt	233	4.2	28	0.9	14.4
Luggage Pt 95	109	0.5	22	0.1	1.8
Myponga	337	8.1	18	0.4	1.5
Oxley	83	7.6	12	1.1	31.5
Port Adelaide	92	3.2	28	1.0	3.9
Port Kembla	378	11.2	38	2.0	2.1
Sandgate	39	9.5	8	1.8	14.4
St Marys	176	22.3	29	3.7	2.6
Victor Harbor	317	3.8	15	0.2	2.0
Wacol	28	2.5	7	0.6	133.8
Werribee 83	281	4.4	35	0.6	1.8
Werribee 97	266	0.5	33	0.1	1.2
West Hornsby	149	15.7	18	1.9	6.1

* CuE as percentage (%) of total biosolid Cu.

If this hypothesis is correct, and if the organic fraction is similarly important for CuE in biosolids as it is in soils, then a strong positive relationship should be observed when the ratio of total Cu: OC is plotted against CuE, which is indeed the case ($R^2 = 0.68$, Figure 2.3.2a). This suggests that OC may play a role in determining CuE in biosolids. However, in terms of predicting CuE, the ratio of total Cu: OC is no more useful as a single parameter than total Cu alone (Figure 2.3.2b, $R^2 = 0.70$) for these biosolids. If the amount of organically bound Cu were determined (*i.e.* excluding that occluded in Fe or Al oxides, etc), then the ratio of this Cu: OC may have a stronger relationship with CuE (in which case the ratio may prove to be a better predictive parameter than total Cu alone). However, this hypothesis could not be tested here because in

order to do so a fractionation procedure would have had to be performed on multiple replicates of all 24 biosolids, which would have been too labour and time intensive considering the possibly limited returns given the inherent flaws of sequential extraction schemes (*i.e.* low selectivity, etc., see Chapter 1 - general introduction). Therefore, this hypothesis was explored with a smaller set of biosolids in Chapter 4 (Section 4.3.7).

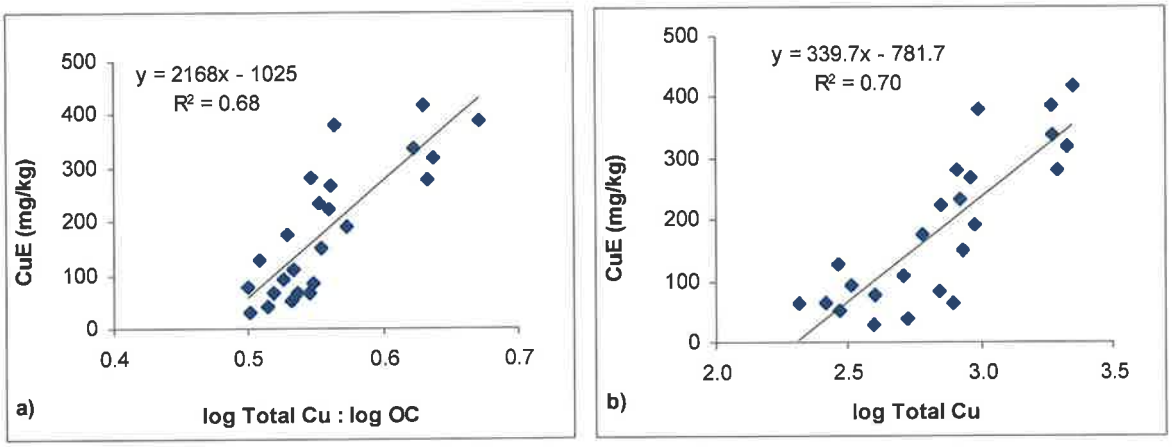


Figure 2.3.2: Relationship between isotopically exchangeable Cu (CuE, in mg/kg) and; a) the ratio of $\log_{10}$ total Cu (mg/kg): $\log_{10}$ Organic Carbon (OC, in mg/kg), and b) $\log_{10}$ total Cu (mg/kg).

Regression analysis of the data (stepwise backwards multiple linear regression) determined total Cu and pCu^{2+} to be variables significantly influencing isotopically exchangeable Cu (CuE), with total Cu having the greatest influence. Together, these variables accounted for 80.6% of the variation in CuE (Eq. 2.3.3). Simple correlation analysis also indicated that Cu^{2+} activity (pCu^{2+}) was related to CuE (Table 2.3.9). None of the other parameters tested, including pH, OC and $CaCl_2$-extractable Cu, were identified as significant ($p \leq 0.05$). Regression of CuE predicted by the model against observed values returned an R^2 of 0.82 (Figure 2.3.3), indicating a strong predictive ability of the model.

$$\text{CuE (mg/kg)} = 281.5\ (40.4)\ \text{Log Total Cu} - 14.9\ (3.96)\ pCu^{2+} - 459\ (137) \qquad (R^2 = 0.81) \qquad \textbf{Eq 2.3.3}$$

where 'logTotal Cu' is $\log_{10}$ total biosolid Cu concentration (mg/kg). Figures in parentheses are standard errors.

ANOVA	*d.f.*	*s.s.*	*m.s.*	*v.r.*	*F pr.*	R^2
Regression	2	286535	143268	48.65	<0.001	0.806
Residual	21	61837	2945			
Total	23	348372	15147			

ANOVA Accumulated	*d.f.*	*s.s.*	*m.s.*	*v.r.*	*F pr.*	*Cumulative* R^2
Log Tot Cu	1	244768	244768	83.12	<0.001	0.689
pCu2+	1	41767	41767	14.18	0.001	0.806
Residual	21	61837	2945			
Total	23	348372	15147			

Table 2.3.9: Correlations for Isotopically Exchangeable Cu (CuE) and other parameters

Parameter	Correlation coefficient (r)
log Total Cu (mg/kg)	0.852*
Log $CaCl_2$ ext. Cu (mg/kg)	-0.019
Log OC (mg/kg)	0.388
pH	-0.383
pCu^{2+}	-0.642*

*significant at the 95% level, double sided T-test.

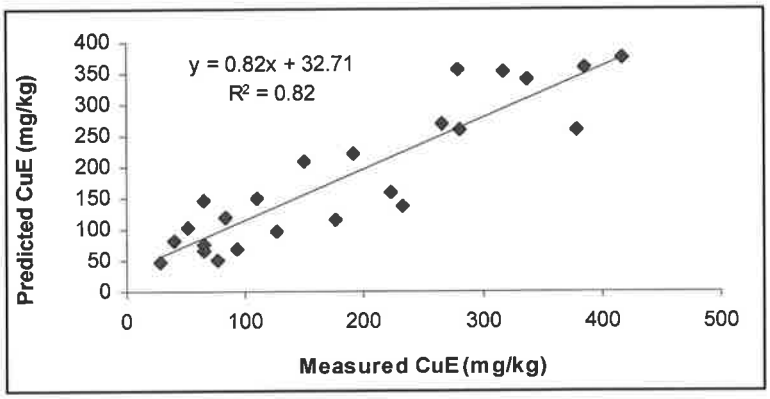


Figure 2.3.3: Predicted v measured CuE values (CuE = 281.5 Log Total Cu – 14.9 pCu^{2+} – 459).

$CaCl_2$ extraction did not extract a consistent percentage of the radio-labile Cu pool (CuE) (Table 2.3.8). Rather, it accessed varying amounts in each biosolid, most likely depending on the nature of surface Cu adsorption sites. $CaCl_2$ extraction cannot therefore be used as an approximation of the radio-labile pool. It may be argued that the two assessment techniques measure different levels of availability, *i.e.* readily available Cu (0.01M $CaCl_2$ extraction) and long-term potentially available Cu (CuE). There is also the possibility that the E-value measurement overestimates the potentially available Cu pool. This would be the case if organically complexed Cu in solution

was not fully exchangeable with the added radio-tracer (^{64}Cu) and surface sorbed Cu, as this would violate the assumptions of the CuE calculation. However, in a preliminary experiment conducted in our laboratories, a Donnan dialysis technique in combination with radioisotope equilibration was used to demonstrate that organically complexed Cu in solution is fully exchangeable (labile). This was found to be the case for Cu complexed with humic acid purchased from Sigma-Aldrich and natural humic acid, as well as for Cu complexes in contaminated soil and biosolid pore water extracts. The details are reported elsewhere (Nolan *et al.* 2003). One issue that still remains, however, is the question of exchange kinetics. It has been shown that in the case of some soils isotopic equilibrium is not completely achieved within 24 hours (Delas *et al.* 1960; McLaren and Crawford 1974), so that measured CuE values may increase if equilibration time is extended (Figure 2.3.4). McLaren and Crawford (1974) concluded that while isotopic equilibrium can be considered virtually complete within 24 hours, there is a slow, secondary equilibration that may continue for longer in some soils. To explain this phenomenon, Delas *et al.* (1960) hypothesized that there is a rapid exchange between colloid surface Cu and solution Cu establishing an apparent equilibrium, but a much slower exchange may continue between solution Cu and Cu adsorbed on internal surfaces and in fine pores. The short half-life of the ^{64}Cu isotope (approximately 12.8 hours) precludes any extension of the equilibration time used in the method to allow for this potential secondary equilibration process. Therefore, it remains a source of potential error. Nevertheless, the procedure utilising the 24-hour equilibration provides a useful measure of the biosolid Cu in rapid equilibration with Cu in the biosolid pore water.

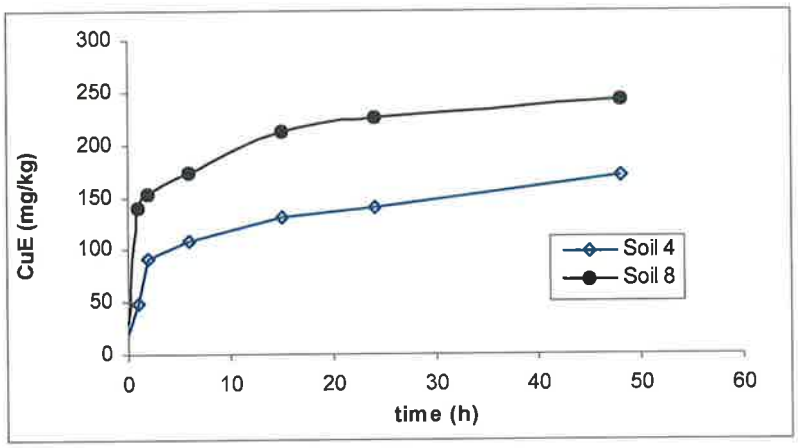
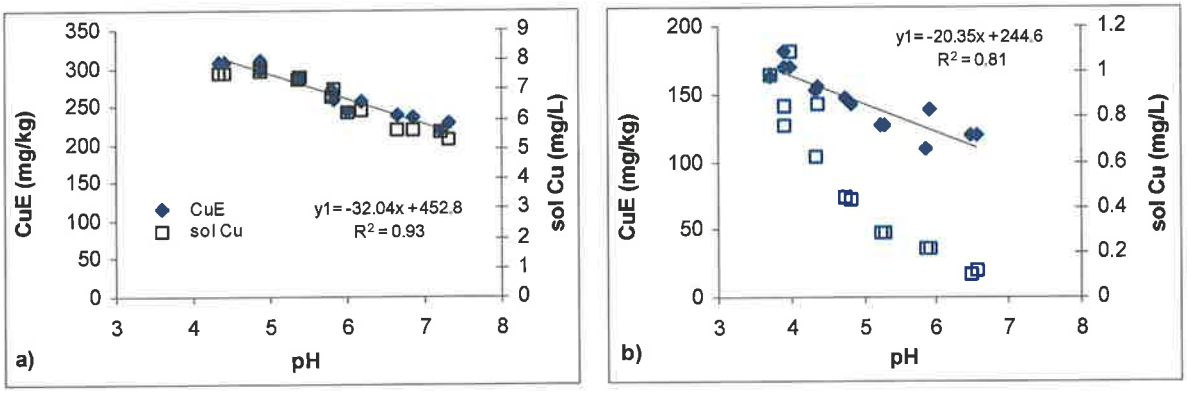


Figure 2.3.4: Isotopically exchangeable Cu as a function of equilibration time for two soils where complete isotopic equilibrium was not achieved within 24 hours (Delas *et al.* 1960).

2.3.4 Effect of pH adjustment on Isotopically Exchangeable Cu

Results in the previous section indicated that, when considered across all biosolids, the E values (CuE) were not significantly related to pH. However, examination of individual biosolids that had been subjected to pH adjustment revealed a different outcome. Strong linear relationships between CuE and pH were identified for all three biosolids tested, with R^2 values of 0.93, 0.81 and 0.78 for Bolivar 95, Chelsea 98 and Port Kembla, respectively (Figure 2.3.5).



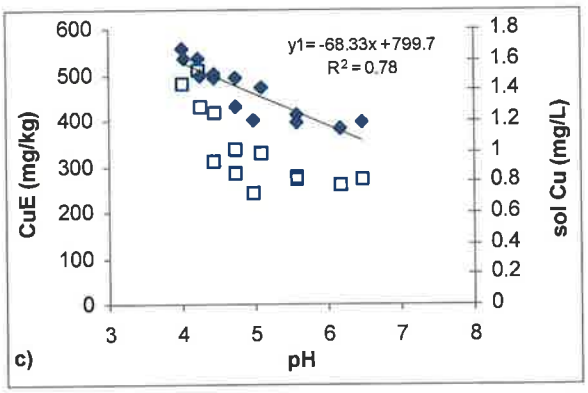
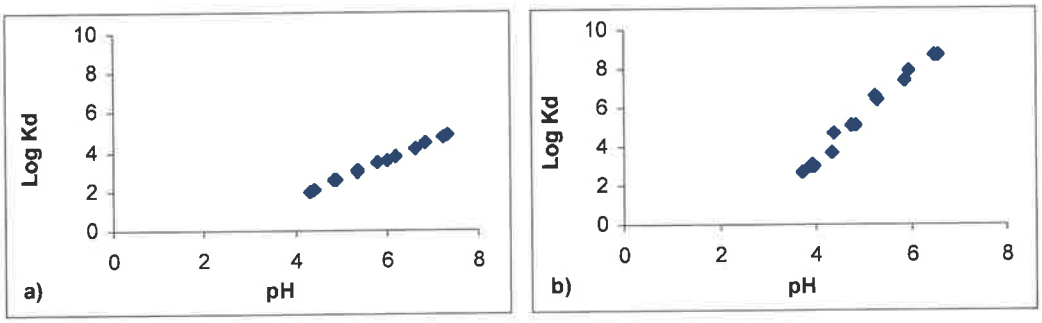


Figure 2.3.5: Relationship between isotopically exchangeable Cu (CuE) and pH, and between extract solution Cu and pH for; a) Bolivar 95, b) Chelsea 98, and c) Port Kembla. The regression equations refer to CuE against pH.

Concentrations of CuE were higher at lower pH, such that between pH 7 and 4 (pH 7.2 for Bolivar 95), CuE values increased 1.4 fold for Bolivar 95, 1.7 fold for Chelsea 98, and 1.5 fold for Port Kembla. The solution Cu concentrations also increased as pH decreased (Figure 2.3.5), which potentially supports the tenet that a decrease in the pH of an individual biosolid increases the amount of exchangeable Cu. The linear increase in Kd with rising pH also supports this (Figure 2.3.6) The fact that CuE is not constant with pH indicates that biosolid Cu is not just held exchangeably on surfaces of minerals or organic matter. The implications of these findings are that reductions in soil pH, such as that potentially brought about by nitrification processes, may lead to increased availability of biosolid Cu in amended soils. Therefore, frequent monitoring and regulation of pH in amended soils may be a necessary addition to sludge use regulations.



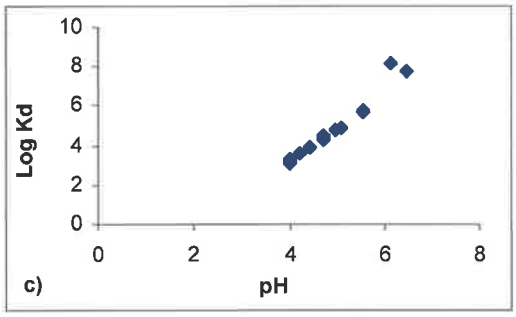


Figure 2.3.6: Log_{10} partition coefficient (Kd = $CuE_{(solid)}$ / Cu^{2+} solution activity), as a function of pH in three pH adjusted biosolids; a) Bolivar 95, b) Chelsea 98, and c) Port Kembla.

In an attempt to identify the likely minerals controlling Cu solubility as pH declined, the log activities of solution Cu^{2+} for the three biosolids were plotted on a solubility product diagram (Figure 2.3.7). Values for Port Kembla exactly matched values for the experimentally determined "soil-Cu" defined by Lindsay (1979), which he speculated was dominated by the mineral cupric ferrite ($CuFe_2O_4$). Bolivar 95 and Chelsea 98 values did not correspond to any of the minerals (Figure 2.3.7), suggesting the release of Cu into solution was controlled by multiple or undefined minerals and/or organic complexes. Examination of the extract solutions (ICP-AES) revealed increases in Fe, Mn, P, Ca, and Zn concentrations with decreasing pH, thus dissolution of minerals, coprecipitates or complexes containing Cu and some of these elements may be the mechanism responsible for the increased CuE observed at lower pH.

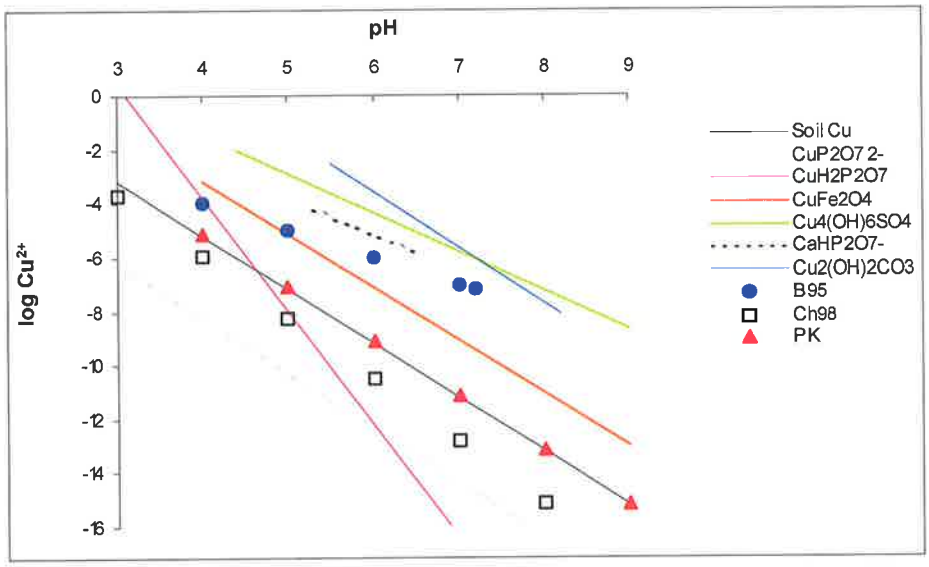


Figure 2.3.7: Log molar Cu^{2+} activities in extract solutions from three pH-adjusted biosolids plotted on a Cu mineral solubility diagram (mineral solubility data from Lindsay 1979).

The effect of pH on Cu availability identified here raises the possibility of setting biosolid re-use regulations based on worst-case scenarios. In keeping with the desire to move away from the use of total metal contents in biosolid regulations towards more relevant regulations based on available concentrations, limits for re-use could be based on CuE values. To account for potential increases in availability with decreasing pH, a pH protection factor could be introduced whereby the CuE is recalculated based on a pH worst-case scenario *(i.e.* pH 4). In order to be protective the steepest slope determined for CuE v pH could be applied to all biosolids (in this case the slope of -68 for Port Kembla, Figure 2.3.5), to produce a predicted CuE value at pH 4. Land application restrictions for each individual biosolid could then be decided according to this value. Subjecting the 24 biosolids examined in this study to this type of manipulation produces pH 4 CuE values ranging from 144 mg/kg to 518 mg/kg (Table 2.3.10).

Table 2.3.10: Biosolid CuE Values Re-calculated at pH 4

Biosolid	pH	Total Cu (mg/kg)	CuE (mg/kg)	CuE as % Total Cu	CuE pH4* (mg/kg)	CuE pH 4 as % Total Cu
Bolivar 95	7.23	701	222	32	442	63
Bolivar 97	6.46	942	190	20	357	38
Chelsea 77	6.21	258	65	25	215	83
Chelsea 87	5.73	291	51	18	169	58
Chelsea 96	5.17	203	64	32	144	71
Chelsea 98	6.19	291	126	43	275	95
Cronulla	6.15	775	65	8	211	27
Fairfield	6.55	398	76	19	250	63
Gumeracha	5.21	1818	385	21	468	26
Hahndorf	5.62	1916	279	15	389	20
Heathfield	5.50	2190	416	19	518	24
Luggage Point	6.67	828	233	28	415	50
Luggage Point 95	4.96	506	109	22	174	34
Myponga	5.62	1852	337	18	447	24
Oxley	6.47	696	83	12	251	36
Port Adelaide	6.80	325	92	28	283	87
Port Kembla	5.63	964	378	39	489	51
Sandgate	6.27	522	39	8	193	37
St Marys	5.76	597	176	29	296	50
Victor Harbor	5.93	2095	317	15	448	21
Wacol	6.41	394	28	7	192	49
Werribee 83	4.11	800	281	35	289	36
Werribee 97	5.06	909	266	29	338	37
West Hornsby	6.71	851	149	18	333	39

* CuE at pH 4 calculated by applying slope of steepest CuE v pH curve (-68, Port Kembla, Figure 2.3.5) to CuE values obtained for each biosolid at natural pH.

2.3.5 Copper Ion Activity (pCu^{2+})

The Cu^{2+} activity observed for the biosolids ranged from pCu^{2+} 6.54 to > 15 (Table 2.3.11). The lowest activity observed during the electrode calibration process was $pCu^{2+} = 15$ (Figure 2.2.2), therefore, although it would be possible to extrapolate lower activities from mV data, it was decided to set $pCu^{2+} = 15$ as the detection limit. Table 2.3.11 indicates that Cu^{2+} activity in some of the extract solutions exceeded levels that have been associated with toxicity symptoms in maize ($pCu^{2+} < 8$) (McBride 2001). Therefore Gumeracha, Hahndorf, Heathfield, Myponga, Victor Harbor, Werribee 83 and Werribee 97 biosolids may all be expected to produce similar symptoms of Cu toxicity should maize be grown with them as the medium. It is interesting to

note that the five biosolids with the greatest total Cu (Gumeracha, Hahndorf, Heathfield, Myponga and Victor Harbor) all have cupric ion activities above this threshold, yet the biosolid with the least total Cu (Chelsea 96, which also has the least organic carbon) also has a value just outside this range. This suggests that the ratio of total Cu: number of potential binding sites may influence pCu^{2+} in solution. Statistically significant ($p \leq 0.05$) relationships were found between pCu^{2+} and biosolid total Cu, pH, and total carbon content (considering all biosolids) (Figure 2.3.8).

Table 2.3.11: Cupric Ion Activity in Biosolid $CaCl_2$ Extracts (± s.e., n=2)

Biosolid	pCu^{2+}	±
Bolivar 95	12.19	0.072
Bolivar 97	10.45	0.203
Chelsea 77	9.76	0.208
Chelsea 87	8.91	0.072
Chelsea 96	8.32	0.029
Chelsea 98	9.22	0.009
Cronulla	13.82	0.423
Fairfield	>15*	-
Gumeracha	6.56	0.171
Hahndorf	7.31	0.080
Heathfield	7.06	0.054
Luggage Pt	>15	-
Luggage Pt 95	10.24	0.062
Myponga	7.98	0.114
Oxley	>15	-
Port Adelaide	12.05	0.026
Port Kembla	8.23	0.074
Sandgate	>15	-
St Marys	13.82	0.402
Victor Harbor	8.22	0.106
Wacol	>15	-
Werribee 83	6.54	0.029
Werribee 97	7.00	0.007
West Hornsby	10.58	0.116

* Values below detection limit (>15 pCu^{2+}).

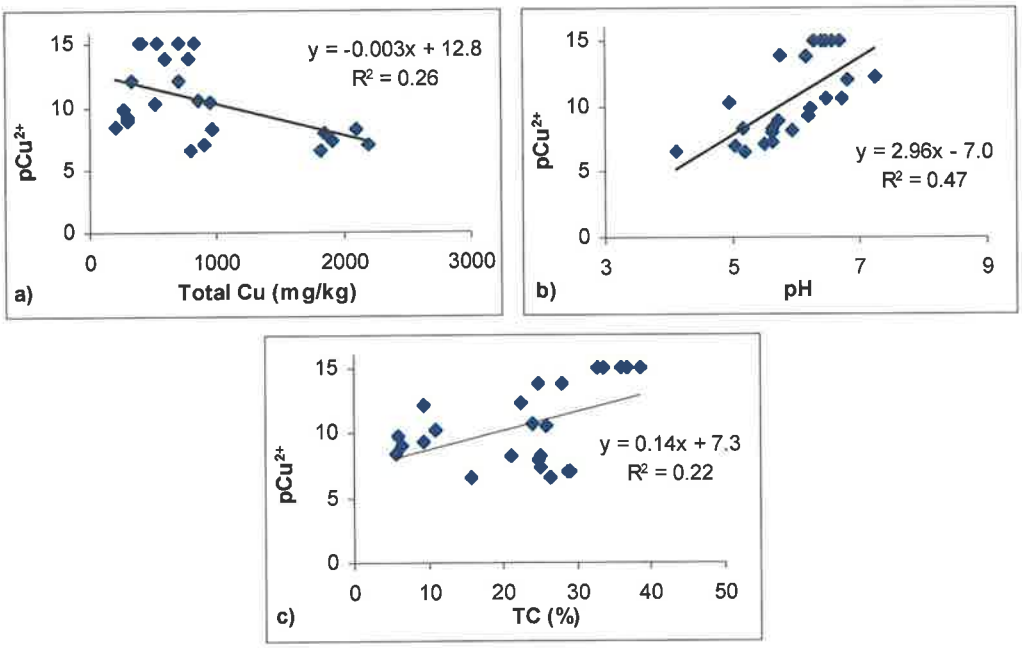


Figure 2.3.8: pCu^{2+} as a function of a) biosolid total Cu, b) pH and c) Total carbon content.

Multiple linear regression analysis confirmed pH, total Cu and total carbon percentage as factors controlling pCu^{2+} (Eq. 2.3.4):

$pCu^{2+} = 1.95\ (0.46)\ pH - 0.003\ (0.01)\ Total\ Cu + 0.15\ (0.03)\ \%TC - 2.27\ (2.76)$ $(R^2 = 0.78)$ **Eq. 2.3.4**

where: Total Cu = total biosolid Cu concentration (mg/kg), and %TC = percent total carbon. Figures in parentheses are standard errors of parameters.

ANOVA						
	d.f.	*s.s.*	*m.s.*	*v.r.*	*F pr.*	R^2
Regression	3	178	59.36	28.2	<0.001	0.78
Residual	20	42	2.11			
Total	23	220	9.57			
ANOVA Accumulated						
	d.f.	*s.s.*	*m.s.*	*v.r.*	*F pr.*	*Cumulative* R^2
PH	1	102	102	48.7	<0.001	0.44
Tot Cu	1	26	27	12.7	0.002	0.55
%TC	1	48	49	23.2	<0.001	0.78
Residual	20	42	2.1			
Total	23	220	9.6			

When plotted against measured pCu^{2+}, the values predicted using Eq. 2.3.4 returned an R^2 of 0.81 (Figure 2.3.9). The model derived here differs to that reported by Sauve *et al.* (1997) for pCu^{2+} in contaminated soils. Their model included only total Cu and pH (Eq. 2.3.5):

$$pCu^{2+} = -1.70\ (0.12)\ \log\text{Total Cu} + 1.4\ (0.08)\ pH + 3.42\ (0.50) \qquad \textbf{Eq. 2.3.5}$$

However, the soils included in their study could mostly be regarded as mineral soils low in organic matter, so it is not surprising that soil C concentration was found to be non-significant. Therefore, the inclusion of soil C as a parameter may become increasingly important when soils of higher organic matter contents are examined. This was recognised in 1999 when, in his PhD dissertation, Sauve reported a model for predicting pCu^{2+} that included a C term (Sauve 1999) (Eq. 2.3.6):

$$pCu2+ = -1.98\ (0.10)\ \log\text{Total Cu} + 1.5\ (0.06)\ pH + 1.08\ (0.31)\ \log OC + 2.81\ (0.44) \qquad \textbf{Eq. 2.3.6}$$

When this model was fitted to data from the current study a regression of predicted and observed values returned an R^2 of 0.64, indicating that OC is influential in determining the pCu^{2+} in solution extracts of soils or biosolids with considerable organic matter contents.

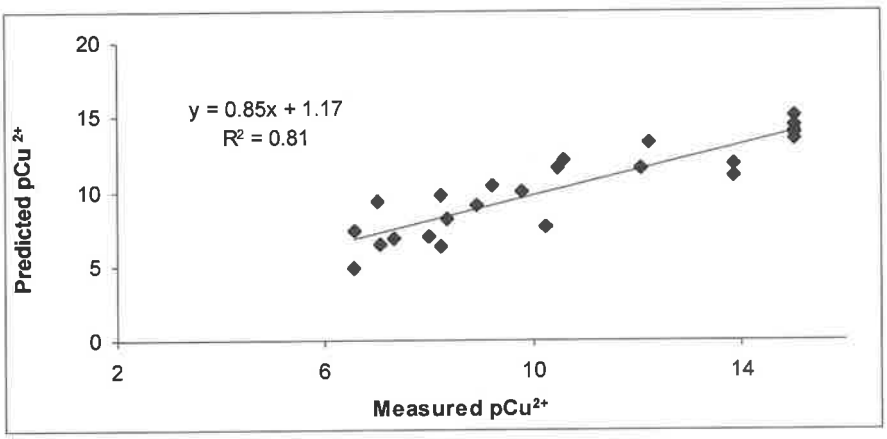


Figure 2.3.9: Predicted v measured pCu^{2+} values ($pCu^{2+} = 1.95pH - 0.003\text{Total Cu} + 0.15C\% - 2.27$).

Equation 2.3.4 provides a means by which pCu^{2+} can be predicted from the parameters pH, total Cu and total C%, while equation 2.3.3 allows prediction of CuE using total Cu and pCu^{2+}. Therefore, by combining these equations, it should be possible to generate a model that will predict CuE from pH, total Cu and total C % alone (Eq. 2.3.7). For 13 out of the 24 biosolids examined, CuE values predicted using Eq. 2.3.7 were within ± 20% of measured CuE values (Table 2.3.12). This suggests that Eq. 2.3.7 may be useful as a guide for estimating CuE from easily measured parameters, but that the model involving direct measurement of pCu^{2+} (Eq. 2.3.4) will be more accurate. The limited accuracy of some of the values predicted using Eq 2.3.7 is most likely due to the compounding of parameter error estimates when models are combined.

CuE (mg/kg) = 281.5Log Total Cu − 14.9[1.95pH - 0.003Total Cu + 0.15TC% - 2.27] − 459 **Eq. 2.3.7**

where; 'logTotal Cu' is $\log_{10}$ total biosolid Cu concentration (mg/kg), Total Cu is in mg/kg, and TC% is total C percentage (parameter error estimates are omitted here for clarity, see equations 2.3.3 and 2.3.4 for values).

Table 2.3.12: Measured CuE (mg/kg, ± s.e.) and Values Predicted from pH, C% and Total Cu (Eq. 2.3.7)

Biosolid	CuE	±	Predicted CuE
Bolivar 95	222	4.6	147
Bolivar 97	190	2.5	209
Chelsea 77	65	6.9	72
Chelsea 87	51	0.7	100
Chelsea 96	64	1.2	71
Chelsea 98	126	3.9	81
Cronulla	65	0.7	182
Fairfield	76	7.2	61
Gumeracha	385	18.2	387
Hahndorf	279	8.5	365
Heathfield	416	13.1	388
Luggage Point	233	4.2	159
Luggage Point 95	109	0.5	190
Myponga	337	8.1	359
Oxley	83	7.6	143
Port Adelaide	92	3.2	78
Port Kembla	378	11.2	238
Sandgate	39	9.5	99
St Marys	176	22.3	160
Victor Harbor	317	3.8	384
Wacol	28	2.5	51
Werribee 83	281	4.4	250
Werribee 97	266	0.5	237
West Hornsby	149	15.7	189

2.4 Conclusions

Australian biosolids have been found to vary widely in terms of total element concentrations, carbon contents, pH, EC, and other chemical and physical properties. Wide variation has also been observed in regard to Cu availability. The analyses revealed that the easily extractable Cu content, measured by neutral salt extraction, was most influenced by pH and total carbon percentage. The isotopically exchangeable Cu (CuE or E value) was predicted by total Cu concentration and Cu^{2+} activity in solution, where a regression of measured v predicted values returned an R^2 of 0.82. Therefore the model produced here provides a potential means to predict isotopic exchangeability of Cu without use of the expensive and difficult to manage ^{64}Cu isotope, though the model will require further testing on larger datasets before this can be confirmed. While found to be not significant when all biosolids were considered as a single dataset, on an individual biosolid basis pH was shown to have a strong influence on CuE. The increase in available Cu with decreasing pH suggests that regulations for the use of biosolids on agricultural land may need to be expanded to include monitoring and maintenance of soil pH on a more frequent basis (currently pH measurements are only required after 10 years of application, according to South Australian biosolids regulations). The CuE values, adjusted to a conservative pH value (*i.e.* pH 4), could be used to represent the potentially available Cu and thus be used in setting biosolid re-use regulations.

The factors found to be most influential for cupric ion activity (pCu^{2+}) were pH, total Cu concentration, and total carbon content. The pCu^{2+} values observed for some of the biosolids examined were in the potentially toxic range for some plants, therefore Cu toxicity symptoms may be expected if plants are grown with these sludges as the growth medium.

3. Temporal Trends of Total and Potentially Available Element Concentrations in Sewage Biosolids: A Comparison of Biosolid Surveys Conducted 18 Years Apart

3.1 Introduction

In 1983 deVries (1983b) conducted a survey to determine the chemical characteristics of biosolids from various locations around Australia. He measured total element concentrations and determined element availability using DTPA and $CaCl_2$ extractions. Since that time numerous factors potentially affecting biosolid properties and characteristics, and their re-use applications, have changed. These include new or improved sewage treatment technologies, establishment of new and decommissioning of old treatment plants, changes to industrial processes and industrial waste disposal regulations, and the enactment of stricter legislation protecting environmental quality. A survey was therefore conducted to evaluate similarities and differences between biosolids produced and analysed in the early 1980's and those examined today, with particular emphasis on total element concentrations and the availability of nutrients and potentially toxic elements. This work therefore compares the results of deVries (1983b), together with those in his unpublished notes, with results obtained in our laboratory from experiments on more recently produced biosolids. A number of the treatment plants were sampled in both surveys, thus some direct comparisons for individual plants were possible.

3.2 Methods and Materials

Details and general information on the 20 biosolids investigated in the original survey were outlined by deVries (1983b). Table 3.2.1 provides a summary. Table 2.2.1 (Chapter 2) gave similar details for biosolids in the current collection (*i.e.* the 2001 survey). Air dry samples of the biosolids surveyed in 2001 were ground to <2mm. Total element concentrations were determined by *aqua regia* (1:3 HNO_3:HCl) digestion. Calcium chloride ($CaCl_2$) extractable

element concentrations were determined following the method used in the 1983 survey (deVries 1983b), whereby duplicate 1.0g biosolid samples were equilibrated with 25 mL 0.01M $CaCl_2$ for three days on an end-over-end shaker. Samples were then centrifuged at 1200*g* for 15 minutes, the supernatants filtered through 0.45 μm syringe filters, and the elemental contents determined by Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES). Results of total and extractable element determinations were compared with the 1983 values to identify any general trends across all biosolids. In addition, because biosolids from several treatment plants were sampled in both surveys, and/or multiple samples of different ages were collected from stockpiled collections, it was possible to examine temporal trends in biosolids from individual plants, namely Werribee, Chelsea, Luggage Point and Bolivar. Therefore, although biosolids are variable by nature, even those from within a single treatment plant (Levi-Minzi *et al.* 1981), some long-term trends were examinable.

Table 3.2.1: Biosolids in the 1983 Survey (deVries 1983)

Biosolid	Origin[#]	Age (at testing)	Major waste input	Treatment or digestion type	Drying system
Bolivar	SA	*	Industrial + domestic	Anaerobic	Evaporation
Angle Park I – bare site	SA	*	Industrial + domestic	Land Filtration	Evaporation
Angle Park II – lush veg	SA	*	Industrial + domestic	Land Filtration	Evaporation
Werribee I	Vic	<2 years	Industrial + domestic	Land Filtration	Evaporation
Werribee II	Vic	15 years	Industrial + domestic	Land Filtration	Evaporation
Werribee III	Vic	>20 years	Industrial + domestic	Land Filtration	Evaporation
Ballarat South I	Vic	2 weeks	Industrial + domestic	Aerobic	Evaporation
Ballarat South II	Vic	1 year	Industrial + domestic	Aerobic	Evaporation
Ballarat North I	Vic	2 weeks	Domestic	Anaerobic	Centrifuge
Ballarat North II	Vic	1 year	Domestic	Anaerobic	Centrifuge
Waragamba	NSW	Unknown	Domestic	*	*
Malabar	NSW	Unknown	Industrial + domestic	*	*
Port Kembla	NSW	Unknown	Heavy industry + domestic	*	*
Griffith	NSW	10 days	Light industry + domestic	*	*
Luggage Point I	Qld	<4 months	Industrial + domestic	Anaerobic	Evaporation
Luggage Point II	Qld	1 year	Industrial + domestic	Anaerobic	Evaporation
Mt. Isa I	Qld	2 months	Heavy industry + domestic	Anaerobic	*
Mt. Isa II	Qld	2 years	Heavy industry + domestic	Anaerobic	*
Wembley	WA	< 2months	Domestic	*	*
Canberra	ACT	*	Domestic	Lime Stabilised	*

[#] origin refers to Australian state: SA = South Australia, Vic = Victoria, NSW = New South Wales, Qld = Queensland, WA = Western Australia, ACT = Australian Capital Territory. * signifies information not given in original text.

3.3 Results and Discussion

3.3.1 All Biosolids – Total Element Concentrations

Figure 3.3.1 shows the maximum, median, minimum and quartile range of biosolid total element concentrations identified in the two surveys. The figure suggests that total concentrations of Cu, Mn, Ni, Na, and Ca in Australian biosolids have changed little over the period examined. Median values of Cd, Mg, Pb, and Zn determined in 2001 were all greatly reduced compared to those of the previous survey (87%, 86%, 77% and 58% reductions respectively). The range of values observed for these elements had also considerably contracted since 1983. The likely cause of the decreased Pb concentrations in modern biosolids is the change to lead-free petrol, which has been shown to decrease atmospheric Pb levels around cities and industrialised areas (Bravo and Torres 2000). Reasons for decreases in other elements are less clear, but may be related to improved industrial processes and the increasingly stringent regulations governing contents of industrial wastes entering sewers (e.g. ARMCANZ 1994). However, it should be noted that the 1983 values for Mg (median approximately 3% by mass, maximum approximately 6%) appear suspiciously high, particularly when compared to other contemporary published works such as that by Sommers *et al.* (1976), where the maximum observed concentration was less than 2%. Median total concentrations of K and P were higher in the 2001 survey than in 1983 (58% and 120% higher, respectively). The higher P levels may possibly reflect an increase in the use of P based detergents since the early 1980's (WRc 2002) and the better recovery of P in treatment processes necessitated by more stringent standards for sewage effluents.

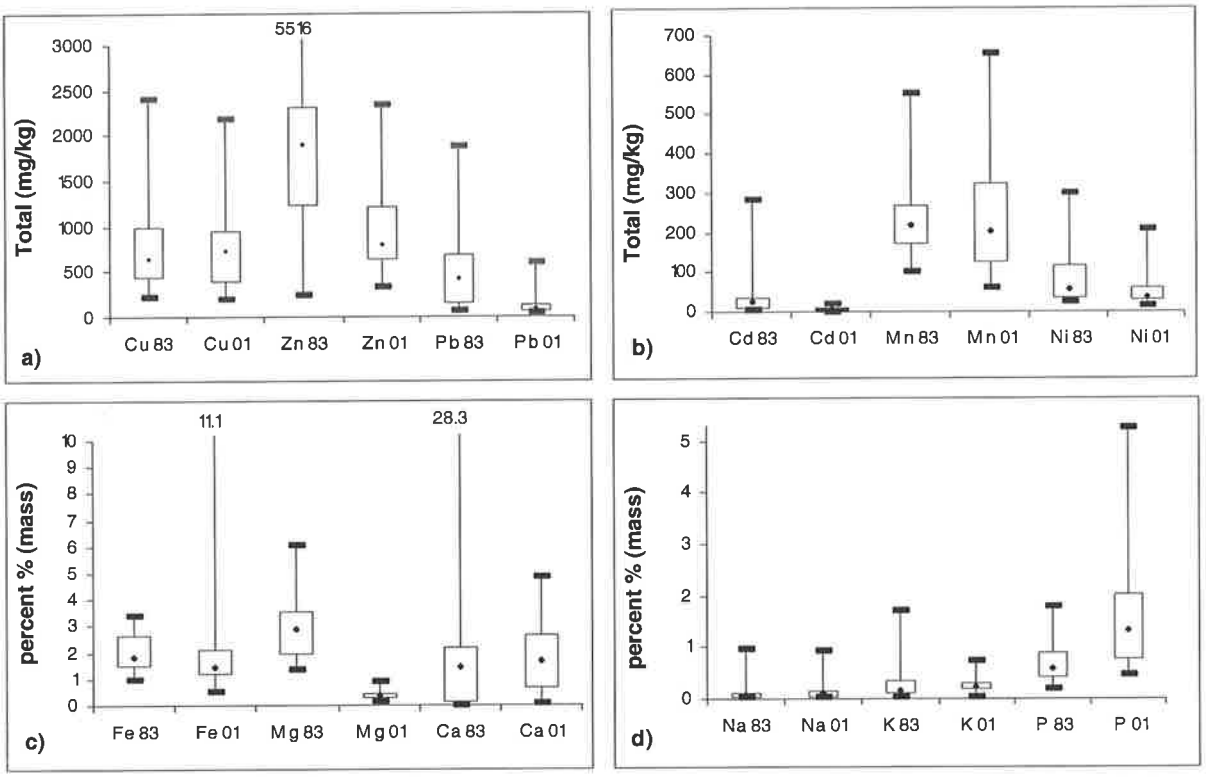


Figure 3.3.1: Box-whisker diagrams showing maximum, median, minimum and quartile ranges for total element concentrations in biosolids surveyed in 1983 and 2001. Maximum values for Zn (a), Ca (c), and Fe (c) exceed the respective y-axes, data labels indicate their values.

The reductions in total metal concentrations identified here (particularly Pb, Cd and Zn) compare to similar reductions observed in biosolids produced in the UK over the last 30 years (Figure 3.3.2) (Environment Agency 1999). In discussion of such concentration decreases in the UK Smith (1996) also cited changes to manufacturing processes and waste content controls as driving forces.

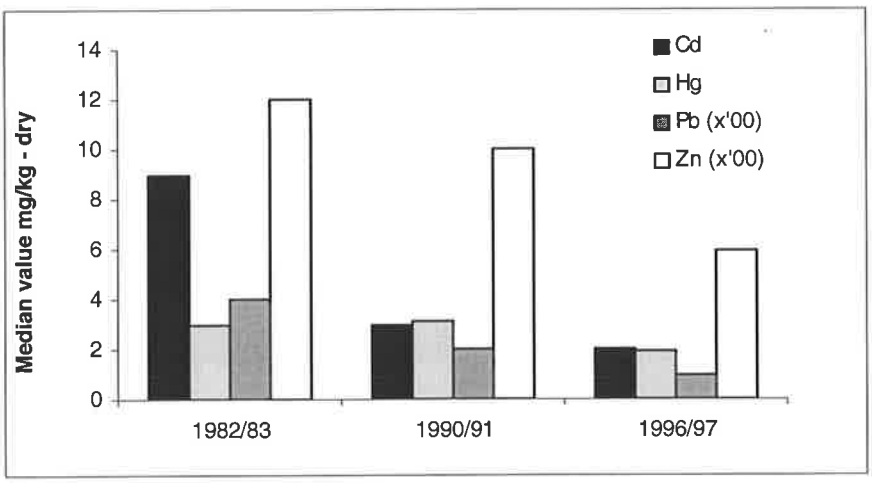


Figure 3.3.2: Total concentrations of selected metals in UK biosolids from 1982 –1997 (Environment Agency 1999).

3.3.2 All Biosolids – Calcium Chloride Extractable Element Concentrations

Extractable metal contents of biosolids determined by de Vries in 1983 (not previously published) are presented in Table 3.3.1, while Table 3.3.2 shows extractable element concentrations determined in the 2001 survey. Table 3.3.3 compares the two sets of results by displaying the median, maximum, minimum and quartile range values for each element determined in the respective surveys. Upon examination of Table 3.3.3 it can be seen that, with respect to median values, extractable concentrations of Cu, Zn, Mn, Cd and Ni all fell by 50 – 95%, while extractable P increased by 65% and Na by 85%. Further investigations into how these elements are bound in sewage biosolids are needed in order to explain why such differences in extractability were observed.

Table 3.3.1: $CaCl_2$ Extractable elements (mg/kg) determined in the 1983 survey*

Biosolid	Cu	Zn	Mn	Cd	Ni	Na	Mg	K	P
Bolivar	37	31	45	1.4	27	7186	2491	7033	1133
Angle Park I	7.6	524	53	4.2	17	90	655	311	195
Angle Park II	3.3	183	29	1.6	7.7	147	900	639	167
Werribee I	2.1	51	52	1.1	8.4	473	980	249	5
Werribee II	1.8	29	26	0.9	4.7	556	2021	321	15
Werribee III	9.6	707	75	6.6	41	314	654	192	26
Ballarat South I	3.8	57	22	1.6	4.7	351	1111	340	46
Ballarat South II	5.9	182	42	8.1	5.1	205	1188	294	248
Ballarat North I	70	4.7	7.9	0.3	4.5	529	2065	534	441
Ballarat North II	8.5	3.8	17	0.4	3.4	586	1363	300	497
Warragamba	14	34	10	1.1	3.7	418	837	343	77
Malabar	45	35	6.8	0.6	87	621	803	217	8
Port Kembla	28	62	10	1	6.4	6028	2851	2235	107
Griffith	2.5	24	36	0.6	0.8	348	1279	373	40
Luggage Point I	35	8.9	8.8	0.6	11	1088	1126	483	81
Luggage Point II	38	15	10	0.6	13	435	1450	212	63
Mt Isa I	32	335	51	4.3	5	842	959	219	28
Mt Isa II	24	266	41	4.9	4.8	288	686	93	37
Wembly	17	27	9.7	0.5	5.9	393	1133	168	73
Canberra	31	0.4	1.6	0.6	3.1	180	2869	122	21

* The precision of replicates was not indicated in the original notes of deVries *et al*, (1983), hence standard errors not given.

Table 3.3.2: $CaCl_2$-Extractable Elements (mg/kg) ± standard errors Determined in the 2001 Survey

Biosolid	Cu	±	Zn	±	Cd	±	Mn	±	Ni	±	Na	±	K	±	P	±	Mg	±
Bolivar 95	39.0	2.24	5.2	3.29	0.06	0.06	3.4	0.30	9.1	0.25	4327	209.2	1137	35.7	71	0.56	1485	65.6
Bolivar 97	44.7	0.29	4.0	0.62	0.03	0.08	7.3	0.04	3.6	0.04	1298	14.5	706	1.5	101	0.98	1178	3.1
Chelsea 77	0.5	0.05	4.5	0.76	0.10	0.03	4.7	0.13	1.5	0.05	791	34.4	329	11.2	94	0.56	1196	38.4
Chelsea 87	0.3	0.07	5.8	0.33	0.19	0.05	8.1	0.25	1.9	0.04	790	32.8	308	10.2	101	0.08	1423	46.7
Chelsea 96	0.8	0.34	11.7	0.15	0.24	0.08	11.3	0.05	2.2	0.11	365	9.9	278	27.2	180	1.90	1116	33.6
Chelsea 98	1.7	0.03	3.3	0.03	0.01	0.01	6.2	0.00	1.9	0.04	1397	31.1	576	10.9	158	17.7	1605	48.6
Cronulla	1.2	0.14	2.2	0.11	b/d*	0.01	12.5	0.37	2.1	0.07	809	8.0	343	6.1	218	1.49	1940	12.8
Fairfield	3.5	0.72	4.4	1.38	b/d	0.04	12.5	0.25	2.5	0.03	1910	4.0	1019	16.5	2064	125.7	1626	89.4
Gumeracha	7.3	0.37	94.3	0.74	0.07	0.01	37.7	1.33	1.1	0.00	90	6.7	322	12.6	94	9.36	369	20.3
Hahndorf	5.8	0.09	98.0	1.05	0.05	0.03	10.6	0.31	0.9	0.04	93	4.5	185	10.0	57	0.78	232	10.4
Heathfield	5.6	0.06	144.7	1.82	0.12	0.07	30.6	0.21	2.6	0.07	217	2.1	311	0.9	100	1.03	925	3.4
Lug Point	4.2	0.59	5.4	0.01	b/d	0.04	10.2	2.30	4.3	0.21	2611	59.4	1510	29.7	3345	478.4	4151	604.7
Lug Point 95	2.6	0.15	59.6	1.31	0.96	0.03	175.8	1.07	6.1	0.13	931	37.6	249	10.1	77	13.09	1598	70.2
Myponga	4.0	0.12	216.4	6.51	0.17	0.07	17.2	0.67	1.2	0.05	233	0.4	163	0.5	22	1.06	390	2.2
Oxley	7.3	0.57	18.2	0.50	0.07	0.01	18.3	0.30	12.2	0.01	954	9.9	908	4.3	1681	77.87	2312	63.2
Port Adelaide	5.4	0.60	2.1	0.55	0.01	0.01	5.0	2.43	2.4	0.27	8095	465.7	976	53.7	137	20.38	2346	151.4
Port Kembla	4.5	0.59	42.1	0.32	0.40	0.03	80.0	0.53	3.6	0.09	441	11.0	71	4.3	76	2.25	2892	4.9
Sandgate	2.4	0.09	5.2	0.01	0.01	0.07	66.2	1.61	4.2	0.09	1398	1.7	699	1.8	639	29.14	1752	48.5
St Marys	1.9	0.09	3.9	0.52	b/d	0.19	12.9	0.71	0.7	0.04	585	7.3	441	10.1	77	3.42	1885	12.0
Victor Harbor	8.7	0.37	48.0	1.42	0.01	0.01	9.3	0.01	1.0	0.04	239	16.1	180	13.4	207	2.10	434	30.4
Wacol	7.9	1.25	4.2	0.73	b/d	0.04	7.1	0.10	0.7	0.03	1430	10.0	5757	14.0	8436	273.6	2925	209.0
Werribee83	9.1	0.13	230.8	0.50	2.90	0.06	13.3	1.76	9.8	0.00	132	1.6	739	14.5	256	7.83	182	5.2
Werribee97	4.7	0.16	116.4	0.97	0.68	0.03	24.7	0.18	7.5	0.04	507	0.6	451	15.9	124	0.00	411	1.7
West Hornsby	2.0	0.45	1.7	0.51	b/d	0.10	11.6	0.04	1.5	0.03	336	1.9	284	3.2	56	5.84	730	7.3

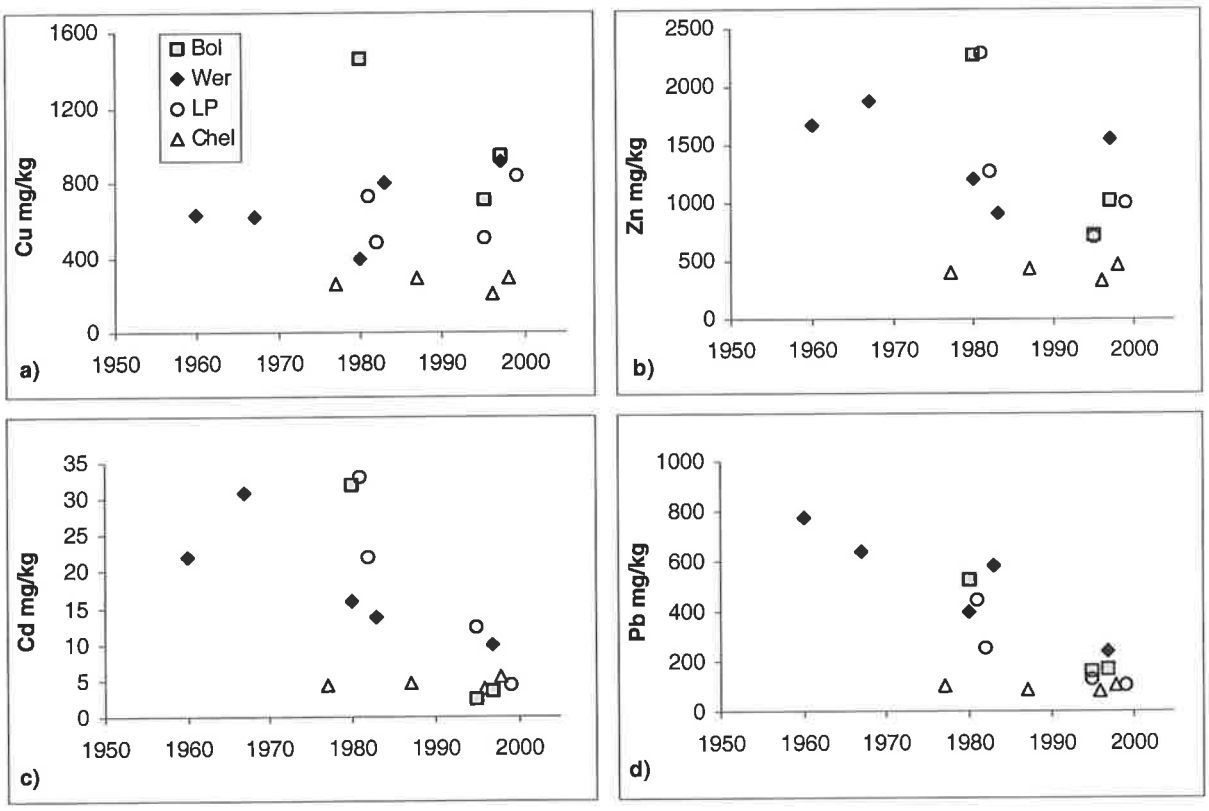
* b/d below detection.

Table 3.3.3: Maximum, Minimum, Median and Quartile Values of Extractable Elements in the Two Surveys

	Cu		Zn		Mn		Cd		Ni		Na		Mg		K		P	
	1983	2001	1983	2001	1983	2001	1983	2001	1983	2001	1983	2001	1983	2001	1983	2001	1983	2001
Min	2	0.3	0.4	2	2	3.4	0.3	b/d	0.8	0.7	90	90	654	182	93	71	5	22
Q1	5	2.0	21.75	4	10	7.9	0.6	0.006	4.7	1.4	308	312	884.3	656	215.8	283	28	77
Med	16	4.3	34.5	6	24	12.0	1.05	0.1	5.5	2.3	427	791	1130	1454	305.5	392	68	113
Q3	33	7.3	182.3	68	43	19.9	2.25	0.178	11.5	4.2	595	1397	1593	1899	400.5	781	174	228
Max	70	44.7	707	231	75	175.8	8.1	2.9	87.0	12.2	7186	8095	2869	4151	7033	5757	1133	8436

3.3.3 Individual Treatment Plants – Total Concentrations

When treatment plants are considered individually, it can be seen that total element concentrations in biosolids have fluctuated over time to varying extents (Figure 3.3.3). Metal concentrations (Cu, Zn, Mn, Fe, Cd, Ni and Pb) in Bolivar biosolids have decreased over time, while P concentrations have remained constant. In the case of Chelsea biosolids, the concentrations of all elements measured have remained stable over the period examined. Werribee and Luggage Point biosolids have shown significantly more variability. In terms of individual elements, Pb and Cd showed the strongest trends, with concentrations decreasing over time in biosolids from most of the treatment plants. This is not surprising considering the results discussed in the earlier section.



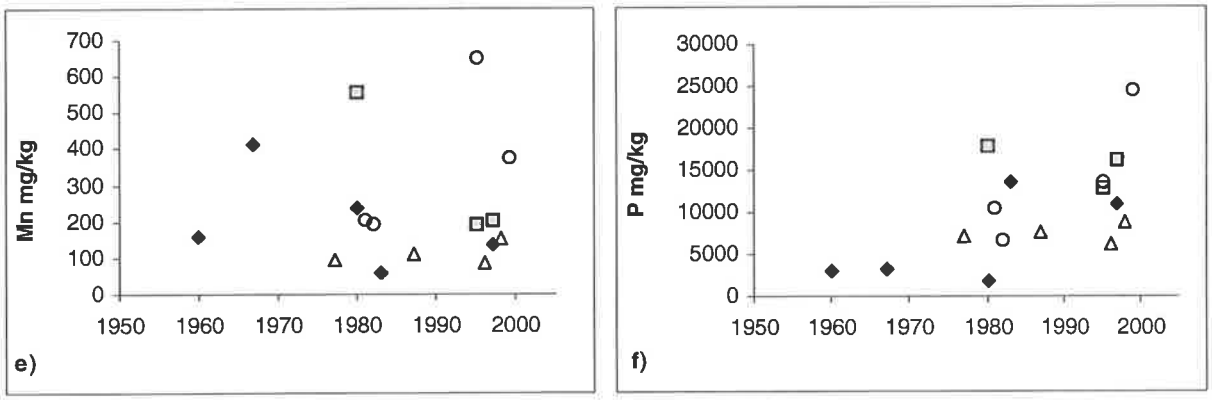


Figure 3.3.3: Total element concentrations in biosolids from individual treatment plants over time. Bol = Bolivar Treatment Plant, Wer = Werribee, LP = Luggage Point, and Chel = Chelsea.

3.3.4 Individual Treatment Plants – Extractable Elements

While extractable Cu remained constant at most of the plants, it decreased 10 fold in the biosolids from Luggage Point over the period examined (Table 3.3.4). This is an interesting point considering that total Cu concentration increased in Luggage Point biosolids during this period (Figure 3.3.3a), which shows that total element concentrations do not necessarily reflect the available portion. This emphasises the need to consider the available fraction of metals in biosolids when assessing their value as a source of plant nutrients, or as a source of environmental contamination. Extractable Zn decreased in biosolids from Werribee and Bolivar, but remained constant in those from Chelsea. The amount of extractable P increased in biosolids from all plants except Bolivar, where the amount had fallen to less than one tenth of that recorded for biosolids from the early 1980's.

Table 3.3.4: Extractable Elements in Biosolids from Individual Treatment Plants Over Time

	Year	Cu	Zn	Mn	Cd	Ni	K	P
Werribee								
	1960	9.6	707	75	6.6	41	192	26
	1967	1.8	29	26	0.9	4.7	321	15
	1980	2.1	51	52	1.1	8.4	249	5
	1983	9.1	231	13	2.9	9.8	739	256
	1997	4.7	116	25	0.7	7.5	451	124
Chelsea								
	1977	0.47	4.5	4.7	0.10	1.5	329	94
	1987	0.28	5.8	8.1	0.19	1.9	308	101
	1996	0.77	11.7	11.3	0.24	2.2	278	180
	1998	1.73	3.3	6.2	0.01	1.9	576	158
Bolivar								
	1980*	37	31	45	1.4	27	7033	1133
	1995	39	5	3	0.06	9	1137	71
	1997	45	4	7	0.03	4	706	101
Luggage Point								
	1982	35	9	9	0.6	11	483	81
	1981	38	15	10	0.6	13	212	63
	1995	3	60	176	1.0	6	249	77
	1999	4	5	10	0	4	1510	3345

* year of production for Bolivar biosolid described in the original publication assumed to be 1980.

3.4 Conclusions

A comparison of the two surveys reveals that total Cu, Mn, Ni, Na and Ca concentrations in Australian biosolids have changed little over time, while Cd, Pb and Zn concentrations have significantly decreased. The concentration of P and K has increased over the same period. In terms of extractable or available elements, Cu, Zn, Mn, Cd and Ni all decreased, while extractable P and Na increased. Examination of biosolids from individual treatment plants over time revealed that although some general trends may be identified, a high level of variability is observed between biosolids of different origin, both in terms of total and available element concentrations. This highlights the need to assess biosolids on a case-by-case basis if a true indication of their potential detrimental environmental impacts and benefits are to be gained.

4. Effect of Incubation Time on Biosolid Properties and Cu Availability

4.1 Introduction

A large proportion of soil and biosolid Cu is often associated with the organic fraction (Emmerich *et al.* 1982; Holtzclaw *et al.* 1978; McLaren and Crawford 1973a) and therefore the question arises as to what happens to this Cu as the organic materials degrade with time. The time bomb theory postulates that once a threshold amount of organic matter is lost the metals previously bound to it will become freely available (McBride 1995), while the concept of reversion theorises that metals may become less available over time through processes of occlusion, solid state diffusion, or other processes (Brown *et al.* 1998, and see Chapter 1, *Section 1.8.4*). However, neither of these theories have been proven conclusively, with evidence for both the importance of organic matter in biosolid metal retention (Hooda and Alloway 1994; McBride 1995) and conflicting evidence indicating greater importance of other components (Brown *et al.* 1998; Li *et al.* 2001) being present in the literature. Therefore, the effect of time on biosolid metal availability remains an area of scientific study that requires urgent attention.

This experiment was undertaken to determine the effect of incubation time on biosolid properties and, importantly, on the location and availability of biosolid metals. Treatments included biosolids in isolation (100% biosolid) and soil biosolid blends (70:30% soil: biosolid). In addition to these, parallel treatments were also set up to which glucose was periodically added to accelerate microbial activity, with the aim of maximising any effects of microbial breakdown of organic matter on metal availability.

4.2 Methods

4.2.1 Biosolid Preparation and Incubation Treatments

Six biosolids from the set described in Chapter 2 were selected to provide coverage of a range of properties and characteristics (Table 4.2.1), including extremes of Cu, Zn and Cd concentrations, as well as variation in organic C % and nutrient content. Due to high salinity, and the potential detrimental effects of this on planned plant growth studies, two of the selected biosolids (Bolivar 95 and Port Kembla) were treated to reduce their salt contents. Bolivar 95 was treated by placing five separate batches of 1.2 - 1.5 kg of the material in dialysis tubing (pre-soaked in deionised water for 24 hours). Deionised water was added to the tubing, making the ratio of liquid: solid inside the tubing between 3:1 and 4:1. The tubing was then sealed and placed in 30L of deionised water, with the external water replaced every two days. After a total of six days dialysis, Bolivar 95 was removed from the dialysis tubing, laid out on plastic trays, and placed in an oven (35°C) to dry. Once dried, the material was combined and homogenised and then ground to pass a 2 mm sieve. This procedure reduced the electrical conductivity (EC) of the material (1:5 suspension) from 6.81 to 2.61 dS/m.

The EC of Port Kembla biosolids was not successfully reduced by the dialysis bag method, thus a fresh batch of the material was leached using an alternative method. Four batches of 600 g were placed in separate plastic bags to which four pore volumes of de-ionised water were added. The plastic bags were pierced 10 times with a needle and drainage was allowed to occur. The leached material was then placed onto filter paper (Whatman no. 1) covered Buchner funnels and a further four pore volumes of de-ionised water passed through under suction. The Port Kembla biosolids were then dried and mixed in the same manner as Bolivar 95 described above. The EC of Port Kembla biosolids was reduced from 4.5 to 3.6 dS/m.

The properties of the two treated biosolids were re-measured using the techniques described in Chapter 2, with the results displayed in Table 4.2.1 along with the properties and characteristics of the other, non-treated biosolids selected for the incubation experiment. The properties of the soil used in biosolid/soil blend treatments are also given.

Table 4.2.1 Initial Properties of Biosolids Selected for Incubation Experiment

Biosolid	Age	pH	$EC_{1:5}$ (dS/m)	Total Cu (mg/kg)	Total Zn (mg/kg)	Total Cd (mg/kg)	Total P (mg/kg)	Total C %	OC %	$CaCl_2$ Ext Cu (mg/kg)	pCu^{2+}
Boliver 95^	5	7.50	2.61	630	723	2.4	13095	19	11	13.0	13.5
Boliver 97	3	6.58	3.68	942	933	3.5	16283	26	16	43.0	10.4
Chelsea 96	4	5.17	2.05	203	323	3.9	6277	6	3	1.1	8.3
Port Kembla^	<5	5.71	3.60	953	1374	16.8	35031	24	16	3.1	13.3
Werribee 97	3	5.03	1.04	909	1407	9.9	10947	29	19	3.3	7.0
West Hornsby	<5	5.72	3.61	851	690	3.1	49837	24	19	9.2	10.6
Soil (RBE*)	na	5.05	0.82	3.2	9.9	0.2	196	1.24	0.6	B/d	9.8

^ Properties shown are those determined after treatment (dialysis or leaching) for high salinity.
* The soil was a sandy red brown earth from Roseworthy Agricultural College, South Australia.

Table 4.2.2 Treatments Imposed in the Incubation Experiment

#	Treatment	Designation	#	Treatment	Designation	#	Treatment	Designation
----------Biosolids----------			----------Soil / Biosolid Blends----------			----------Soil----------		
1	Bolivar 95	B 95	13	Soil/ Bolivar 95	B 95/Soil	25	Roseworthy	Soil
2	Bolivar 97	B 97	14	Soil/ Bolivar 97	B 97/Soil	26	Roseworthy + G	Soil+G
3	Chelsea 96	Ch 96	15	Soil/ Chelsea 96	Ch 96/Soil	27	Reference Soil 1	RS 10^
4	Port Kembla	PK	16	Soil/ Port Kembla	PK/Soil	28	Reference Soil 2	RS 20
5	Werribee 97	Wr 97	17	Soil/ Werribee 97	Wr 97/Soil	29	Reference Soil 3	RS 50
6	West Hornsby	WH	18	Soil/ West Hornsby	WH/Soil	30	Reference Soil 4	RS 100
7	Boliver 95 + G*	B 95+G	19	Soil/ Bolivar 95 + G	B 95/Soil+G	31	Reference Soil 5	RS 200
8	Boliver 97 + G	B 97+G	20	Soil/ Bolivar 97 + G	B 97/Soil+G	32	Reference Soil 6	RS 400
9	Chelsea 96 + G	Ch 96+G	21	Soil/ Chelsea 96 + G	Ch 96/Soil+G			
10	Port Kembla + G	PK+G	22	Soil/ Port Kembla + G	PK/Soil+G			
11	Werribee 97 + G	Wr 97+G	23	Soil/ Werribee 97 + G	Wr 97/Soil+G			
12	West Hornsby + G	WH+G	24	Soil/ West Hornsby +G	WH/Soil+G			

* + G indicates treatment received monthly allocations of glucose (450 μg/g). Dose based on Dahlin and Witter (1998).
^ Reference soils were created by dosing the Roseworthy soil with $CuSO_4$ at 10, 20, 50, 100, 200 and 400 μg Cu/g soil.

Treatments shown in Table 4.2.2 include biosolids (# 1-12) as well as soil / biosolid (70%: 30%) blends (# 13-24). The soil used was a sandy soil from Roseworthy Agricultural College, in South Australia (Table 4.2.1). It was decided to include soil/biosolid blends to assess any temporal changes where soil could also influence biosolid metal availability (*i.e.* biosolid-amended soils). However, in order to maximise the possibility of observable effects occurring, the biosolid content of blended treatments was set much higher than the common application rates used in Australian agriculture (ca. 8 – 15 t/ha). A blend of 70% soil: 30% biosolid

equates to an application rate of approximately 500 t/ha, if the top 10cm of soil is considered. The blended treatments were prepared by hand mixing in plastic buckets. Soil only treatments (# 25 and 26) were also included. In order to enhance any effects microbial activity may have had on metal bioavailability during incubation, one set of treatments received glucose every month at a rate of 450 μg/g soil. The dose was based on that used by Dahlin and Witter (1998). The aim of these treatments was to accelerate microbial breakdown of biosolid organic matter, testing the hypothesis that this may lead to the release of metals associated with the organic fraction.

Reference soils were prepared by dosing samples of the Roseworthy soil with $CuSO_4$ solution to concentrations 10 – 400 mg Cu/kg soil (Table 4.2.2). Dosed soils were allowed to equilibrate for one week, after which $CaCO_3$ was added and mixed through to adjust the pH back to that of the original soil. The aim of the reference soils was to provide a point of comparison for the biosolid treatments. That is, to be able to relate determinations of available Cu in biosolid treatments to an equivalent amount of freshly added, inorganic Cu. The reference soils would also make it possible to compare any effects of time on Cu availability in biosolid treatments with effects observed in inorganically dosed soils (*i.e.* to compare ageing of biosolid bound Cu to that of inorganic Cu added to soil).

For all treatments (including samples and reference soils) 1600 g were placed in separate plastic buckets. De-ionised water was added and mixed through to raise the moisture content of each treatment to 60% of that at field capacity (determined by two days drainage at 100 cm water suction). The lid of each bucket was pierced four times with a large nail, in order to allow air flow, before being secured. The mass of each bucket was recorded, and all were placed inside a dark incubation chamber where the temperature was maintained at 25-30°C. Once per week moisture loss was replaced by adding de-ionised water (mass basis). Once per

month glucose was added to the appropriate treatments during the watering process. Subsamples of each treatment were taken after 6 months incubation by mixing through the contents of each bucket and removing half of the volume. These 6-month samples were dried by laying them out on trays and placing them in ovens at 35°C. Once dried, the samples were ground to pass a 2 mm sieve and stored in sealed containers for testing. The moisture content of the dried material was determined (by heating at 105°C for 48 hours) and used to calculate the oven dry equivalent mass tested in all subsequent analyses. After the 6 month sample was removed, the portion remaining in each treatment bucket was returned to the incubation chamber and maintained under the set conditions for a further 15 months. At the end of the incubation period, a total of 21 months, the remaining portion of each treatment was collected and processed as per the procedure used at the 6 month collection stage.

4.2.2 Measures of pH, Carbon content, and Abiotic determinations of Bioavailability

At each of the three sampling times (0, 6 and 21 months), several procedures were conducted so that any changes in biosolid properties and/or metal availability could be detected. Determinations of pH, organic carbon content, and hot water extractable carbohydrate were made on all samples following procedures outlined in Chapter 2, while total carbon measurements by LECO combustion were performed on biosolid treatments only (*i.e.* not on soil/biosolid blends). To assess any changes in the nature and composition of organic carbon in the biosolids over time, the biosolids were analysed by solid-state ^{13}C nuclear magnetic resonance spectroscopy (^{13}C NMR). Analysis by NMR was performed on both whole biosolids, and on biosolids treated with hydrofluoric acid (HF) to remove the mineral fraction (Skjemstad *et al.* 1994), using both cross polarization (CP) and Bloch Decay (BD) methods. Further details of the HF treatment and the NMR procedures are given in the papers produced from this work (Smernik *et al.* 2003a; Smernik *et al.* 2003b). The HF treatment and NMR analyses were performed by Dr Ronald Smernik of the University of Adelaide.

The availability of Cu was determined for all samples using the isotopic dilution (E value), ion selective electrode (pCu^{2+}) and $CaCl_2$ extraction methods outlined in Chapter 2. As a further assessment of metal availability EDTA extractions were performed on the biosolid treatments, following the methods of Clayton and Tiller (1979) with slight modification. To 5 g samples 25 mL 0.05M EDTA were added and equilibrated for seven days by end-over-end shaking. After the seven day extraction samples were centrifuged at 1200 *g* for 10 minutes. The supernatants were transferred to a second set of tubes and centrifuged again at 37100 *g* for 15 minutes, then filtered through Whatman No. 1 filter paper, and centrifuged a third time at 16300 *g* for 10 minutes. The filtered, centrifuged solutions were then analysed for Cu, Cd, Pb and Zn concentration by flame atomic absorption spectrophotometry (AAS). The EDTA and $CaCl_2$ extraction procedures were used to investigate changes in the availability of other metals, such as Zn and Cd, in addition to Cu.

4.2.3 Biosolid Fractionation and Cu Distribution

To determine the distribution of Cu between organic and inorganic fractions in the biosolids, a NaClO oxidation procedure was used to isolate the mineral component. The method followed that of Li *et al.* (2001), with additional steps as suggested by Merrington and Smernik (2004). Subsamples (1 g, n = 8) of each biosolid were treated with 20 mL 0.7M NaClO and heated for two hours in a water bath at 100°C. Samples were then centrifuged for 20 minutes at 1200 *g*, the supernatant decanted off, and a further 20 mL NaClO added followed by another two hours at 100°C. After the second NaClO treatment, samples were centrifuged again and the supernatant decanted off. The residual solids were washed by adding 30 mL distilled water, mixing on a vortex mixer, centrifuging at 1200 *g* for 20 minutes, and discarding the supernatant. Three washings were performed on every sample. The initial and final oven-dry weights of each sample were recorded so that the percentage of mass recovered after

treatment could be determined. Following the steps taken by Merrington and Smernik (2004), a second set of samples was treated with non-reactive 0.7 M NaCl rather than NaClO. The purpose of this being to determine the fraction of biosolid Cu that is removed by the liquid extraction and washing procedures, so as to not overestimate the fraction removed by oxidation of organic matter. The dried residues, and samples of the untreated biosolids, were digested by boiling *aqua regia* extraction. Prior to digestion, all residues were finely ground, homogenised, and dried at 105°C. The Cu concentrations observed were used to determine the organic/inorganic distribution. This entire procedure was performed on biosolids sampled at time 0 and time 21 months, in order to determine whether Cu distribution had changed over the incubation period.

4.2.4 Plant Uptake Study

A plant uptake study was conducted in order to include a biological measure of availability. Treatments in their original condition (time 0) and those subjected to the full incubation period (time 21 months) were used for the plant assay. Small pots (210 mL, in triplicate) were filled with sample to approximately 2/3 capacity, with the exact mass recorded. This equated to between 50 and 90 g for all biosolid and biosolid/soil treatments. For the reference soils dosed with inorganic Cu, 100g were used. Controls were set up using 150 g of acid washed sand. All pots received 10 mL Ruakura nutrient solution (Smith *et al.* 1983), minus the micronutrient supplement (Table 4.2.3). De-ionised water was then added to raise the moisture content of the samples to field capacity, being defined as the moisture content after two days drainage at 100 cm water suction. The control pots, having sand only, were watered to 80% saturation capacity. One small spoonful (approximately 0.5 g) of rye grass seeds (*Lolium multiflora*) was scattered across the surface of the sample, which was then covered with white polythene beads to reduce evaporation. The final mass of each pot was recorded, and all were placed inside a growth chamber (Phoenix Biosystem). The growth chamber

conditions were set at 14 hours daylight, 20°C, and 10 hours darkness, 16°C. Water loss was replaced daily by adding sufficient de-ionised water to raise pots to their initial weight. After five days in the growth chamber each pot received an additional 5 mL nutrient solution. Plants were harvested after 21 days. Harvesting was performed by cutting plants at 0.5 cm above the surface of the polythene beads. All plants within a single pot were grouped together and placed inside a paper envelope, which was then placed in an oven to dry for three days at 70°C. The envelopes were then transferred to a desiccator until the plant material was weighed and digested using concentrated nitric acid.

Table 4.2.3 Nutrient Solution Applied in Plant Assay

Nutrient	Ca	K	Mg	Na	Cl	NO_3-N	NH_4-N	P	S
Concentration (mg/L)	120.5	226.7	19.9	13.9	8.9	181.8	47.8	37.9	57.5

The plant trial described above was not the first option chosen. Initially it was decided to follow the double pot method of Stanford and DeMent (1957). Briefly, this method involves germinating and growing plants in sand held in small pots with detachable bases. Once the plants have developed to a desired stage the bases of the pots are removed and each pot is placed on a second pot containing the growth medium of interest (*i.e.* soil, or in this case biosolids or biosolid/soil blends). The nutrient/contaminant being investigated is withheld from the plants in the initial stage, thus the roots grow rapidly into the growth medium of the second pot. The plant uptake of nutrients or contaminants from the growth medium can then be determined by harvesting and analysing the plant material. This method was chosen because it offers a number of potential benefits; 1) seeds are able to germinate in a less hostile environment than that of a contaminated soil (or biosolid, which may inhibit germination), 2) all plants are in a similar condition when introduced to the growth medium, and 3) roots from the upper (sand) pot can be harvested and analysed without contamination from the soil or biosolids. This attribute was of particular interest because Cu is known to be largely retained in plant roots (Loneragan 1981; Mitchell *et al.* 1978). Using sorghum (*Sorghum bicolour*), the

method was successful when tested in a preliminary trial with one biosolid. However, when performed on all samples from the initial period (time = 0), the results were not good. Root penetration was difficult to determine in many cases, and was absent in others (*i.e.* often roots did not penetrate the growth medium or only did so to a very shallow extent) thus uptake from the growth medium could not be measured with certainty. Due to this problem the double pot method was abandoned in favour of the rye grass assay described above.

4.3 Results and Discussion

4.3.1 Carbon

During the course of the incubation period Chelsea 96 and Werribee 97 showed no change in total C% (as measured by LECO combustion), while Bolivar 95, Bolivar 97, Port Kembla and West Hornsby all showed reductions, particularly between times 0 and 6 months (Figure 4.3.1 and Table 4.3.1). The negative C loss shown by Chelsea 96 at the 21-month sampling (Table 4.3.1) is within the standard errors of zero change. Treatments receiving glucose showed no significant difference in total C% from those not receiving glucose, suggesting that stimulation of microbial activity did not enhance degradation of biosolid C.

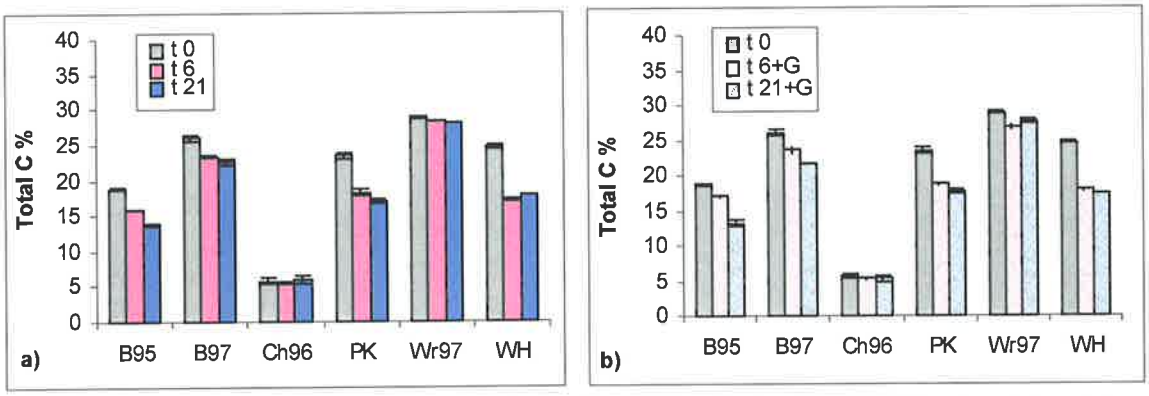


Figure 4.3.1: Total C% during a 21month incubation for a) biosolids and b) biosolid treatments receiving monthly doses of glucose. Error bars indicate 2x standard error.

Table 4.3.1: % Initial Total C Lost During Incubation

	6 months	21months	*LSD*	6 months	21months	*LSD*
				+ Glucose treatments		
B95	15.6	26.8	0.70	9.3	29.6	2.81
B97	10.5	13.2	2.40	9.3	17.0	2.66
Ch96	3.3	-4.6	18.32	5.5	6.0	10.10
PK	22.2	27.2	3.22	20.2	25.0	2.19
Wr97	1.9	3.0	0.36	7.1	4.3	1.04
WH	30.2	27.6	0.71	27.4	29.8	0.85

Organic C results, measured by wet dichromate oxidation, followed those of total C% in the case of Bolivar 97, Port Kembla, Chelsea 96 and West Hornsby, but were at odds in the case of Bolivar 95 and Werribee 97 (Figure 4.3.2). According to the wet dichromate oxidation measurements OC% declined significantly in the first 6 months for Werribee 97, while total C% measured on the LECO indicated no change (Figure 4.3.1). The initial value for total C%

in Werribee 97 was 29%, while OC% determinations returned a value of only 19%. The pH of Werribee 97 is approximately 5, thus it is unlikely to contain many carbonates that could otherwise explain the discrepancy. Therefore, the difference in values for the two initial C measurements must be due to a fraction of the biosolid organic C not being oxidised during the dichromate treatment, which is susceptible to incomplete oxidation (Navarro *et al.* 1991; Nelson and Sommers 1982). It is possible that the proportion of this resistant fraction increased during the incubation period, whilst total C% remained stable. For the soil/biosolid blends, only Port Kembla showed a significant change with time (Figure 4.3.2-c).

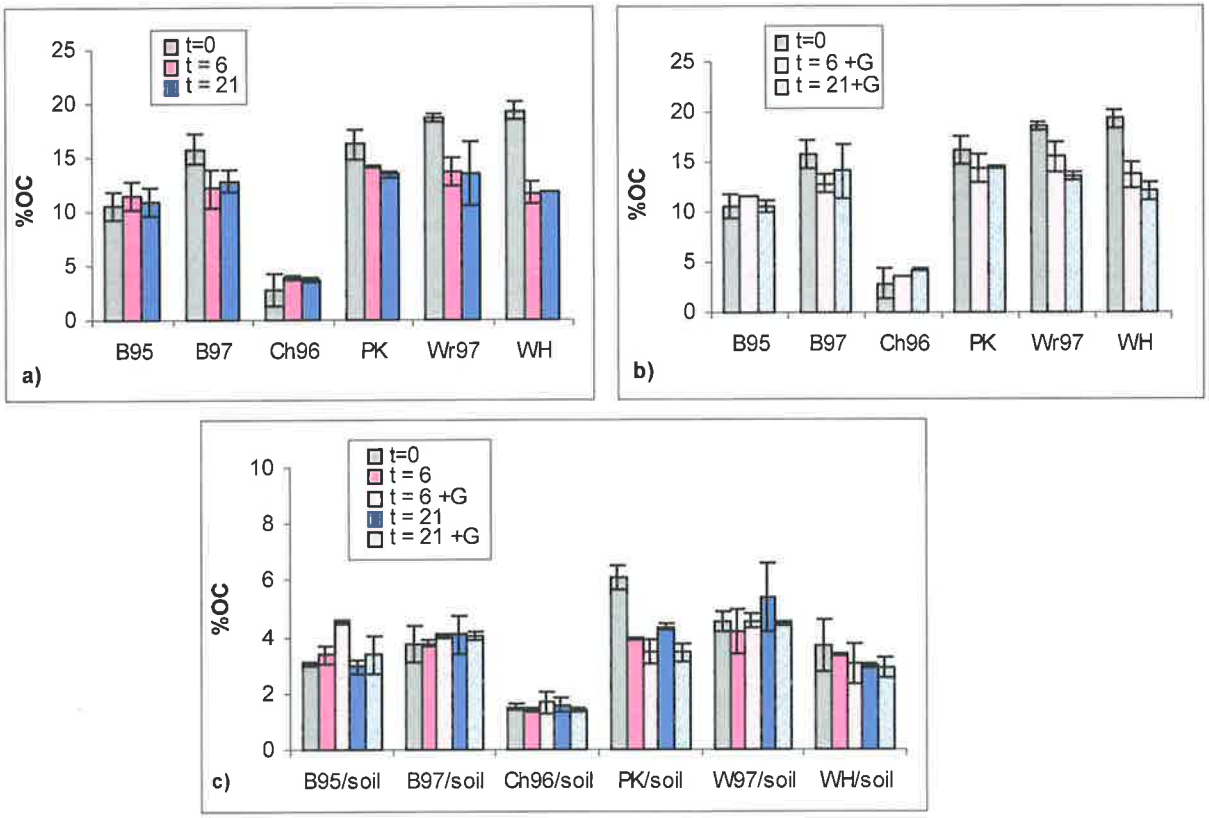


Figure 4.3.2: Organic C% measured by wet dichromate oxidation for a) biosolids, b) biosolids + glucose treatments, and c) soil/biosolid blends. Error bars indicate 2x standard error.

Nuclear Magnetic Resonance Spectroscopy (^{13}C NMR) performed using the Bloch decay (BD) and cross polarization (CP) techniques found BD to be more quantitative than CP. Spin counting procedures (Smernik *et al.* 2003b) found BD to have approximately double the C NMR observability (C_{obs}) of CP (Table 4.3.2). The hydrofluoric acid (HF) treatment further improved C_{obs}, raising the values to near unity (Table 4.3.2), and had C recoveries of 80-90%

for all six biosolids. Due to this improved signal response only the HF-treated samples from the time = 21 month period were subjected to NMR analysis. Four chemical shift regions were identified within the NMR spectra, and, although still operationally defined, were assigned to carbonyl (190-165ppm), aryl (165-110ppm), *O*-alkyl (110-45ppm) and Alkyl (45-0ppm) C moieties (Smernik *et al.* 2004) (Table 4.3.3).

Table 4.3.2: NMR %C Observabilities (C_{obs}) Determined From Spin Counting and %C Recovered After HF Treatment for Original (t = 0) Samples

	whole sludge		HF treated		C recovered
	$^{+}C_{obs}$ CP^	C_{obs} BD*	C_{obs} CP	C_{obs} BD	post HF
B95	34	69	73	92	80
B97	38	89	70	92	87
Ch96	31	ND	75	98	79
PK	30	58	67	99	86
Wr97	51	101	69	100	85
WH	23	56	67	91	81

$^{+}C_{obs}$ = Proportion of potential ^{13}C NMR signal intensity (C observability) detected in NMR spectrum.
^CP = Cross Polarization. *BD= Bloch Decay.

Table 4.3.3: Percent of Total Nuclear Magnetic Resonance (NMR) Signal Contained in Four Chemical Shift Regions in ^{13}C Bloch Decay (BD) NMR Spectra of Whole and Hydrofluoric Acid (HF)-treated Biosolids at time = 0 months

	Carbonyl	Aryl	O-alkyl	Alkyl
	---------- Whole Biosolids ----------			
B95	11.6	12.1	39.6	36.7
B97	6.2	13.8	42.1	38
Ch96	ND^	ND	ND	ND
PK	8.8	21.5	34.9	34.8
Wr97	7.1	22.5	41.3	29.1
WH	11.5	21.9	36.2	30.3
	---------- HF-treated Biosolids ----------			
B95	8	11.9	39.6	40.6
B97	8.5	12.6	40.5	38.3
Ch96	7.5	15.4	40.1	36.9
PK	7.9	14.6	38	39.6
Wr97	5.4	17.6	40.4	36.7
WH	9.3	9.7	36.4	44.6

^ ND = Not Determined. No spectra acquired due to low C content

The percentages of the total NMR signal contained within the four chemical shift regions for the 21 month samples (Table 4.3.4) were different to those at time 0 (Figure 4.3.3), with increases in Aryl C and decreases in Alkyl C over time. The relative reduction in Alkyl C was generally between 4 and 8 % (Figure 4.3.3), which extends outside the 2% precision

limitation (error margin) for the NMR signal distribution analysis (Baldock and Smernick 2002). Therefore, although minor, a real change can be considered as having occurred. However, the extent of the change is put into perspective by looking at it in terms of mass change in each of the chemical shift fractions (Figure 4.3.4). In these terms it can be seen that the reductions in Alkyl C (and indeed all other changes) are less than 4%. Therefore, because reductions in total C % were generally much greater than this 4% figure (Table 4.3.1), it must be concluded that microbial decomposition of biosolid C during the incubation period was not particularly selective for any of the chemical shift regions identified by NMR.

Table 4.3.4: Percent of Total NMR Signal Contained in Four Chemical Shift Regions in ^{13}C Bloch Decay (BD) NMR Spectra and % Obsevabilities (C_{obs}) for Hydrofluoric Acid (HF)-treated Biosolids at time = 21 months

	Carbonyl	Aryl	*O*-alkyl	Alkyl	C_{obs}
B95	9	18	36	36	109
B97	9	18	39	34	99
Ch96	8	21	40	31	99
PK	8	17	35	32	98
Wr97	6	22	40	37	97
WH	9	16	39	39	111

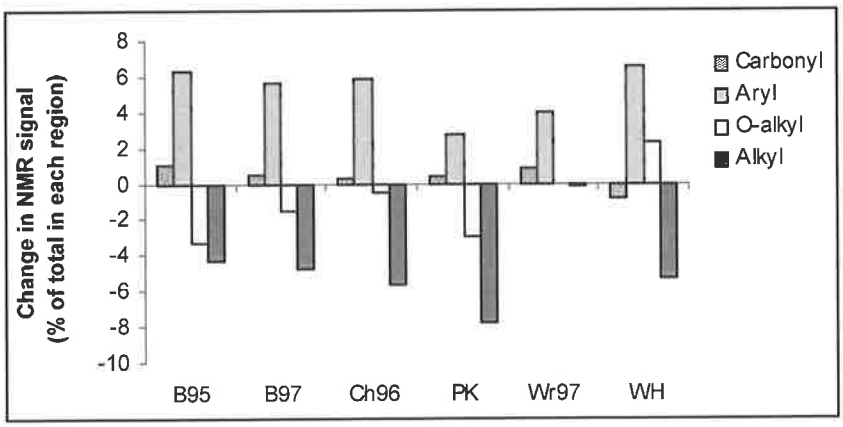


Figure 4.3.3: Change in percentages of NMR signal distribution amongst four chemical shift regions between time 0 and time 21 month samples (negative values indicate relative reductions).

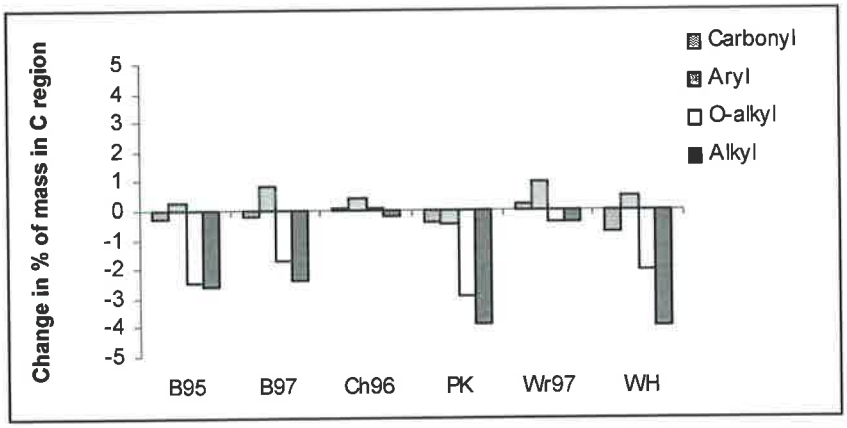
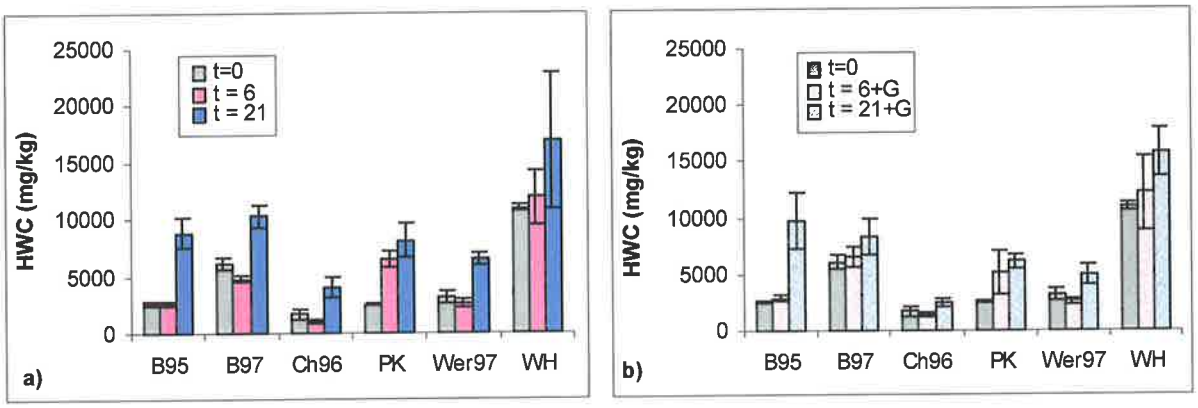


Figure 4.3.4: Change in percentages of NMR signal distribution amongst four chemical shift regions, expressed as mass % in each region, between time 0 and time 21 month samples.

In contrast to the minimal changes identified by NMR, hot water extractable carbohydrate (HWC), a measure of the easily decomposable C present in the sample, showed a general increase over time for the biosolid samples (Figure 4.3.5). This may reflect an accumulation of dead microbial biomass during the incubation, which indicates that decomposition was indeed occurring. The addition of glucose did not produce significantly different HWC values. The soil/biosolid blends displayed similar trends, but, due to greater standard deviations than the biosolids, only Bolivar 95/soil and West Hornsby/soil samples had statistically significant differences (Figure 4.3.5).



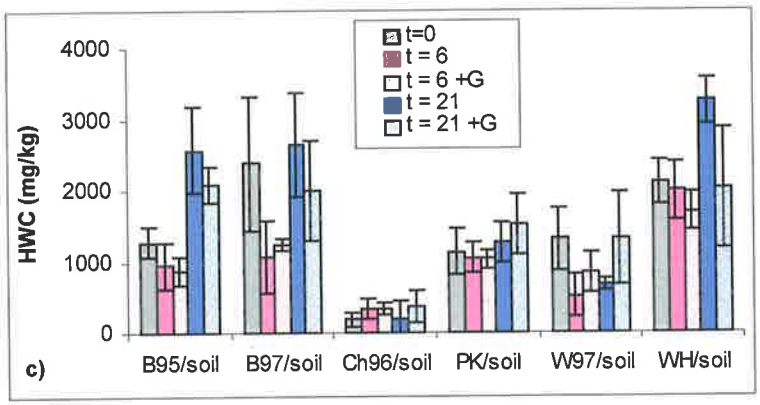
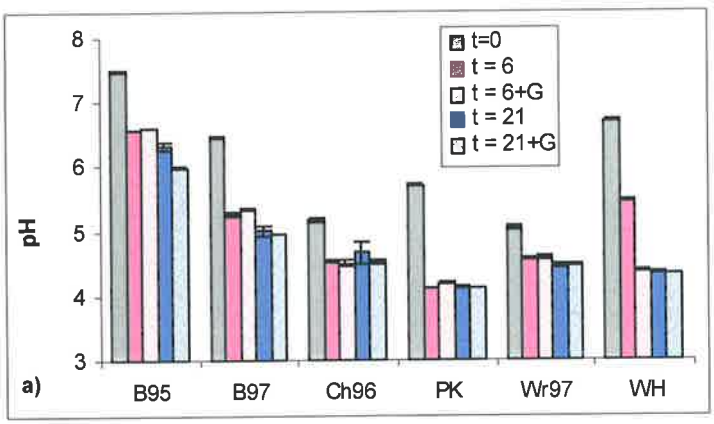


Figure 4.3.5: Hot water extractable carbohydrate (HWC) over time for a) biosolids, b) biosolids + glucose treatments, and c) soil/biosolid blends. Error bars indicate 2x standard error (n=3).

4.3.2 pH

During the incubation pH values declined in all treatments, including control and reference soils (Figure 4.3.6). Microbial processes such as nitrification are the likely causes of the pH reductions, as this has been noted in other investigations involving the incubation of biosolids (Dudley *et al.* 1986; Wong *et al.* 2000). Reductions were greater in the biosolids than in soil/biosolid blends, with West Hornsby recording the greatest reduction of 2.35 pH units over the 21-month period (Figure 4.3.6). The 400 mg Cu/kg treatment showed the greatest pH reduction (0.75 units) of the inorganically dosed reference soils.



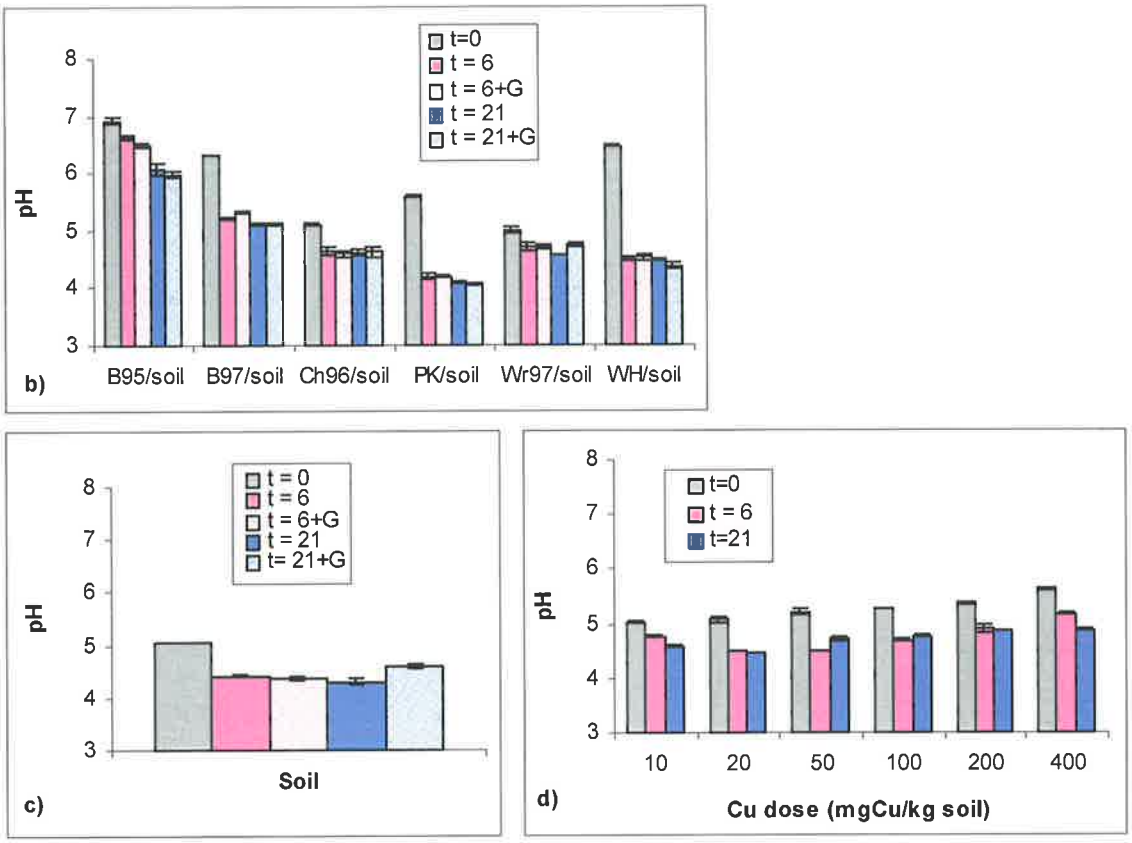


Figure 4.3.6: pH values for t = 0, 6 and 21 month samples for a) biosolids, b) biosolid/soil blends, c) control soil and d) Cu dosed reference soil. Error bars indicate 2x standard error (n=2).

4.3.3 Isotopically Exchangeable Cu

The absolute values of isotopically exchangeable Cu (the E value, or CuE) increased over time for several of the biosolid and biosolid/soil blend treatments (Figure 4.3.7). Statistically significant increases were also recorded for four out of the six biosolids when CuE values were viewed in terms of percentage of total Cu (Figure 4.3.8). These results differ from those reported for Cd and Zn in biosolids incubated for up to 3.5 months (Stacey *et al.* 2001) and for Cu in six soils incubated for 10 months (Williams and McLaren 1982), where no significant changes in E values were observed. Here, in contrast to the biosolids, dosed reference soils showed decreases over time at all dose concentrations (Figure 4.3.7), and thus exhibited signs of reversion, the process whereby metals become less available with time, as has been found by others working with soils dosed with inorganic salts (Almas *et al.* 1999; Brennan *et al.* 1986; Ma and Uren 1998). Due to this contrast, when the CuE values of the biosolid treatments are considered in terms of an equivalent amount of inorganic Cu (*i.e.* the

inorganic Cu dose required to produce an equivalent CuE value) an increase over time is observed in every case (Figure 4.3.9). This inorganic equivalent is determined by a regression of CuE against Cu dose in the reference soils, with the resulting equation applied to the CuE values of the biosolid samples (Figure 4.3.9c).

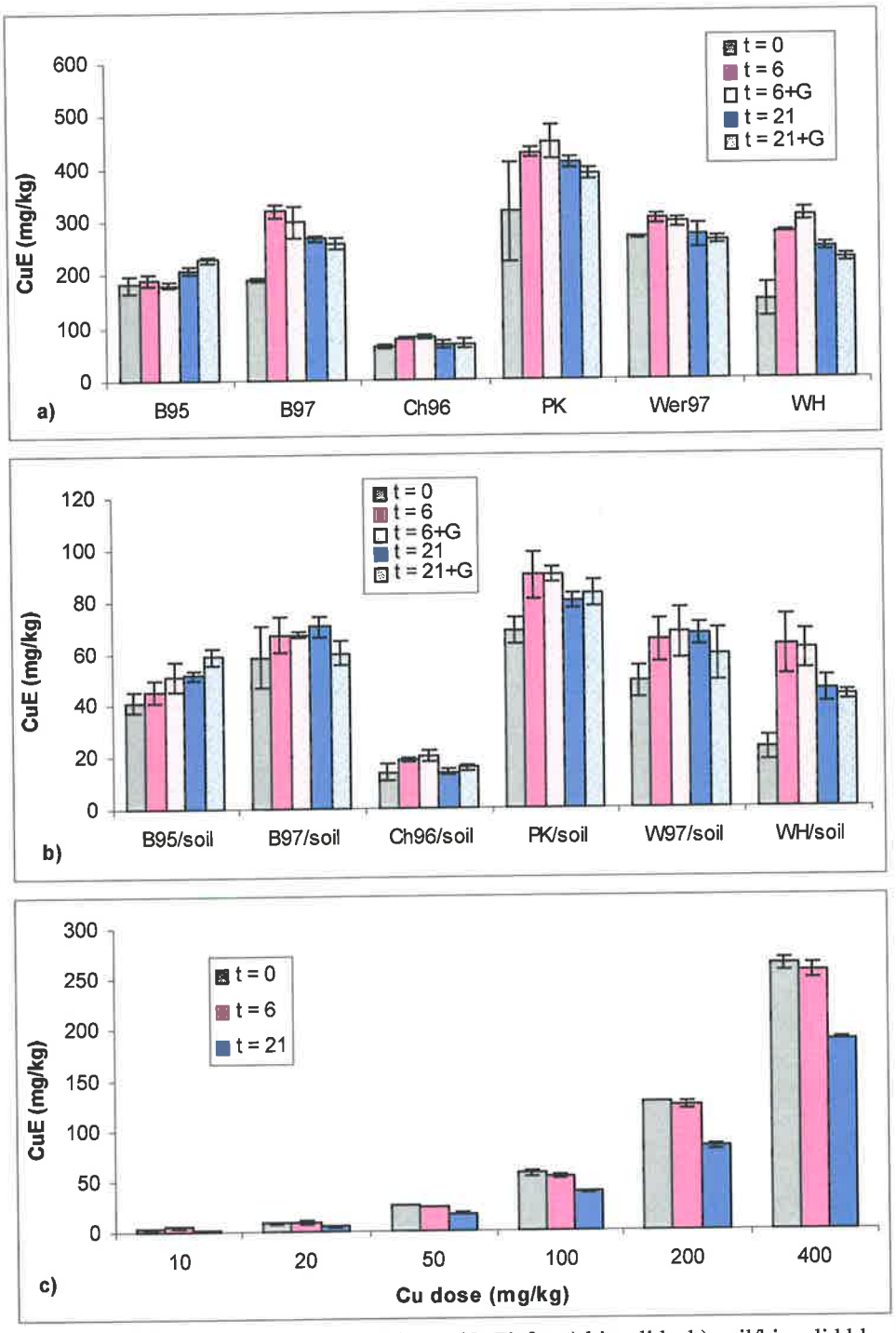


Figure 4.3.7: Isotopically exchangeable Cu (CuE) for a) biosolids, b) soil/biosolid blends and c) inorganically dosed reference soils. Error bars indicate 2x standard errors (n=3).

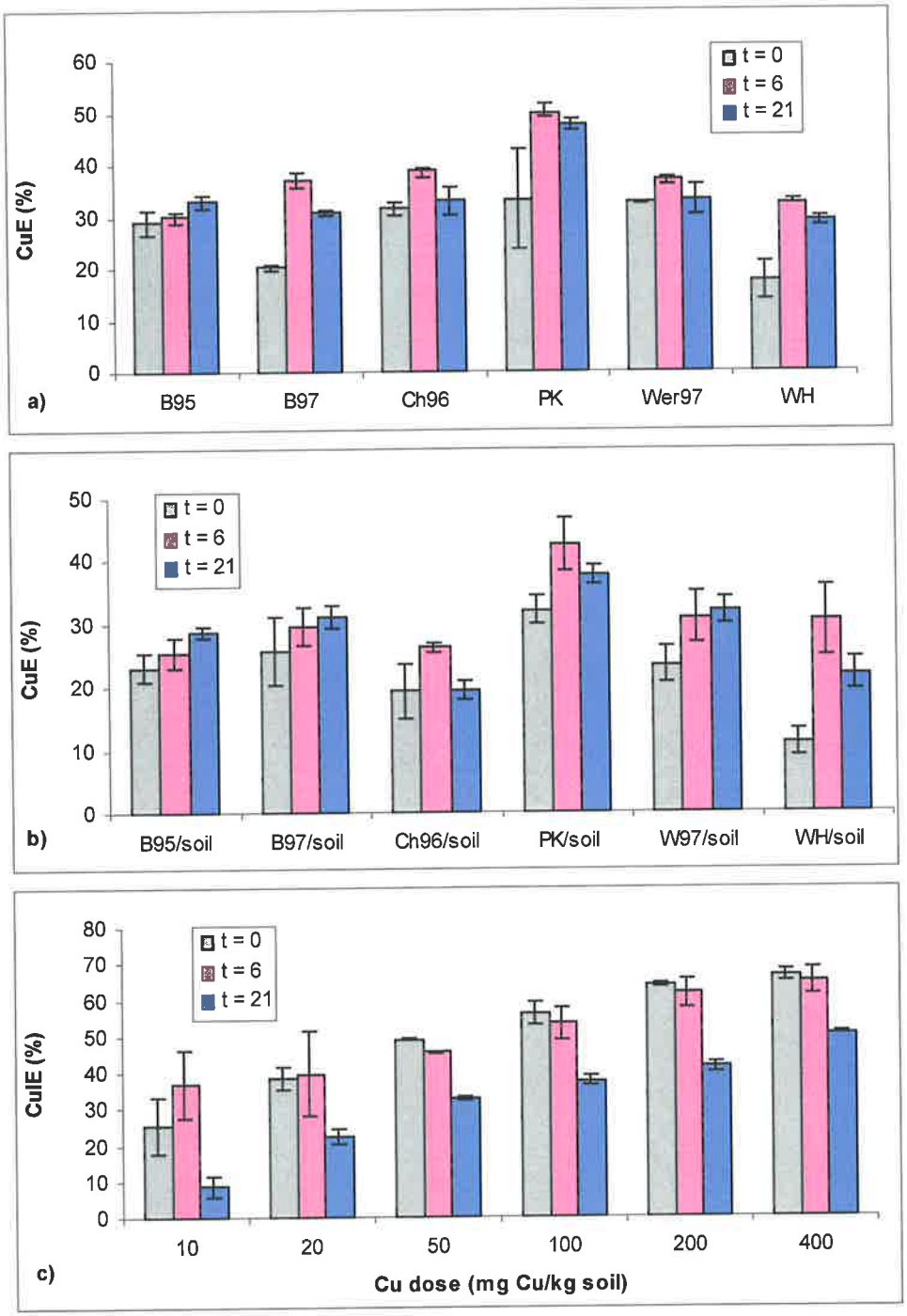


Figure 4.3.8: Isotopically exchangeable Cu (CuE) expressed as % of total Cu for a) biosolids, b) soil/biosolid blends and c) inorganically dosed reference soils. Error bars indicate 2x standard errors (n=3).

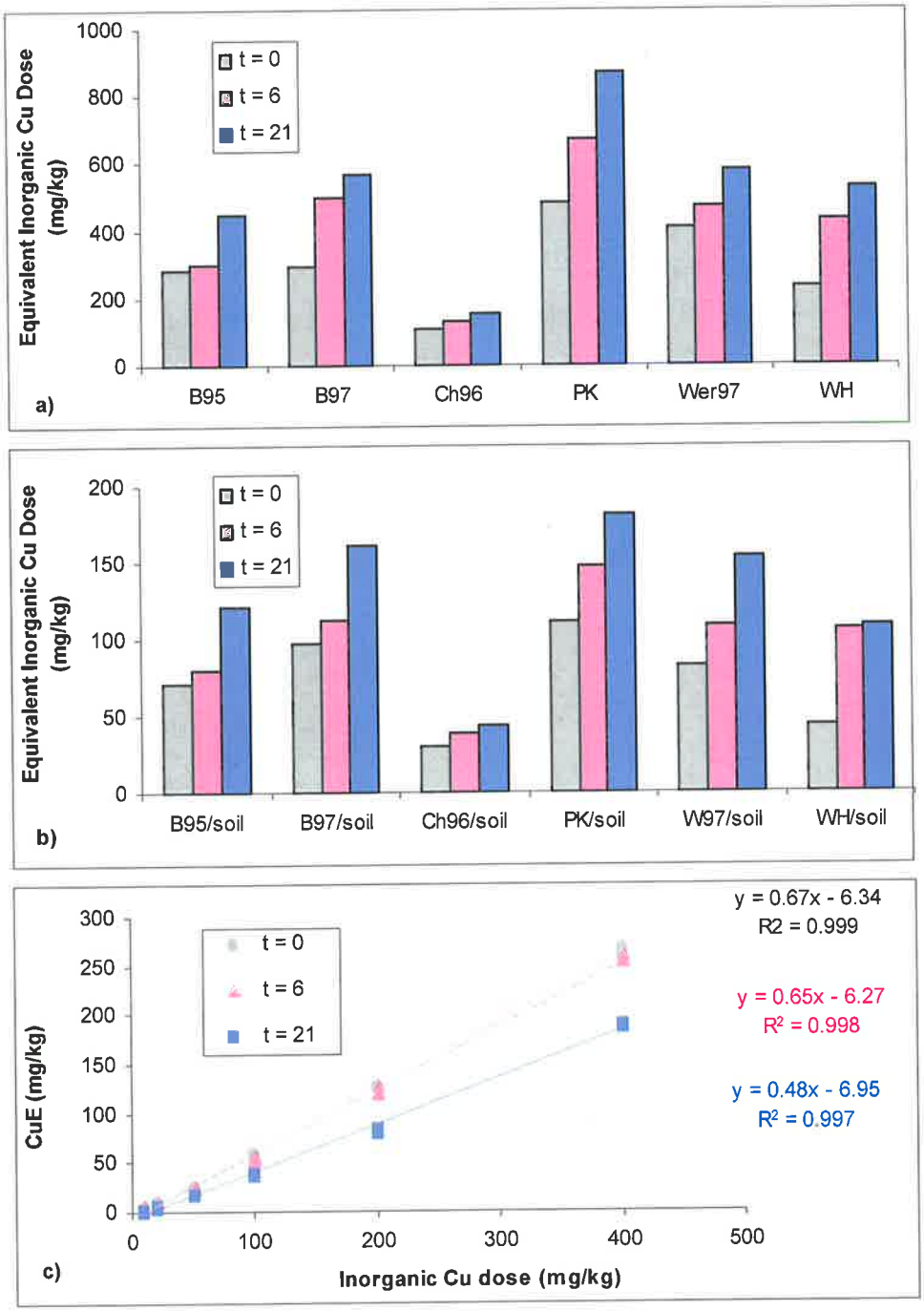


Figure 4.3.9: Equivalent dose of inorganic Cu required to produce CuE values of a) biosolids and b) soil/biosolid blends at time 0, 6 and 21 months. Graph c) displays regression equations for CuE against Cu dose for inorganically dosed reference soils at the 3 sample times.

At first glance the data seem to support the notion of sludge-borne metals becoming more available with time, most likely due to organic matter decomposition and subsequent metal release (as per the time bomb theory). Plotting the relationship between change in E value (ΔCuE) and percent of initial C lost over time appears to lend further support to this tenet

(Figure 4.3.10). From such plots clear, positive relationships can be seen for the three biosolids showing increases in CuE over time; Bolivar 97, Port Kembla and West Hornsby.

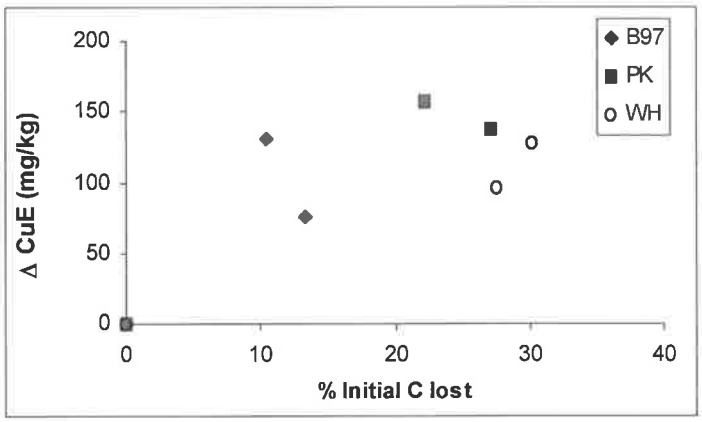


Figure 4.3.10: Change in isotopically exchangeable Cu (ΔCuE) versus percent of initial C lost for biosolids showing increases in CuE between time 0, 6 and 21months; Bolivar 97, Port Kembla and West Hornsby.

However, before any evidence for the time bomb theory can be claimed, the decrease in pH over the course of the incubation period and its potential effects must be considered. It has already been established that decreasing the pH of a biosolid can increase the isotopically exchangeable fraction of Cu (*c.f.* Chapter 2), therefore it is possible that any increases in CuE observed here are simply the results of this pH effect. Plotting the change in CuE against change in pH during the incubation reveals a clear relationship for the three biosolids showing increases in CuE (Figure 4.3.11). Therefore, given the apparent relationships between both ΔCuE and ΔpH and between ΔCuE and %C lost, the question remains as to whether the increases in CuE observed for these biosolids over time are related to C mineralisation, simple pH effects, or an interaction between pH and C. The dataset, consisting of three replicates at three measurement times per parameter per biosolid, is not large enough to separate out any interaction effects statistically (Kate Dowling, Biometrics SA, *pers comm.*). However a plot of ΔpH v %C lost over time confirms the existence of a relationship (or a confounding effect) between the two parameters (Figure 4.3.12). Therefore the underlying cause of the CuE increases remains unidentified, necessitating a separate investigation to determine whether the

increases are related to an organic matter mineralisation process (*i.e.* the time bomb effect) or to simple pH change. This additional investigation is the subject of a later chapter (Chapter 5).

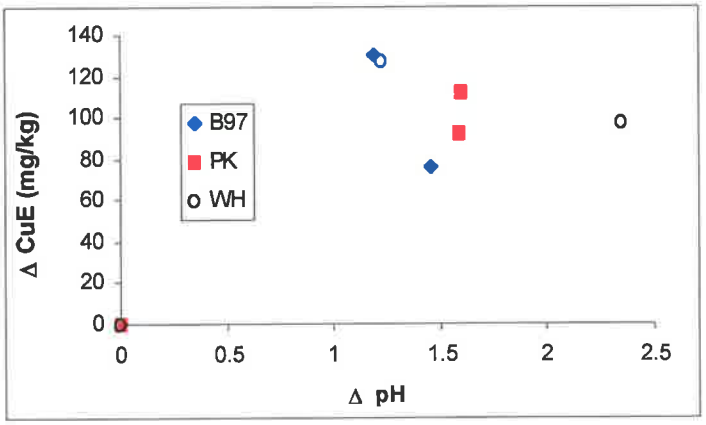


Figure 4.3.11: ΔCuE v ΔpH during the 21-month incubation for biosolids showing increases in CuE; Bolivar 97, Port Kembla and West Hornsby.

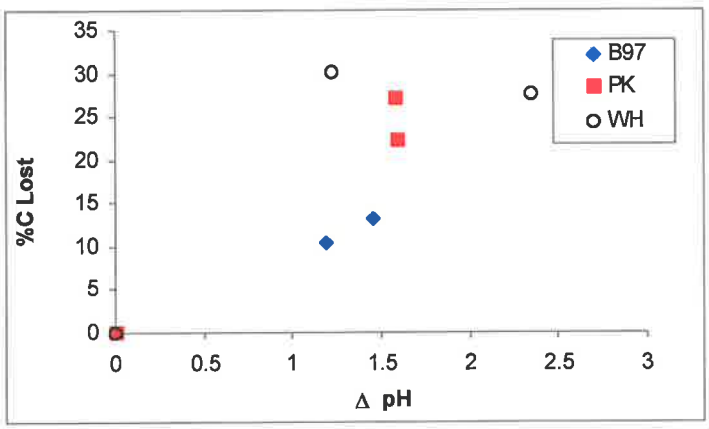


Figure 4.3.12: Relationship between % initial C lost and ΔpH at time 0, 6 and 21 months for Bolivar 97, Port Kembla and West Hornsby.

4.3.4 $CaCl_2$-Extractable Metals

Extraction of 0, 6 and 21 month samples with 0.01M $CaCl_2$ revealed that, in general, the extractable-Cu fraction remained stable or declined with time (Figure 4.3.13). The most drastic result was that observed for Bolivar 97, where the amount of extractable Cu fell by a factor of five (43 mg/kg – 8 mg/kg). The other biosolid from Bolivar, Bolivar 95, declined by only 25%, but it is likely that much of the easily solubilized Cu was removed from this sludge by the leaching process imposed on it prior to the incubation experiment (*c.f. section 4.2.1*). Extract procedures were repeated on numerous occasions for the Bolivar biosolids (3 times, 3 replicates each time) to check the validity and consistency of the results, with each set confirming the reported outcome. Therefore the decline in the $CaCl_2$ extractable Cu content of the Bolivar 97 biosolids was a real effect, possibly caused by the decrease in OC which would have reduced the dissolved OC in solution extracts and thus decreased Cu mobilisation. Results for Port Kembla were opposite to the general trends observed, with an increase in extractable Cu with time. The amount doubled in the +glucose treatments (3 mg/kg – 6 mg/kg) and more than tripled (to 11 mg/kg) in the standard treatments between times 0 and 21 months (Figure 4.3.13). For the soil/biosolid blends the general result was stability over time, with the exception of Bolivar 97/soil which, as in the case for the corresponding biosolids only treatment, showed a major reduction (14 mg/kg – 3 mg/kg). The results agree to some extent to those of Mashhady (1984), who found concentrations of water-extractable Cu in biosolid amended soil was the same after 6 weeks incubation as at the beginning. Williams and McLaren (1982) also failed to identify any effects of time on $CaCl_2$-extractable Cu in six soils (10 month incubation). The reference soils showed no change except at the highest dose (400 mg/kg), where an increase with time was observed. However, this increase was minor, from 10 to 13 mg/kg, and so would have little environmental relevance (Figure 4.3.13). The lack of increase with time for $CaCl_2$-extractable Cu, despite the pH reductions observed, suggests that the pH change did not affect the fraction of weakly surface sorbed Cu

targeted by this extractant (Figure 4.3.14), whereas the isotopically exchangeable fraction was affected (previous section). This agrees with the results of Sanders *et al.* (1987), who also found that $CaCl_2$-extractable Cu was not influenced by pH changes occurring during incubation of biosolid amended soils.

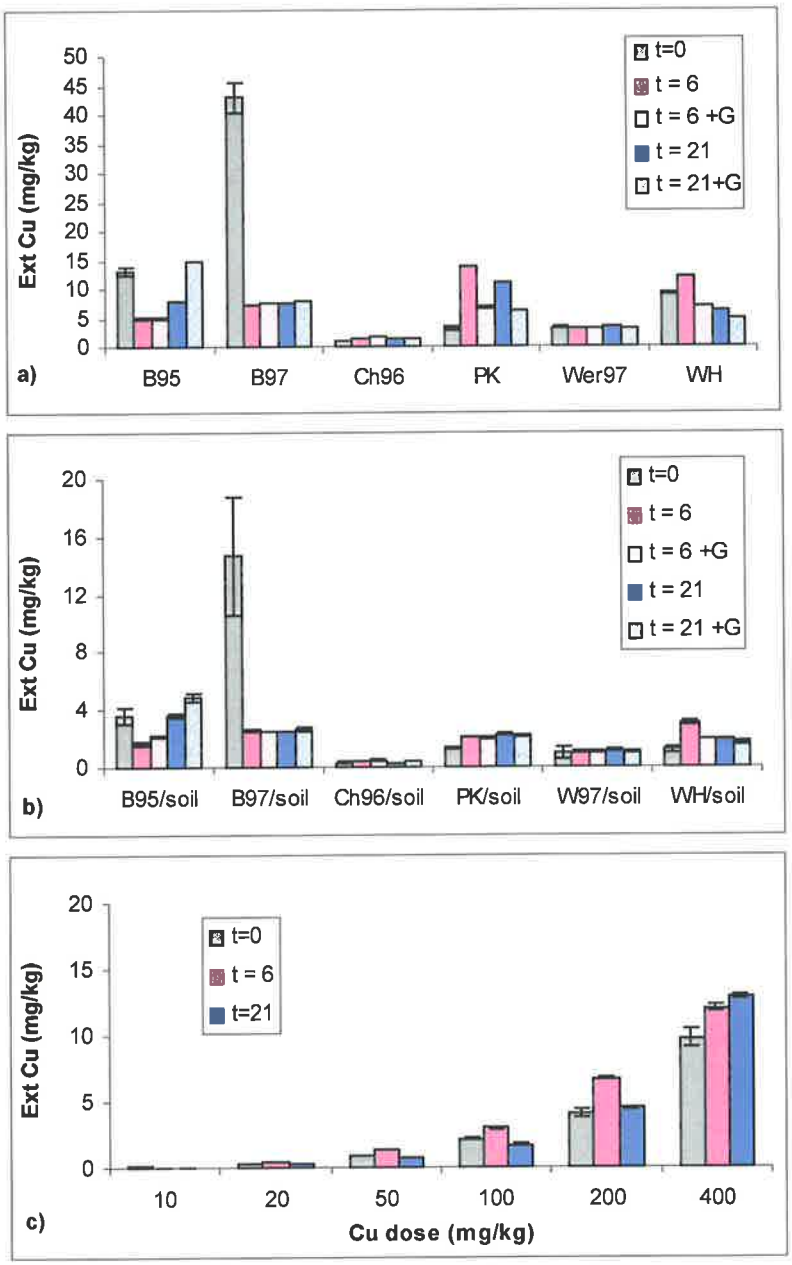


Figure 4.3.13: $CaCl_2$-extractable Cu at time 0, 6 and 21 months for a) biosolids, b) soil/biosolid blends and c) Cu dosed reference soils. Error bars indicate 2x standard error (n=3).

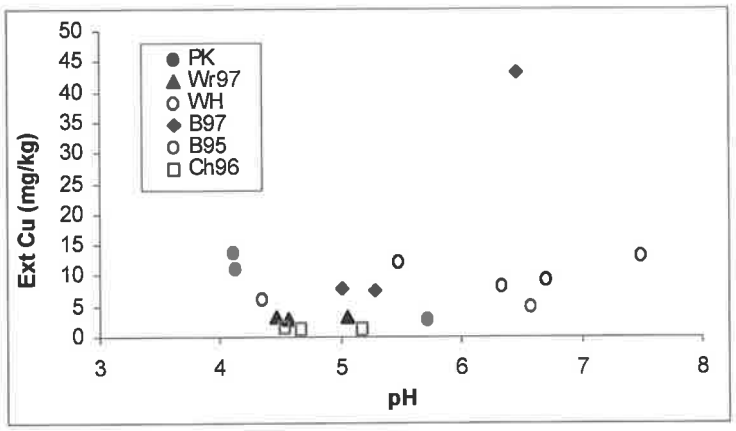


Figure 4.3.14: Relationship between $CaCl_2$-extractable Cu and pH at times 0, 6 and 21 months for biosolids Bolivar 95, Bolivar 97 and Chelsea 96, Port Kembla, Werribee 97 and West Hornsby.

In contrast to Cu, $CaCl_2$-extractable Zn increased over the incubation period in all biosolid treatments (Figure 4.3.15). This increase was likely due to the decrease in pH during the experiment, as evidenced by the strong relationship between pH and extractable Zn identified when the pH values from times 0, 6 and 21 months were plotted against the values of extractable Zn for each of the biosolids (Figure 4.3.16). The relationship was exponential, with an apparent critical point between pH 5 and pH 5.5. This relates well to the results of Adams and Sanders (1984), who found that increased amounts of biosolid Zn were released to solution as pH decreased below a threshold value of 5.8. In that study the authors determined a pH threshold value of 4.5 for Cu, which may explain why in this study Port Kembla was the only sludge treatment to exhibit increased $CaCl_2$-extractable Cu with time and pH change (*i.e.* Port Kembla was the only biosolid for which pH fell appreciably below 4.5, see Figure 4.3.6).

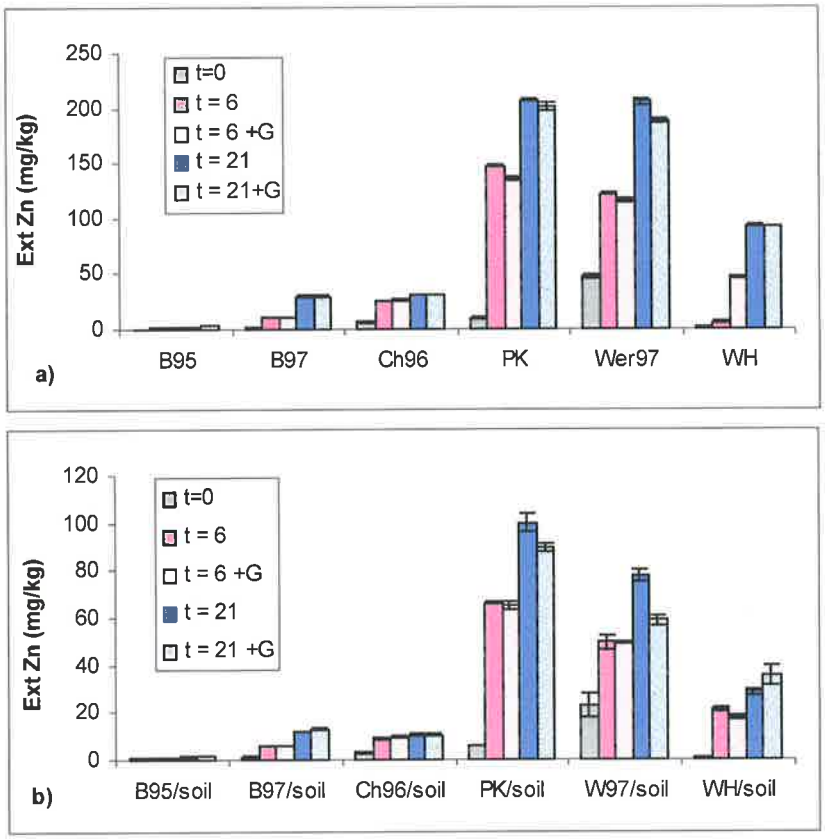


Figure 4.3.15: $CaCl_2$-extractable Zn at time 0, 6 and 21 months for a) biosolids and b) soil/biosolid blends. Error bars indicate 2x standard errors (n=3).

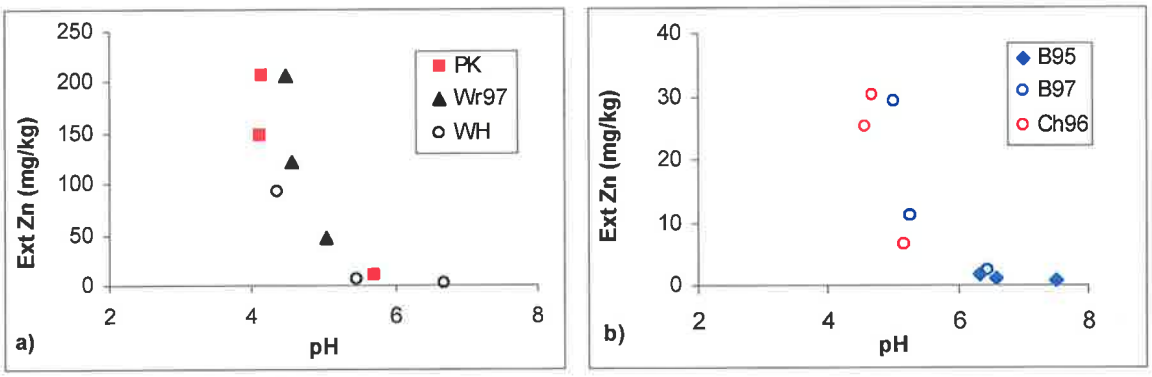


Figure 4.3.16: Relationship between $CaCl_2$-extractable Zn and pH at times 0, 6 and 21 months for biosolids a) Port Kembla, Werribee 97 and West Hornsby, and b) Bolivar 95, Bolivar 97 and Chelsea 96.

Extractable Ni showed two distinct patterns; decreases with time in the two sludges from Bolivar while all other sludges recorded increases (Figure 4.3.17). The fall in $CaCl_2$-extractable Ni in the Bolivar samples mirrors the fall in extractable Cu observed for them, suggesting some processes were occurring that reduce the extractability of certain metals in these biosolids (*i.e.* occlusion or solid state diffusion, etc). The increase with time in all other

biosolids (and their soil blended treatments) is once again most likely explained by pH decreases. Sanders and Adams (1984) observed a critical pH threshold of 6.3 below which Ni was readily released from biosolids to solution, and here the pH of Chelsea 96, Port Kembla, Werribee 97 and West Hornsby all fell below pH 5 during the course of the incubation.

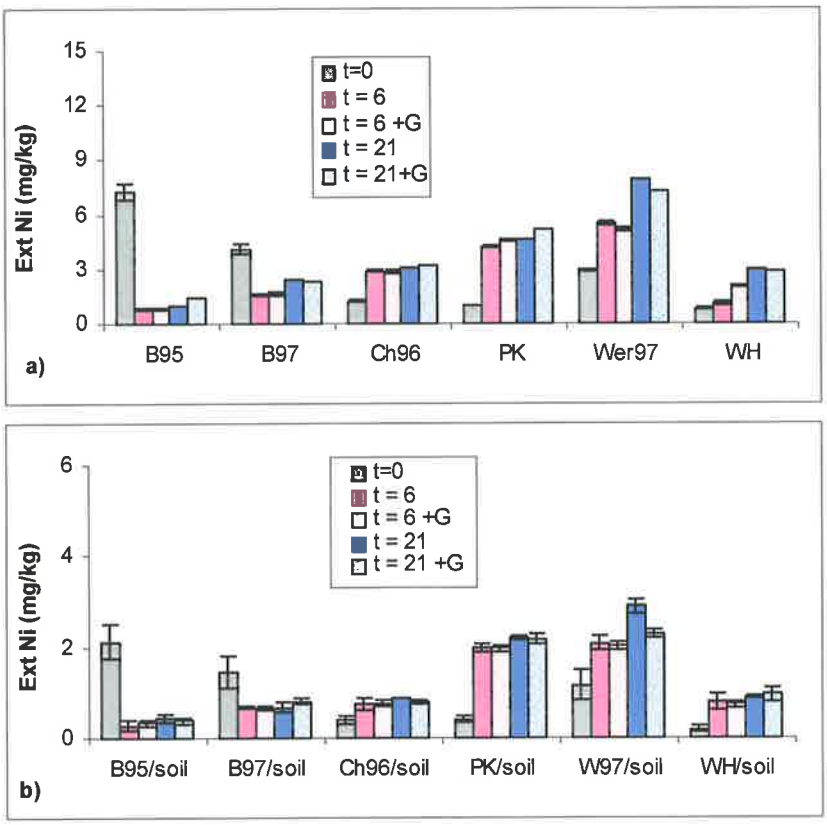


Figure 4.3.17: $CaCl_2$-extractable Ni at times 0, 6 and 21 months for a) biosolids and b) soil/biosolid blends. Error bars indicate 2x standard errors (n=3).

4.3.5 EDTA-Extractable Metals

EDTA-extractable Cu did not vary significantly ($p \leq 0.05$) with time for Werribee 97 biosolids, and, while statistically significant, varied less than 16% for Chelsea 96 (Figure 4.3.18). These were the biosolids for which pH and total C varied the least during the incubation, thus a lack of change in EDTA-extractable Cu was not surprising. However, the large increases observed for Bolivar 95, Port Kembla and West Hornsby were less predictable. All three of these biosolids had EDTA-extractable Cu values below 5 mg/kg at time 0, but by 6 months had values in excess of 500, 300 and 200 mg/kg for Bolivar 95, Port Kembla and West Hornsby,

respectively. Low values could have been expected for Bolivar 95 and Port Kembla at time 0 due to the pre-treatment imposed on them prior to incubation (dialysis and leaching), but a low value for West Hornsby cannot be accounted for by this. However, values below 5 mg/kg EDTA extractable Cu were not expected for any of these biosolids because each recorded values of isotopically exchangeable Cu at time 0 of 150 mg/kg or above, and it has been found that EDTA extraction often yields higher values than isotopic dilution techniques (Williams and McLaren 1982). Therefore the entire procedure (7 days extraction with 0.05M EDTA followed by centrifugation and filtering, etc.) was repeated for these time 0 samples. The replicated procedure confirmed the initial results, with the outcome showing all three had EDTA-extractable Cu less than 3 mg/kg (thus were within 2 standard errors of previous). The Fe and Al concentrations were not included in AAS measurements of the EDTA extracts, so it cannot be said for certain whether it is possible that these metals out competed Cu for chelation with the ligand in time 0 extracts. The changes observed over time could not be due to instrument error or to a change in operator, because all samples (time 0, 6 and 21 months) were extracted and analysed concurrently using archived samples (stored dry in air tight containers). It is possible that extractability decreased further during dry storage, as was found by Williams and McLaren (1982), but this is unlikely to account completely for the very low time 0 values observed. Therefore, although the absolute values for some of the biosolids at time 0 may be questionable, the increases observed must be real and due to changes in one or more biosolid properties during incubation.

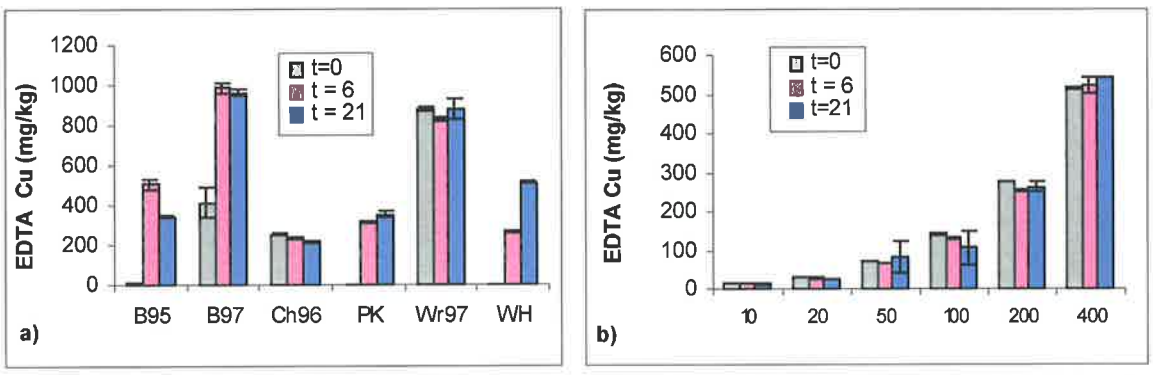


Figure 4.3.18: EDTA-extractable Cu at times 0, 6 and 21 months for a) biosolids, and b) inorganically dosed reference soils (10, 20 50, 100, 200 and 400 mg Cu/kg). Error bars indicate 2x standard errors (n=3).

Comparison of these results with those for pH (Figure 4.3.6) leads to the conclusion that pH was the property most likely to be the driving force behind the changes in EDTA-extractable Cu. That is, the patterns of pH change observed for the biosolids reflect those of EDTA-extractable Cu. For example, the EDTA-extractable Cu for Bolivar 95, Bolivar 97 and Port Kembla all showed large increases between 0 and 6 months, followed by no or only minor changes at 21 months. The pH change pattern was the same, with a large change between 0 and 6 months (1 unit decrease for Bolivar 95, 1.2 units for Bolivar 97, and 1.6 for Port Kembla) followed by minimal further pH change between 6 and 21 months (0.2 for Bolivar 95, 0.27 for Bolivar 97, and no change for Port Kembla). The patterns also matched for West Hornsby, the other biosolid recording increases in EDTA-extractable Cu. The pH change for West Hornsby showed two virtually equal, large declines between 0 and 6 months (1.2 units) and between 6 and 21 months (1.1 units). The change in EDTA-extractable Cu followed this pattern exactly, with two large increases of over 200 mg/kg between 0 and 6 months and between 6 and 21 months (Figure 4.3.18).

The results seen here are consistent with those of Silviera and Sommers (1977) who observed that DTPA-extractable Cu more than doubled during a 44 week incubation for two sludge/soil combinations where the pH was known to have decreased. By contrast, for their soil/sludge combination that did not experience pH decline the extractable Cu content decreased. Similarly, Parkpain *et al.* (2000) found that DTPA-extractable Cu decreased by 30-40% in biosolid-amended soils incubated for 12 weeks were the pH remained stable.

The dosed reference soils showed no significant changes in EDTA-extractable Cu with time (Figure 4.3.18). This contrasts with the results for isotopically exchangeable Cu, which showed clear evidence of fixation with time despite pH reductions (Figure 4.3.7). The EDTA-extractable Cu was consistently greater than the E value, being between 2 and 5 times greater

at time 0, between 2.0 and 3.3 times at 6 months, and 3 – 11 times greater at 21 months. This suggests that EDTA extracts soil Cu not in the isotopically exchangeable pool, and therefore may overestimate the labile fraction. Such a conclusion was also drawn by Williams and McLaren (1982), who dosed 6 soils with $CuCl_2$ and measured EDTA-extractable Cu and CuE values over a 44-week incubation period. They found that EDTA-extractable Cu was 1.4 to 1.9 times greater than the amount of isotopically exchangeable Cu.

Concentrations of EDTA-extractable Pb did not change with time for any of the biosolids (Figure 4.3.19), whereas EDTA-extractable Zn and Cd remained stable in all biosolids except Port Kembla, for which extractable Zn and Cd concentrations increased by 35% and 165%, respectively. Port Kembla had the lowest pH at the end of the incubation period (pH 4.13), which may account for this biosolid being the only one showing increases for these metals. The results for Pb were similar to those of Silviera and Sommers (1977), who observed no change in DTPA-extractable Pb in any of the soil/biosolid combinations (2 soils, 3 biosolids) they examined during two incubation experiments lasting 12 and 44 weeks. Their results for Cd and Zn were not dissimilar to those of the present study either, with stability or increases observed depending on the biosolid, with the greatest increases (up to 250%) recorded in soil/biosolid mixtures showing the greatest pH reductions (Silviera and Sommers 1977). Parkpain *et al.* (2000) found DTPA-extractable Cd remained constant over a 12-week period in sludged soils, while DTPA-extractable Zn remained constant or decreased by up to 20%. However, in all of their samples the pH remained stable. Therefore pH may be a factor in the results obtained here, with the various outcomes for the different biosolids likely to be due to different net results of the opposing forces of metal fixation, organic matter decomposition, and dissolution reactions caused by pH changes.

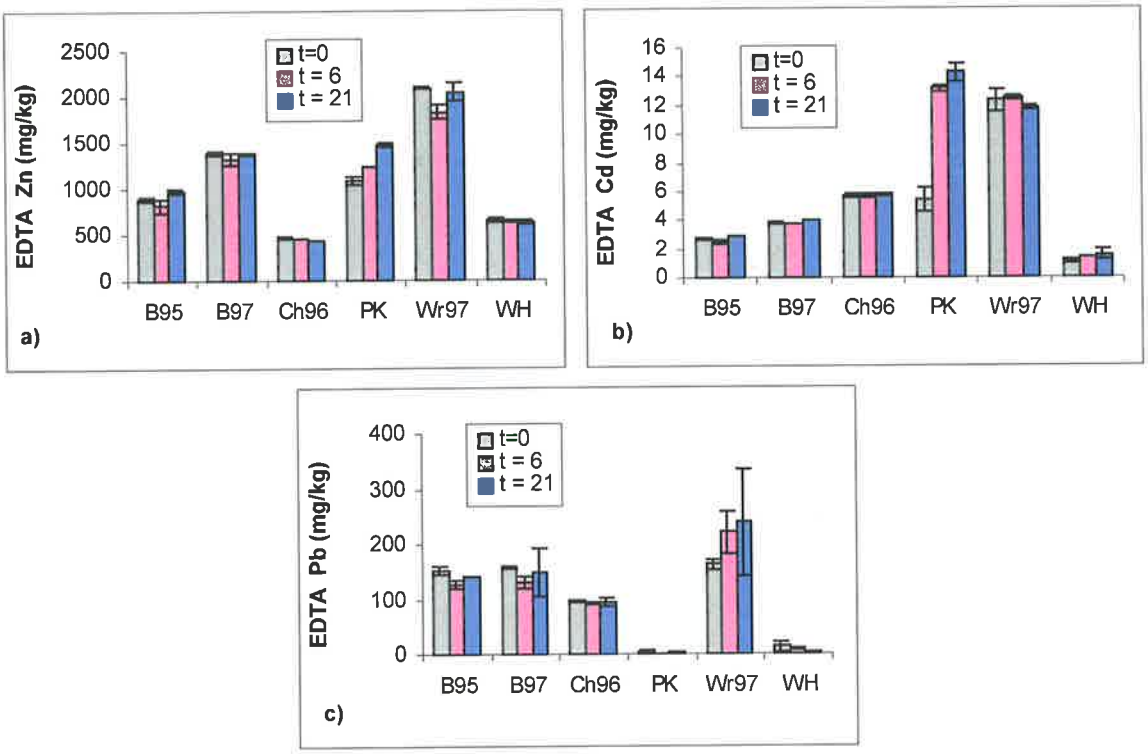


Figure 4.3.19: EDTA extractable Zn, Cd and Pb in incubated biosolids at times 0, 6 and 21 months. Error bars indicate 2x standard errors (n=3).

4.3.6 Cupric Ion Activity (pCu^{2+})

The pCu^{2+}, or negative log of the free Cu^{2+} activity, declined in all biosolids during the incubation (Figure 4.3.20), signifying an increase in the amount of free ion activity in solution extracts. The greatest change was observed for Port Kembla biosolids, while Werribee 97 and Chelsea 96 showed only minimal change. Results for the soil/biosolid blends mirrored those for the biosolids (Figure 4.3.20). The Cu dosed reference soils showed some decline by the 6 month sampling stage, but after 21 months were generally the same as at time 0 (Figure 4.3.20).

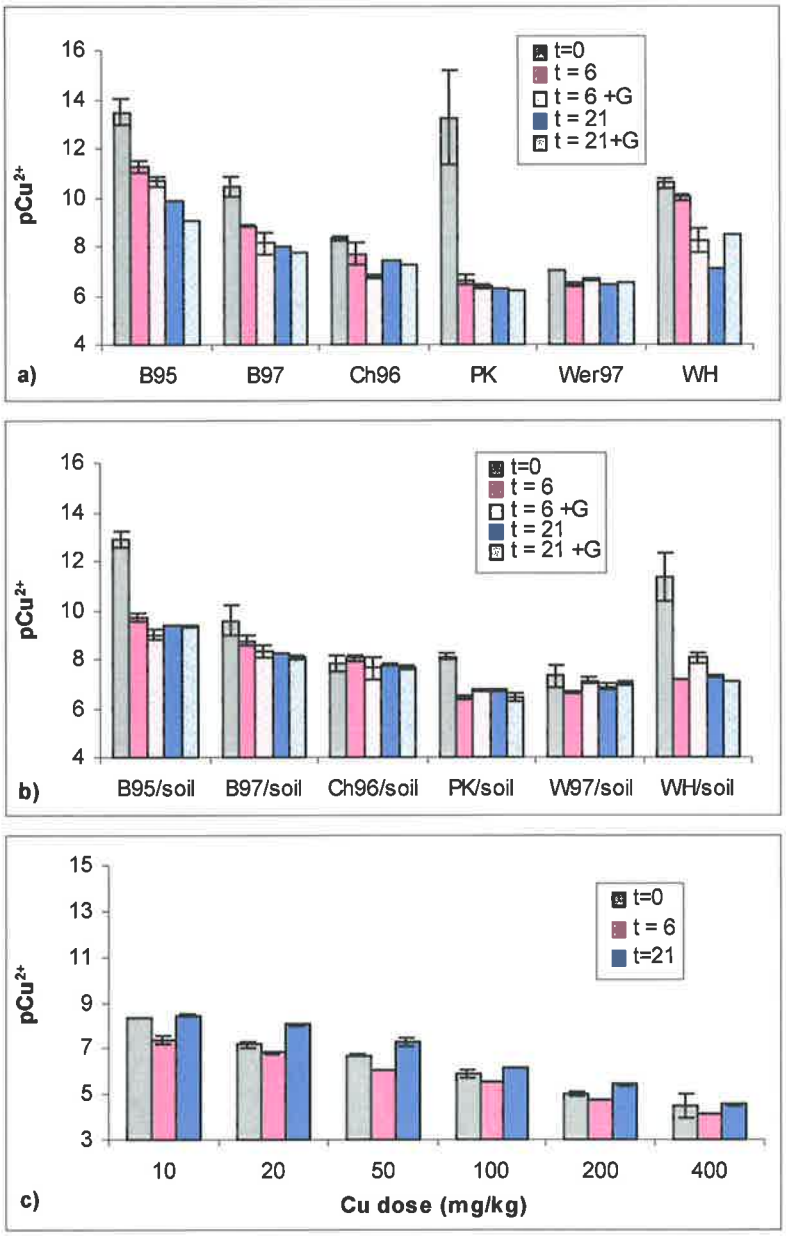


Figure 4.3.20: Cupric ion activity (pCu^{2+}) in $CaCl_2$ extracts at times 0, 6 and 21 months for a) biosolids, b) soil/biosolid blends and c) reference soil dosed with inorganic Cu. The +G labels indicate glucose treatments, and error bars indicate 2x standard errors (n=2).

In Chapter 2, stepwise regression revealed pH, total Cu and total C % to be the factors most influencing pCu^{2+} when all biosolids were considered together (*section 2.3.5*). Treating the biosolids from the present incubation study as a group once again, the pCu^{2+} of each biosolid was plotted against its corresponding pH for each of the three measurement times (Figure 4.3.21). The regression line shows an R^2 of 0.78, indicating a strong relationship between pCu^{2+} and pH. If the initial pCu^{2+} value for Port Kembla, which had wide standard errors (Figure 4.3.20), is omitted from the data, the R^2 value rises to 0.92, further indicating the

strong relationship. When pCu^{2+} is examined in the same way in relation to total C%, no such relationship was identified (Figure 4.3.21). Indeed, the regression returned an R^2 value of <0.001. Therefore, the overriding or dominant variable driving change in pCu^{2+} with time was pH. This was confirmed by examining the pCu^{2+} v pH relationship for each of the biosolids individually over the incubation period, where R^2 values were all 0.77 or higher (Table 4.3.5).

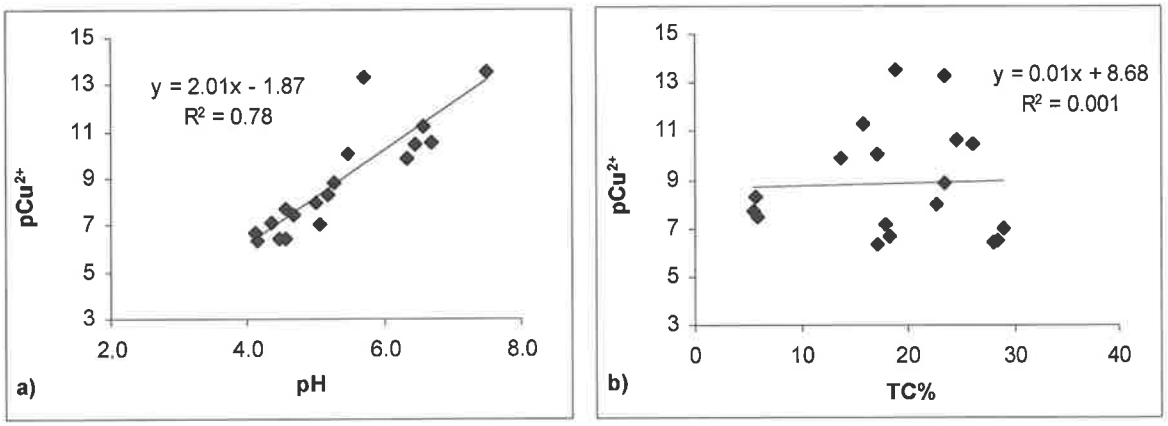


Figure 4.3.21: Cu ion activity (pCu^{2+}) plotted against corresponding pH (a) and total C% (b) for all 6 biosolids at the three measurement times.

Table 4.3.5: Linear Regression Equations for pCu^{2+} v pH Over the 21-month Incubation

Biosolid	pCu^{2+} v pH	R^2
B95	pCu^{2+} = 2.92pH - 8.31	0.97
B97	pCu^{2+} = 1.59pH + 0.24	0.97
Ch96	pCu^{2+} = 1.19pH + 2.09	0.77
PK	pCu^{2+} = 4.27pH -11.08	1.00
Wr97	pCu^{2+} = 1.02pH + 1.85	0.98
WH	pCu^{2+} = 1.46pH + 1.20	0.85

4.3.7 Biosolid Fractionation and Cu Distribution

The masses recovered after oxidative treatment of the six biosolids with 0.7M NaOCl ranged from 60 to 80% (Table 4.3.6), while for the non-oxidative NaCl treatments recovery masses were 90 to 95%, as was expected. These percentage mass recoveries and the Cu concentrations of the isolated residues (determined by *aqua regia* digestion) were used to calculate the proportions of Cu in the mineral and organic phases of the biosolids in their

initial condition (Table 4.3.7). Mineral phase Cu was calculated as mass proportion (α) recovered from the NaOCl treatment multiplied by the Cu concentration of the residue;

$$\text{Mineral phase Cu } (Cu_{min}) = \text{NaOCl treatment } \alpha \text{ mass recovered} \times \text{NaOCl residue [Cu] in mg/kg}$$

Organic phase Cu was calculated by first multiplying the mass α recovered from the non-oxidative NaCl treatment by the Cu concentration of the residue (being both mineral and organic Cu), then subtracting the amount calculated for the mineral phase;

$$\text{Organic phase Cu} = (\text{NaCl treatment } \alpha \text{ mass recovered} \times \text{NaCl residue [Cu] in mg/kg}) - Cu_{min}$$

The amount lost through simple solubilisation during the fractionation procedure (*i.e.* the readily extractable fraction that may include both organic and inorganic Cu), was calculated by difference.

Table 4.3.6: Percentage Mass Recoveries After Treatment with 0.7M NaOCl and NaCl

Biosolid	Total C%[+]	NaOCl % recovery		NaCl % recovery		% mass lost
B95	19	72	(0.84)[$]	93	(0.28)	7
B97	26	61	(0.22)	90	(0.20)	10
Ch96	6	80	(1.07)	94	(0.27)	6
Pk	24	66	(0.37)	90	(0.45)	10
Wr97	29	57	(1.00)	95	(0.17)	5
WH	25	63	(0.86)	91	(0.36)	9

[+] Total C% measured by LECO combustion, shown here for comparison.
[$] Parentheses indicate 2x standard error.

Table 4.3.7: Distribution of Biosolid Cu Between Mineral, Organic and Readily Extractable Phases

	% Mineral	% Organic	Total	% Readily Soluble[*]
B95	85	27	112	0
B97	77	11	87	13
Ch96	82	0	82	18
Pk	60	32	91	9
Wr97	70	19	89	11
WH	79	16	95	5

[*] Calculated by difference (100% - Mineral + Organic fraction).

Chelsea 96 biosolids have only 5-6% total C (Table 4.3.6), and it is possible that some of this was removed by the NaCl treatment and washing procedures, so it is perhaps not surprising

that no Cu was identified in the non-readily extractable organic fraction (Table 4.3.7). However, it was expected that more organic Cu would have been determined in the other biosolids. Even if the readily soluble (or readily extractable) fraction lost during treatment was mostly organic in origin, the fraction of organic Cu in these biosolids (18 - 41%) is less than the 55% observed by Scancar *et al.* (2000) in biosolids from Europe. Tao *et al.* (2003) also determined approximately 50% of the Cu in some biosolid-amended soils from China to be in the organic fraction. The lower values observed here may be due to shifts in Cu partitioning with ageing, as the studies cited above investigated relatively fresh biosolids.

Changes in Cu partitioning over time were investigated here by examining the absolute values determined for the various treatment residues. That is, absolute values for the Cu concentrations in the isolated mineral fraction (NaOCl treated residue) and the non-readily extractable fraction (NaCl residue) were compared for the two time periods (Figure 4.3.22). For Bolivar 95 and Port Kembla biosolids, Cu in the non-extractable component decreased, suggesting the amount of extractable Cu increased with time. However, the mineral component showed an increase in the amount of Cu present, suggesting that any loss of Cu from the non-extractable pool into the extractable pool came from the organic fraction (*i.e.* Cu moved from the organic fraction into the easily soluble and mineral fractions over time). The proportion of Cu in the mineral fraction of Chelsea 96 remained constant. Similarly, the Cu distribution was stable over time for both Bolivar 97 and Werribee 97. For the biosolids from West Hornsby, reductions in the mineral fraction Cu and non-readily extractable Cu were observed, with losses from the former being of lower proportions than the latter. This indicates movements from both organic and mineral components into the readily extractable pool, which supports the CuE results that found Cu availability increased with time.

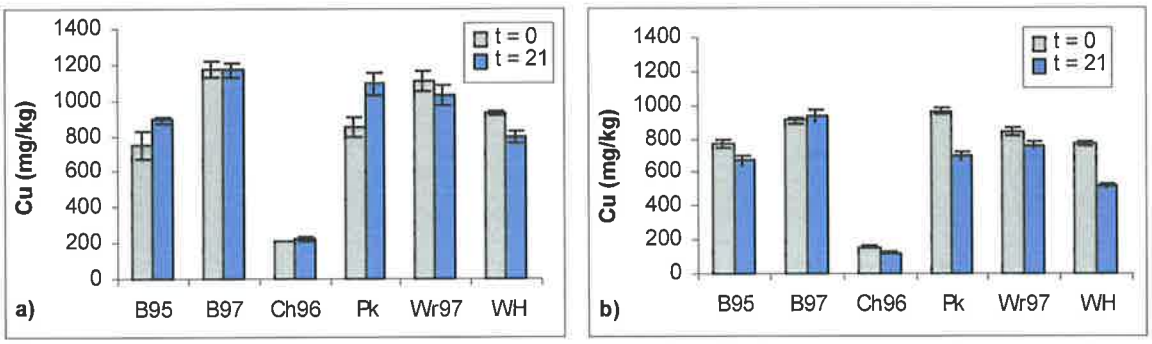


Figure 4.3.22: Cu concentration in a) mineral fraction (NaOCl residue), and b) non-readily extractable fraction (NaCl residue; mineral and organic Cu) at 0 and 21 months incubation. Error bars show 2x standard errors (n=2).

Changes in the proportion of Cu present in the mineral fraction can also be examined by calculating the time enrichment ratios, being the final concentration divided by the initial. Supporting the conclusions drawn from Figure 4.3.22, the enrichment ratios for Port Kembla and Bolivar 95 were both 1.2 or above, indicating a net shift into the mineral fraction. Chelsea 96, Bolivar 97 and Werribee 97 all had values approximating 1.0, indicating their lack of change, while West Hornsby had an enrichment ratio of 0.85, signifying a loss from the mineral phase during the incubation.

In Chapter 2 (*section 2.3.3*) a hypothesis was raised that the ratio of organically bound Cu: organic carbon may be a better predictor of isotopically exchangeable Cu (CuE) than the parameter of total Cu alone. This notion was not investigated at that point because of the large number of samples involved in that investigation and the amount of labour required for the necessary fractionations. However, the fractionation of the six biosolids examined in this incubation experiment offered an opportunity to test this hypothesis. The larger dataset of Chapter 2 produced an R^2 value of 0.70 for the regression of Log_{10} Total Cu (mg/kg) against CuE, and the same regression for the six biosolids in this incubation experiment returned an R^2 of 0.67 (Figure 4.3.23). However, when the ratio Log_{10} organic Cu: Log_{10} organic carbon was regressed against CuE for these biosolids, as hypothesised, an improved R^2 of 0.89 was returned. This increased amount of variance accounted for suggests that the organically bound

Cu is chiefly involved in isotope exchange reactions, and thus is important in determining the labile pool. Therefore, although the greatest proportion of biosolid Cu was found to be in the mineral fraction (Table 4.3.7), the organically bound Cu may have a disproportionately large influence on isotopic exchange reactions. Such a conclusion is consistent with that drawn by McLaren and Crawford (1974), who found the majority of isotopically exchangeable Cu in 24 British soils was associated with the organic fraction. Therefore, for this limited set of samples, the hypothesis was upheld, and so justifies further investigations to determine whether the hypothesis can stand up to wider examination. Such investigations are necessary before this hypothesis can be declared proven.

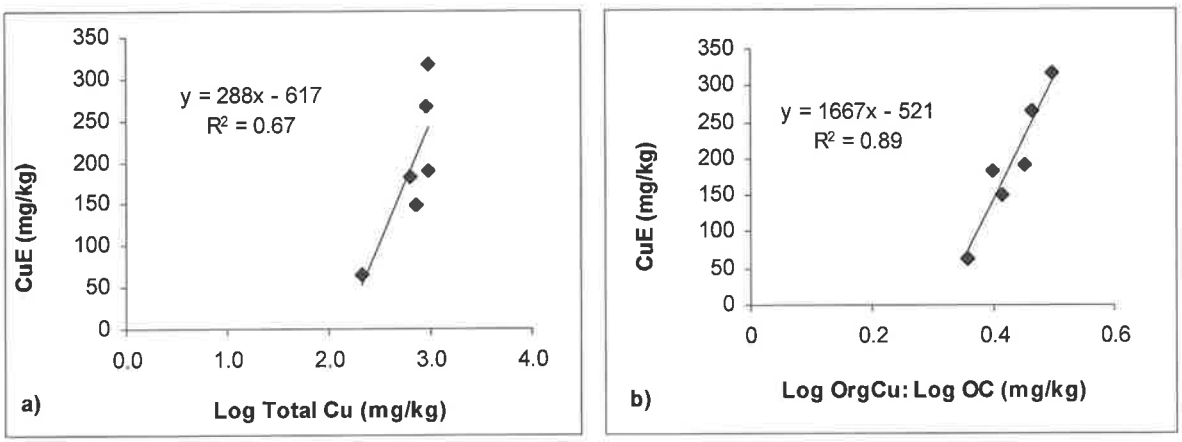


Figure 4.3.23: Isotopically exchangeable Cu (CuE) against a) Log_{10} total Cu concentration and b) the ratio of Log_{10} organic fraction Cu: Log_{10} organic carbon.

4.3.8 Plant Uptake

Yields for plants grown in biosolid only treatments were generally less than that achieved in their soil-blended counterparts (Figure 4.3.24). The exceptions were Chelsea 96, for which there were no significant differences in yield between biosolid and soil/biosolid blend, and Werribee 97, where greater yields were observed in the biosolid treatment (Figure 4.3.24). In terms of percentage of control yield, the biosolids from Bolivar produced less than 50%, while Port Kembla and West Hornsby returned less than 30%. This indicates that the biosolid

treatments were generally a more hostile environment for the plants than the soil/biosolid blends. The Cu concentrations in the above ground plant tissues were the same regardless of whether the plants were grown in biosolids or in soil/biosolid blends (Figure 4.3.25), suggesting that either poor growth led to sub-optimum Cu assimilation in the biosolids treatments or that the plants had already achieved their maximum uptake and thus could not assimilate additional Cu despite there being a greater supply. Such physiological limitation to uptake was observed and discussed by Hamon *et al.* (1999), while Dowdy *et al.* (1978) similarly noted no further increases in Cu and Zn concentrations in edible portions of snap beans (*Phaseolus vulgaris*) despite increases in biosolid loading. However, the results for the Cu-dosed reference soil only partially support this inference, because even though a plateau-type effect was observed in terms of shoot Cu concentration (Figure 4.3.26), statistically significant differences were found for shoot Cu concentrations among several of the various soil Cu concentrations imposed (Figure 4.3.25). Therefore, plant maximum uptake rate limitations cannot be the main reason for the lack of shoot concentration differences observed between biosolid and soil/biosolid blend treatments. For the reference soils dosed at 50-400 mg Cu/kg soil the relative increases in shoot Cu concentration per unit of added soil Cu were much smaller than at lower soil Cu concentrations (Figure 4.3.26), and thus it is a much finer line when distinguishing between available Cu levels in this concentration range using the rye grass assay. Biosolid and soil-blend treatments with Cu availabilities corresponding to within this range would therefore be very difficult to separate given the confounding effects of differing growth responses (see Figure 4.3.4) on plant tissue concentrations. Therefore growth limitation and variability, more so than maximum Cu uptake rate limitations, would likely account for why the rye grass trial did not identify clear differences in available Cu amongst the various biosolid treatments.

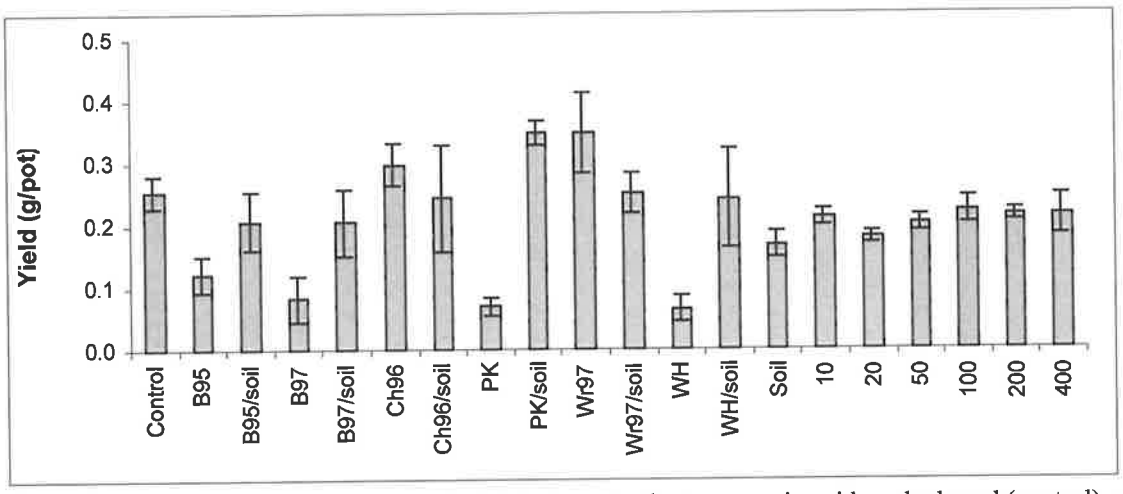


Figure 4.3.24: Yields (oven dry mass, g/pot) for rye grass plants grown in acid washed sand (control), and all biosolids, soil/biosolid blends, soil, and dosed reference soil (10, 20, 50, 100, 200, 400 mg Cu/kg) treatments at time 0 in the incubation experiment. Error bars indicate 2x standard error (n=3).

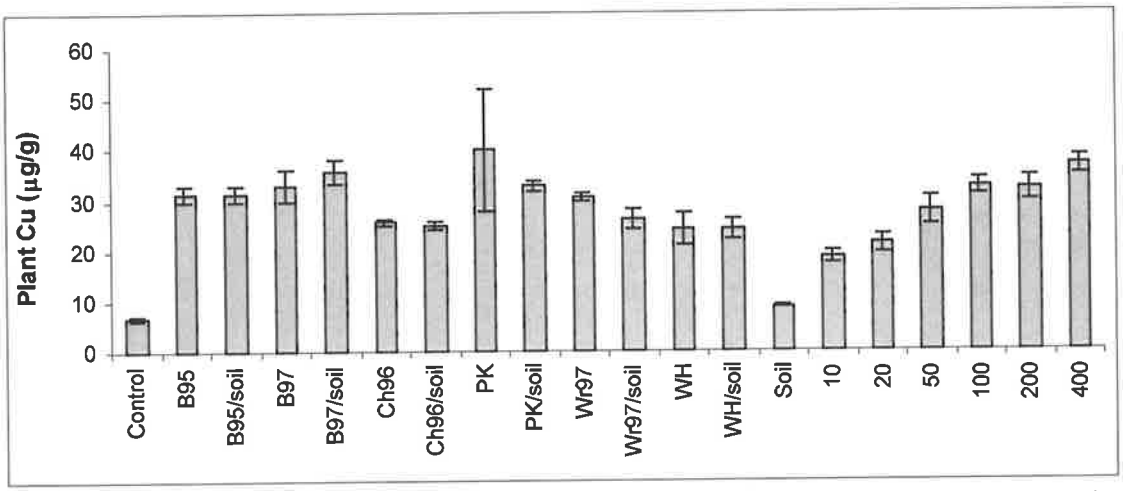


Figure 4.3.25: Copper concentration (µg/g) in above ground tissue of rye grass grown in control sand and all treatments from time 0 of the incubation experiment. Error bars indicate 2x standard errors (n=3).

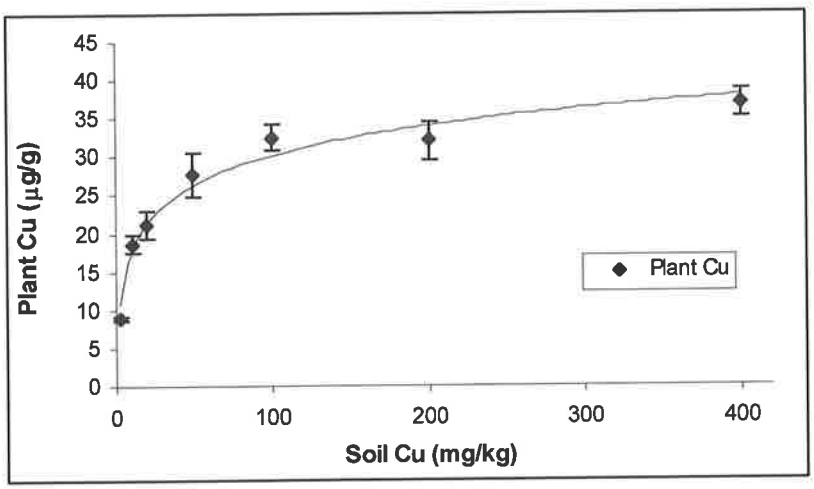


Figure 4.3.26: Copper concentration (µg/g) in above ground tissue of rye grass grown in Cu-dosed reference soils (0, 10, 20, 50, 100, 200, and 400 mg Cu/kg). Error bars indicate 2x standard errors (n=3).

There was no relationship between total Cu and plant tissue Cu for either biosolid or soil/biosolid blend treatments, but a typical logarithmic relationship did exist for the Cu-dosed soils (Figure 4.3.27). This follows the hypothesis that biosolid metals are not well represented by metal salts in plant uptake experiments (Brown *et al.* 1998). In the case of the soil/biosolid blends, concentrations of $CaCl_2$-extractable Cu were correlated to plant Cu returning an R^2 of 0.66 when the $CaCl_2$-extractable Cu values were log-transformed (Figure 4.3.28). The relationship was also strong for the Cu-dosed reference soils (Figure 4.3.28), where the R^2 was 0.97, being equal to the R^2 value for total Cu v plant Cu. However, there was no relationship between $CaCl_2$-extractable Cu and plant tissue Cu in the biosolid treatments (Figure 4.3.28).

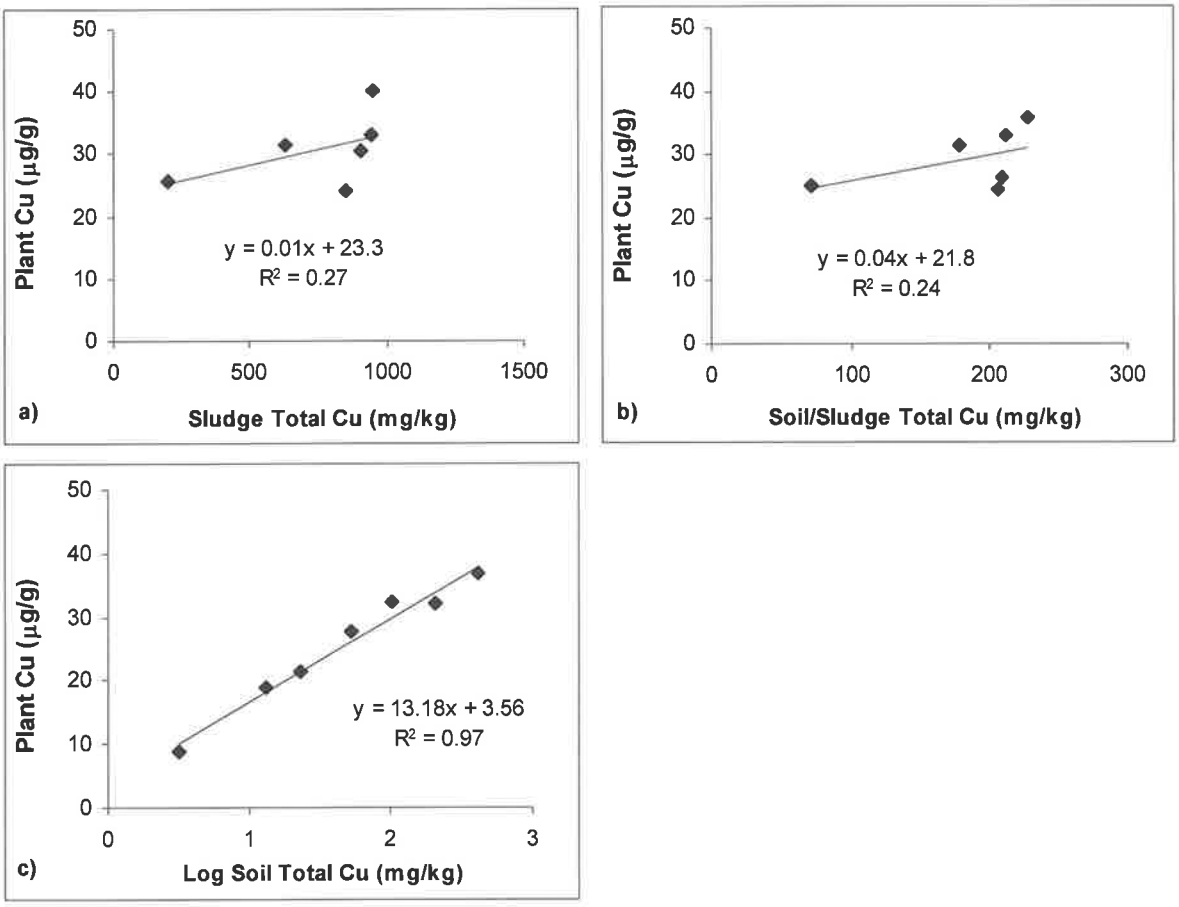


Figure 4.3.27: Plant Cu concentration (µg/g) v total Cu for time 0 samples; a) biosolid treatments, b) soil/biosolid blends, and c) Cu-dosed reference soils.

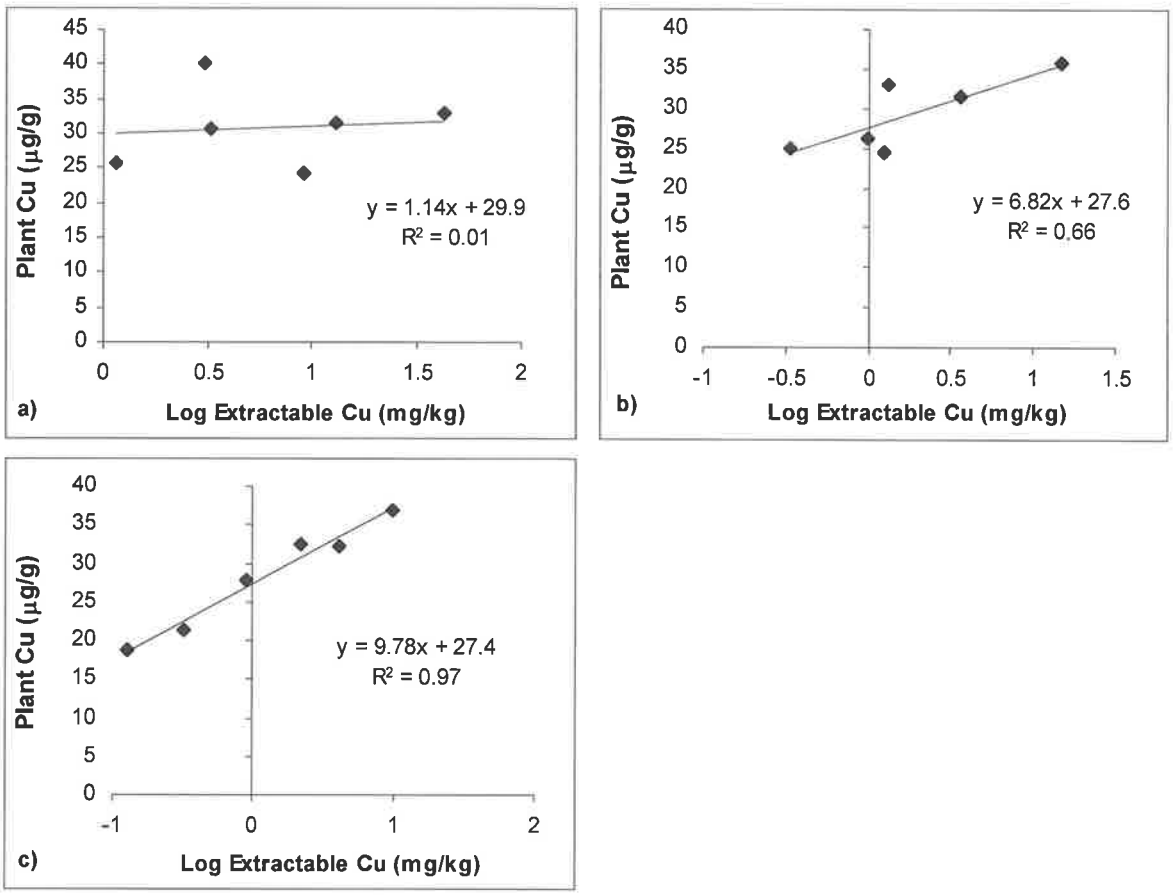


Figure 4.3.28: Plant Cu concentration v. $CaCl_2$-extractable Cu for a) biosolids, b) soil/biosolid blends, and c) Cu-dosed reference soils.

Isotopically exchangeable Cu (CuE) also showed a relationship with plant Cu for soil/biosolid blends and for the dosed reference soils (Figure 4.3.29). The R^2 values were very similar to those between plant Cu and $CaCl_2$-extractable Cu, but, unlike the $CaCl_2$-extractable Cu values, the values for CuE were normally distributed. Importantly, CuE was the only measure to show any relationship with plant tissue Cu in the case of biosolids only treatments (*i.e.* no soil), returning an R^2 of 0.57 (Figure 4.3.29). There was no relationship between EDTA-extractable Cu and Cu concentrations in plants in these biosolid treatments (Figure 4.3.30a), but there was a relationship for the Cu-dosed reference soils (Figure 4.3.30b). Similarly, there was a strong relationship between pCu^{2+} and plant Cu ($R^2 = 0.92$) for the dosed reference soils, but there was no relationship between the factors for the biosolids or soil/biosolid blend treatments (Figure 4.3.31).

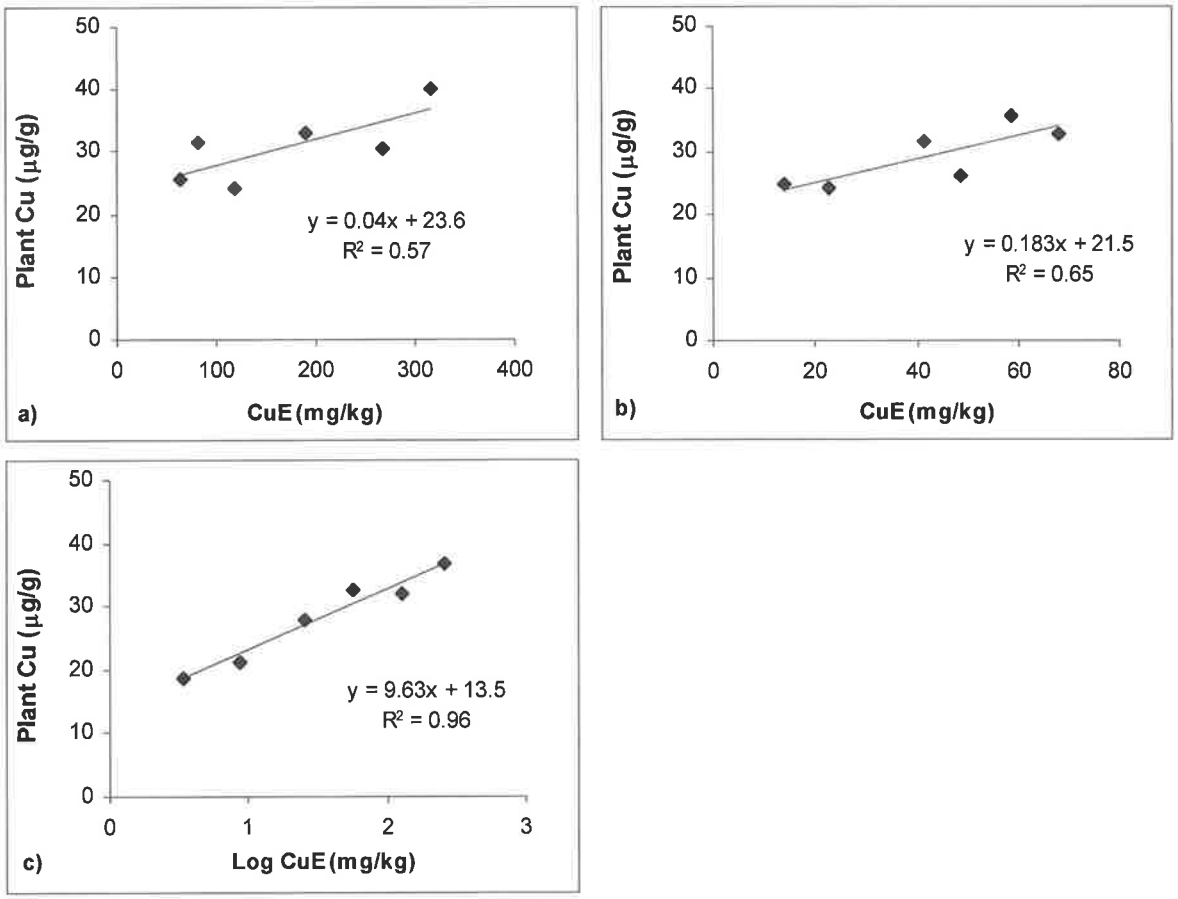


Figure 4.3.29: Plant tissue Cu v. isotopically exchangeable Cu (CuE) for a) biosolids, b) soil/biosolid blends, and c) Cu-dosed reference soil.

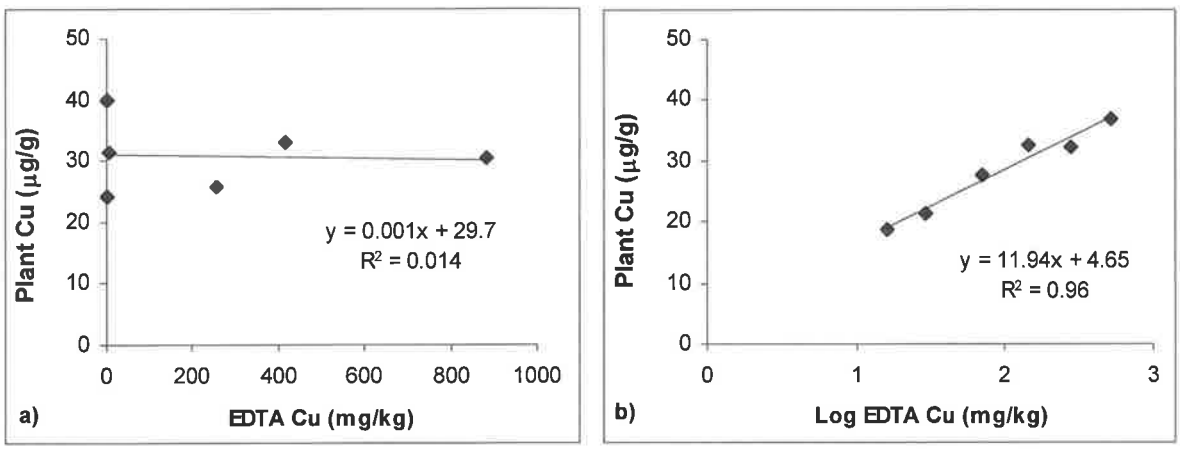


Figure 4.3.30: Plant Cu v. EDTA-extractable Cu for a) biosolids and b) Cu-dosed reference soils.

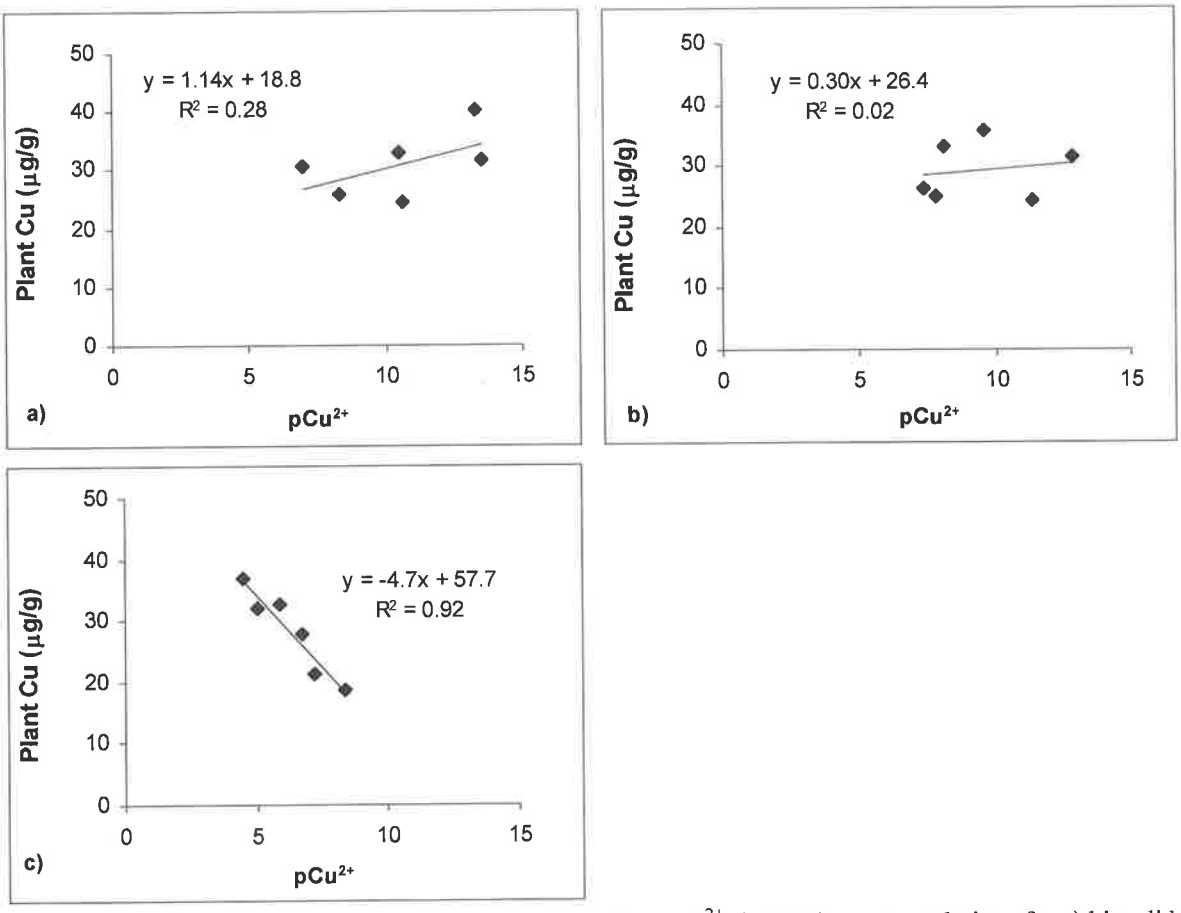


Figure 4.3.31: Plant Cu concentration v. cupric ion activity (pCu^{2+}) in $CaCl_2$ extract solutions for a) biosolids, b) soil/biosolid blends and c) Cu-dosed reference soils.

Inspection of the regression equations for plant tissue Cu concentrations v. the various chemical measures employed (Figures 4.3.27 – 4.3.31, and summarised in Table 4.3.8), reveals that isotopically exchangeable Cu was the only method to account for an appreciable amount of the variation observed in plant Cu concentrations in the biosolid treatments, but that the percentage variance accounted for was still only 57%. Therefore, with less than 60% of the accumulated variation in plant Cu concentrations accounted for, even for the best soil measure, the data suggests that plant shoot Cu concentration for the biosolids samples was not a good measure of the available Cu fraction. The failure was due most probably to poor plant growth in biosolid treatments restricting Cu assimilation and translocation rates. This highlights the need for non-plant based measures of availability in environments that may be hostile to the growth of common test plants, or in circumstances where contaminant levels may exceed those producing the maximum assimilation rate in plants. For the soil/biosolid

blends, which generally had better growth, isotopically exchangeable Cu and $CaCl_2$-extractable Cu were better related to plant Cu than in the case of the biosolid treatments (Table 4.3.8). This outcome adds further support to their use as measurements of available metals. However, it must be borne in mind that the plant Cu concentrations recorded for all biosolids and soil/biosolid blend treatments fell within a very narrow range (90% of treatments were within a span of 10 μg Cu/g plant material, while standard errors were commonly between 1 and 2 μg/g), thus over interpretation of the results of this plant trial must be resisted.

Table 4.3.8: Simple Linear Regression Equations for Plant Tissue Cu Concentration (y) Against Various Chemical Measures (x)

x variable	Biosolids Only	R^2
Total Cu	y = 0.01x + 23.3	0.27
$CaCl_2$ extractable Cu	y = 1.14Logx + 29.9	0.01
CuE	y = 0.04x + 23.6	0.57
EDTA Cu	y = 0.001x + 29.7	0.01
pCu^{2+}	y = 1.14x + 18.8	0.28
x variable	Soil/biosolid blends	R^2
Total Cu	y = 0.04x + 21.8	0.24
$CaCl_2$ extractable Cu	y = 6.82Logx + 27.6	0.66
CuE	y = 0.183x + 21.5	0.65
EDTA Cu	N/A	
pCu^{2+}	y = 0.30x + 26.4	0.02
x variable	Dosed Reference soils	R^2
Total Cu	y = 13.18Logx + 3.56	0.97
$CaCl_2$ extractable Cu	y = 9.78Logx + 27.4	0.97
CuE	y = 9.63Logx + 13.5	0.96
EDTA Cu	y = 11.94Logx + 4.65	0.96
pCu^{2+}	y = -4.7x + 57.7	0.92

When plants were grown in the time 21-month samples the yields were smaller than for the corresponding time 0 samples in many of the treatments, and in the case of Bolivar 97, Port Kembla and West Hornsby biosolids treatments plant growth failed completely (Figure 4.3.32). For the remaining biosolid treatments, and for the majority of the soil/biosolid blends and the reference soils, the Cu concentration in above ground plant tissue was not statistically different than at time 0 (Figure 4.3.33), and thus showed no changes with time in terms of plant uptake. Port Kembla and West Hornsby soil/biosolid blends did not follow this pattern, but rather they recorded lower plant tissue Cu concentrations in the 21-month samples than in

the time 0 samples. However, as was the case with the time 0 samples, the spread of plant tissue Cu concentrations was within a very narrow band (only 10 μg/g variation across all biosolid and soil/biosolid blend treatments), thus caution is required when making inferences from these data. As was the case at time 0, the tissue Cu concentrations for plants grown in the dosed reference soils at time 21 months were strongly related to all chemical measures, returning R^2 values of > 0.95 when regressed against the $\log_{10}$ transformed values of total Cu, $CaCl_2$-extractable Cu, EDTA-extractable Cu and isotopically exchangeable Cu. However, unlike at time 0, the time 21 month plant trial showed no relationship between plant tissue Cu concentration and any of the chemical measures of availability ($R^2 < 0.1$) for soil/biosolid blend treatments. Due to growth failure in three out of the six biosolid treatments, the available dataset was considered too small to produce statistically relevant results for this group (*i.e.* the validity of a three point regression would be very dubious).

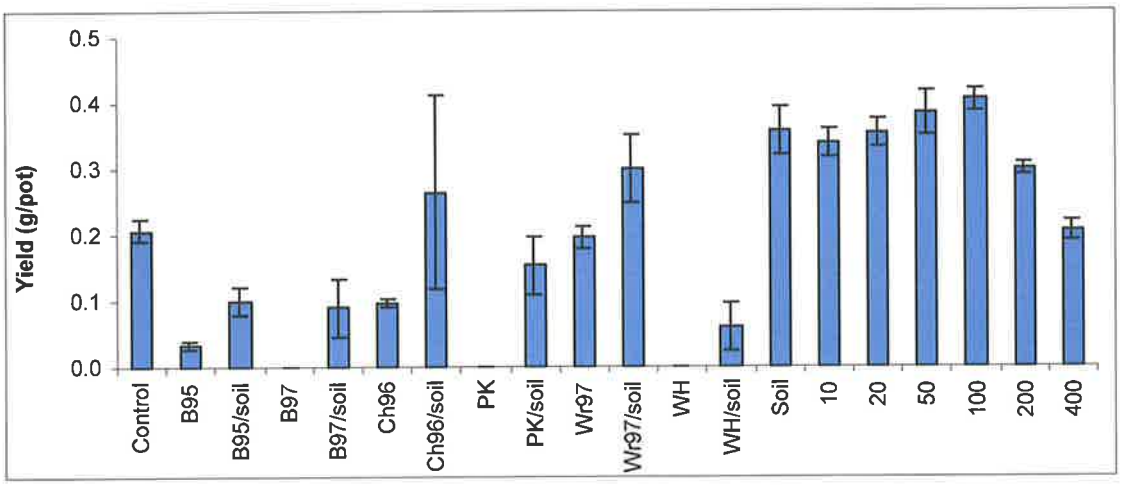


Figure 4.3.32: Yield of rye grass (g/pot) grown in all treatments after 21 months of incubation. Error bars indicate 2x standard errors (n=3).

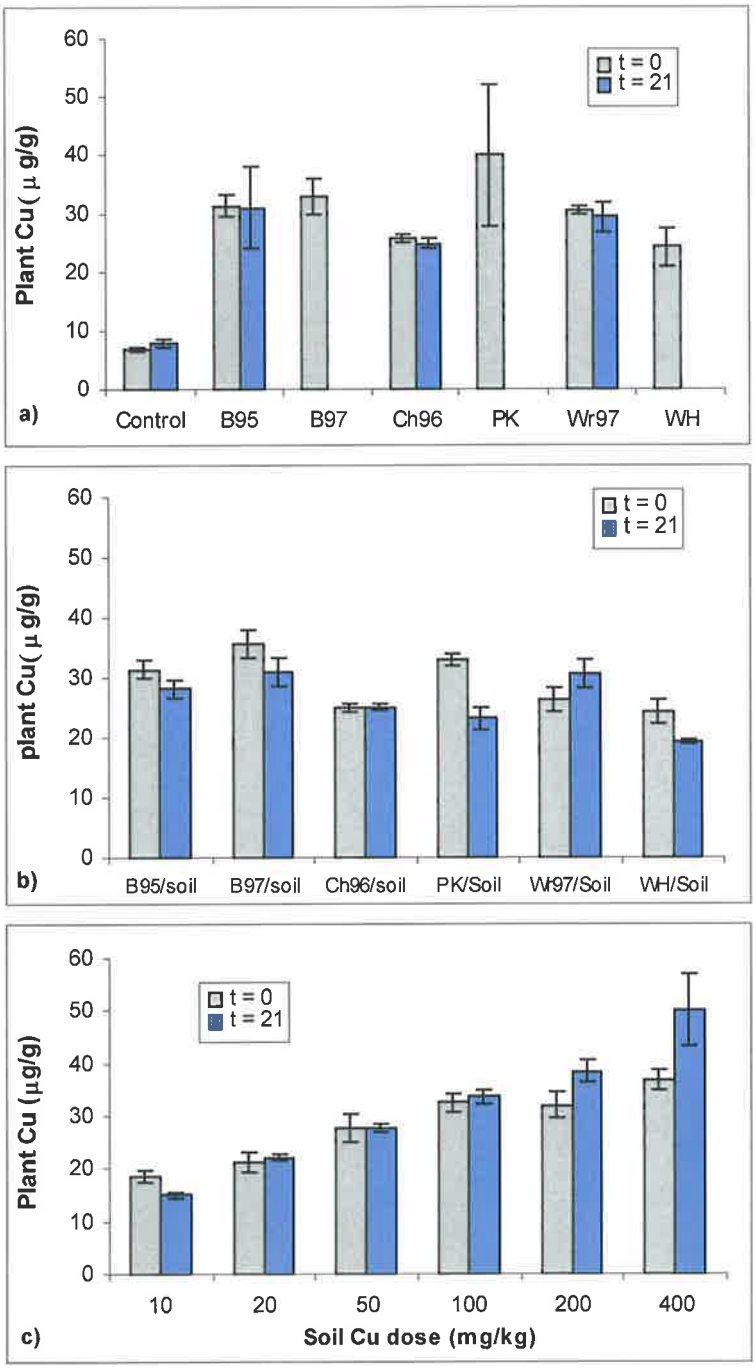


Figure 4.3.33: Copper concentration (µg/g) in above ground tissue of rye grass grown in control sand and all treatments after 0 and 21 months of incubation; a) biosolids, b) soil/biosolid blends and c) Cu-dosed reference soils (10 – 400 mg Cu/kg). Error bars indicate 2x standard errors (n=3).

Failure to correlate chemical measures with plant tissue Cu concentrations is not uncommon in soil research (Beckett *et al.* 1983; Davies *et al.* 1987; Haq *et al.* 1980; Jarvis and Whitehead 1981). However, in this case, the lack of consistent relationships is more likely to be due to plant assimilation limitations than to any failure of the chemical measures to target the available fraction. This argument is based on the fact that total Cu and isotopically

exchangeable Cu both varied by an order of magnitude between the different biosolid treatments and $CaCl_2$-extractable Cu varied by two orders of magnitude, whereas plant shoot Cu concentrations were all virtually within a range of 10 μg Cu/g plant material. It is unlikely that across such a broad range of values for these various techniques that the available portion in each of the biosolid treatments would be almost the same, as implied by the nearly uniform plant tissue concentrations. In addition to the effects of sub-optimal growth discussed previously, the lack of variation in shoot tissue Cu concentration observed here may be due to the low translocation rate of Cu from roots to shoots (Brams and Fiskell 1971; Sauerbeck 1991). Kozlov *et al.* (2000) found leaf tissue Cu concentration in Birch trees was only raised by 3 – 9 μg/g above that of plants grown in control soil despite contamination levels in the soil of up to 1000 mg Cu/kg. They found the great majority of assimilated Cu was retained in the root. If root concentrations had been analysed here it is possible that stronger or more consistent relationships with the measured parameters may have been identified. However, it was impossible to separate biosolid residues from the roots, hence such an analysis could not be done.

4.4 Conclusions

The results show that C decomposition occurred during the incubation experiment, with up to approximately 30% of the initial total C lost from the biosolids. However, the NMR analyses revealed that decomposition was not specific to any particular type of C, but rather losses were consistent across all defined C groups. Isotopically exchangeable Cu (CuE), EDTA-extractable Cu, and pCu^{2+} all indicated Cu availability increased with time for several of the biosolids examined. Similarly, the fractionation procedure indicated that Cu can migrate from the organic phase, and possibly also from the mineral phase, into the readily extractable fraction over time. The fractionation procedure also pointed out the possible importance of the

organically bound Cu in determining CuE. However, the increases in available Cu with time appear to have been mainly due to the pH decreases that occurred during the incubation, rather than to any release of metals from the decomposition of organic matter. Strong relationships between pH and measures of available Cu were consistently found and therefore lend strong support to this possibility. However, relationships were also identified between changes in CuE and changes in total C%. To further complicate the issue, pH changes over time were also found to be related to changes in total C%. Therefore the question remains as to whether the observed increases in metal availability were caused by microbial decomposition or were simply a function of pH reduction, and thus a further investigation is required to separate these factors (see Chapter 5). Nevertheless, be it a question of organic matter mineralisation or simple pH change, the outcome remains that metal availability increased with time in this investigation, and so has implications for biosolids use in the field. If indeed the increases were due to pH change, as appears most likely, then it may become necessary to increase the frequency of pH monitoring on sites receiving biosolids applications (re-testing of soil pH is only required after 10 years of applications, and only if amendments are to continue, under current South Australian regulations (SAEPA 1996). This may be a necessary adjustment to ensure low risk conditions are maintained on such sites.

5. Distinguishing Between pH and OC Effects by pH Normalisation

5.1 Introduction

Isotopically exchangeable Cu (E value or CuE) increased over time for some of the biosolids and soil/biosolid blends examined in the 21-month incubation experiment described in Chapter 4. However, changes in both pH and total C % occurred during the incubation, with the increases in CuE seemingly being related to both. To further complicate the issue, the pH and C changes were related to each other, indicating an interaction or confounding effect. Therefore the increased availability of Cu with time may have been due to either mineralisation of organic matter and subsequent release of previously bound metals (*i.e.* the time bomb concept), or simply to pH change. It is necessary to separate the effects of the pH and C factors in order to identify the driving variable behind the change in metal availability. This is important because each would have different implications for the long-term fate of biosolid metals, and for the management of biosolid-amended soils. Therefore a study was conducted to determine the effect of pH reduction on the CuE of the biosolids and biosolid-amended soil examined in the previous incubation experiment, with the results used to pH normalise the CuE values recorded at the different measurement times of that experiment. By normalising the recorded CuE values for pH the extent of any changes caused by OC mineralisation during the incubation could then be assessed.

5.2 Methods

Acid titrations were performed on archived time = 0 samples of the biosolids and soil/biosolid blends to determine the doses of acid required to lower the pH to the levels observed during the incubation experiment. For each of the biosolids and soil blends multiple 4 g samples were equilibrated with a 0.01M $CaCl_2$ solution (40 mL) containing various amounts of hydrochloric acid. Equilibration was achieved by end-over-end shaking for 40 hours, after which the samples were centrifuged and the pH determined in the supernatant solution using

an Orion pH electrode and meter. The results were used to construct pH titration curves (Figure 5.2.1). To conserve samples the biosolid treatments for Port Kembla and Bolivar 95 were not titrated here, rather the titration curves determined for their non-leached counterparts (Chapter 2) were used as a guide for the acid additions to follow.

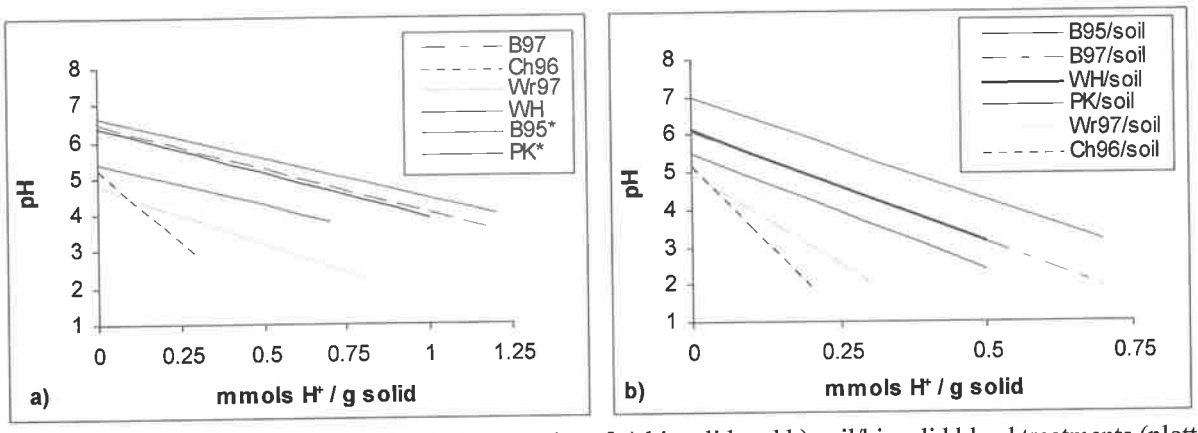


Figure 5.2.1: Acid titration curves for time 0 samples of a) biosolid and b) soil/biosolid blend treatments (plotted equations shown). *Titration curves for Bolivar 95 and Port Kembla biosolids are those determined in Chapter 2.

Using information gained from the acid titrations a procedure was set up to determine the isotopically exchangeable Cu (CuE) in pH adjusted samples of all biosolids, soil/biosolid blends, and Cu-dosed reference soils examined in the incubation experiment documented in Chapter 4. Measurements of CuE were performed as before, with 4 g samples equilibrated with 40 mL 0.01M $CaCl_2$ for 40 hours on end-over-end shakers after which the radioisotope ^{64}Cu was added (in 0.1 mL solution containing approximately 2 MBq activity). After a further 24-hour equilibration period, samples were centrifuged for 10 minutes at 1200 *g*, the supernatant solutions filtered through 0.45 μm filters, and then measured for radioactivity (gamma counter) and total solution Cu (ICP-AES). The difference in this procedure was that for each biosolid, soil blend and Cu-dosed reference soil, samples were equilibrated with 40 mL 0.01M $CaCl_2$ containing various doses of acid (added as 1M HCl prepared in 0.01M $CaCl_2$). Five acid dose levels, in duplicate, were imposed on each of the biosolids, blends and reference soils. A further additional step in this CuE procedure was that 5mL of the supernatant solution was subsampled for pH determination (*i.e.* a separate subsample from

those used for ICP analysis and gamma counting). The resulting values of the CuE and pH determinations were used to construct plots of change in CuE (ΔCuE) v change in pH (ΔpH), the equations from which were used to pH normalise the CuE values recorded during the Chapter 4 incubation experiment. This allowed the effect of pH change during incubation to be accounted for, thus making it possible to determine the extent of the effects of organic matter mineralisation on Cu availability.

5.3 Results and Discussion

Strong relationships were observed between CuE and pH change for Bolivar 97, Port Kembla, Werribee 97 and West Hornsby biosolids (Figure 5.3.1 and Table 5.3.1). Clearly, for these biosolids, decreases in pH bring more Cu into the isotopically exchangeable pool. The pH reductions imposed on Bolivar 95 and Chelsea 96 caused minimal changes to CuE (Figure 5.3.1), which is consistent with the minimal or zero change in CuE observed throughout the incubation experiment for these biosolids (Chapter 4, Figure 4.3.7). This differs, however, to the results obtained for the unleached Bolivar 95 biosolids that were subjected to pH alterations in Chapter 2 (*section 2.3.4*). In that investigation a strong relationship was noted for CuE and pH change ($R^2 = 0.94$). However, in that Chapter 2 investigation, the aim was to examine the general relationship between pH and CuE, thus the pH was reduced by up to 3 units. By contrast, for the current investigation the pH was only altered so as to span the range observed during the incubation study (1.2 units for Bolivar 95). Thus it is possible that if a greater range of pH change was imposed, a relationship approaching that observed in Chapter 2 may have been noted. However, that would not be relevant to the issue of pH normalisation being dealt with here.

The strength of the ΔpH v ΔCuE relationship varied among the soil/biosolid blends (Figure 5.3.2). There was a poor relationship between ΔpH and ΔCuE for both Bolivar biosolid blends

(R^2 <0.5) (Table 5.3.1), which matches the lack of significant change in CuE values seen for these during the incubation experiment (Figure 4.3.7). By contrast, there was a strong relationship for ΔpH v ΔCuE (R^2 >0.93) for the Port Kembla/soil and Werribee 97/soil blends, which accords with the increases in CuE observed for these treatments in the incubation study. There were linear relationships for ΔpH v ΔCuE at 50 mg Cu/kg and above for the Cu-dosed reference soils (Figure 5.3.3). For the 10 and 20 mg Cu/kg reference soils the CuE values varied less than 0.5 mg/kg across the entire pH range imposed (3±0.3 and 6.5±0.3, respectively). Therefore regression equations of ΔpH v ΔCuE are not displayed in the results tables or figures for the 10 and 20 mg/kg reference soils.

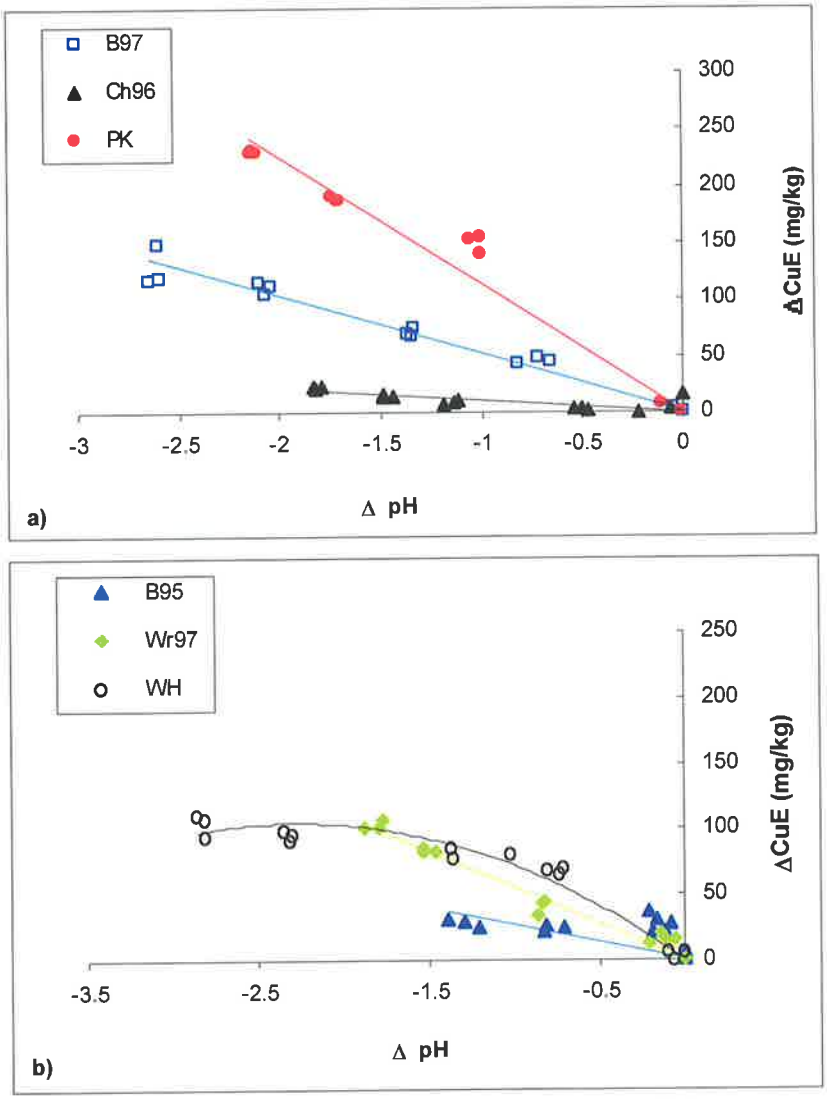


Figure 5.3.1: Change in CuE (ΔCuE, mg/kg) v. change in pH (ΔpH) for biosolids; a) Bolivar 97, Chelsea 96 and Port Kembla, and b) Bolivar 95, Werribee 97 and West Hornsby.

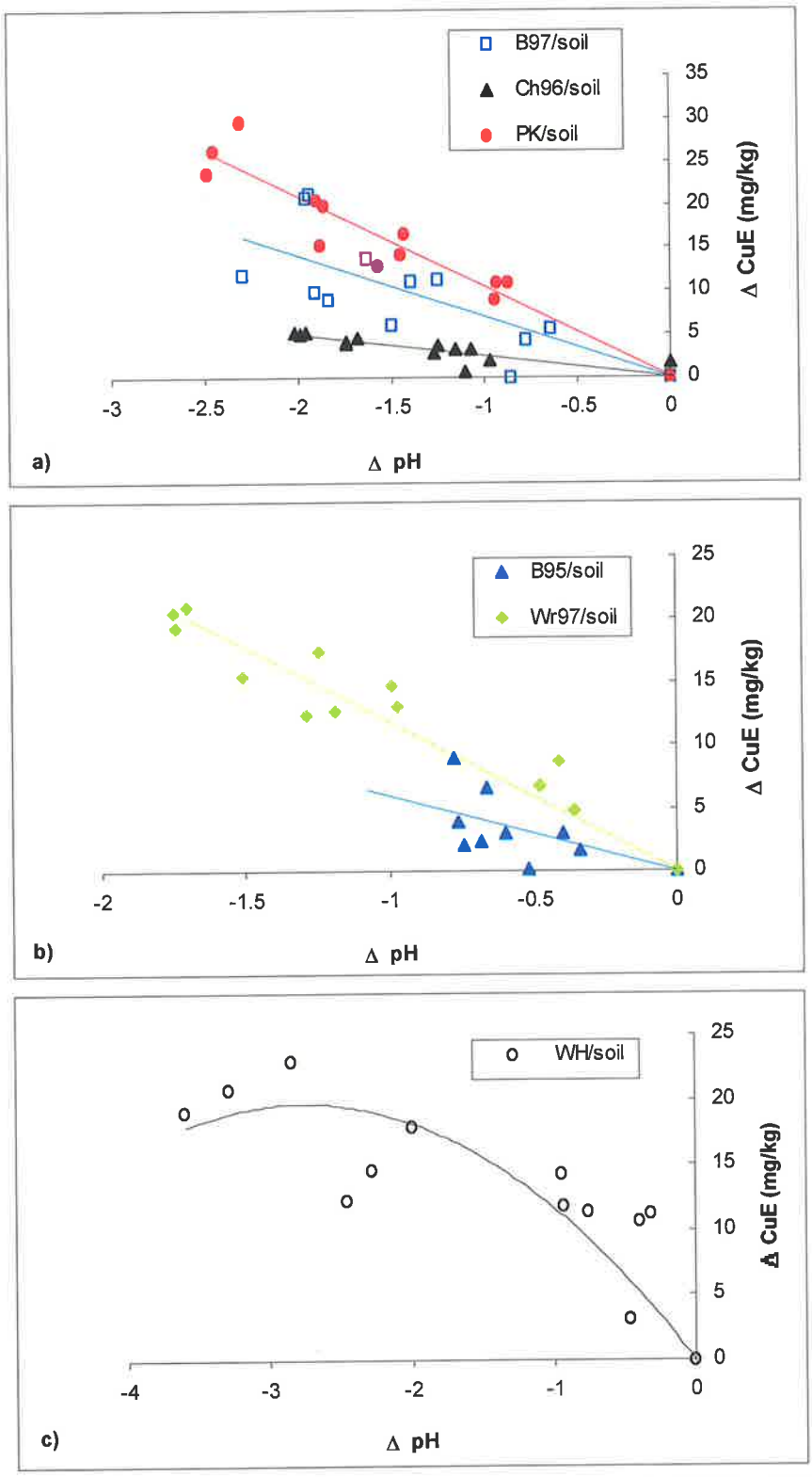


Figure 5.3.2: Change in CuE (ΔCuE, mg/kg) v. change in pH (ΔpH) for soil/biosolid blends; a) Bolivar 97, Chelsea 96 and Port Kembla, b) Bolivar 95and Werribee 97, and c) West Hornsby.

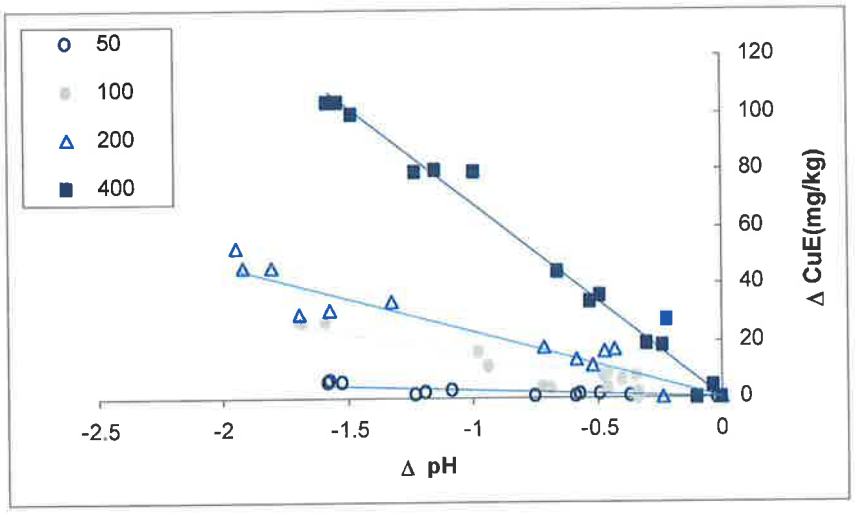


Figure 5.3.3: Change in CuE (ΔCuE, mg/kg) v. change in pH (ΔpH) for reference soil dosed at 50, 100, 200, and 400 mg Cu/kg soil.

Table 5.3.1: Regression Equations for ΔCuE v ΔpH for Biosolids, Soil/Biosolid Blends and Cu-Dosed Reference Soils

Biosolid	Regression Equation	R^2
B95	$\Delta E = -26.43\Delta pH$	-1.50
B97	$\Delta E = -50.96\Delta pH$	0.958
Ch96	$\Delta E = -10.38\Delta pH$	0.565
PK	$\Delta E = -113.22\Delta pH$	0.944
Wr97	$\Delta E = -54.98\Delta pH$	0.962
WH*	$\Delta E = -20.19\Delta pH^2 - 91.9\Delta pH$	0.941
Soil blend		
B95/soil	$\Delta E = -5.98\Delta pH$	0.489
B97/soil	$\Delta E = -7.08\Delta pH$	0.472
Ch96/soil	$\Delta E = -2.49\Delta pH$	0.715
PK/soil	$\Delta E = -10.48\Delta pH$	0.937
Wr97/soil	$\Delta E = -11.84\Delta pH$	0.931
WH/soil	$\Delta E = -2.56\Delta pH^2 - 14.2\Delta pH$	0.762
Reference soil		
50mgCu/kg	$\Delta E = -2.80\Delta pH$	0.755
100	$\Delta E = -14.80\Delta pH$	0.869
200	$\Delta E = -23.16\Delta pH$	0.923
400	$\Delta E = -68.13\Delta pH$	0.975

* West Hornsby data was curvilinear and best fit by a quadratic function.

The equations derived from the pH adjustment procedure (Table 5.3.1) were used to pH normalise the CuE values determined for each of the treatments in the incubation experiment (Figure 5.3.4). After pH normalisation it can be seen that t = 0 and t = 21 month CuE values were not significantly different in any of the biosolids or soil/biosolid blends. Therefore, it can be generally concluded that as long as pH is maintained in these biosolids or biosolid-

amended soils the available Cu pool (as measured by CuE) will be the same after 21 months incubation as at the beginning. However, this is not the same as stating that available Cu remains stable, because that was not the case for all of the treatments. The West Hornsby and Bolivar 97 biosolids and the West Hornsby soil/biosolid blend did have equivalent t = 0 and t = 21 month CuE values after pH normalisation but their 6 month values were elevated (Figure 5.3.4), indicating an initial rise in availability followed by a decrease down to original levels. This pattern could be explained by a release of Cu through organic matter mineralisation that was subsequently re-sorbed by mineral or resistant organic fractions. Both Bolivar and West Hornsby biosolids experienced significant C loss during the first 6 months of the incubation (Table 4.3.1, Chapter 4), which lends support to this hypothesis. This raises the question as to what binds the Cu in biosolids and biosolid amended soils, and how the binding capacity of the various components of the materials change with time (a question that is investigated in Chapter 6).

Normalisation of the reference soils for pH revealed the extent of Cu fixation to be more pronounced than previously noted (Figure 5.3.4). Once the effects of pH were accounted for, it became even more clear that the amount of Cu in the labile pool decreased with time, as has often been noted for metals added to soils in salt form (Almas *et al.* 1999; Brennan *et al.* 1980; Ma and Uren 1997; Tye *et al.* 2003). The mechanisms responsible for this reduction in the available pool are likely to include diffusion into soil pores not accessible to the bulk solution, solid-state diffusion, or other slow soil-metal fixation reactions (Cook *et al.* 1999; Ma and Uren 1997; Tye *et al.* 2003).

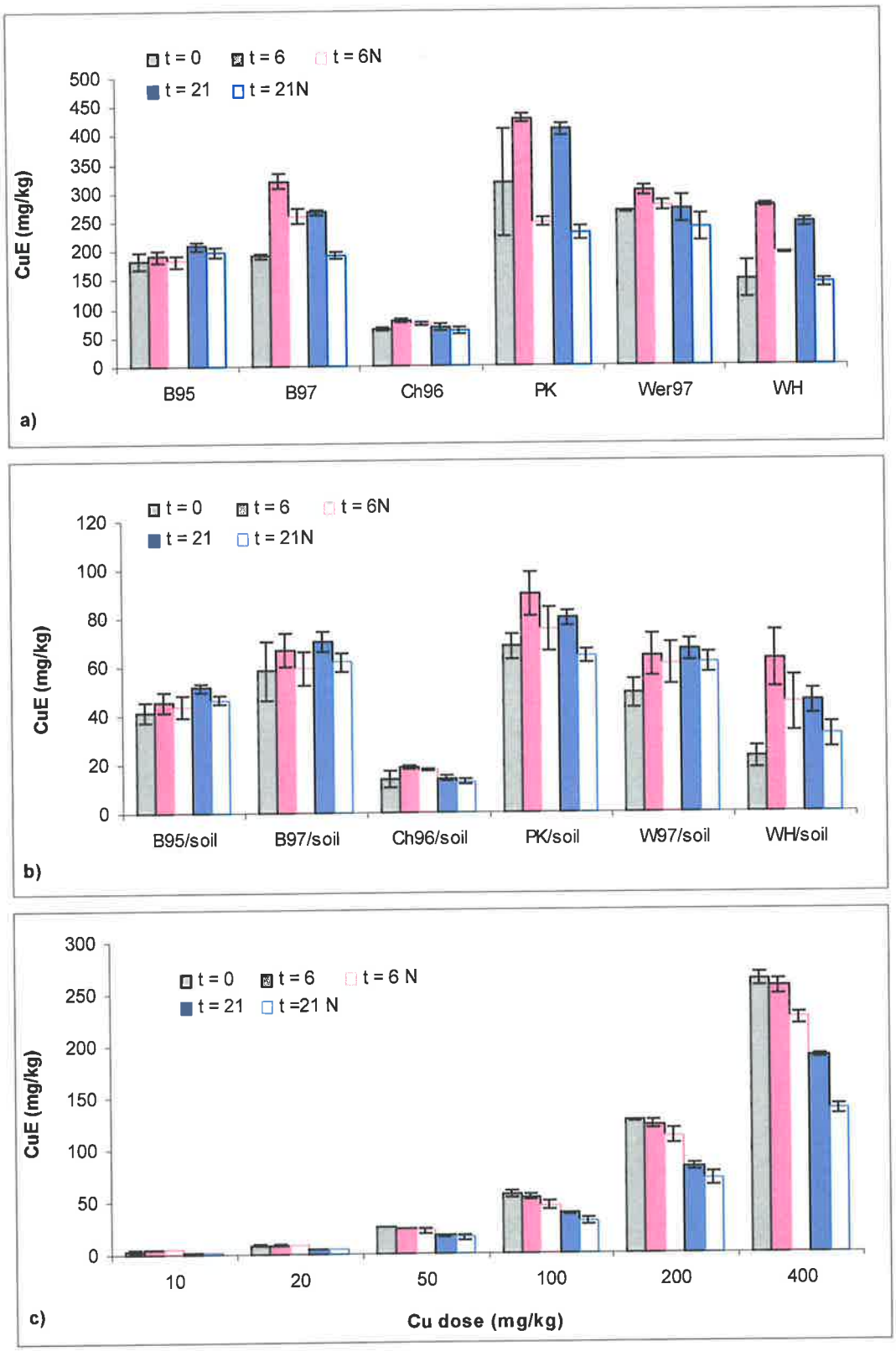


Figure 5.3.4: Measured (solid bars) and pH-normalised (open bars) CuE values from the 21-month incubation experiment; a) biosolids, b) soil/biosolid blends and c) Cu-dosed reference soil. Error bars are 2x standard errors (n=3).

The results indicate that pH was principally responsible for the changes in CuE observed for the biosolids and biosolid-amended soil, while fixation processes led to CuE decreases in soils

dosed with metal salts. However, this raises the question as to what mechanisms are operating as pH declines, *i.e.* what processes bring about the CuE value increases. Analysis of the supernatant solutions from the CuE procedure where pH was altered allowed changes in the concentration of other elements, occurring as pH declined, to be examined. Increases in solution P, Ca, Mg, Mn and Zn concentrations were highly correlated with pH decreases (Table 5.3.2), while Al concentrations were related to a lesser extent. Solution Fe and S concentrations were not consistently related to pH change (Table 5.3.2).

Table 5.3.2: Linear Correlation Co-efficients (r) for ΔpH v ΔSolution Element Concentration (mg/L) in pH Adjusted Biosolids

Biosolid	P	S	Al	Fe	Ca	Mg	Mn	Zn
B95	-0.95	-0.14	-0.86	0.70	-0.98	-0.98	-0.95	-0.92
B97	-0.97	-0.28	-0.29	0.56	-0.97	-0.99	-0.95	-0.86
Ch96	-0.98	-0.11	-0.81	-0.87	-0.97	-0.98	-0.98	-0.94
PK	-0.97	0.99	-0.77	0.41	-1.00	-1.00	-0.97	-0.93
Wr97	-0.98	0.46	-0.92	-0.88	-0.98	-0.99	-0.99	-0.98
WH	0.88	0.27	0.16	-0.08	-0.98	-0.98	-0.98	-0.89

As solution P concentrations were strongly related to pH decreases in all biosolids except West Hornsby (Figure 5.3.5), this suggests that dissolution of P-containing substances could be involved in CuE increases as pH declines. Chloride salts of Cu are too soluble to explain the pH related changes in CuE, while sulphate salts were generally thought not to be important given the data in Table 5.3.2. The possibility that changes in P solubility could explain changes in CuE was examined further by plotting increases in solution P concentration against increases in CuE resulting from the pH changes imposed during the pH adjustment procedure (Figure 5.3.6). That is, for the five pH adjustments made in this experiment, the values for ΔCuE calculated from the equations in Table 5.3.1 were plotted against the values of Δsolution P calculated from the regression equations displayed in Figure 5.3.5. The resulting plots (Figure 5.3.6) reveal a relationship between the two variables, furthering the hypothesis that P may be important in determining the extent of CuE change when pH is altered. The importance of P to sludge metal availability has been noted

previously by Jing and Logan (1992), who observed a highly significant negative relationship between Cd phytoavailability and biosolid P ($R^2 = 0.59$) in a study of 16 biosolids. The authors found no such relationship between plant Cd and either biosolid Al or Fe concentration, or Al + Fe concentration.

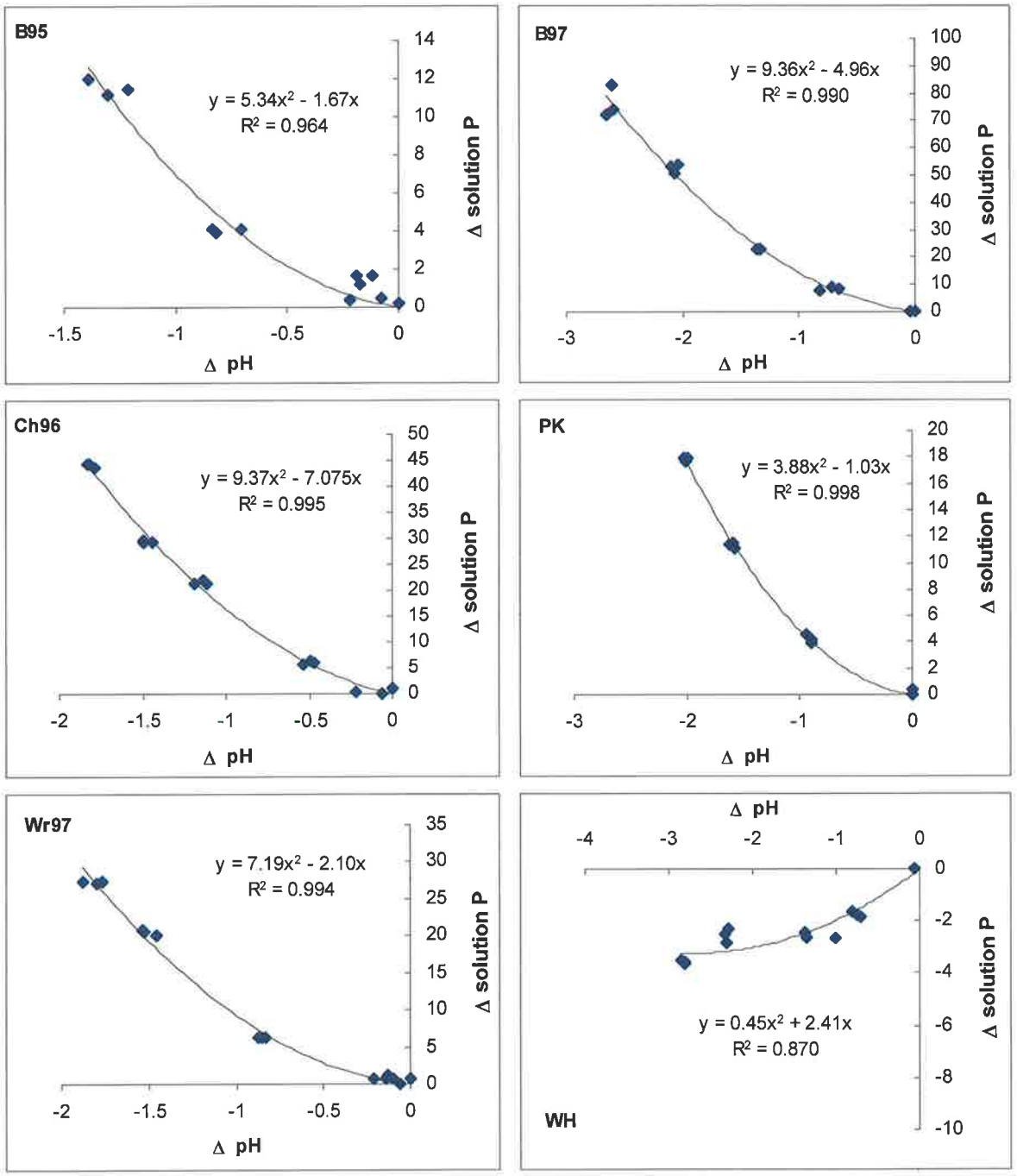


Figure 5.3.5: Change in supernatant solution P concentration (Δsolution P, mg/L) v. change in pH (ΔpH) for biosolids examined in the pH normalisation procedure.

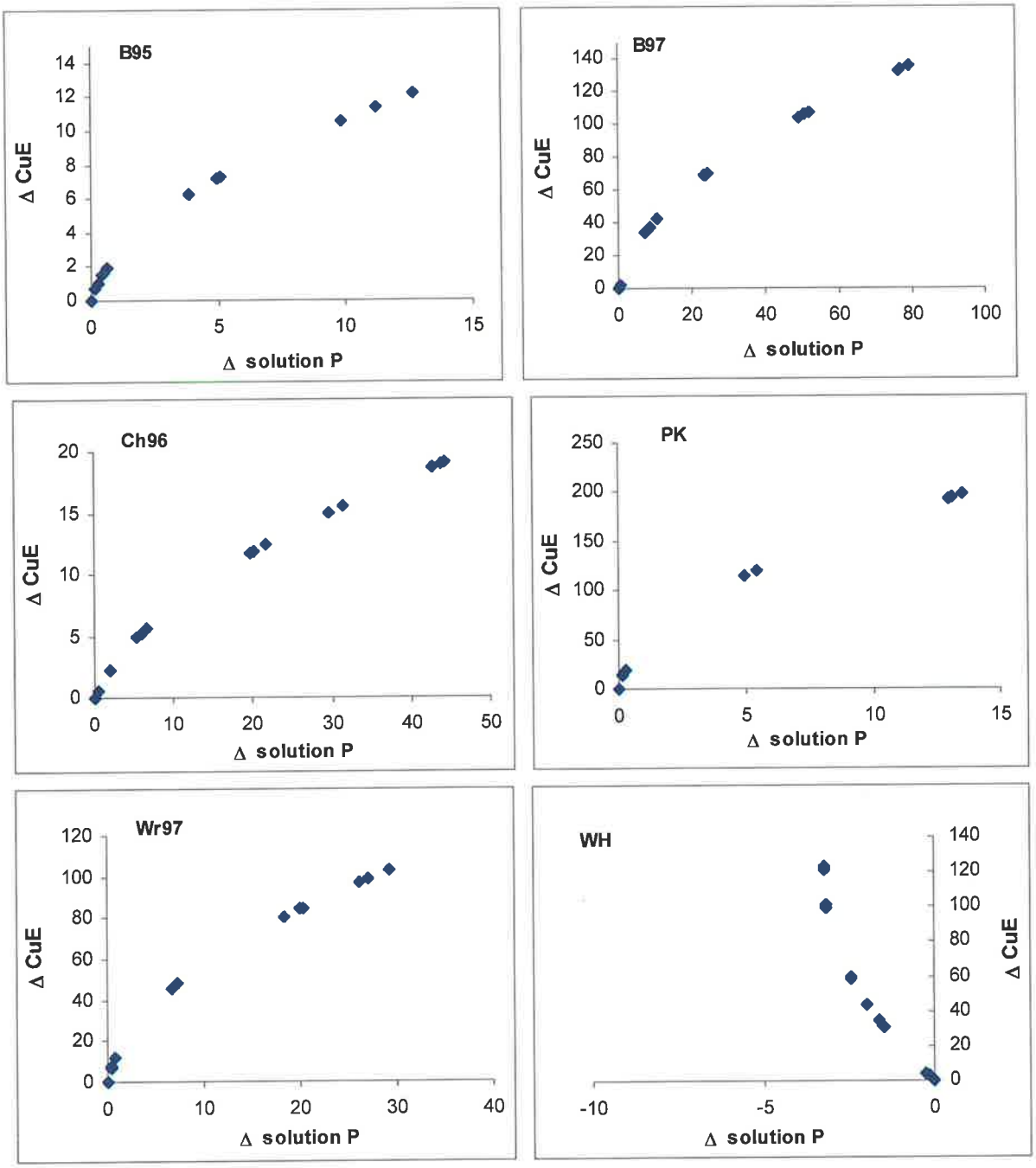


Figure 5.3.6: Change in CuE (ΔCuE, mg/kg) v. change in supernatant solution P concentration (Δsolution P, mg/L) for the pH adjustments imposed during the pH normalisation procedure.

Correlations between solution element concentrations do not however prove causality. It is possible that the increases in solution P and in CuE values may be coincidental, occurring separately as the results of distinct processes triggered by pH change. For example, solution Ca concentration increased as pH declined (Table 5.3.2), thus it may be that dissolution of co-precipitates of Cu and Ca led to CuE increases as pH declined. This possibility was explored by plotting the changes in solution Ca concentration during pH adjustment against

changes in CuE (Figure 5.3.7). The resulting plots indicate a possible relationship between increases in solution Ca and increases in CuE (except in the case of Bolivar 95 biosolids). Therefore either P or Ca (or both) may be involved in controlling changes in CuE, thus an investigation is required to examine the hypothesis that P is important to the binding of Cu in biosolids. This will be a focus of the following chapter (Chapter 6).

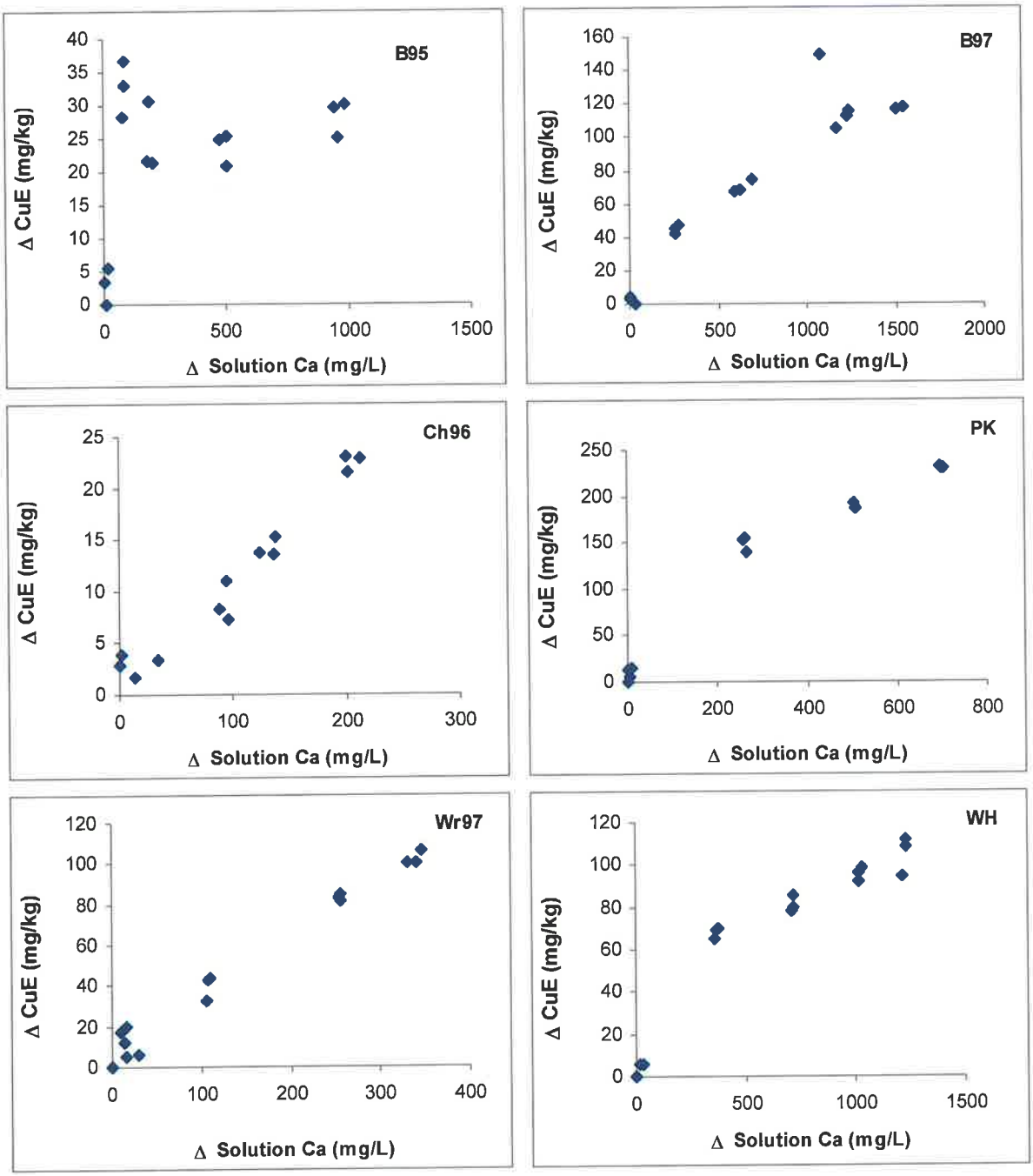
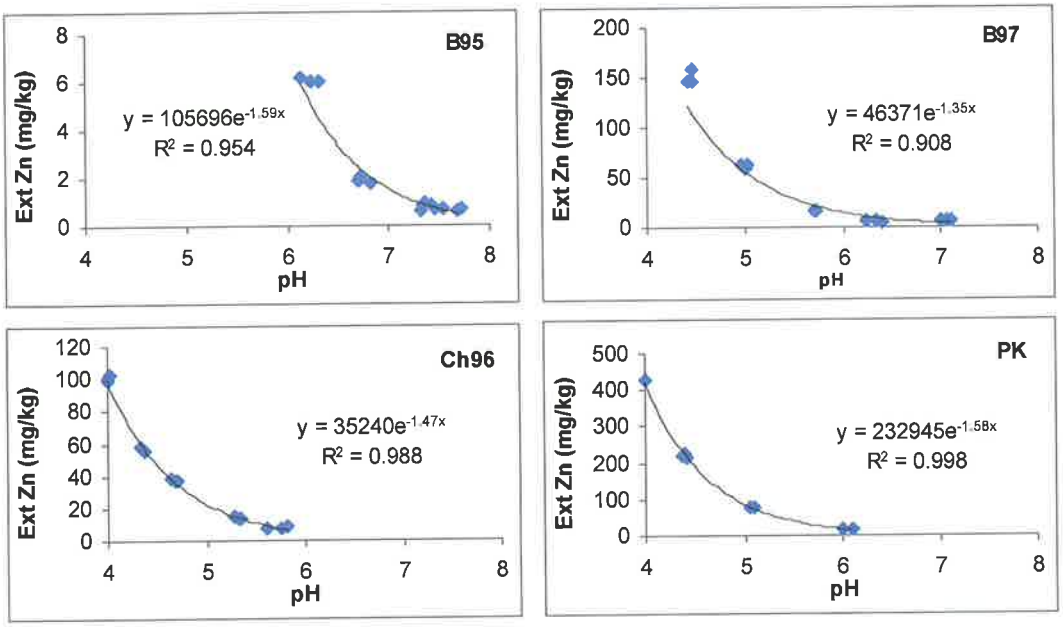


Figure 5.3.7: Change in CuE (ΔCuE, mg/kg) v. change in supernatant solution Ca concentration (Δsolution Ca, mg/L) for the pH adjustments imposed on the biosolids during the pH normalisation procedure.

Analysis of the supernatant solutions also allowed the extractable Zn concentration to be examined. In Chapter 4 it was found that $CaCl_2$-extractable Zn increased with incubation time, apparently in response to pH reduction (*section 4.3.4*). The CuE procedure also uses 0.01M $CaCl_2$ as the extractant, thus the Zn concentrations in the pH-adjusted supernatant solutions produced here can be used to calculate extractable Zn, allowing the effects induced by pH change to be definitively determined. The solid: solution ratio differs to that used in the straight $CaCl_2$ extracts of Chapter 4 (1:10 for CuE and 1:5 for $CaCl_2$ extracts), but the pH – extractable Zn relationship can still be examined from the present data. As was the case when pH v $CaCl_2$-extractable Zn was examined across the three time periods in Chapter 4 (Figure 4.3.15), the relationship between the two variables was best described here by an exponential curve (Figure 5.3.8). The R^2 values for these curves were all above 0.9, indicating a very strong relationship between extractable Zn and pH, as has been found previously (Adams and Sanders 1984). Therefore it is most likely that the increases in $CaCl_2$-extractable Zn observed during the incubation experiment were due to pH reduction, rather than to changes related to decomposition of organic matter.



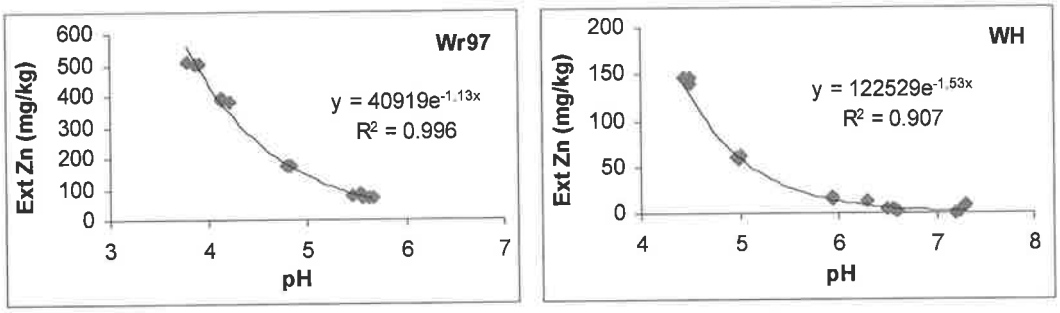


Figure 5.3.8: $CaCl_2$-extractable Zn (Ext Zn, mg/kg) v. pH for pH-adjusted biosolids.

5.4 Conclusions

Through pH normalisation it can be concluded that the increases in isotopically exchangeable Cu (CuE) observed during the previous incubation experiment were principally due to pH change, brought about by processes such as nitrification, rather than to organic matter mineralisation. The mechanisms involved in the pH driven CuE increases remain unclear though, but solution P increased concurrently with increases in CuE values. Therefore the importance of P to Cu binding in biosolids needs to be investigated further. This study provided evidence that the increases in extractable Zn noted in the incubation experiment were also likely due to pH reductions. Therefore, with pH shown to be the driving variable behind metal availability changes in biosolids and biosolid-amended soil, this adds weight to the argument for increasing the frequency of pH monitoring on biosolid-amended sites, and for developing a measure of the potential acidification index for biosolids.

However, the effects of organic matter mineralisation cannot be completely dismissed because two of the biosolids showed surges in CuE at the 6 month sampling stage before values returned to original levels, perhaps indicating a release and re-sorption of Cu over time. Therefore, although a minor player in Cu release for the most part, organic matter mineralisation may still be a determining factor in some biosolids, and thus an investigation is needed to determine what binds Cu in biosolids and how this may change with time.

6. Cu Partitioning Amongst Biosolid Fractions – What Binds the Cu?

6.1 Introduction

Experiments reported in previous chapters identified the characteristics of indigenous biosolid Cu in terms of organic and inorganic partitioning, and investigated whether the proportions changed with time. However, it is also important to determine how added metals bind to biosolids, and whether the relative importance of different biosolid fractions varies as the biosolids age. Such knowledge would allow prediction of the fate of metals added in further contaminations of biosolid-amended soils. Importantly, it would also provide an understanding of the possible fate of metals released from within biosolids themselves. For example, whether metals released from the breakdown of organic materials in biosolids would be re-adsorbed by other organic components or by the mineral fraction. Changes over time in the binding capacity of mineral and organic components, and of whole biosolids, may also give an indication as to the stability of the bonds that hold metals, and thus may indicate the long-term ability of the biosolids to retain metals. Hooda and Alloway (1994) observed initial increases in Cd and Pb retention capacity in a range of Indian and English soils amended with biosolids, but this declined with time and had reverted to near control soil values after 450 days. The reduction was attributed to losses of organic matter, and its associated sorption sites, through microbial decomposition. However Li *et al.* (2001) found up to 61% of Cd sorption capacity was controlled by the inorganic biosolid fraction, and thus this capacity would not be expected to decline over time due to processes such as organic decomposition. Supporting this, Merrington and Smernik (2004) found that the mineral fraction dominated Cd sorptivity, and that weathering in the field increased the sorption capacity of biosolid-amended soils. Hettiarachchi *et al.* (2003) also noted increased Cd sorption ability in sludged soils, and further reported that both organic and inorganic biosolid fractions contributed significantly to the increase. However, long-term effects were not investigated. Less work has been done on Cu, but it was noted by Petruzzelli *et al.* (1978) that the

destruction of organic matter significantly reduced the Cu sorbing ability of four Italian soils. Thus the questions of which components within biosolids sorb the metals, and in particular Cu, and whether sorption capacity can be retained with time, remain unresolved. Therefore an investigation to determine the solid-solution Cu partition coefficient (Kd) for whole biosolids and biosolid fractions, and how this may change with incubation time, was conducted.

6.2 Methods

Three of the biosolids from the incubation experiment described in Chapter 4 (Bolivar 95, Port Kembla and West Hornsby) were selected for this investigation. The selection was based on their comparatively high proportion of total Cu in the organic fraction (Table 4.3.7, Chapter 4), and, in the case of West Hornsby, a counter-intuitive relationship between solution P concentration and isotopically exchangeable Cu that goes against the trend followed by all the other biosolids examined (Figure 5.3.6, Chapter 5). Samples from time 0 and time 21 months were treated in order to isolate the mineral and organic fractions.

6.2.1 Isolation of inorganic fraction

A NaClO oxidation procedure was used to isolate the mineral component. The method followed that of Li *et al.* (2001). Subsamples (1 g, n = 8) of each biosolid were treated with 20 mL 0.7M NaClO (pH 8.5) and heated for two hours in a water bath at 100°C. Samples were then centrifuged for 20 minutes at 1200 *g*, the supernatant decanted off, and a further 20 mL NaClO added followed by another two hours at 100°C. After the second NaClO treatment, samples were centrifuged again and the supernatant decanted off. The residual solids were washed by adding 30 mL 0.01M $Ca(NO_3)_2$ (pH 7), mixing on a vortex mixer, and centrifuging at 1200 *g* for 20 minutes. The supernatant washing solutions were then discarded. Three washings were performed on every sample, after which samples were oven dried

overnight at 105°C. The initial and final oven-dry weights of each sample were recorded so that the percentage of mass recovered after treatment could be determined.

When following this procedure, Merrington and Smernik (2004) observed a 60% loss of sorptivity that was unaccounted for in their biosolid samples. They concluded that the loss of highly sorptive, water soluble species during the treatment process was the likely cause, and that soluble phosphate may be the leading candidate. Further, in Chapter 5 of this dissertation, changes in solution P were related to changes in isotopically exchangeable Cu (Figure 5.3.6), indicating that P may be important in the binding of Cu in biosolids. Therefore in this investigation additional treatments were established to determine whether the replacement of lost reactive P could account for any discrepancies arising between the Kd of whole biosolids and the sum of the Kd's of their respective fractions (as occurred in the work of Merrington and Smernik 2004). Further batches of NaOCl treatments were set up for each of the biosolids (again, 1 g/sample, n = 8). The treatments were performed identically to that described above, except that there was an additional step after the third wash of the residual material before drying. The residues were treated with a KH_2PO_4 solution to replace the lost soluble reactive P. The maximum 0.01M $CaCl_2$-extractable P concentration observed for each of the biosolids during the pH adjustment procedure detailed in Chapter 5 (150 mg P/kg for Bolivar 95, 250 mg/kg for Port Kembla, and 100 mg/kg for West Hornsby) was considered to represent this potentially reactive P. Therefore, to each of the residues (equivalent to 1 g whole biosolid), the required amount of P was delivered by adding 20 mL P solution (7.5 mg P/L for Bolivar 95, 12.5 mg/L for Port Kembla and 5 mg/L for West Hornsby), mixing on a vortex mixer, and equilibrating by shaking end-over-end for 24 hours. After 24 hours shaking the samples were centrifuged at 1200 *g* for 20 minutes, the supernatants discarded and the residual materials placed in an oven at 105°C to dry. As with all treatments, once dried and weighed the samples were ground and stored until Kd measurement.

6.2.2 Isolation of organic fraction

The organic matter fraction was isolated using the hydrofluoric acid (HF) method (Skjemstad *et al.* 1994). Briefly, this involves successive treatments with 2% HF solution, where 3g samples are shaken in 50mL 2% HF solution for 1 hour (x5), 16 hours (x3) and 64 hours (x1). Between treatments samples were centrifuged and the supernatant discarded then replaced with fresh 2% HF. After the final treatment, the residues were rinsed three times with de-ionised water and freeze-dried.

6.23 Determination of partition coefficients (Kd)

Sorption of Cu by the organic and inorganic fractions and by whole biosolids was determined using batch experiments employing the radioactive Cu isotope ^{64}Cu. Oven dry 1 (±0.005)g samples, in triplicate, were suspended in 20 mL 0.01M $Ca(NO_3)_2$ solution and shaken end-over-end for 12 hours. After 12 hours shaking the pH of the suspensions were measured and adjusted to equal those of the time 0 whole sludge treatments (7.3 for Bolivar 95, 5.7 for Port Kembla, and 6.29 for West Hornsby, ±0.05 units) using 0.01M NaOH and HNO_3. Due to the low pH of HF treatment residues (~2.8), 0.1M NaOH was used to adjust pH in those samples. The volume added to each sample during the pH adjustment process was recorded, allowing the volume of 0.01M $Ca(NO_3)_2$ required to raise the solid: solution ratio to 1:25 to be calculated and subsequently added. This solid: solution ratio follows that commonly used for Kd measurements (Hettiarachchi *et al.* 2003; Hooda and Alloway 1994; Li *et al.* 2001; Merrington and Smernik 2004). All samples were then subjected to a further 24 hours equilibration on the end-over-end shakers, after which carrier free ^{64}Cu was added (0.1 mL, 650 KBq) and the samples shaken again for 24 hours. Samples were then centrifuged for 20 minutes at 1200 *g* and the supernatants filtered through 0.45 μm syringe filters. The

radioactivities of the filtered supernatants were measured on a gamma counter, and the partitioning coefficient (Kd) determined (equation 6.2.1):

$$Kd\ (L/kg) = \text{Cu sorbed / Cu solution} = R\text{-}r/r \times L/S \qquad \textbf{Eq 6.2.1}$$

where: R = total radioactivity added (Bq/L), r = solution radioactivity after equilibration (Bq/L), and L/S = liquid to solid ratio (L/kg) (Collins *et al.* 2003).

6.3 Results and Discussion

The percentage mass recoveries from NaOCl treatments ranged from 73 to 87% (Table 6.3.1), while those recovered from HF treatments were between 29 and 50%.

Table 6.3.1: Biosolid C Contents Prior to Treatment and Percentage Mass Recoveries (± s.e.) After Fractionation Treatments

	Pre-treatment	Fractionation Treatment Mass Recovery %				
Biosolid	C content (%)*	NaOCl	±	NaOCl +P	±	HF
$B95_{t=0}$	19	86.7	0.15	75.1	0.38	50.3
$B95_{t=21}$	14	84.0	0.11	74.8	0.23	46.4
$PK_{t=0}$	24	85.9	0.17	73.2	0.21	44.0
$PK_{t=21}$	17	82.5	0.12	73.6	0.15	33.5
$WH_{t=0}$	25	86.0	0.20	84.8	0.23	46.2
$WH_{t=21}$	18	78.2	0.14	78.3	0.17	29.6

* Determined by LECO combustion, *see Chapter 4*.

The percentages of mass recovered from the HF treatments were greater than the proportion of C previously measured for the biosolids (Table 6.3.1), even if a conversion factor of 1.7 for C to organic matter content is considered (Weber *et al.* 2004). This suggests that the HF treatment did not remove all the mineral components in the biosolids. Skjemstad *et al.* (1994) suggested that in this situation the residual mineral matter is most likely to be composed of HF-resistant minerals containing quartz, zirconium, and ilmenite. The retention of these minerals in the HF residue may account for the greater than 100% recoveries observed if the HF and NaOCl recovery percentages are summed. Thus it must be noted that these techniques to remove mineral and organic fractions from biosolids and soils are operationally defined.

The measured Kd values of the whole biosolids and their various fractions (Table 6.3.2) were made comparable by converting the values for the fractions to 'proportional Kds' (Table 6.3.3), whereby the converted value indicates the Kd contributed by that fraction in the equivalent of 1 g whole sludge. These proportional Kd values were calculated by multiplying the measured Kd by the proportion of mass recovered from each fraction;

Proportional Kd for fraction Q = measured Kd for Q * (mass recovery % for Q/100) **Eq 6.3.1**

Table 6.3.2: Measured Kd Values (L/kg ± s.e., n=3) for Whole Biosolids and Biosolid Fractions

	$B95_{t=0}$		$B95_{t=21}$		$PK_{t=0}$		$PK_{t=21}$		$WH_{t=0}$		$WH_{t=21}$	
Fraction	Kd	±	Kd	±	Kd	±	Kd	±	Kd	±	Kd	±
whole	278	4	297	9	1031	16	389	4	724	76	320	7
HF	1172	73	628	99	2213	28	1428	117	814	41	359	72
NaOCl	620	11	579	3	202	10	137	3	256	6	196	5
NaOCl+P	1038	24	1095	8	118	5	146	8	305	4	201	1

Table 6.3.3: Proportional Kd Values (L/kg ± s.e., n=3) for Whole Biosolids and Biosolid Fractions

	Whole	±	HF	±	NaOCl	±	NaOCl+P	±
$B95_{t=0}$	278	4	590	37	538	10	779	18
$B95_{t=21}$	297	9	292	46	487	2	819	6
$PK_{t=0}$	1031	16	974	13	173	9	86	3
$PK_{t=21}$	389	4	478	39	113	2	108	6
$WH_{t=0}$	724	76	376	19	220	5	258	4
$WH_{t=21}$	320	7	106	21	153	4	157	1

The Kd values for HF residues from the 21-month samples were all greatly reduced compared to those of the time 0 samples (Table 6.3.3), indicating a loss of metal sorption capacity from the organic fraction. However, the Kd of whole biosolids in the case of Bolivar 95 did not decrease with time, suggesting that for this biosolid the sorption capacity lost from the organic fraction was not important in terms of maintaining overall sorption capacity. Therefore, for the Bolivar biosolid, the mineral fraction is the principal component responsible for Cu sorption. This could be due to Bolivar 95 having a much greater Al concentration than both Port Kembla and West Hornsby (29025 mg/kg compared to 10949 and 17397 mg/kg respectively). Thus in Bolivar 95 the Al content and other mineral components may compensate for any sorption capacity lost from the organic phase. This supports the notion of

biosolid mineral fractions shielding the environment from metal releases upon decomposition of organic matter, sometimes referred to as the protection theory (Brown *et al.* 1998; Chaney and Ryan 1993).

By contrast, the whole sludge Kd values for Port Kembla and West Hornsby decreased with incubation time (Table 6.3.3). The reduction was greater than 60% for Port Kembla and greater than 50% for West Hornsby. In both biosolids, the reduction in overall sorption capacity with time was most likely due to the decreases in sorption capacity of the organic fraction (mentioned above), because the percentage reductions in Kd values over time were greater for the HF samples than for the NaOCl samples (51% v 34% for Port Kembla, and 70% v 30% for West Hornsby, see Table 6.3.3). Therefore, the organic fraction plays an important role in Cu sorption for both of these biosolids, and, accordingly, their capacity to sorb additional metals decreases over time as their organic matter degrades. However, the remaining capacity may still be sufficient to retain within the sludge matrix any metals released from biosolid components (*i.e.* metals released through microbial breakdown of organic matter). The lack of increase in available Cu over time recorded in Chapter 5 of this dissertation (pH normalised values, Figure 5.3.4) supports this suggestion. However, the length of time that this diminishing sorption capacity remains sufficient to retain metals is a question that needs to be addressed. This would be less of an issue for West Hornsby than for Port Kembla, because as West Hornsby aged the inorganic constituents became more dominant in terms of Cu sorption (Table 6.3.3). Therefore the rate of Kd decline in West Hornsby biosolids would be expected to decrease, and possibly stabilise, as the mineral fraction becomes increasingly dominant.

Replacement of lost P increased the Kd of Bolivar 95 biosolids, but had either no effect or only a marginal effect on the Kd of Port Kembla and West Hornsby biosolids (Table 6.3.3).

Therefore P appears to be an important sorbing agent for Cu in the Bolivar biosolids, but not in Port Kembla or West Hornsby. This agrees with the results of Chapter 5, where West Hornsby was found to have a negative relationship between solution P concentration and isotopically exchangeable Cu (Figure 5.3.6).

The sum of biosolid fraction Kd values does not exactly match those of the whole biosolids (Table 6.3.3). For Port Kembla, the summed fractions amount to 111% of the whole sludge Kd value (time 0), while for West Hornsby the summed figure is 82% of the whole sludge. The under representation in the case of West Hornsby suggests a component involved in Cu sorption was removed by the treatment processes. Contrary to the speculation of Merrington and Smernik (2004), who found sums of biosolid fraction Kd values for Cd to equal less than 30% of whole sludge values, soluble P was not the missing component (as discussed above). The results of Chapter 5 of this thesis suggested Ca may be important in Cu binding in these biosolids, but as Ca was added during the rinsing process performed on all treatments (3x 20 mL 0.01M $Ca(NO_3)_2$) it is an unlikely candidate for explaining the under representation in the West Hornsby summation. Hettiarachchi *et al.* (2003) found the NaOCl treatment removed between 19 and 31% of the Mn in four biosolid-amended soils, therefore it is possible that removal of Mn minerals from the biosolids in the present investigation could account for the gap between the summed Kd of the fractions and that of the whole sludge for West Hornsby. For Bolivar 95 the summed values far exceed the Kd of the whole biosolid, with the difference being more than 4 fold for time 0 samples. This suggests that for this biosolid the treatments exposed surfaces not involved in Cu sorption in the whole sludge. Alternatively, it is possible that during the HF treatment some F was specifically adsorbed onto the surface layers of aluminosilicates and Al oxy/hydroxides in the biosolid, which was not removed by the rinsing process. Such ligand exchanges are favoured by low pH conditions (McBride 1994; Slavek *et al.* 1984), and thus would be quite possible during the

acid treatment. Being a surface potential determining ion, such chemisorption of F^- on Al surfaces would lower the surfaces' point of zero charge, and thus would enhance the sorption of Cu from solution (resulting in greater Kd values). The Bolivar biosolid would be the most susceptible to this F retention, due to its much greater Al content than the other biosolids examined (see above). However, this does not explain the results of the NaOCl-treated samples for Bolivar 95, which also had elevated Kd values relative to whole biosolids.

In addition to comparisons with the work of Merrington and Smernik (2004) discussed above, it would be useful to compare the level of agreement between the summed fraction Kds and the Kd of whole samples in this investigation with the agreement found in other studies. Unfortunately, such comparisons cannot be made with the work of Li *et al.* (2001) because in their investigation the organic fraction was not isolated, and therefore sorption by that fraction was not specifically determined. However, Hettiarachchi *et al.* (2003) did provide data in graphic format for one of their biosolid-amended soils (fine sandy loam amended with lime-composted biosolids) that can be used to calculate a comparative summation of sorption coefficients for the different fractions isolated. Cadmium sorption values from their NaOCl treatment (-OC treatment), $NH_2OH{\cdot}HCl$ treatment (-reducible Fe and Mn) and NaOCl + $NH_2OH{\cdot}HCl$ treatment (-OC and -Fe and Mn) at the highest rate of Cd addition (72 μg Cd in 25 mL solution with 1 g soil solid equivalent) can be summed to yield a figure equal to 109% of the whole, intact sample. This result is not dissimilar to the 111% observed here for the summed Cu Kd values of the various fractions for Port Kembla biosolids. Unfortunately, Hettiarachchi *et al.* (2003) did not provide results for their other biosolid/soil treatments, thus further comparisons cannot be made.

6.4 Conclusions

The relative importance of the mineral and organic fractions to Cu sorption varies between biosolids, and in some cases can vary over time. The inorganic fraction was of principal importance to Cu sorption in the case of Bolivar biosolids, and accordingly the Kd value remained stable over the incubation period. By contrast, the organic fraction was found to dominate Cu sorption in the Port Kembla biosolids, with the Kd value being much lower in samples incubated for 21 months than in un-incubated samples. Presumably, the decrease was due to reductions in the size of the organic fraction over time. Further studies should be conducted to determine at what point this loss of sorption capacity becomes important in regard to the retention of biosolid metals within the biosolid matrix. Sorption of Cu in West Hornsby biosolids was initially dominated by organic components, but the inorganic fraction became increasingly important with time. Therefore, decreases in Kd of West Hornsby biosolids would be expected to level out and stabilise as Cu sorption becomes dominated by the mineral fraction. Phosphorus was found to be important in Cu binding in Bolivar biosolids, but not in Port Kembla or West Hornsby biosolids. The lack of agreement between Kd values for whole biosolids and the sum of biosolid fractions (particularly in the case of Bolivar 95) points out the difficulties of isolating the target fractions, and also the shortcomings of current fractionation techniques.

7. Copper Availability in 7 Soils from Israel incubated with and without biosolids

7.1 Introduction

Land application of sewage biosolids is becoming increasing popular because of its potential benefits to soil fertility and structural stability (Hall and Coker 1981; Johansson *et al.* 1999; Joshua *et al.* 1998; Mosquera-Losada *et al.* 2001; Oberle and Keeney 1994; Peverly and Gates 1994). However, such benefits come with a price; that being the risks of accumulation of metals and other potentially toxic elements in soils that can pose a risk to environmental health (Bhogal *et al.* 2003; Chaudri *et al.* 2000; King and Hajjar 1990; MacLean *et al.* 1987; McGrath 1987). Copper (Cu) is one of the metals of chief concern in this regard, as it has been shown to be toxic to microbes, with many species of fungi and N-fixing bacteria being especially sensitive (Chaudri *et al.* 1992; Dahlin *et al.* 1997; McGrath *et al.* 1988). It is vital to determine the likely long-term impact of Cu and other sewage biosolid metals on soils and ecosystems so that applications of the material can be kept below levels which may exert a toxicological effect. Long-term biosolid land application studies conducted in Europe and the United States (Aitken and Cummins 1997; Brown *et al.* 1998; Chang *et al.* 1987; Dowdy *et al.* 1991; Jarausch-Wehrheim *et al.* 1996; McGrath 1987) have drawn conflicting conclusions regarding metal availability over time. A further problem is that conclusions drawn from these temperate regions may not be wholly applicable to low rainfall 'Mediterranean' climatic conditions, such as those existing in parts of southern Australia and Israel. Thus, as is the case in the drier states of Australia, researchers in Israel and other countries of the Middle East face a dearth of knowledge on the long-term fate of biosolid metals in soils from Mediterranean climates. Acknowledgement of this mutual problem led to a research collaboration whereby the techniques developed here for assessing Cu availability (Chapters 2 – 6) were applied to Israeli soils and biosolids incubated for 7 years. The main benefits of this work to the current PhD project were that it provided access to samples incubated for much

longer periods, and therefore allowed for greater examination of the long-term availability and fate of biosolid Cu. The techniques employed include radio-isotopic dilution, with ^{64}Cu, and measurements of ion activity using a Cu^{2+} ion selective electrode.

7.2 Methods

7.2.1 Soils and Biosolids

Seven soils were selected for the study that encompass a range of soil and climatic conditions and parent materials in Israel; 1) a dune sand, 2) a loamy Calcic Haploxeralf (Nahal-Oz, from loessial desert dust origin), 3) a fine loamy, calcareous, Typic Xerochrep (Mitzpe Masuah, a rendzina soil formed on soft limestone), 4) a fine silty, Typic Rhodoxeralf (Netanya 6, formed by gradual accumulation of aeolian deposits into a sand matrix), 5) a fine clayey Vertic Palexeralf (Terra Rossa, a clayey soil formed on hard limestone, the specimen is a deep variant that forms on plateaus), 6) a fine clayey, halloysitic mesic Typic Rhodoxeralf (Golan 37; formed on young basaltic surfaces), and 7) a very fine clayey, montmorillonitic Chromic Haploxerert (Golan 4, on old basaltic surfaces). Soils were collected from the A or Ap horizon except in the case of the Netanya soil, for which the Bt horizon was taken. Soils were air dried and passed through a 2 mm sieve. Dry biosolids were obtained from the drying beds at the municipal sewage treatment plant in Haifa, Israel. The biosolids had been anaerobically digested before drying to a water content of 100 g/kg. Biosolids were crushed to pass a 1-mm sieve and stored in sealed containers at 4°C until use. Total contents of N and P in the biosolids were 15.4 and 15.0 g/kg respectively, and the C/N ratio was 14. Selected properties of the soils and biosolids are shown in Table 7.2.1.

Table 7.2.1: Selected Properties of Soils and Biosolids Prior to Incubation

Soils and biosolids	Code	Clay	Silt	Sand	Carbonates	OC	pH [#]	CEC[$]	Dominant clay[§§]
		g/kg						$cmole_c/kg$	
Dune sand	DS	3	12	985	18	<1	7.80	1.0	-
Nahal-Oz	NO	181	302	517	129	4	7.54	30	M, I
Mitzpe Masuah	MM	196	590	214	572	29	7.39	26	M, I
Netanya 6	Net	352	145	503	0	3	6.29	22	K, M
Terra Rossa	TR	350	550	100	56	15	7.37	45	K, M
Golan 37	G37	296	536	168	0	12	6.15	21	H, A
Golan 4	G4	744	106	150	8	7	7.25	72	M
Biosolids					70	210	6.46	38	

[#] pH in 1:5 solid: 0.01M $CaCl_2$. [$]CEC determined by the NH_4-Na acetate method (Thomas 1982).
[§§] H= halloysite; I= Illite; K= kaolinite; M= montmorillonite.

Total metal concentrations in soils and biosolids were determined using a modification of US-EPA method 3051A. Five hundred mg samples (crushed to pass 0.25 mm) were microwave digested in 12 mL *aqua regia* (9 ml concentrated HNO_3 + 3 ml concentrated HCl) plus 1 ml perchloric acid. The digestion was performed as follows; 10 minutes at 580 watts then ventilation of the microwave chamber for 10 minutes, followed by another 10 minutes operation at 580 watts and ventilation for 10 minutes. The tubes were allowed to cool to room temperature, and the contents quantitatively transferred to 50 mL volumetric flasks containing yttrium at 1 mg/L as an internal standard. Solutions were then analysed by ICP-AES, and total metal contents determined (Table 7.2.2). Sample collection, textural analysis, microwave digestions and the ICP analyses were conducted by Dr. Pinchas Fine and Amir Hass of the Institute of Soil, Water and Environmental Sciences, Volcani Centre, Israel.

Table 7.2.2: Total Metal Concentrations (± standard errors, n=3) in Soils and Biosolids

Soil	Code	Zn	Cu	Ni	Pb	Cd
		mg/kg				
Dune sand	DS	20 ± 8	2 ± 0.1	2 ± 0.1	0.5 ± 0.0	0.3 ± 0.05
Nahal-Oz	NO	42 ± 3	21 ± 0.3	24 ± 0.7	3.6 ± 0.1	0.6 ± 0.04
Mitzpe Masuah	MM	78 ± 5	29 ± 1.5	43 ± 0.7	3.4 ± 0.2	1.7 ± 0.12
Netanya	Net	43 ± 1	18 ± 0.3	29 ± 0.6	2.0 ± 0.0	0.6 ± 0.11
Terra Rossa	TR	60 ± 1	19 ± 1.2	38 ± 0.4	8.5 ± 3.5	0.6 ± 0.01
Golan37	G37	103 ± 2	36 ± 0.4	103 ± 0.8	13.0 ± 0.0	1.1 ± 0.04
Golan4	G4	68 ± 2	37 ± 1.1	52 ± 1.8	8.7 ± 0.7	0.5 ± 0.01
Biosolids		2468 ± 351	633 ± 108	81 ± 8.0	136 ± 16	6.4 ± 0.53

7.2.2 Incubation procedure

One (1) kg oven dry equivalent masses of soils and mixtures of soil and biosolids (9:1 soil: biosolid ratio, equivalent to an application of 250 t/ha considering the upper 20 cm plough layer) were placed in 2 L pots. All samples were wetted to 60% of the water holding capacity, as determined at -33 kPa, and thoroughly mixed. They were then incubated for 7 years in a constant temperature controlled room (30°C). Moisture loss during the incubation was minimised by placing the pots in closed 50 L containers with free water at the base. The containers were also aerated with moistened air. The moisture contents of the soils and soil/biosolids mixtures were monitored every 3-4 weeks, and water was added if needed (water losses did not exceed 5% at any stage). The incubation was conducted at the Volcani Centre, Israel.

7.2.3 Changes in Physical Properties and Copper Availability

Archived time 0 samples (air-dried and stored under desiccation) and samples from after the 7-year incubation were ground to pass a 2 mm sieve and tested for organic carbon (OC) content via the dichromate oxidation method (Allison 1965) (n=2). The pH was measured (n=2) in 1:5 solid: 0.01M $CaCl_2$ solution extracts using an Orion pH electrode and meter. Isotopically exchangeable Cu (CuE) was determined following a slightly modified method of McLaren and Crawford (1974). Triplicate samples (3 g) were equilibrated with 30 mL 0.01M $CaCl_2$ (containing 3 drops of toluene to inhibit microbial activity) for 40 hours on end-over-end shakers, after which the radioisotope ^{64}Cu was added (in 0.1 mL solution containing approximately 2 MBq activity). After a further 24-hour equilibration period, samples were centrifuged for 10 minutes at 1200 *g*, the supernatant solutions filtered through 0.45 μm filters, and then measured for radioactivity (gamma counter) and total solution Cu (Graphite Furnace Atomic Absorption Spectrometer - GFAAS). Isotopically exchangeable Cu (the E-value, or CuE) was calculated as follows:

$$CuE = \frac{Cu(sol) * TA}{CR * M} \qquad \textbf{Eq. 7.2.1}$$

where: CuE = Isotopically exchangeable Cu (mg/kg); Cu(sol)= Concentration of Cu in 0.01M $CaCl_2$ solution (mg/L); TA= Total activity of ^{64}Cu added (Bq); CR= Count rate in solution (Bq/L); M= Mass of sample (kg).

Cupric ion activity (pCu^{2+}) was measured in solution extracts using a cupric ion selective electrode (Orion 9429), a silver-silver chloride double junction reference electrode (Orion 900200), and a mV meter (Orion 720). Duplicate 3 g samples were equilibrated with 30 mL 0.01M $CaCl_2$ solution for 24 hours by end-over-end shaking. Samples were then centrifuged for 10 minutes at 1200 *g*, the supernatant decanted, and the pCu^{2+} (negative log molar Cu^{2+} concentration) determined. Calibration standards were prepared using ethylenediamine dihydrochloride (EN) (Ma *et al.* 1999), $Cu(NO_3)_2$, NaOH, and deionised H_2O. All standards had 35 mL of 0.1 mg/L $Cu(NO_3)_2$ solution plus 5 mL 0.1M EN. The pH was adjusted to achieve the desired Cu^{2+} activity in the standards by adding varying volumes of 0.01M NaOH (Minnich and McBride 1987). Deionised water was used to maintain a consistent volume (60 mL) across standards. The activity of Cu^{2+} in the standards was calculated using GEOCHEM-PC (V2), and a calibration curve of pCu^{2+} and mV produced (Figure 7.2.1).

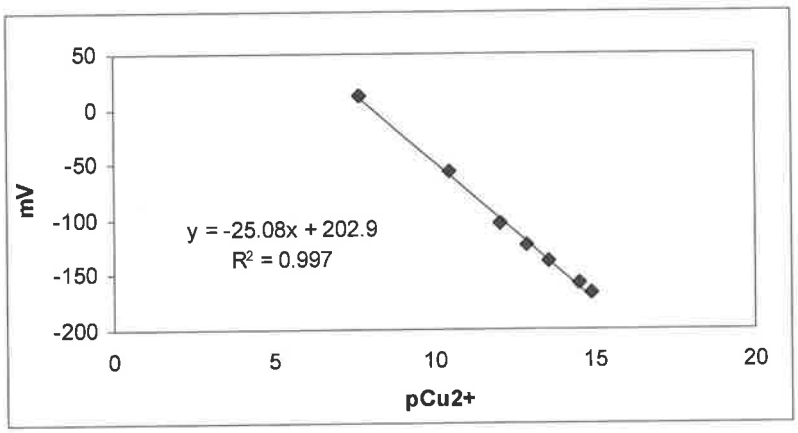


Figure 7.2.1: Calibration curve constructed to determine cupric ion activity (pCu^{2+}) from mV readings. The slope deviates slightly from the ideal Nernstian response of –29.58mV.

7.2.4 Statistical Analysis

Statistical analyses were performed using Genstat 5 for Windows (release 4.1) (Anon. 1998) and Microsoft Excel.

7.3 Results and Discussion

7.3.1 Changes in Soil Organic C and pH

The Golan soils (G4 and G37), Mitzpe Masuah (MM) and the Terra Rossa (TR) all showed significant reductions ($p \leq 0.05$) in OC by the end of the 7-year incubation (Figure 7.3.1), while the other non-amended soils showed no change. In terms of the percentage of initial OC lost, the reductions amounted to 55, 35, 39 and 50% for Golan 4, Golan 37, Mitzpe Masuah and Terra Rossa soils respectively. All of the biosolid-amended soils lost significant amounts ($p \leq 0.05$) of OC during the incubation (Figure 7.3.1), with the percentage of initial OC lost ranging between 28% for Nahal-Oz to 53% for Golan 4. In the case of Golan 4, Mitzpe Masuah and Terra Rossa biosolid-amended soils, the OC content had decreased back to that of the original unamended soils by the end of the 7-year incubation (Table 7.3.1), while for the remaining amended soils the OC levels were still elevated relative to their unamended states. This compares to the study by Brown *et al.* (1998), where approximately 80% of the OC added in biosolid amendments to a fine sandy loam soil in Maryland USA (pH 5.5-6.5, and application rates 100-224 t/ha) had been mineralised after 14 years. By contrast, the study by Hyun *et al.* (1998) found added biosolid OC had only declined by 30-40% after 10 years in a sandy loam soil in California USA (application rates 135-1080 t/ha).

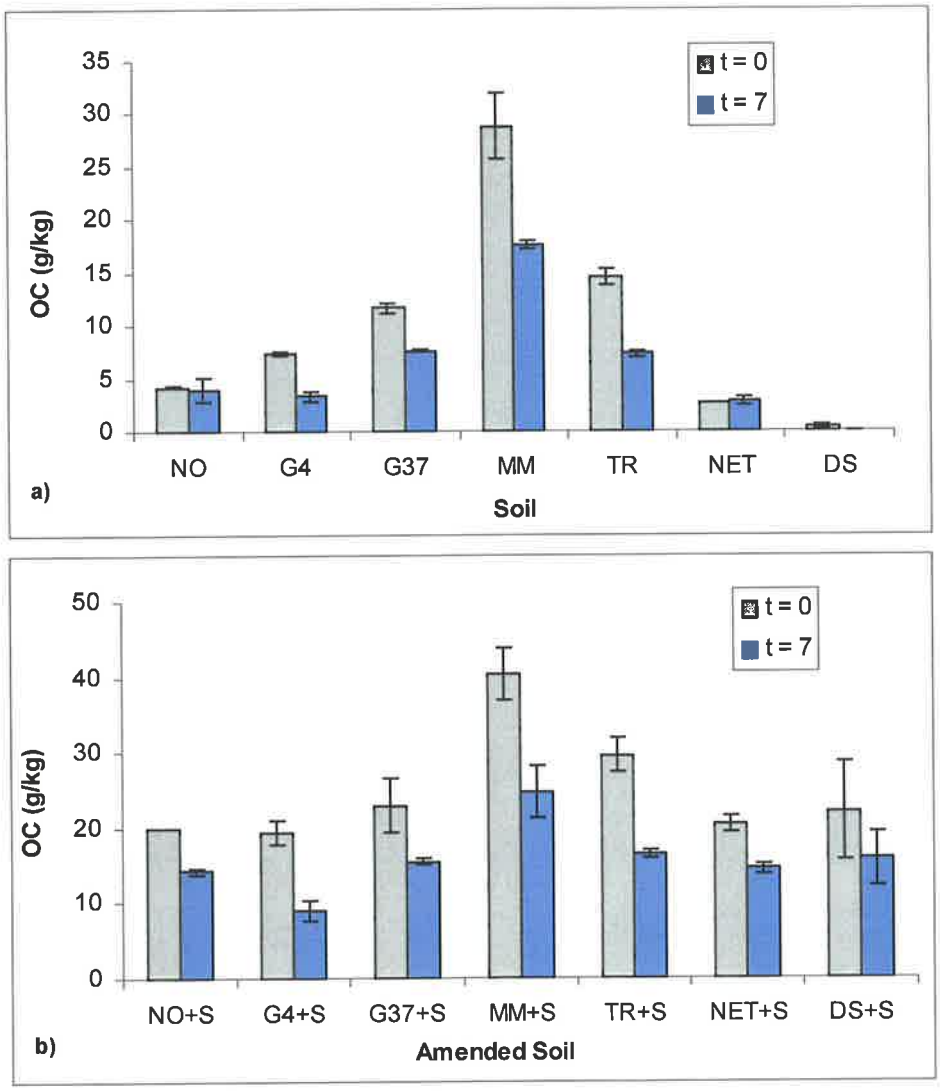


Figure 7.3.1: Organic Carbon content (g/kg) before and after 7 years incubation for a) soils and b) soil/biosolid mixtures (amended soils). Error bars indicate 2x standard errors (n=2).

Table 7.3.1: Organic C Content of Original Unamended Soils and Amended Soils After Incubation

Soil	Code	Unamended Soil OC (g/kg)	Amended Soil OC post incubation (g/kg)	$LSD_{(0.05)}$
Nahal-Oz	NO	4.3	14.3	0.7
Golan 4	G4	7.4	9.1	ns*
Golan 37	G37	11.7	15.5	1.1
Mitzpe Masuah	MM	28.8	24.8	ns
Terra Rossa	TR	14.5	16.5	1.6
Netanya 6	NET	2.7	14.5	1.3
Dune sand	DS	0.4	15.9	5.4

* ns – not significant

By the end of the incubation the pH had increased marginally in Nahal-Oz, dune sand, Golan 4 and Golan 37 soils, whereas the Netanya 6 soil recorded a very large pH increase of 1.5 units (Figure 7.3.2). This soil may have a low buffering capacity due to its lack of

carbonates and low OC (Table 7.2.1), making it susceptible to pH change. The Mitzpe Masuah and Terra Rossa soils showed no change in pH. For the soil/biosolid mixtures all pH values were essentially the same after 7 years as at time 0 (Figure 7.3.2). Increases in soil pH can occur as the result of organic matter decomposition, because mineralisation processes release OH^- ions and consume H^+ ions (Ritchie and Dolling 1985). However, decreases in pH are often observed in studies involving biosolid-amended soils (*i.e.* Brown *et al.* 1998; Dowdy *et al.* 1978; Hooda and Alloway 1994) due to the greater effect of nitrification processes on soil pH. The oxidation of ammonium (NH_4^+) to nitrate (NO_3^-) is an acid forming process, and thus commonly leads to pH reductions in soil (Kennedy 1986);

$$NH_4^+ + 2O_2 \rightarrow NO_3^- + H_2O + 2H^+ \qquad \textbf{Eq. 7.3.1}$$

Dudley *et al.* (1986) provided a good example of these effects, as they found pH continually increased in one biosolid-amended soil during the first 4 weeks of incubation (pH 5.34 – 8.28), but then decreased steadily until termination of the experiment at 30 weeks (final pH 4.63). The pH decline was attributed to soil nitrification, as soil nitrate levels were constant for the first 4 weeks but had increased by two orders of magnitude by week 10 (Dudley *et al.* 1986). Therefore the lack of pH decline in the samples of the present study may be due to low nitrification rates caused by low numbers of nitrifying bacteria in the soils and or the relatively low N content of the biosolids (~1.5% $_{w/w}$). In some cases, the buffering capacity of the soils and/or the biosolids may have also played a role in resisting pH change, as OC, clay, and carbonates were high in several of the soils and in the biosolid (Table 7.2.1).

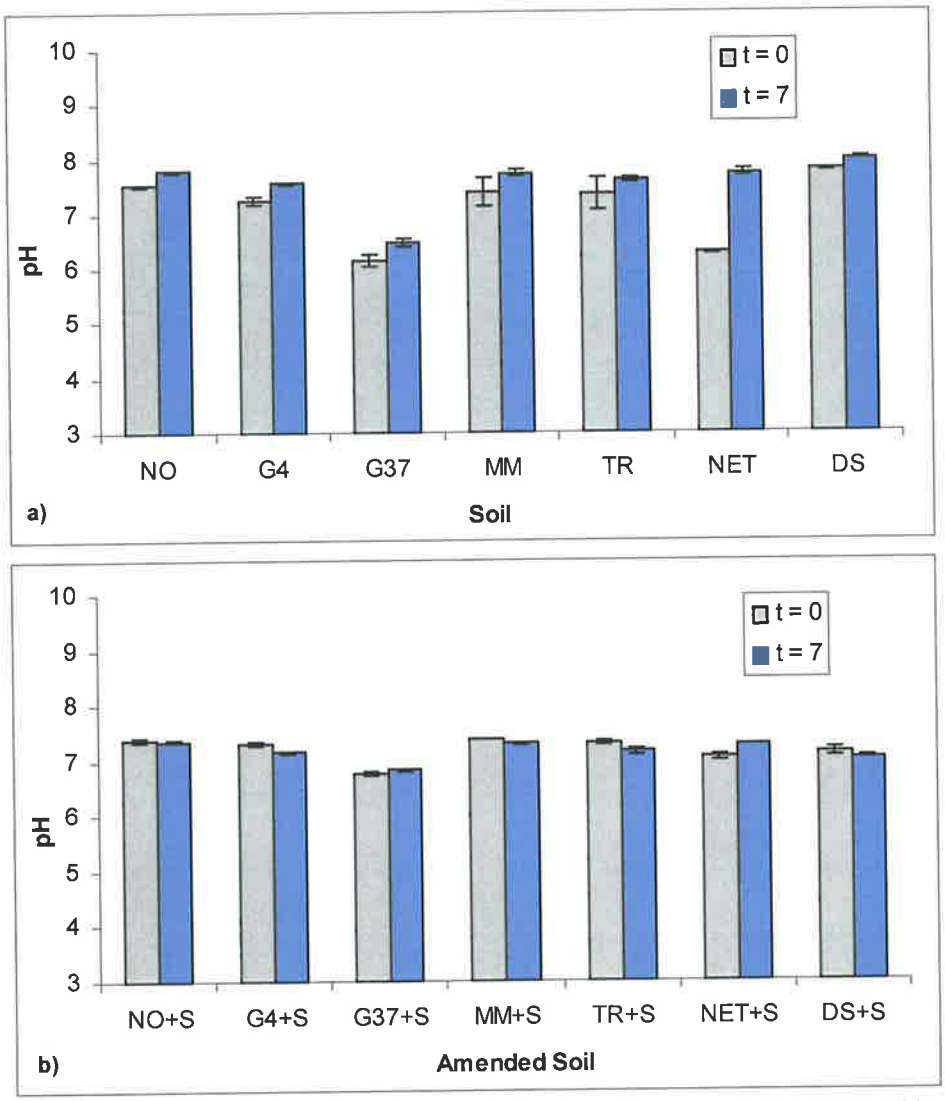


Figure 7.3.2: pH before and after 7 years incubation for a) soils and b) soil/biosolid mixtures (amended soils). Error bars indicate 2x standard errors (n=2).

7.3.2 Isotopically Exchangeable Cu

For 5 out of the 7 unamended soils isotopically exchangeable Cu (CuE) was not significantly different ($p \leq 0.05$) between original samples and those incubated for 7 years (Figure 7.3.3), while for the 2 soils showing statistical differences, Terra Rosa and Golan 4, the changes were less than 1 mg/kg, so are unlikely to have any environmental relevance. The Netanya 6 soil and the Dune sand (both in original condition and after incubation) had solution extract Cu concentrations below the level quantifiable by GFAAS (0.001 mg/L), so CuE values calculated for them can only be viewed as estimates. The CuE values for the unamended soils

(0.15 – 2 mg/kg, representing 0.8 – 7% of the total soil Cu) were similarly low to those measured by McLaren and Crawford (1974) for 24 English soils. They found a spread of 0.19 – 12 mg/kg, or from 2 - 19 % of the total Cu, with half of the soils having less than 10% of their total Cu as isotopically exchangeable.

The CuE of the biosolid-amended soils at time 0 ranged from 1.4 mg/kg for Netanya 6 to 10.9 mg/kg for Mitzpe Masuah (Figure 7.3.3). When put in terms of the percentage of total Cu that was isotopically exchangeable, the spread was between 2 and 12%. These low percentages indicate that the great majority of Cu added to the soils by biosolid application was in non-labile forms. In contrast to the unamended soils, the majority of the biosolid-amended soils did show significant ($p \leq 0.05$) changes in CuE over the incubation period (Figure 7.3.3). The amended Golan soils, Mitzpe Masuah, Terra Rossa and Netanya 6 all showed increases in CuE with time. Because pH remained stable during the incubation period for these amended soils, the increases in CuE observed are most likely due to losses of organic matter through mineralisation (which were considerable in terms of percentages of initial OC). However, the increases in CuE were only between 2 and 5 mg/kg (representing a maximum change of only 5% in terms of the amount of total Cu that was isotopically exchangeable), thus their likely environmental impact would be small. A similar lack of environmentally relevant change in metal availability was noted for Cd by Brown *et al.* (1998), who observed no difference in Cd uptake by lettuce (*Lactuca sativa*) in the 14 years following biosolid application to a fine sandy loam. Similarly, Hyun *et al.* (1998) had still failed to find any changes in Cd availability 10 years after biosolid application ceased, using both plant uptake and solubility measures.

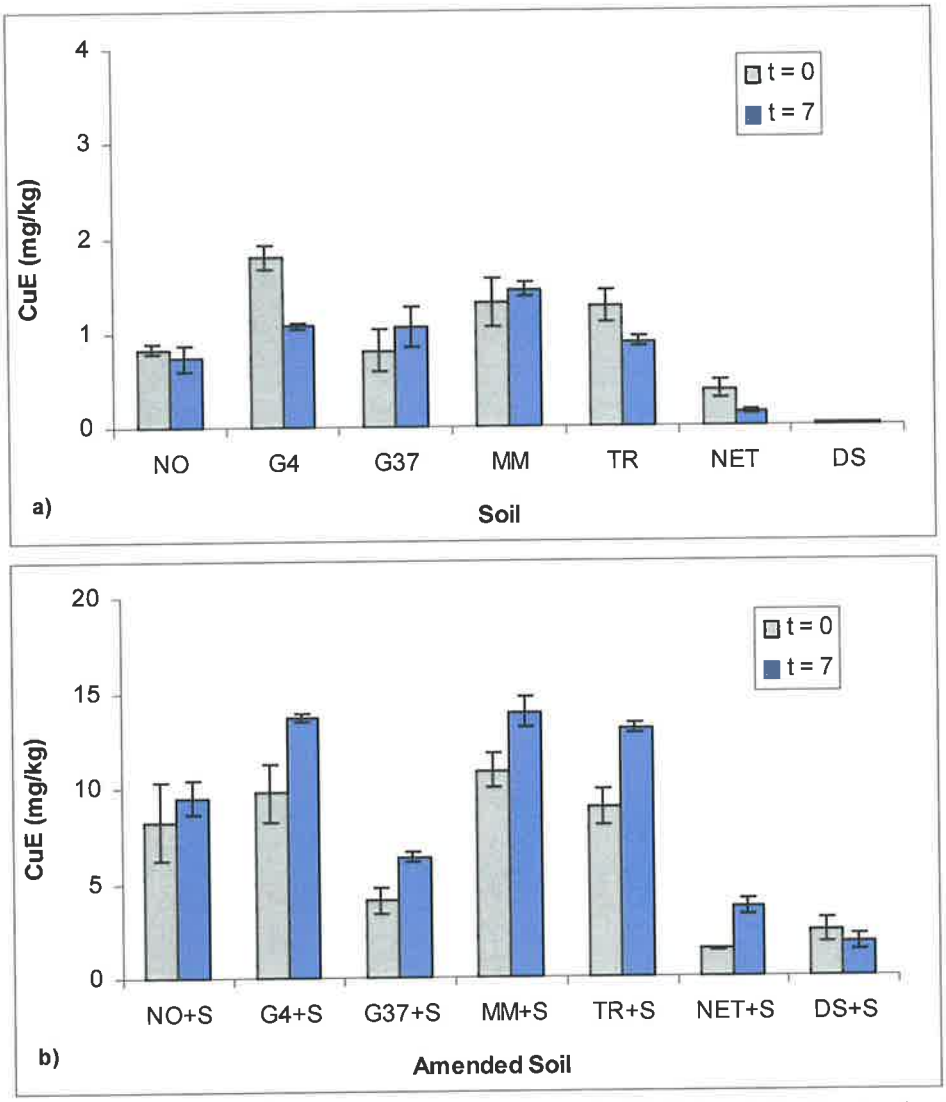


Figure 7.3.3: Isotopically exchangeable Cu (CuE, mg/kg) before and after 7 years incubation for a) soils and b) soil/biosolid mixtures (amended soils). Error bars indicate 2x standard errors (n=3).

7.3.3 Changes in Cu^{2+} Ion Activities

As would be expected given the minimal changes in pH observed during the incubation, cupric ion activities (pCu^{2+}) in $CaCl_2$ extract solutions were generally consistent for pre- and post-incubation samples (Figure 7.3.4), with the exceptions of Nahal-Oz and Netanya 6 for the unamended soils (no significant differences were observed for any of the amended soils). The differences between pre- and post-incubation pCu^{2+} were only just significant for the unamended Netanya 6 soil ($p \leq 0.05$), with values of 10.4 and 10.7 respectively, thus the change is unlikely to be of any real consequence for the environment. The change observed

for the Nahal-Oz soil was greater (0.8 units), but may still be of little environmental relevance because pCu^{2+} values were already well above those found to cause toxicity to plants (pCu^{2+} < 8, McBride 2001). However it is interesting that the Nahal-Oz soil showed a change in pCu^{2+}, because it showed no changes in any of the other parameters measured (OC, pH, or CuE).

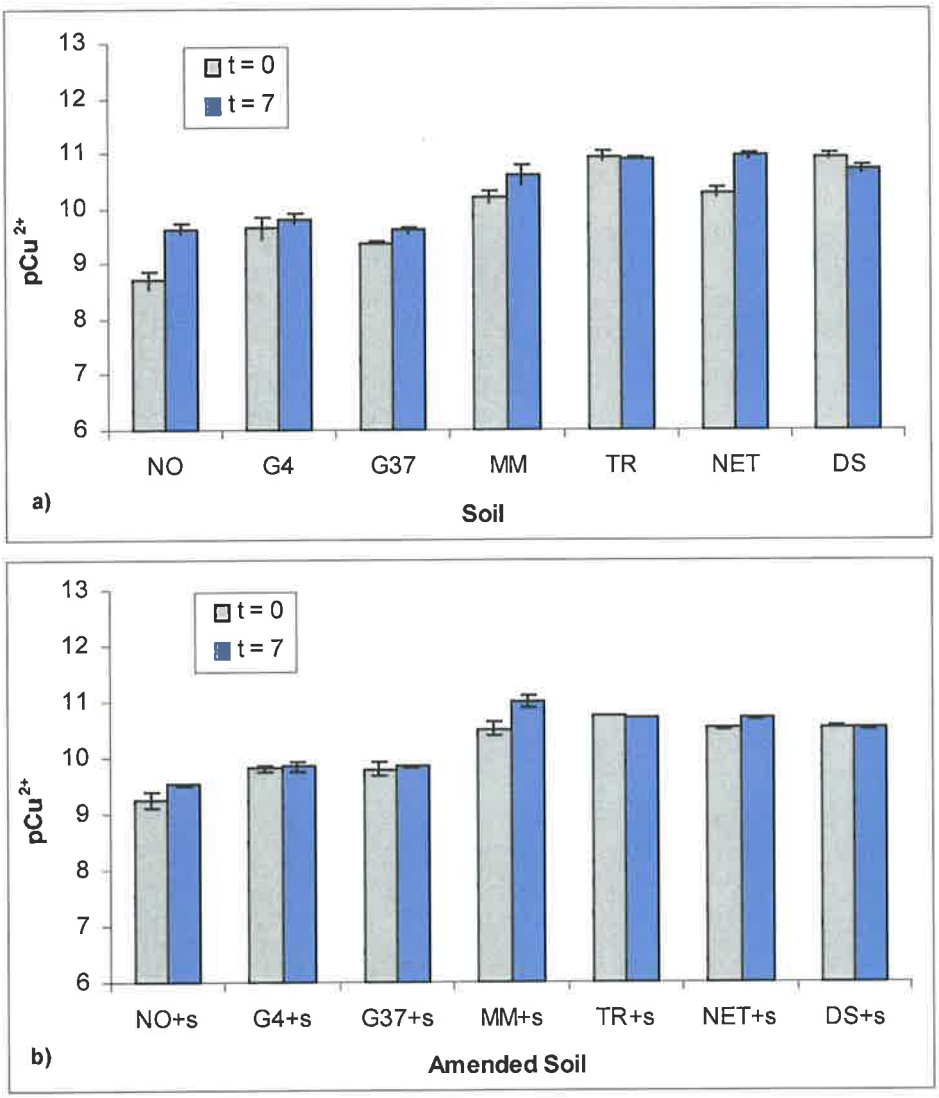


Figure 7.3.4: Cupric ion activity (pCu^{2+}) in $CaCl_2$ extracts before and after 7 years incubation for a) soils and b) soil/biosolid mixtures (amended soils). Error bars indicate 2x standard errors (n=2).

7.4 Conclusions

Unamended soils incubated for 7 years lost up to 55% of their initial OC content, while soils amended with biosolids lost between 28 and 53%. The pH remained relatively constant in

most treatments but increased by 1.5 units in one soil. Despite the losses of OC, isotopically exchangeable Cu (CuE) did not change by more than 5 mg/kg in any of the unamended or amended soils (with the changes representing less than 5% of total soil Cu in every case). The pCu^{2+} results also revealed essentially no changes over time, thus from an environmental impact viewpoint the availability of Cu remained low and constant. I conclude therefore that, in these samples, Cu was either not associated with the readily degradable OC or was able to be retained by other biosolid or soil components once released. Therefore, as was shown to be the case for the Australian biosolids previously examined (Chapter 5), organic C degradation did not lead to increased Cu availability, and thus no evidence for the time-bomb hypothesis has been found for Cu. Therefore, with respect to Cu, application of such biosolids to these soils at rates of up to 250 t/ha are not likely to pose any environmental risks in the short to medium term. Conclusions on the potential longer-term risks to the environment (*i.e.* >10 years) cannot be drawn directly from these results, however, given the changes in OC already observed and the apparent chemical distribution of Cu into more stable forms, it seems unlikely that the biosolid Cu applied to these soils will pose a long-term threat.

8. Conclusions and Future Directions

This work determined, using isotopic exchange techniques, that the available Cu fraction in the Australian biosolids examined ranged between 7 and 43% of their total Cu contents. This supports the findings of other researchers who have determined available metal fractions to be substantially less than total metal concentrations (Bhogal *et al.* 2003; Sims and Kline 1991; Sloan *et al.* 1997; Tao *et al.* 2003), and thus adds to the case for basing future biosolids re-use regulations on the available fraction rather than on totals. A model was produced for predicting the available Cu in biosolids without the use of the expensive and difficult to manage ^{64}Cu isotope (Eq. 8.1), and future work should involve investigations to test the robustness of this model with other soils and biosolids.

$$CuE\ (mg/kg) = 281.5 Log\ Total\ Cu - 14.9 pCu^{2+} - 459 \quad (R^2 = 0.806) \qquad \textbf{Eq. 8.1}$$

where CuE = isotopically exchangeable Cu, and logTotal Cu is $\log_{10}$ total biosolid Cu concentration (mg/kg).

Because this study also found that pCu^{2+} can be predicted using pH, total Cu concentration and total C%, an alternative model in which pCu^{2+} is replaced by these parameters was also derived (Eq. 8.2). However, the accuracy of the model involving direct measurements of pCu^{2+} is likely to be greater.

$$CuE\ (mg/kg) = 281.5 Log\ Total\ Cu - 14.9[1.95pH - 0.003 Total\ Cu + 0.15 TC\% - 2.27] - 459 \qquad \textbf{Eq. 8.2}$$

where 'logTotal Cu' is $\log_{10}$ total biosolid Cu concentration (mg/kg), Total Cu is in mg/kg, and TC% is total C percentage.

Availability assays (isotopic dilution, ion activity measurements and EDTA extraction) performed on the Australian biosolids examined showed that biosolid Cu can become increasingly available over time (Chapter 4). However, the increases observed were principally due to decreases in pH (most probably resulting from nitrification processes), rather than to any release of metals following organic matter mineralisation. Therefore, although not a predictive variable for available Cu when all biosolids were considered together (Equation 8.1 above), pH was found to have a strong effect on Cu availability when

the pH of an individual biosolid was altered. Due to this pH effect it was concluded that the proposed sludge re-use regulation system, that based on available fractions rather than totals, should include a pH protection factor. The system would involve determining available metal concentrations (using isotopic tracer or other techniques) and then those defined concentrations would be adjusted to a worst-case pH scenario using a pH-available metal slope (*i.e.* Chapter 2, Table 2.3.5). Biosolid classification and re-use regulations could then be based on this metal concentration. More study is required to determine the range of slopes in pH-available metal relationships for biosolids, so that a suitably protective equation can be selected.

Research needs to be done to determine the mechanisms responsible for increases in available Cu as pH declines. This project identified dissolution of P containing substances and Ca-Cu coprecipitates as potential mechanisms leading to the release of Cu (Chapter 5), but Kd measurements on whole and fractioned biosolids suggested other mechanisms must also be involved in Cu sorption and dissolution (Chapter 6). A better understanding of the processes controlling such metal release will allow for more accurate predictions or calculations of the long-term fates of biosolid metals, particularly in the case of land use changes that can potentially lead to soil pH reductions.

Partition coefficient (Kd) experiments found the importance of mineral and organic fractions to Cu sorption varied between biosolids, with one being dominated by the mineral phase, another by the organic, and a third by a combination of the two (Chapter 6). However, despite the importance of the organic fraction no evidence was found in this PhD thesis for the 'time bomb effect', which hypothesises that metals will become more available once decomposition processes reduce organic matter contents to below a critical level (McBride 1995). Rather, the results show that although C levels may decline substantially over time, availability can

remain constant provided pH remains stable. This is in line with the sludge protection theory (Chaney and Ryan 1993), which declares that the mineral components of biosolids can compensate for any retention capacity lost from the organic fraction as it degrades. It is possible, however, that if the Australian biosolids and biosolid-amended soils examined here were incubated for longer an effect of organic matter mineralisation on available Cu content may have been observed. This is possible because in the 21-month incubation study only up to 30% of the initial organic C was mineralised, which may be an insufficient reduction to see any substantial effects on biosolid metal availability. Therefore longer-term studies of metal availability in Australian biosolids are required. However, such studies may need to continue for periods in excess of 10 years, because the biosolid-amended soils from Israel still showed no evidence for the time-bomb effect after 7 years of incubation, despite significant losses of initial organic C (Chapter 7). One aspect that should be incorporated into future long-term trials is the possibility of tracking biosolid organic matter in sludge-amended soils using NMR techniques. That is, organic matter in soils derived specifically from sludge amendments could be monitored as it degrades over time, and this could be examined in relation to long-term changes in metal availability. This will be possible because work arising from this PhD project has found soil and biosolid organic C to be distinct (Smernik *et al.* 2003), and therefore distinguishable.

While isotopic dilution, ion activity measurements and EDTA extraction all detected the pH-driven Cu availability changes in the 21-month incubation experiment (Chapter 4), $CaCl_2$ extraction did not. This indicates a possible shortcoming of the $CaCl_2$ extraction technique for assessing available Cu, as it appears to only identify the easily solubilized fraction and not a consistent proportion of the exchangeable pool. Uptake of Cu into above ground tissue of rye grass was also found to be of limited use in determining Cu availability in heavily amended soils or straight biosolids. Plant assays found shoot Cu concentrations in all biosolid

treatments were within a very narrow range (~10 μg/g), despite widely varying substrate properties (including total and available Cu, as measured by the abiotic techniques listed above). In contrast to this, soils dosed with varying amounts of inorganic Cu salts did show a plant tissue Cu concentration response. Therefore, poor plant development in biosolids media, which would limit uptake and exacerbate the low translocation rate of Cu from root to shoot, is the most likely contributor to the poor performance of the plant-based assays. If it were possible to determine plant root Cu concentrations the plant assays may have proven more useful. In future work investigations should take advantage of the technique developed by Chaignon and Hinsinger (2003), where the root mass is kept separate from the substrate via a polyamide mesh and therefore can be analysed without fear of contamination. Their technique has many of the benefits of the Stanford and DeMent (1957) method that was tried here unsuccessfully (Chapter 4), but may not suffer the same problems because a much smaller mass of soil is used (a 1 mm layer only). A further avenue of research should involve the use of Diffusive Gradients in Thin films (DGT) devices. These resin-based devices show promise as potential surrogates for plant root uptake, because they are able to measure pore-water metal concentrations and resupply fluxes from the soil (Davison *et al.* 2000; Harper *et al.* 1998). The use of DGT would overcome many of the problems encountered in the plant Cu uptake assays of this project, such as root Cu retention, root contamination, and poor plant growth. Recently Zhang *et al.* (2001) demonstrated that DGT techniques accurately predicted tissue Cu concentrations in plants grown on soils contaminated with inorganic Cu (R^2 0.95), while Degryse *et al.* (2003) found DGT procedures gave good predictions of pore-water Zn concentration in field contaminated soils. Therefore the next logical step will be to apply the DGT technique to investigations on biosolids.

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