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Transformation and plant availability of copper in pasture soils

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ABSTRACT

The response of pasture to copper (Cu) fertilisers in most soils is very short-lived necessitating frequent applications of Cu fertilisers. The short-term response to Cu by plants is attributed to the ready adsorption of Cu by organic matter and other soil components. Cu distribution among these different fractions and the relative availability of these fractions for plant uptake, are fundamental to an understanding of the transformation of Cu in soil. As Cu has not been routinely analysed in the past, there is no standard soil test extractant in New Zealand. The use of single chemical extractions in routine soil analysis is a fast and simple way to evaluate the availability of soil nutrients to plants. Farmers require accurate information on the length of time that Cu applications remain fully effective in order to supply the Cu required for the grazing animal. Pasture provides the main source of Cu for grazing animals. There is a need to define the rates of change in the effectiveness of Cu fertiliser over the range of soil and climatic conditions encountered in New Zealand.

The specific objectives of the study were: (i) to investigate the effect of soil components on the sorption and desorption of added and native Cu in soils; (ii) to examine the soil and fertiliser properties that influence the effectiveness of Cu topdressing in terms of increasing Cu uptake by pastures; (iii) to determine the transformation of Cu added through fertiliser applied to soils; (iv) to quantify the forms of Cu in soils using a sequential fractionation procedure; (v) to identify the forms of Cu in various soil test extractants and to assess the efficiency of these soil test extractants in predicting Cu uptake; (vi) to estimate the effects of N and P fertilisers on the uptake of native Cu by ryegrass; (vii) to examine at the residual effectiveness of two Cu source fertilisers as influenced by N fertiliser, lime and EDTA additions; and (viii) to evaluate the seasonal influence on the availability of native and added Cu to pasture.

Copper sorption and desorption isotherms were determined for a number of soils (Manawatu, Tokomaru, Ramiha, Ngamoka and Mangamahu) before and after the removal of various soil components. A series of glasshouse and field trials were carried out using three Cu sources, five soils and four Cu levels. The dry matter yields of ryegrass and Cu concentration in the herbage were monitored over a number of harvests. The soil was collected from the glasshouse trial at various intervals and analysed for different fractions (exchangeable, organic, oxide and residual) and were

extracted with various soil test extractants. Copper extracted from the soils was correlated with the Cu concentration in the herbage.

A second glasshouse trial with two soils, four levels of nitrogen (N) and five levels of phosphate fertiliser was conducted. The dry matter (DM) yield and the Cu concentration in the ryegrass were measured. The effects of N fertiliser, lime and EDTA addition on the availability of residual Cu was investigated in a separate glasshouse trial. A series of field trials were conducted, in the same paddocks, to examine the effect of season on the uptake of Cu from two Cu sources.

The differences in the chemical characteristics of the soils resulted in some variation in the sorption and desorption of Cu between the soils. Soil pH, organic carbon, iron and aluminium oxides play a major role in the sorption and desorption of Cu in soils. Organic matter and oxides are important in adsorption reactions, but differences exist in their relative importance. Increasing levels of Cu increased the Cu concentration in plants. Sources of Cu fertiliser have a significant effect on DM yield, and Cu concentration at all harvests. Soil pH, organic matter, CEC and clay content correlated with Cu concentration in plants. Cu uptake in grasses decreased with time after fertiliser application. Organic and oxide bound Cu contributed >80% of total Cu in all the soils. The organically bound Cu fraction was highest in soils with high levels of organic matter. Both the organically bound and the oxide bound fractions of Cu decreased with time after fertiliser application, indicating a possible decrease in the availability of Cu. Soil exchangeable, organic and oxide bound fractions of Cu were correlated with soil organic matter, CEC and clay content. Both the organic and oxide bound Cu were correlated with plant Cu uptake. The major forms of Cu extracted by the soil test reagents include organically bound, followed by oxide bound, residual and exchangeable forms. The ratios of different forms of Cu strongly suggest that Cu is residing mainly in the organic form and increases in this order: exchangeable < residual < oxide < organic. The efficiency of chemical extractants in extracting the Cu from the soil followed: TEA-DTPA > Mehlich-3 > Mehlich-1 > 0.02M SrCl₂ > 0.1M HCl > 1.0M NH₄NO₃ > 0.01M CaCl₂ > 0.1M NaNO₃ > 0.01M Ca(NO₃)₂.

Increasing levels of both N and P fertilisers increased both the DM yield and the uptake of native Cu. Increasing levels of N increased both the DM yields and the Cu

concentration in soils with residual Cu. The effect on Cu concentration persisted beyond the first cut only at the highest N addition. Increasing levels of lime increased the DM yield of pasture, but decreased the Cu concentration in pasture at the highest level of lime addition. Increasing levels of EDTA increased the Cu concentration in soils and thereby increased the Cu concentration in the pasture. The application of 1000 kg lime ha⁻¹ and 50 kg N ha⁻¹ was very effective in enhancing plant availability of residual Cu in soils, but EDTA increased the plant available Cu to toxic levels. The highest application rate of lime and N fertiliser decreased the exchangeable and free Cu in the Ngamoka soil, but EDTA showed the opposite effect.

In the field experiment Cu levels have no significant effect on DM yield during all seasons. The field study shows differences in seasonal response to added Cu. Increasing levels of Cu increased the Cu concentration in pasture. Types of Cu fertiliser have a significant effect on Cu concentration. The differences in pasture growth and Cu concentrations in plants seasonally could be attributed to the differences in air and soil temperature, soil moisture content and solar radiation patterns within the trial period.

Adsorption and desorption reactions are likely to be the major factors controlling the availability of Cu to plants. The major forms of Cu that can be extracted by soil test extractants are the organically bound, followed by oxide bound, residual and exchangeable forms. Organic and oxide bound Cu were the main sources of plant available Cu. The uptake of native Cu and residual Cu from soils showed that N and lime at 50 kg N ha⁻¹ and 1000 kg lime ha⁻¹ levels increased the Cu concentration, and EDTA also increased the plant available Cu to toxic levels. The effect of N, lime, and EDTA on the availability of residual Cu in ryegrass needs further investigation. Both the glasshouse and field trials indicate that Cu uptake is internally regulated by the growth of pasture and externally affected by the transformation of Cu in soils.

DEDICATED

to my

BELOVED PARENTS

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CHAPTER 1 GENERAL INTRODUCTION

1.1 INTRODUCTION

Copper (Cu) is one of the most important essential elements for plants, animals and humans. Cu was confirmed as an essential element for plants in the 1930s. In nature, Cu forms sulphides, sulphates, carbonates and other compounds, and also occurs under reducing environments as the native metal. Cu is associated with soil organic matter, oxides of iron and manganese, soil silicate clays and other minerals (McLaren *et al.*, 1983b; Shuman, 1991; Swift and McLaren, 1991; McBride, 1994). Cu in the soil solution occurs as free ionic Cu and organically complexed species (Hodgson *et al.*, 1966; Baker and Senft, 1995; Fotovat and Naidu, 1997; McBride *et al.*, 1998).

The bioavailability of Cu is related to the chemical potential of the respective species in the soil solution. The level and distribution of total and extractable Cu in the soil profile varies with soil type and parent material. Cu is specifically adsorbed or fixed in soils, making it one of the trace elements that moves the least. Higher concentrations of Cu in the surface horizon of a soil is an indication of increased retention of Cu added from various sources. Cu is added as smelter ash, fertilisers, sewage sludges and other wastes, fungicides or bactericides, manures from pigs and poultry, fed selected Cu containing compounds for increased feed efficiency and greater growth rates. Generally Cu concentrations in plants range from 5 to 20 mg kg⁻¹, which may extend from 1 to 30 mg kg⁻¹. Cu accumulation differs among plant species and cultivars. Therefore, it is not possible to give single values for Cu deficiency or toxicity concentrations. Cu in plants functions as part of the prosthetic group of enzymes, and as a facultative activator of enzyme systems.

In animal nutrition, Cu deficiency is almost entirely confined to the grazing animal due to low levels of Cu in the herbage/forage. Or to the normal to be low normal levels of Cu accompanied by elevated levels of Mo, S and Fe, that are sufficient to limit Cu absorption and retention. In many parts of the world, under a wide range of soil and climatic conditions, the occurrence of Cu deficiency in grazing animals may be attributed to: (i) inherently low soil and herbage Cu concentrations, such as in the area of the falling disease of cattle and neonatal ataxia of lambs in western Australia; (ii)

dual deficiency of Cu and Co on coastal calcareous-leached soils of granite origin, such as in Florida and southern Australia; and (iii) conditioned deficiency by the use of lime (CaCO_3) and from a high intake of antagonists to Cu metabolism, such as Mo in the pumice and peat soils of New Zealand and the humic peat soils of Europe (Underwood, 1981).

In New Zealand, the pastoral system is legume based and nitrogen fertiliser is used in pastures for encouraging out of season pasture growth. Phosphate fertilisers are regularly used in legume based pasture systems. Application of a high N supply may cause rapid plant growth and accentuate Cu deficiency by exhausting the Cu in the soil solution (Alloway and Tills, 1984; Robson and Reuter, 1981). Reuter *et al.* (1981b) reported that interaction between P and Cu was indirect and positive in its effect on growth in subterranean clover, and when the P supply increased from a marginally deficient level to an adequate level, there was depressed Cu absorption and an accentuated Cu deficiency.

There has been some conflict of opinion as to the best means of correcting the Cu deficiency in stock. The effectiveness of pasture topdressing with Cu depends on the nature of the pasture and on soil properties. The residual effects of Cu topdressing also differs between soils. It has often been observed that in most New Zealand soils, the response to Cu fertilisers in pasture is very short lived. In Cu deficient soils, application of Cu fertilisers increases the Cu uptake by plants only for the first few harvests and the Cu concentration in the herbage decreases to the background level within a short time (Sherrell and Rawnsley, 1982; Willimott, 1995; Khan *et al.*, 1996).

Copper added to the soil is rapidly immobilised and plant utilisation is very dependent on exploitation of the soil by roots. There is a need to define the rates of decline in the effectiveness of Cu fertilisers over the range of conditions encountered. This information is required so that the needs of crops, pastures and the grazing animal can be met and agricultural production maintained. Cu in the soil solution occurs mainly as Cu organic matter complexes. There is still insufficient knowledge concerning the reactions controlling the availability of Cu to plants, although it is generally assumed that adsorption and desorption reactions control the levels of Cu in the soil solution.

Therefore it is difficult to predict changes in the supply of plant available Cu in field situations from the existing knowledge of the chemistry of Cu in soils.

In this study the adsorption and desorption reactions of Cu in a range of pasture soils were examined. The effects of different Cu sources as fertilisers, other nutrients (nitrogen and phosphorus), soil amendments (lime and EDTA) on the plant availability and transformation of Cu were examined. Finally the seasonal responses on the availability of Cu in pasture plants is examined under field conditions. The overall structure of this thesis is presented in Figure 1.1.

1.2 OBJECTIVES OF VARIOUS EXPERIMENTS

A number of laboratory, glasshouse and field experiments were conducted with the following objectives.

1.2.1 *Adsorption and desorption of Cu in pasture soils*

- To determine the adsorption capacity of a range of pasture soils.
- To examine the effect of pH on Cu adsorption.
- To quantify the contribution of the different soil components to Cu adsorption.
- To examine the effects of pH, the incubation period of Cu with soil and the contact time on the desorption of Cu.

1.2.2 *Plant availability of Cu from different Cu fertilisers in pasture soils.*

- To examine the effectiveness of a range of Cu fertilisers on plant growth and the raising of pasture Cu concentration.

1.2.3 *Transformation and plant uptake of Cu in different soils*

- To determine the transformation of Cu fertilisers with time, which might account for the changes in Cu availability to plants.
- To evaluate the plant availability of Cu from the various soil Cu fractions.

- To evaluate the efficiency of different extracting reagents for the determination of Cu availability in pasture soils.

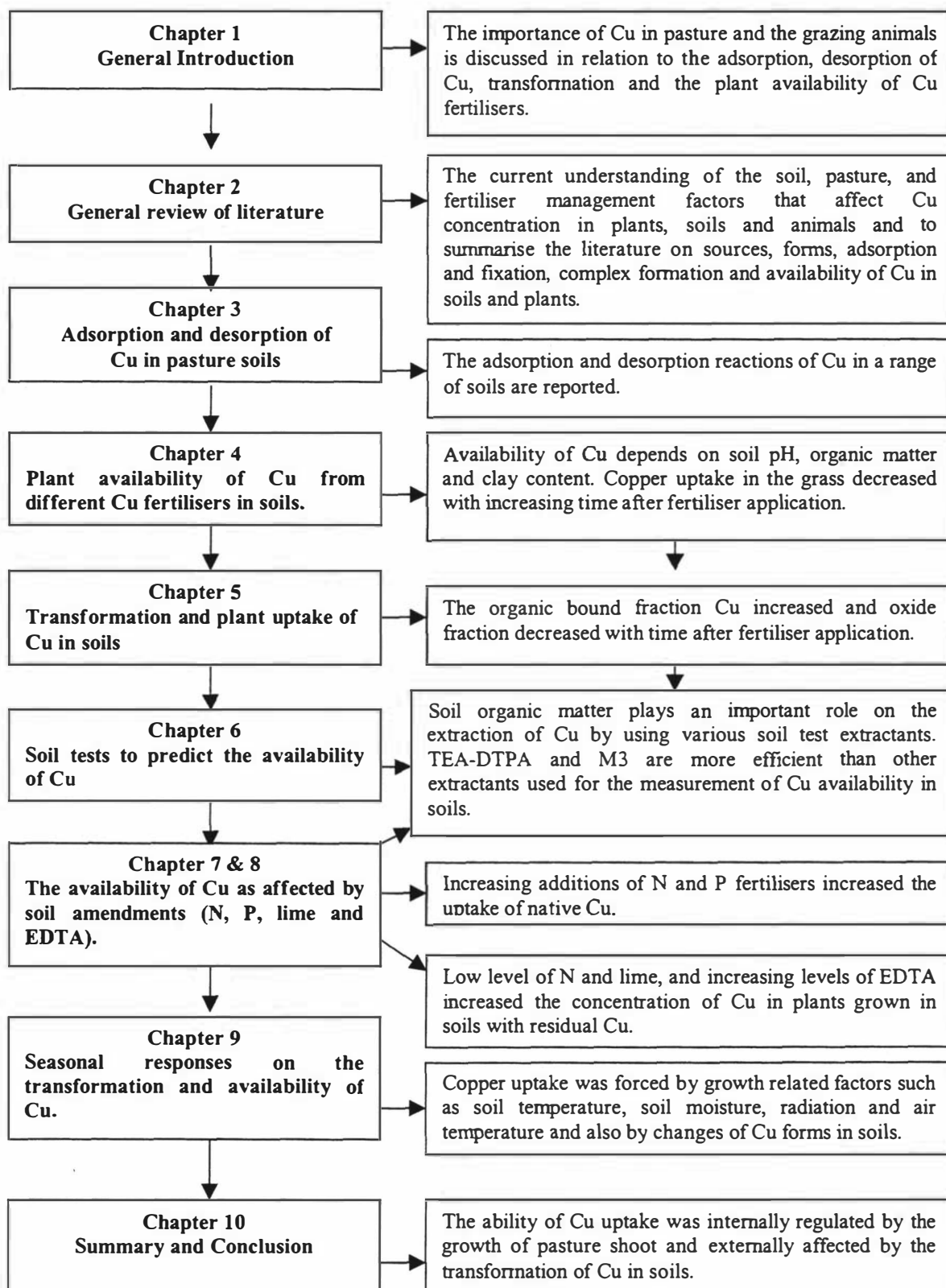
1.2.4 Effect of nitrogen, phosphorus fertilisers, lime, and EDTA on the availability of native Cu and residual Cu.

- To evaluate the effects of nitrogen and phosphorus fertilisers on the uptake of native Cu by ryegrass in two contrasting soils.
- To determine the residual effectiveness of fast and slow release Cu fertilisers as influenced by N fertiliser, lime and EDTA addition.

1.2.5 Seasonal response of copper availability in pasture

- To examine the seasonal effect on the ability of two different Cu fertilisers (CuSO_4 and CuO) in raising the Cu concentration in pasture and the transformation of Cu in soils.

Figure 1.1 The flow diagram / structures of the thesis.



CHAPTER 2 LITERATURE REVIEW

2.1 INTRODUCTION

The objective of this review is to describe the current understanding of the soil, pasture, and fertiliser management factors that affect Cu concentration in plants, soils and animals. And to summarise the literature on the Cu sources of additions to soils, the forms of Cu in soils, adsorption and desorption of Cu in soils, and organic binding and plant availability of Cu in soils.

2.2 SOURCES OF COPPER IN SOILS

2.2.1 *Soil parent materials*

Typical Cu contents of the major rock types are given in Table 2.1. The average Cu concentration in the earth's crust ranges from 24 to 55 mg kg⁻¹ and the average Cu range for soils is 20 to 30 mg kg⁻¹. Aubert and Pinta (1977) and McBride (1981) however reported that C horizon soils derived from a parent material of crystalline schists (garnet, granulite) containing an intrusion of norite with 1000 mg kg⁻¹ Cu, contain up to 100 mg kg⁻¹ Cu. Typical Cu concentrations found in soils derived from different parent materials are presented in Table 2.2. Copper is translocated with clays and tends to be most abundant when the clay content is high (Fagbami *et al.*, 1985). Fractionation (Shuman, 1979) and sorption studies (McBride, 1981; McLaren *et al.*, 1983b) have shown that Cu may be tightly adsorbed on Al, Mn, and Fe oxides. Soils derived from volcanic ash and pumice may be deficient in plant available Cu (Nyandat and Ochiing, 1976).

Table 2.1 Copper content of major rock types (mg kg⁻¹).

Rock type	Average concentrations	Range	References
Igneous rocks	-	10-100	Krauskopf (1972)
Limestone and dolomite	6	0.6-13	Cox (1979)
Shale and clay	35	23-67	Cox (1979)
Earth's crust	70	-	Parker (1981)
Soils	30	2-250	Bowen (1979)

Table 2.2 Typical Cu concentrations in soils from various parent materials (Viets, 1962).

Soils	Parent materials	Concentrations (mg kg ⁻¹)
Peat	Peat (Histosols)	15-40
Sandy soil	Drift (Aresols, Podzols)	2-10
Sandy soil	Granite	10
Silty clay loams	Shales (Gleysols, Cambisols)	40
Clays	Clay developed on clay rocks (Gleysols)	10-27
Loams	Developed on basalt rock (Cambisols)	40-150
Humic loams	Developed on chalk	7-28
Organic- rich loams	Loess (Chernozems)	1-100
Pumice soil	Pumice (Lithosols, Arenosols)	3-25
Tropical soil	Ferralsols	8-128

2.2.2 *Ore minerals*

In nature, Cu occurs both in the free state and in combination with other elements in a host of minerals. Copper usually occurs as the metal or associated with sulphur in most rock types. Two distinct assemblages of sulphides are recognised. The magnetic sulphides include Cu-Fe sulphides and are represented by chalcopyrite, the most important mineral of this group. The second assemblage is characterised by the complex sulphides and sulpho salts, and is the source of up to 70% of the world's copper production.

Schroeder (1979) reported that chalcopyrite (CuFeS₂) is the most abundant. Although Cu occurs in approximately 150 minerals, only few are considered important ore minerals (Table 2.3).

Table 2.3 Copper ore minerals (Hignett and McClellan, 1985).

Group	Minerals	Chemical formula
Complex sulphides	Chalcopyrite	CuFeS_2
	Bornite	Cu_5FeS_4
	Enargite	Cu_3AsS_4
	Tetrahedrite	$(\text{Cu, Fe})_{12}\text{Sb}_4\text{S}_{13}$
Simple sulphides	Chalcocite	Cu_2S
	Covellite	CuS
Oxides	Cuprite	Cu_2O
Carbonates	Malachite	$\text{CuCO}_3 \cdot \text{Cu}(\text{OH})_2$
	Azurite	$2\text{CuCO}_3 \cdot \text{Cu}(\text{OH})_2$

2.2.3 Fertilisers

A wide range of Cu materials are used to supply Cu to soils. The various sources of Cu used for crop production are presented in Table 2.4. The most usual Cu fertiliser is $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, although other compounds, mixtures and chelates are also used (Table 2.5). Hydrated CuSO_4 is compatible with most fertiliser materials. Man made inputs of Cu to land are very diverse. Soil levels of Cu are affected by soil and crop treatments including fungicides and fertilisers; not used exclusively to rectify Cu deficiencies, such as livestock manures, sewage sludges and atmospheric deposition.

Table 2.4 The principal copper materials used in Cu fertilisers.

Material	Formula	Cu ^a g kg ⁻¹
Copper sulphate pentahydrate	$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	250
Copper sulphate monohydrate	$\text{CuSO}_4 \cdot \text{H}_2\text{O}$	350
Basic copper sulphate	$\text{Cu}_4(\text{OH})_6\text{SO}_4$	530
Copper carbonate	CuCO_3	550
Copper hydroxide	$\text{Cu}(\text{OH})_2$	300
Copper oxide	CuO	250-750

^aThe g kg⁻¹ copper listed is that of the generally marketed grade, and not necessarily that of the pure compound.

Table 2.5 Copper minerals and Cu containing fertiliser materials (Netzer and Beszedits, 1979).

Source	Formula	Cu (g kg ⁻¹)	Water solubility
Cuprite	Cu ₂ O	890	Insoluble
Tenorite	CuO	750	Insoluble
Covellite	CuS	660	Insoluble
Chalcocite	Cu ₂ S	800	Insoluble
Chalcopyrite	CuFeS ₂	350	Insoluble
Malachite	CuCO ₃ . Cu(OH) ₂	570	Insoluble
Azurite	2CuCO ₃ . Cu(OH) ₂	550	Insoluble
Chalcanthite	CuSO ₄ . 5H ₂ O	250	Soluble
Copper sulphate monohydrate	CuSO ₄ . H ₂ O	350	Soluble
Basic copper sulphate	CuSO ₄ . 3Cu(OH) ₂	130-530	Insoluble
Copper nitrate	Cu(NO ₃) ₂ . 3H ₂ O	-	Soluble
Copper acetate	Cu(C ₂ H ₃ O ₂). H ₂ O	320	Slightly
Copper oxalate	CuC ₂ O ₄ . 0.5H ₂ O	400	Insoluble
Copper oxychloride	CuCl ₂ . 2CuO. 4H ₂ O	520	Insoluble
Copper ammonium phosphate	Cu(NH ₄)PO ₄ . H ₂ O	320	Insoluble
Copper chelate	Na ₂ Cu EDTA	130	Soluble
Copper chelate	NaCu EDTA	90	Soluble
Copper polyflavanoids	-	50-70	Soluble
Copper-sulphur frits	-	Varies	Varies
Copper-glass fusions	-	Varies	Varies
Sewage sludge	-	0.4-10	Slightly
Animal manures (no Cu supplements)	-	0.02-0.00	Slightly
Animal manures (With Cu supplements)	-	0.6-1.9	Slightly

2.2.4 Pesticides

Copper compounds have been used for many years as fungicides and bactericides. In the past, CuSO₄.5H₂O was mixed with lime to control plant pathogens, recently Cu(OH)₂ has been used in fairly large amounts in sprays (Bordeaux mixture) as a blanket preventive treatment (Walsh *et al.*, 1972; Florez-Vélez *et al.*, 1996). Copper sprays have been recommended for disease control. These sprays can enrich Cu in the soil by as much as 11.2 to 16.8 kg ha⁻¹ yr⁻¹. Copper toxicity may develop in sandy soils,

because they have a low CEC ($<5 \text{ cmol kg}^{-1}$) and often contain $<1\%$ organic matter. Continued overuse of Cu containing fungicides and bactericides can result in the accumulation of Cu in soils to phytotoxic levels (Delas *et al.*, 1960; Lepp *et al.*, 1984).

2.2.5 Sewage sludge

Copper concentrations in sewage sludge vary widely. Page (1974) and Sommers (1977) have reported significant levels of Cu in sludge. Sewage sludge contains appreciable amounts of Cu, but application of sludge to soils generally results in only slight to moderate increases in the Cu content of plants (CAST, 1976). In Pennsylvania, USA it has been found that many sludges from waste water treatment plants cannot meet the 1000 mg kg^{-1} maximum threshold limit for Cu, but can meet a 1200 mg kg^{-1} Cu limit. Maximum Cumulative metal loading of soils from sludges is regulated in many countries through resource management legislation. In New Zealand, the New Zealand Dairy Board has recently introduced restrictions on the use of sewage sludge in pastures soils. But recent studies have showed that the addition of bio-solid composts increases the formation of organic-metal complexes and thereby reduces the bioavailability of heavy metals (Bolan *et al.*, 2001). New Zealand guidelines for the application of sewage sludge to soils contains maximum annual permissible applications of Cu to land and maximum allowable concentrations in soils are 12 kg ha^{-1} and 140 mg kg^{-1} , respectively (Tiller *et al.*, 2000). The regulated level of Cu of 60 ppm in the plough layer (15 cm) is intended to prevent long-term adverse effects on biological processes essential for soil fertility; as well as possible adverse health effects to sheep and cattle on treated pastures (Hornick *et al.*, 1976 and Baker *et al.*, 1985). Because of the relatively high concentrations of Cu and other metals, compared to non-sludge treated soils, sewage sludge should be analysed before use as a soil amendment. Under no conditions is it considered practical to use sewage sludge as a soil conditioner without consideration of the metal and pathogen concentrations in the sludge. In case the resultant loading of Cu and other metals to the soil damage the crops grown. A Cu rich sludge (6000 mg kg^{-1} on a dry basis with 2300 mg kg^{-1} extractable in 0.005 M EDTA) addition to land at 125 t ha^{-1} in the first year and 31 t ha^{-1} in each of 3 subsequent years, raised the total soil Cu from 30 to 600 mg kg^{-1} and the 0.05 EDTA extractable Cu from 8 to 280 mg kg^{-1} , of which 180 mg kg^{-1} was still EDTA extractable after 4 years (Stevenson and Fitch, 1981).

While sewage sludges are capable of substantially increasing the soil levels of Cu, there have been no reports of plant toxicity's to sludge Cu, when grown in fertile limed soils. Soil organic matter appears to be the dominant factor controlling Cu retention (Harter, 1986). Thus, the rate of decomposition of sludge organic matter becomes a prime consideration for sludge treated soils.

2.2.6 Composts and agricultural wastes

Garcia *et al.* (1995) and Ciavatta *et al.* (1993) reported that the concentration of Cu in sewage sludge increases during composting. Japenga *et al.* (1992) pointed out that complex formation involving high molecular weight dissolved organic matter is the most important driving force for heavy metal solubilisation. Barbera (1987) found that the application of compost to agricultural soils increased both the total and available concentrations of metals in the soils. Arnesen and Singh (1998) investigated three sources of organic material (cow manure, pig manure and peat) on the extractability of Cu in soil, and Cu uptake by wheat and barley plants. They reported that increasing rates of all three organic materials increased the Cu concentration. The increase in Cu uptake by plants suggests that a change in the speciation of Cu in the soil solution may have enhanced its plant availability.

2.2.7 Wood preservatives

The wood preservative industry is one of the major markets for Cu (Loebenstein, 1993). McLaren *et al.* (1994) examined the leaching of Cu, Cr, and As (CCA) from the timber treatment solution through free draining coarse textured surface and subsurface soils (Typic Ustipsamment and Udic Ustochrept) using undisturbed soil lysimeters. Cumulative amounts of Cu leached through the lysimeters ranged from 4 to 30% of the total Cu applied (90 mg Cu) from a CCA solution. The amounts of Cu leached from the CCA solution were generally higher than those recorded from sewage sludge addition, which is attributed to the presence of Cu in relatively immobile forms in the latter (McLaren *et al.*, 1994).

2.2.8 *Atmospheric deposition*

Atmospheric inputs of Cu to soils from both rain and dry deposition vary considerably according to the proximity of industrial emissions containing Cu, and the type and quantities of wind-blown dust. In the UK, the total annual deposition of Cu from dust was found to vary between 100 g ha⁻¹ and 480 g ha⁻¹. While crop removal was estimated at 50 to 100 g ha⁻¹, the deposition of dust was not sufficient to correct Cu deficiency in crops or livestock (Shorrocks and Alloway, 1987). The lack of response to this deposition was attributed either to the form of Cu or to soil adsorption or both. Duce *et al.* (1976) estimated the annual natural fluxes of Cu to the atmosphere to be 14.3×10⁶ kg. This is in reasonable agreement with Nriagu's (1979) estimate of the total quantity of atmospheric emission of 3.2×10⁶ t Cu (about 1% of the 307×10⁶ t produced) since 3800 BC. This amount is about three orders of magnitude greater than the present day atmospheric Cu burden.

2.3 COPPER IN SOILS

2.3.1 *Total Cu in soils*

Gilbert (1952) reported that the total Cu content in normal agricultural soils is expected to range from 1 to 50 mg kg⁻¹. Copper content of the world's soils has been characterised by Aubert and Pinta (1977) according to various climatic zones as follows: a) temperate and boreal regions - total Cu ranges from trace to several hundred mg kg⁻¹, (lowest content of 8 mg kg⁻¹ was reported on the fluvioglacial and alluvial sands of the lower Volga Valley in the USSR; some of the highest values, 50 to 200 mg kg⁻¹ in the USSR are found on the Kola Peninsula); b) arid and semi arid region soils, in these regions generally contain from average to high total Cu; and c) tropical humid regions soils have fluctuating total Cu contents, with values from trace to 200-250 mg kg⁻¹. The data in Table 2.6 gives concentrations of Cu in uncontaminated surface layers of the major soil groups.

Table 2.6 Ranges and mean concentrations of Cu in surface soils calculated on the world scale (Kabata-Pendias and Pendias, 1992).

Name of soils	Range (mg kg ⁻¹ dry wt.)	Mean (mg kg ⁻¹ dry wt.)
Podzols (sandy soil)	1 - 70	13
Cambisols (silty and loamy soil)	4 - 100	23
Rendzinas	6.8 - 70	23
Kastanozems and Chernozems	6.5 - 140	24
Histosols (organic soil)	1 - 113	16

2.3.2 Forms of soil Cu

Total Cu in soils includes six 'pools', classified according to their physico-chemical properties. The pools include: soluble ions, inorganic and organic complexes in the soil solution; exchangeable Cu; stable organic complexes in humus; Cu adsorbed by hydrous oxides of Mn, Fe and Al; Cu adsorbed on the clay-humus colloidal complex; and crystal lattice-bound Cu in soil minerals. When dilute salt solutions such as 0.01M CaCl₂ are used to extract soils, the soil solution and exchangeable Cu are considered as one fraction (Shorrocks and Alloway, 1987). In surface soils, the total Cu concentration in the soil solution is normally only 0.01 to 0.6 μ M, owing to its high affinity for sorption by organic and inorganic colloids (Shorrocks and Alloway, 1987).

The dissolved and exchangeable forms are of special importance in plant nutrition. Cu in the soil solution, exchangeable, specifically adsorbed and organically bound Cu are considered in equilibrium. They represent the available forms for plant uptake. Oxide-bound and residual forms of Cu are relatively unavailable to plants (McLaren and Crawford, 1973a). The amount of Cu in any given form can vary with time, and the extractants used. McLaren and Crawford (1973a) found that the distribution of Cu between the main soil constituents could be influenced considerably by the presence of free manganese oxide. They found that the bulk of the 'available' soil Cu reserves reside in the organically bound fraction. Mathur and Lévesque (1983) concluded that the organically bound Cu could be the dominant form in Histosols. Apparently, enrichment of the soil with Cu, from either organic or inorganic sources, can be reflected by increases in most fractions, with the exception of the residual form. Most of the increases occurring in the organic fraction. In some cases, however, Cu associated

with Fe and Mn oxides may constitute the major fraction of total Cu in soils (Kuo *et al.*, 1983).

2.3.2.1 *Soil solution Cu*

Although Cu is one of the least mobile heavy metals in soils, this metal is abundant in the soil solution of all types of soil. Concentration of Cu in the soil solution obtained by different techniques from different soils varies from 3 to 420 $\mu\text{g L}^{-1}$, which corresponds to Cu concentrations of 0.047 to 6.58 μM (Table 2.7). Williams (1981) reported that soil solution Cu concentrations ranged from 3 $\mu\text{g L}^{-1}$ to 240 $\mu\text{g L}^{-1}$ in soils receiving regular additions of Cu. Soil solution Cu can be classified into three categories.

- Inorganic complexes of Cu.
- Cu complexed with soluble organic matter.
- Uncomplexed Cu or ionic Cu.

The stability constants of the inorganic Cu complexes show that the formation of significant quantities of these complexes does not occur in most soils (Lindsay, 1972). Gardea-Torresdey *et al.* (1996) observed that the amount of Cu bound to soluble organic matter is dependent on the solution pH. Only hydroxy and carbonate species are expected to exist in significant quantities in the soil solution. The solubility of Cu^{2+} becomes negligible around pH 7.0 - 8.0, while above this pH Cu carbonate complexes and CuOH^+ become significant (McBride, 1981). Mattigod and Sposito (1977) observed that the soluble carbonate forms would be the dominant inorganic Cu species in highly alkaline soils. Nitrate and phosphate species would not be expected to contribute significantly to total inorganic Cu in the soil solution. McBride (1981) mentioned that, among the anions (nitrate, chloride, sulphate, and phosphate) in soil solutions, sulphate and chloride may form complexes with Cu^{2+} in saline soils. Mattigod and Sposito (1977) concluded that the major inorganic form of complexed Cu^{2+} in neutral and alkaline soil solutions would be CuCO_3 . In highly alkaline soils, $\text{Cu}(\text{OH})_4^{2-}$ and $\text{Cu}(\text{CO}_3)_2^{2-}$ become the predominant soluble species. McBride and Bouldin (1984) estimated that at least 99.5% of the Cu in the soil solution of a surface loamy soil (5.3% organic matter content) was in an organically complexed form. Hodgson *et al.* (1966) observed that as the pH was raised there was little change in the total concentration of Cu in the soil solution, but the partitioning between uncomplexed and complexed Cu

changed. At low pH, a higher proportion of Cu was present as the uncomplexed ion and with increasing pH, soluble Cu-organic complexes became more significant. They showed that the log ratio of complexed Cu to uncomplexed Cu was positively correlated to the organic matter in solution ($r = 0.88$, $p < 0.01$). As illustrated in Table 2.8, hydrated forms of Cu ions are unlikely to exist in aqueous systems with a pH below about 6.0. As the pH approaches neutrality, however, Cu is rapidly hydrolysed to $\text{Cu}(\text{OH})_2^0$. Harter (1983) reported that the amount of $\text{Cu}(\text{OH})_2$ species in the soil solution at pH 6 accounted for only 2% of the Cu^{2+} ion, but at pH 8 for 92% of the Cu^{2+} ion.

Brümmer *et al.* (1986) and McBride (1989) consider that at pH values close to 7, more than 99% of the Cu in solution is complexed by organic matter. Wu *et al.* (2000) investigated the activity of Cu^{2+} ions and analysed the dependence on pH and other soil properties. In saturated soil extracts, the activity of free Cu^{2+} ions varied from 4.78×10^{-8} to $11.4 \times 10^{-8} M$, which constituted 0.002 to 0.008% of total soil Cu. The activity of Cu^{2+} ion in soils fell in the range of 10.9×10^{-11} to $4.0 \times 10^{-9} M$, which accounted for 0.1 to 7.8% of the dissolved Cu. The dominant species of Cu in soil solutions is Cu^{2+} and CuOH^+ ions, which are about 10 times greater than CuHCO_3^+ . They obtained a highly significant correlation coefficient between soil properties (pH and DOC) and the activities of Cu^{2+} and CuOH^+ , which implies that the concentration of free Cu^{2+} ions in the soil system is strongly pH dependent.

Table 2.7 Copper in the natural soil solution of different soils.

Techniques used for obtaining soil solution	Soils/Country	Copper concentration ($\mu\text{g L}^{-1}$)	References
Suction from soil paste	30 series of soil, USA	40	Bradford <i>et al.</i> (1971)
Pump off 0.01N CaCl_2 solution	5 series of soil, New York, USA	3 - 18	Hodgson <i>et al.</i> (1965)
Centrifugation	Contaminated soil	28 - 135	Kabata-Pendias (1972)
do	Three field soil, Japan	37	Yamasaki <i>et al.</i> (1975)
do	Contaminated soil, Canada	3.04 - 7.42	Wu <i>et al.</i> (2000)
Ceramic plate or porous Cup suction	Two forest soils, Germany	18 - 27	Heinrichs and Mayer (1980)
do	Contaminated soil, Japan	14 - 44	Itoh and Yumura (1979)
Zerotention lysimeter	Temperate forest soil.	1.8 - 22	Bergkvist <i>et al.</i> (1989)
Unspecified technique	Different paddy soils, Australia	<1 - 3	Tiller (1981)
do	Contaminated soil, Russia	29 - 116	Upitis and Gubar (1986)
Centrifugation	Dutch forest and arable soils	15 - 150	Römkens and Salomons (1998)
Ion exchange resin	11 different soils, Australia	5.2 - 74.4	Fotovat and Naidu (1997)
Centrifugation	Dutch sandy soils	46-117	Römkens <i>et al.</i> (1999)
Deionised water	Surface soil, USA	60 - 420	Reddy <i>et al.</i> (1995)

Table 2.8 Effect of pH on solution composition of Cu, expressed as percent in solution (Harter, 1983).

Soil pH	4.0	5.0	6.0	7.0	8.0
Cu^{2+}	100	100	96	33	1
CuOH^+	-	-	2	7	1
$\text{Cu}(\text{OH})_2^0$	-	-	2	56	92

2.3.2.2 *Exchangeable Cu*

Copper held by electrostatic attraction (non specific adsorption or ion exchange mechanisms) onto exchange sites in soils is termed as exchangeable Cu. Exchange sites comprises the negatively charged surfaces of clays and iron, aluminium and manganese oxides and also the functional groupings of organic matter (Hodgson, 1963). Variable charge of clay minerals results from the deprotonation of hydroxyl groups, while isomorphous substitution of ions in the clay structure accounts for the permanent charge associated with these materials. The negative charge on iron, aluminium and manganese oxides results from the deprotonation of surface hydroxyl groupings (Parfitt, 1980). Similarly, the dissociation of protons from mainly carboxyl and phenol functional groups of organic matter contributes to its negative charge (Parfitt, 1980).

2.3.2.3 *Specifically adsorbed Cu*

Specific adsorption of Cu is a term, which refers to the formation of stable complexes between Cu ions and specific functional groups at the surface of organic and inorganic soil colloids. Copper can be “specifically” adsorbed by layer silicate clays, oxides of Fe, Mn, and Al, and organic matter. In specific adsorption, ions are held much more strongly by the surface charge, as these ions penetrate the co-ordination shell of the structural atoms and are bonded by covalent bonds via O atoms (Cu-O-Fe or Cu-O-Al bonds) or OH groups to the structural cations (Huang, 1980; Wu *et al.*, 1999). Stevenson and Fitch (1981) observed that organic colloids and clays play a major role in Cu retention by soil. In most mineral soils, Cu may be bound as clay-metal-organic complexes, since in these soils, organic matter is intimately bound to clay. Specific adsorption of Cu is measured in the presence of a weak electrolyte solution of one of the major soil cations (calcium or magnesium) thereby minimising the occurrence of non-specific (exchange) reactions (Swift and McLaren, 1991).

2.3.2.4 *Occluded Cu*

Soil oxides have a reactive surface capable of adsorbing certain amounts of Cu. Over time the oxide component of soils builds up, and is capable of occluding increasing amounts of Cu. Warnant *et al.* (1981) used Tamm’s reagent (ammonium oxalate and oxalic acid) to extract Fe, Al and Cu associated with oxidic material. In both the A and

B horizons there was a positive linear relationship between either the amount of iron or aluminium extracted and the amount of Cu extracted. McLaren and Crawford (1973b) also observed that acid oxalate-extractable Cu was correlated ($r=0.44$, $p<0.05$) to free Fe.

2.3.2.5 *Cu in the structure of silicate clay and primary minerals*

Copper may be incorporated into the structure of clay minerals by isomorphous substitution of Cu into the clay lattice. In 2:1 layer silicate clays such as montmorillonite, 5-6 member chelates, which are stable to hydrolysis, may form in the inter layer spaces (Yariv and Cross, 1979). Copper is most abundant in basaltic rocks, where there is an accumulation of ferromagnesian minerals and sulphides. Strong covalent bonds form between Cu^{2+} and S^{2-} making copper sulphide one of the most common primary minerals (McBride, 1981). Some of the carbonate forms of Cu minerals (malachite ($\text{CuCO}_3 \cdot \text{Cu}(\text{OH})_2$) and azurite ($2\text{CuCO}_3 \cdot \text{Cu}(\text{OH})_2$) are also reasonably abundant in an oxidising regime. In unweathered arid soils, the pool of Cu associated with primary minerals tends to be larger and includes such minerals as sulphates, chlorides and also carbonates. Under strong leaching conditions in relatively unweathered soils (recent soils), the pool tends to be small and most Cu minerals do not persist (McBride, 1981).

2.3.2.6 *Cu associated with biomass*

A proportion of soil Cu is associated with biological residues, microbial material and decomposing organic matter. The return of crop residues to the soil, where they are decomposed through microbial action increases the amount of Cu in the soil. However, Cu may be immobilised by incorporation into microbial tissue and hence becomes unavailable to plants (Hodgson, 1963). The flush of carbon and nitrogen mineralisation that occurs after rewetting of dried soils can be attributed to dead soil microorganisms (microbial biomass). Sparling and Berrow (1985) observed that air-drying, chloroform fumigation and γ -irradiation had little effect on extractable Cu associated with the microbial biomass. The amount of biomass Cu was found to be small and did not contribute significantly to the amounts of extractable Cu.

2.3.3 *Determination of soil Cu by a sequential fractionation scheme*

The elemental distribution in different fractions, and the contribution of a nutrient to plant uptake is fundamental knowledge required for an understanding of soil chemistry. The distribution of nutrients between the different forms of Cu is not always experimentally clear. Fractionation provides an insight into their form and their availability. The proportions of different Cu forms vary considerably, depending on the soil and fractionation technique used.

Most fractionation data for Cu show changes with time in the ability of chemical reagents to recover applied copper from the soil (Luo and Christie, 1996; Ma and Uren, 1998). For example, McLaren and Ritchie (1993) did not notice any loss of Cu from soil in a long-term trial (20 year period), however they observed changes in the fractions of Cu. Initially, Cu added to the soil at lower rates appeared to be mainly organically bound, whereas at the highest application rate, the added Cu was distributed evenly between the organic and the iron oxide fractions. At the lowest Cu application rate, the proportion of applied Cu present in the organically bound fraction decreased markedly during the trial period, and was mirrored by an increase in the residual Cu fraction. Liang *et al.* (1991) also studied the fate of added Cu in seven Saskatchewan soils, and found that sesquioxides and organic matter were the major components responsible for the adsorption of added Cu. Similarly Mullins *et al.* (1982a) observed that organically bound and sesquioxide occluded Cu held most of the added Cu. These forms of soil Cu are considered unavailable to plants. The effect of pH on Cu distribution has been reported by McLaren and Crawford (1973b) and Jarvis (1981b). These workers found that Cu adsorption was directly related to soil pH, which in turn, was primarily attributed to a greater association with soil oxides.

Iwasaki *et al.* (1997) investigated the depthwise distribution of total Cu in selected agricultural soils and in a natural soil, near an abandoned Cu mine. They observed that Cu was mainly adsorbed onto, or occluded by the organic matter, and the oxides and hydroxides of Fe and Mn. Ma and Rao (1997) examined nine contaminated sites in USA and reported that Cu distribution in various chemical fractions depended on the total Cu content of the soils. As the total Cu concentration in soil increased, the percentage of total Cu in the water soluble, carbonate, Fe-Mn oxide, and organic

fractions increased, and Cu in the exchangeable and residual fraction decreased. Fractionation procedures used by different scientists are presented in Table 2.9 and comparisons of the relative extracting abilities of sequential Cu fractionation schemes are presented in Table 2.10.

Table 2.9 Various fractionation schemes used by different authors.

Extractants	Forms of soil Cu	References
1.0 M ammonium acetate pH 7 0.05M lead nitrate + 0.5M ammonium acetate pH 6 0.44M acetic acid pH 2.6 0.1M hydroxylamine hydrochloride + 0.1M nitric acid pH 2 0.1M sodium pyrophosphate pH 10 0.175M ammonium oxalate + 0.1M oxalic acid (dark) pH 3.5 0.175M ammonium oxalate + 0.1M oxalic acid + 0.1M ascorbic acid pH 3.1 Sulphuric + nitric +perchloric acid mixture (1:5:20)	Exchangeable Pb-displaceable Acid soluble Manganese oxides Organic Amorphous iron oxides Crystalline iron oxide Residual	Iwasaki <i>et al.</i> (1997)
0.01M calcium nitrate 0.7M sodium hypochlorite (pH=8.5) 0.2 M ammonium oxalate + 0.2 M oxalic acid + 0.1M ascorbic acid Concentrated nitric, perchloric and hydrofluoric acid	Exchangeable Cu Organic bound Cu Copper associated with iron oxides Residual Cu	McLaren and Ritchie (1993)
0.5 M calcium nitrate 0.05M lead nitrate + 0.1M calcium nitrate 0.44M acetic acid + 0.1M calcium nitrate 0.01M hydroxylamine hydrochloride + 0.1M nitric acid 0.1M potassium pyrophosphate 0.113M ammonium oxalate + 0.087M oxalic acid (dark) Hydrofluoric acid + nitric acid	Exchangeable+ water soluble Pb-displaceable Acid soluble Manganese oxides Organic Iron oxides Residual	Liang <i>et al.</i> (1991)
Water 0.5M calcium nitrate 0.05M lead nitrate + 0.1M calcium nitrate 0.44M acetic acid + 0.1M calcium nitrate 0.01M hydroxylamine hydrochloride + 0.1M nitric acid 0.1M potassium pyrophosphate 0.175M ammonium oxalate + 0.1M oxalic acid (dark) 0.175M ammonium oxalate + 0.1M oxalic acid (UV) Aqua regia + hydrofluoric acid	Soluble Exchangeable Carbonate Carbonate Manganese oxides Organic Amorphous oxides Crystalline oxides Residual	Miller <i>et al.</i> (1986)

1M magnesium nitrate pH 7 0.7M sodium hypochloride pH 8.5 0.1M hydroxylamine hydrochloride pH 8 0.2M ammonium oxalate + 0.2M oxalic acid pH 3 (dark) 0.2M ammonium oxalate + 0.2M oxalic acid + 0.1M ascorbic acid Aqua regia + hydrofluoric acid + perchloric acid	Exchangeable Organic Manganese oxides Amorphous oxides Crystalline oxides Residual	Shuman (1985)
0.5M potassium nitrate Deionised water 0.5M sodium hydroxide 0.05M disodium salt of EDTA 4 M nitric acid	Readily soluble (exchangeable+sorbed) Organic Precipitated Residual	Sposito <i>et al.</i> (1982)
Water 0.1M magnesium chloride pH 7 1M sodium acetate + acetic acid pH 5 0.02M nitric acid + 30% hydrogen peroxide + 3.2 M ammonium acetate pH 2 0.04M hydroxylamine hydrochloride + 25% acetic acid pH 2 Hydrofluoric acid + perchloric acid	Soluble Exchangeable Carbonate + clay Manganese oxides Crystalline oxides Residual	Tessier <i>et al.</i> (1979)
0.05M calcium chloride 2.5% acetic acid 1 M potassium pyrophosphate 0.175M ammonium oxalate + 0.1M oxalic acid pH 3.5 (UV) Hydrofluoric acid + perchloric acid	Exchangeable Clay Organic Crystalline oxides Residual	McLaren and Crawford (1973a)

Table 2.10 Comparison of the relative extracting abilities of sequential copper fractionation schemes.

Soils	Crop grown/ Crop field	Total Cu/ Applied Cu	Soluble/ Exchangeable (mg kg ⁻¹)	Organic (mg kg ⁻¹)	Manganese oxide (mg kg ⁻¹)	Amorphous iron oxide (mg kg ⁻¹)	Crystalline iron oxide (mg kg ⁻¹)	Oxide/ Precipitated (mg kg ⁻¹)	Residual (mg kg ⁻¹)	Remarks	References
Dystric dark red soil	Typical paddy field	122 mgkg ⁻¹	6.0	60	45	25	40		150	Native Cu	Iwasaki <i>et al.</i> (1997)
Typic Udifluent Rhodic Paleudult		16.61 mgkg ⁻¹	0.09	1.10	2.17	4.83	8.0	-	-	Native Cu	Shuman (1985)
Typic Rhodudult		15.93 mgkg ⁻¹	0.22	0.43	0.33	1.0	3.17				
Typic Hapludult		33.68 mgkg ⁻¹	0.10	0.78	2.0	2.17	8.50				
		15.18 mgkg ⁻¹	0.18	1.03	0.5	0.83	5.17				
Lateritic sandy soil	Wheat field	⁺ 11 kg Cu ha ⁻¹	-	1.1				0.8	1.8	Organic and oxide fractions decreased with time.	McLaren and Ritchie (1993)
Thermo Xerochrepts		470 mgkg ⁻¹	0.05	3.87	-	-	-	0.93	1.39	Organic fraction increased and oxide fractions decreased with time.	Schalscha <i>et al.</i> (1999)
Saskatchewan soils	Bean	21 mgkg ⁻¹	0.45	1.5	0.88	2.45	-	-	15.03	Mean of 27 soils. Some soil showed decrease in organic and oxide fractions.	Liang <i>et al.</i> (1991)
Thermic Typic Umbraquults	Wheat	⁺ 15 mg Cu kg ⁻¹ soil	0.1	1.8	0.5	0.9	1.1	-	-	Copper added as CuSO ₄ .5H ₂ O	Sims (1986)
Typic Hapludults			0.1	0.2	0.5	1.2	1.3	-	-		
Thermic Aquic Hapludult	Corn	⁺ 65.6 kg Cu ha ⁻¹	0.23	7.9*	1.0	-	-	2.9	4.1	Copper added as hog manure.	Mullins <i>et al.</i> (1982b)
Mesic Aquic Hapludult			0.24	12.9*	1.9	-	-	8.2	7.4		
Typic Rhodudult			0.08	13.4*	3.7	-	-	17.7	31.6		

*Specifically adsorbed and organic bound fractions, ⁺ represent the added Cu.

2.3.3.1 *Exchangeable fraction*

As with water soluble Cu, exchangeable Cu was found to represent only a small fraction of the total soil Cu. Exchangeable Cu has been measured by the solution of different neutral salts of Mg and Ca. An electrolyte containing chloride ions is often preferred for the extraction of exchangeable Cu because it will not cause an appreciable pH change or affect other fractions. McLaren and Crawford (1973a) extracted 0.01-0.50 mg Cu kg⁻¹ from soils using 0.05M CaCl₂. They observed that the CaCl₂ extractable Cu was dependent on soil pH. As soil pH is increased the amount of CaCl₂ extractable Cu decreased. This is expected as the adsorption of Cu by soil increases with increasing soil pH. However, Cl⁻ is a stronger complexing anion than NO₃⁻, and for that reason NO₃⁻ salts have been used (Sposito *et al.*, 1982; Shuman, 1985; Singh *et al.*, 1988). Pickering (1986) noted that NO₃⁻ salts have received little attention, and are at least as effective as Cl⁻ salts without the uncertainties introduced by the complexing of Cu by Cl⁻ ions. Acetate is very commonly used with a solution pH of 7.0. Shuman (1985), in a fractionation sequence, used 1M Mg(NO₃)₂ as an extractant (pH=7.0) and the level of Cu removed ranged from 0.01 to 0.18 mg Cu kg⁻¹.

2.3.3.2 *Organic fractions*

Organic fraction procedures are difficult, because they often dissolve other fractions. The usual approach oxidises the organic matter to release the metals. These methods also oxidise sulphides, and thereby result in the overestimation of the organically bound fraction. Some procedures use chelating agents to extract the metals chelated by the soil humic materials. Bascomb (1968) was one of the first to suggest pyrophosphate (K₄P₂O₇) to extract the organic fraction (McLaren and Crawford, 1973a; Miller *et al.*, 1986). Acid peroxide has often been used to destroy organic matter from clays prior to mineralogical analysis. Another oxidising agent, NaOCl at pH 8 to 9.5, has found widespread use in sequential procedures. It has been found to be more effective than H₂O₂ for extracting organic matter with less destruction of carbonates and oxides (Omueti, 1981). Uren *et al.* (1988) presented data to indicate that NaOCl dissolves ≤0.1% of the Mn in lithiophorite and birnessite, the common forms of Mn oxides in soils. Thus, NaOCl seems to be a good compromise as an organic extractant, dissolving the majority of metals of the organic fraction, while leaving the other metal fractions largely intact (Shuman, 1983).

2.3.3.3 *Oxide fractions*

One of the earliest and most popular methods for extracting the amorphous or microcrystalline Fe oxides is that of McKeague and Day (1966). The extractant is 0.2M ammonium oxalate at pH 3. The soil samples are shaken in the dark for 4 hr to prevent photolytic reduction. Another popular method uses oxalate solutions with UV light. Kawai (1977) used a fluorescent lamp with 0.1 M oxalic acid to remove Fe oxides. Grimme (1967) used Zn as a reductant with oxalate for the crystalline oxide fraction, a method suggested by Haldane (1956) that produces no precipitation and does not attack layer alumino silicates. Shuman (1985) and McLaren and Ritchie (1993) employed ascorbic acid as a reductant with oxalate, bypassing the problems of standardising UV methods and the problems associated with the citrate/bicarbonate buffer.

2.3.3.4 *Residual fractions*

The remaining silicates and other resistant minerals are brought into solution by digesting with HF, HNO₃, HCl, or HClO₄ in heat resistant plastic containers, either open or in bombs under pressure. A 90% HNO₃ /10% HCl digestion was found adequate for total metals in a U.S. Bureau of Standards new industrial river sediment, but for selected estuarine sediments, the recovery was low (Sinex *et al.*, 1980). Aitang and Häni (1983) reported that 2 M HNO₃ solubilised 59 and 68% of total Cu and Zn, respectively, and 3M HCl solubilised 59 and 85% of the total Cu and Zn, respectively. Thus, total element concentrations are well approximated by methods that do not destroy the sample completely.

2.3.4 *Determination of total Cu*

The measurement of total Cu in soils involves complete digestion of the soil by strong acids or alkalis. Total Cu determinations are used mostly as a reference between soils and not usually as an index of availability. The most common method of total Cu determination is the hydrofluoric (HF) and perchloric acid (HClO₄) digestion. Soil samples are ignited for five hours at 450⁰C, digested in concentrated HF and HClO₄ and then taken up in HCl (Dolar *et al.*, 1971, McLaren and Crawford, 1973a, Tessier *et al.*, 1979; Haynes and Swift, 1984; Liang *et al.*, 1991).

A comparison between 3 M HCl extractable Cu, 2M nitric acid extractable Cu and total Cu (HF/ HClO₄) was carried out by Aitang and Häni (1983). Between 20-90% of the total Cu was extracted by nitric acid and this depended on soil type. Hydrochloric acid extracts more Cu than nitric acid mainly because it destroys the secondary minerals. Römken and Salomons (1998) observed that total metal content in arable soils was significantly higher than in forest soils determined by hot acid extraction with a mixture (1:4:0.1) of concentrated H₂SO₄, HNO₃ and HClO₄ acid. Iwasaki *et al.* (1997) investigated the depth wise distribution of total contents of Cu in selected agricultural soils and in a natural soil, near an abandoned Cu mine. They digested the soil with a mixture (1:5:20) of H₂SO₄ : HNO₃ : HClO₄ and observed Cu was mainly adsorbed onto or occluded by the organic matter and the oxides and hydroxides of Fe and Mn. Scott and Thomas (1977) measured total Cu in some artificial soils using acid digestion and fusion methods (Table 2.11). Significant losses of Cu occurred through volatilisation with the fusion method. There is not enough information available regarding the relationship between the total Cu content of soils and plant Cu content. Dolar *et al.* (1971) found that total Cu, as measured by HF/ HClO₄ digestion, was significantly correlated with Cu uptake by oats.

Table 2.11 Total soil Cu recovered from soils by three methods of digestion.

Method	Amount of Cu recovered (%)
Sodium bicarbonate fusion	40-47
H ₂ SO ₄ / HNO ₃ / HClO ₄ digestion	89-95
HF/ HClO ₄ digestion	82-90

2.3.5 *Factors affecting forms of Cu in soils*

Most fractionation studies indicated that Cu is found largely in unavailable forms. Cu in all fractions generally decreases with soil depth (Kuo *et al.*, 1983). Cu in the water soluble and exchangeable forms is considered to be plant available, whereas Cu associated with organic matter may be so tightly bound as to be relatively unavailable (Mathur and Lévesque, 1983). Shuman (1985) stated that Cu was found mainly in the silt and clay fractions of 16 acid topsoils, none of which are immediately plant available. Increases in oxidation of organic matter generally results in the redistribution

of Cu from the exchangeable and organic fractions into the Fe oxide fractions, reducing plant availability (Sims and Patrick, 1978). The water soluble and exchangeable forms are considered to be the intensity factor, which when added together, could make a good soil test for plant available Cu (Mathur and Lévesque, 1988). They also indicated that Cu resided generally in nonavailable forms and has less mobility and bioavailability. Cu mobilised due to the reduction of oxides after submergence is associated with the water soluble and organic fractions.

2.3.5.1 *Cu addition to soils*

Cu has a strong ability to bind with organic matter. Cu is usually high in the carbonate fraction in alkaline soils and in the Fe-oxide fractions in acid soils, due to occlusion and strong adsorption (Sposito *et al.*, 1982; Kuo *et al.*, 1983; Sims, 1986). Adsorbed and exchangeable Cu are the smallest fractions (Sanders *et al.*, 1986; McGrath *et al.*, 1988). Chang *et al.* (1984) observed that in untreated soil, Cu was highest in the sulphide residual fraction, but with sewage sludge addition, the Cu level in the carbonate and organic fractions increased more than in the other fractions. Liang *et al.* (1991) observed that most of the added Cu accumulated in forms strongly bound to sesquioxides, organic matter and clay minerals in seven Saskatchewan soils. McLaren and Ritchie (1993) reported that organically bound and iron oxide bound Cu fractions account for most of the applied Cu, irrespective of the rate of application. Shuman (1988a) reported that added P shifted Cu from the residual to the exchangeable fractions in four fine textured soils, presumably making it more plant available.

2.3.5.2 *Soil pH*

Soil pH influences Cu distribution among the various fractions (Sims, 1986; Shuman, 1986; Mathur and Lévesque, 1988). El-Kherbawy and Sanders (1984) found that pH did not influence Cu in the soil solution, but increasing the pH decreased Cu in the exchangeable fractions, making it less bioavailable. Increasing soil pH redistributed Cu from the exchangeable and organic fractions to the Mn-oxide and Fe-oxide fractions (Sims and Patrick, 1978). Sanders and Adams (1987) suggested that increases in soil pH enhanced the occurrence of Cu complexing with soluble organic matter in the soil solution. Thus Cu lost from the exchangeable fractions was changed into less soluble, less bioavailable fractions as the pH was increased. Sims (1986), who reported that lime

additions reduced exchangeable and sorbed Cu, and increased the Cu into more tightly bound fractions, corroborated this finding.

2.3.5.3 *Organic matter amendments*

Since Cu is associated with organic matter, it would be expected that organic matter additions would cause a redistribution of Cu among various fractions, especially the organically bound Cu fractions. The organically bound and the oxide bound Cu fractions are related to soil organic matter content. An increase in the organic Cu and a decrease in the oxide fraction with organic matter additions have been observed (McGrath *et al.*, 1988), indicating a possible increase in bioavailability. As expected the organic fraction Cu was highest in organic soils compared with mineral soils. Since Cu is associated with the organic fraction, its complexing by organic ligands and subsequent release by microbes should be emphasised in chemical fractionation studies (Sims, 1988).

2.3.5.4 *Effect of contact time*

Copper added to organic soils, or added in sewage sludge or manures will revert to the more insoluble and plant unavailable fractions with time. Mathur and Lévesque (1988) observed that the Cu added to peat soils changed to more and more nonreactive forms, causing less replacement of weakly held metal ions. Cu in Cu-enriched manures added to soils initially existed in specifically adsorbed forms, but after 12 days, some was found in the Mn and amorphous Fe oxides, indicating a shift away from plant availability (Miller *et al.*, 1987). Sposito *et al.* (1983) found that Cu added in sewage sludge to arid zone soils increased the carbonate fraction Cu, accompanied by a decrease in the sulphide residual Cu with time. They explained that this reversion was due to CuCO_3 formation accompanying a mineralisation of the organic matter in the sludge. Schalscha *et al.* (1999) reported that added Cu increased exchangeable Cu in soils only slightly, however, significant increases in the carbonate, reducible (associated with oxide form) and oxidisable (bound to organic matter) fractions in soils were observed.

2.4 REACTIONS OF COPPER IN SOILS

Plant available, sometimes called 'extractable', Cu refers to the amount of this element in a soil that is readily absorbed and assimilated by plants. It is widely accepted that the trace element uptake can be correlated with some extractable fractions of the element in the soil. However the chemical activity of Cu is not constant; and therefore, the plant uptake is not well correlated with either the amounts in the various pools, or the amounts removed by various extractants. Since most soils contain only 20 to 30 mg kg⁻¹ total Cu, it is likely that Cu exists mostly as Cu(H₂O)₆²⁺ ions adsorbed at mineral surfaces, occluded or co-precipitated by silicate and nonsilicate clays and as organically complexed ions (Pickering, 1979; Shuman, 1979; McBride, 1981). Mattigod and Sposito (1977) stated that soluble free Cu²⁺ is the dominant ion in the solution phase of acid soils. Soil solutions over a range of pH contain several forms of Cu including Cu²⁺, CuSO₄, Cu(OH)₂, CuCO₃, Cu⁺, CuCl, Cu(Cl₂), and numerous organic complexes of Cu.

Lindsay (1979) and McBride (1981) stated that most Cu containing minerals are too soluble to control the very low activities of Cu²⁺ in the soil solution. McBride (1981) also concluded that the simple precipitation of Cu as, Cu(OH)₂, CuO or Cu₂(OH)₂CO₃ is not generally responsible for the 'adsorption' of Cu⁺ added to soils. The bioavailability of Cu depends on its activity and speciation in soils (Buffle *et al.*, 1990; Lehman and Mills, 1994).

From the solubility relationship, Lindsay (1979) concluded that 'soil-Cu may indeed be cupric ferrite'. McBride (1981) indicated that the tetrahedral co-ordination of Fe³⁺ required for this structure might inhibit its formation under soil conditions. In soils containing appreciable amounts Fe and Al oxides with high specific surface areas, Cu²⁺ could exist as chemisorbed or occluded ions rather than as separate phases, which renders a lower activity of Cu²⁺ in the soil solution than that predicted for pure Cu²⁺ minerals. Since Cu²⁺ can exist in the octahedral co-ordination of silicate clays, as occluded carbonate, some soil Cu²⁺ can only be removed by the dissolution of these clays (Shuman, 1979). Non-diffusible Cu in calcareous soils, that is soluble in dilute acid, is probably present as an impurity within carbonate minerals, and is another form of non-labile Cu immobilised by other soil minerals (McBride, 1981). Many kinds of organic substances form both soluble and insoluble complexes with Cu, thus Cu binding

capacities of soils, and Cu solubility are highly dependent on the kind and amount of organic matter in soils.

Overall solubility of both cationic and anionic forms of Cu decreases at pH 7 to 8. It has been estimated hydrolysis products of Cu [CuOH^+ and $\text{Cu}_2(\text{OH})_2^{2+}$] are the most significant species below pH 7, while above pH 8 anionic hydroxy complexes of Cu become important. Solubility of metals in soils and at mineral oxide surfaces as a function of pH is often dictated by the presence of organic and inorganic ligands (McBride, 1989; Sparks, 1995; Harter and Naidu, 1995).

2.4.1 *Cu adsorption and fixation by soils*

Adsorption mechanisms of Cu have been extensively studied by many scientists and comprehensive publications concerning the physical and chemical behaviour of the metal in soils are available (McLaren and Crawford, 1973a; McBride, 1981; James and Barrow, 1981). All soil minerals are capable of adsorbing Cu ions from solution, and the extent of adsorption depends on the surface charge carried by the soil minerals. In variable charge soils, the surface charge is strongly controlled by pH, therefore the adsorption of Cu ion species can be presented as a function of pH. This type of Cu adsorption is likely to be the most important in soils with a large content of variable-charge minerals (James and Barrow, 1981). When pH is low (with a higher H^+ concentration) the metal oxide surfaces in soils gain H^+ ions resulting in an excess of positive charge. Similarly, when the pH is high the water molecules tend to lose H^+ , resulting in an excess of negative charge (Figure 2.1).



Figure 2.1 Development of variable surface positive charge through the dissociation or association of H^+ on a mineral surface (Bolan *et al.*, 1999).

Bodek *et al.* (1988) stated that of two mobile Cu species, CuOH^+ is preferentially sorbed over Cu^{2+} . Literature data on Cu adsorption by soils and soil components are presented in Table 2.12. Quantification of the plant availability of Cu in soil suspensions requires

the determination of labile Cu (ions in the solid and solution phases in equilibrium with free Cu forms in the soil solution), the activity of Cu^{2+} in the soil solution of acid soils and $\text{Cu}(\text{OH})_2$ in neutral or alkaline soils.

McLaren and Crawford (1973b) also contended that for the relatively small amounts of Cu normally present in soils, specific adsorption is a more important process than the non-specific adsorption (through the cation exchange process) in controlling the concentration of Cu in the soil solution. The Cu sorption capacity of soils may follow closely the extent of CEC, and the amounts of the oxides of Fe and Mn and organic matter in the soil. Organic matter alone may not be a good indicator of the sorption capacity of soil (Kuo and Baker, 1980; Dhillon *et al.*, 1981). Since the solution activity of Cu^{2+} increases with the amount of specifically adsorbed Cu, labile Cu will also increase. But the relationship among the fractions varies with soil pH, organic matter, quantity and composition, and soil mineral abundance and composition. Peat and mineral soils with high amounts of organic matter are most prone to Cu deficiency. Stevenson and Fitch (1981) stated that the maximum amount of Cu^{2+} that can be bound to humic and fulvic acids is approximately equal to the content of the acidic functional groups. Even in soils with normal levels of organic matter (1-8%) most of the Cu not in the mineral phase is organically bound.

McLaren and Crawford (1973a) carried out a fractionation study of the Cu in representative samples of 24 different English soil series, and showed that the bulk of the metal was held in the lattices of weatherable minerals (over 50%), about 30% was bound by organic matter and 15% was adsorbed by hydrous oxides of Fe and Mn. The combined amounts of water soluble and exchangeable Cu, which relates to the activity of Cu^{2+} in the soil solution, was very small (1-2%). While the basic knowledge of Cu retention by clays, soils and sediments continues to accumulate at a steady rate, soil management decisions can be made from the measurement of total sorbed Cu, labile Cu and the activity, or chemical potential, of Cu^{2+} in the soil solution, when the soil pH is in the range desired for crops (Pickering, 1979; Dragun and Baker, 1982). McLaren *et al.* (1990) reported that the adsorption of Cu is strongly influenced by the amount of added Cu and the soil pH. Similarly, the speciation of Cu in equilibrium solutions is affected by these factors. Lal *et al.* (1971) incubated several alkaline soils at 20⁰C, 30⁰C and 40⁰C. They found that the adsorption of Cu by the soils increased with increasing

temperature. But independent examination of the adsorption data revealed no distinct trend. Also, it is improbable that temperatures as high as 40°C would be encountered in soils. However, Assad and Nielsen (1984) found that adsorption of Cu by soils decreased with an increase in temperature from 5 to 25°C. Thermodynamic measurements indicated that adsorption of Cu was an exothermic reaction. Further information is necessary to understand the influence of temperature on adsorption. Especially important is the interaction between pH and temperature, and the influence on the adsorption of Cu by different soil types. Microbial fixation plays an important role in the binding of Cu in certain surface soils. The amount of Cu fixed by the microbial biomass is widely variable, and is affected by various factors, such as metal concentration, soil properties, and the growing season. Microbial fixation of Cu is an important step in the ecological cycling of this metal (Kovalskiy and Letunova, 1974).

Table 2.12 Adsorption of Cu in soils and soil components.

Name of soil/ adsorbent	Electrolyte	pH range	Approximate concentration	Cu	References
Wetland-taro soil	0.005 M CaCl ₂	5 - 8	0-2000 mg Cu Kg ⁻¹ soil		Hue <i>et al.</i> (1997)
Clay, USA	0.01M CaCl ₂	6.0	2.5×10 ⁻⁶ to 1.25×10 ⁻³ M CuCl ₂		Wu <i>et al.</i> (1999)
Six soils, Japan	0.1 M NaClO ₄	2 - 9 (>6)	1.11×10 ⁻⁴ to 1.11× 10 ⁻³ M Cu (II)		Choi <i>et al.</i> (1999)
Orthic humic gleysols	0.005 M CaCl ₂	2.74 -6.74	0 and 200 mg Cu L ⁻¹ as CuCl ₂		Yuan and Lavkulich (1997)
Typic Fragiochrept	0.02 M CaCl ₂	4 - 7	300 μM Cu		Cavallaro and McBride (1984)
Haplic podzol, Fimic Anthrosol, Eutric Cambisol and Calcaric Regosol	0.01M Ca(NO ₃) ₂	2 - 8	0.001-100 mg Cu L ⁻¹ as Cu(NO ₃) ₂		Welp and Brümmer (1999)
Various soil	0.1 M CaCl ₂ and variable	4 - 6	10 ⁻⁷ to 10 ⁻⁴ M		Cavallaro and McBride (1978)
Goethite, α-alumina, silica and kaolinite	0.001M to 0.1 M NaCl	4 - 10	0.0001M Cu		Spark <i>et al.</i> (1997)
Georgia, kaolinite	0.1 M NaCl and variable	4 - 6	10 ⁻⁶ to 10 ⁻³ M		McBride (1978)

() indicates Cu hydroxide formation.

2.4.1.1 *Formation of Cu complexes in soils*

A number of studies have shown that Cu^{2+} in soil solution, especially at higher pH, exists primarily in a form complexed with soluble organic matter (Hodgson *et al.*, 1966). In soils amended with organic residues, such as sewage sludge, organically complexed Cu may be expected to be the predominant form (Behel *et al.*, 1983). Stevenson and Fitch (1981) reported that, based on a commonly used soil Cu fractionation scheme, organically bound Cu accounts for about 20% to 50% of the total soil Cu. The amount of organically complexed Cu in solution generally increases when soil pH is 7 or higher, because of the greater solubility of soil organic matter at higher pH values (McBride and Blasiak, 1979), while the concentration of free ionic Cu at a higher pH is much less, usually in the range of 10^{-9} to 10^{-8} M (McBride and Blasiak, 1979).

Organic enriched surface horizons usually contain higher concentrations of Cu than the deeper horizons that generally contain less organic matter. Thus complexing of Cu by organic matter, in the form of humic and fulvic acids, has long been recognised as an effective mechanism of Cu retention in soils (McBride, 1981). Stevenson and Fitch (1981) provided the following evidence for the complexing of Cu^{2+} by humic and fulvic acids.

- The inability of K^+ and other monovalent cations to replace adsorbed Cu^{2+} from mineral and organic soils.
- The correlation between Cu^{2+} retention and humus content.
- The ability of chelating agents to extract Cu, while solubilising part of the soil humus.
- The selective retention of Cu^{2+} by humic and fulvic acids in the presence of cation-exchange resin.

The complexing ability of humic and fulvic acids is due to their high oxygen-containing functional groups, such as carboxyl, phenolic hydroxyl, and carbonyls of various types. Because of the high acidity and relatively lower molecular weights, metal complexes with fulvic acids are more mobile than those of humic acids. Fulvic acids are also more efficient in complexing metals than humic acids, and so metal ions may also be more available to plants than humic acid-complexed metals. Colloidal organic matter in soils

provides 'specific' adsorption sites for Cu, thereby causing Cu deficiency in organic soils. The fraction of soil Cu associated with organic matter should be expected to be high in soils rich in organic matter.

Stevenson and Fitch (1981) summarised the following effects on soils of the formation of Cu-organic matter complexes:

- The soil solution Cu concentration can be decreased by clay-humus complexes, and by the formation of insoluble complexes with humic acids. Soluble ligands may be of considerable importance in transforming solid phase forms of Cu into dissolved forms.
- In high pH soils (eg. calcareous soils) complex formation will promote maintaining Cu in dissolved forms.
- In the presence of excess Cu, organic complexes may reduce the concentration of Cu^{2+} to non-toxic levels.
- Natural complexing agents may be involved in the transport and mobility of Cu in soils.

2.4.1.2 *Adsorption of Cu by soil components*

The major soil components involved in the adsorption of Cu include soil organic matter, hydrous oxides of Fe, Al and Mn, and clays. The Cu adsorption ability of soils is affected by the pH, the amount of organic matter, Fe and Al oxides and clay minerals (McLaren *et al.*, 1983b; Stevenson *et al.*, 1993; Wu *et al.*, 1999). Soil pH is the major factor for the control of Cu sorption, and many authors confirmed that the sorption of Cu onto soils increases with an increase in pH (Cavallaro and McBride, 1978; Kuo and Baker, 1980; Harter, 1983, McLaren *et al.*, 1983a; Hue *et al.*, 1997; Choi *et al.*, 1999).

2.4.1.2.1 *Sorption by organic matter*

Organic matter plays a major role in the retention of Cu in soils (Stevenson and Fitch, 1981; Kabata-Pendias and Pendias, 1992, McBride, 1994). The organic fraction, in particular, seems to be a source of specific Cu sorption sites in pasture soils (McLaren and Crawford, 1973a; Kadlec and Keoleian, 1986), perhaps because the ion is unique in its ability to form inner sphere complexes at a wide range of pH levels (McBride, 1981).

Organic complex formation could lead to an increased mobility in the soil, since Cu is known to form stable complexes with fulvic acid (Kabata-Pendias and Pendias, 1992). The complexing of metals by organics can also affect retention by mineral surfaces. Goh *et al.* (1986) demonstrated increased retention of Cu by Al precipitation products, when tannic acid was present during precipitation. They attributed the increased sorption capacity to exposed edges as well as to the presence of carboxylate and phenolic groups. Small amounts of organics in solution have been shown to increase solution Cu concentrations, above that which can be expected for ionic Cu in equilibrium with inorganic sorption sites or precipitated forms (McLaren *et al.*, 1981). The complexing of metals by carboxylate groups is commonly proposed as a mechanism of retention (Figure 2.2). Boyd *et al.* (1981) found from electron spin resonance evidence that Cu^{2+} is sorbed to humic acid by forming two inner-sphere bonds with oxygen atoms. Subsequent infrared evaluation of Cu-humic acid complexes indicated that the two bonds were unlikely to result from a complex with a single carboxylate group. They proposed the formation of a chelate ring of either two adjacent carboxylate groups or a carboxylate and an adjacent phenolic OH group, with an oxygen atom from each group forming a single bond with Cu^{2+} .

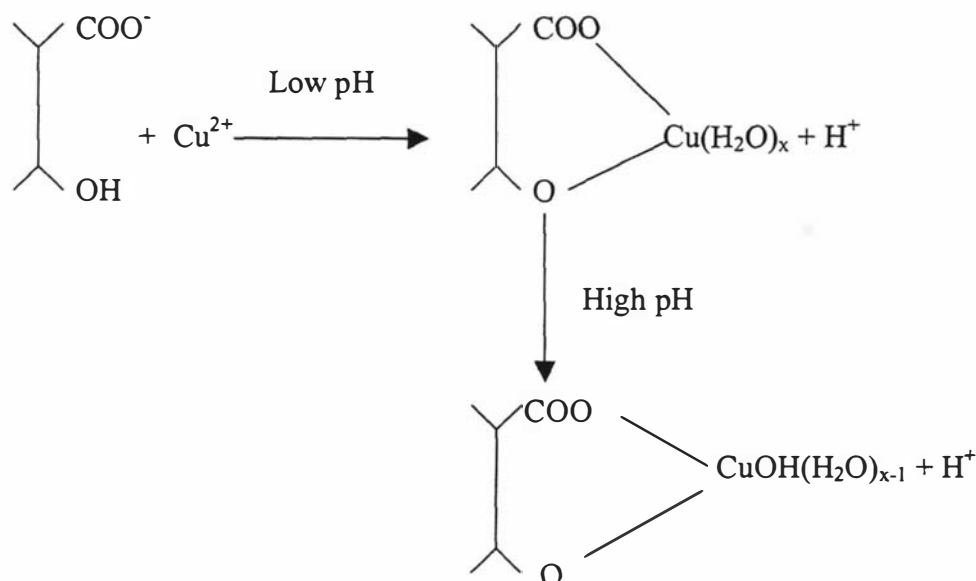


Figure 2.2 Complex formation of Cu ions by humic acid according to pH (Van Dijk, 1971).

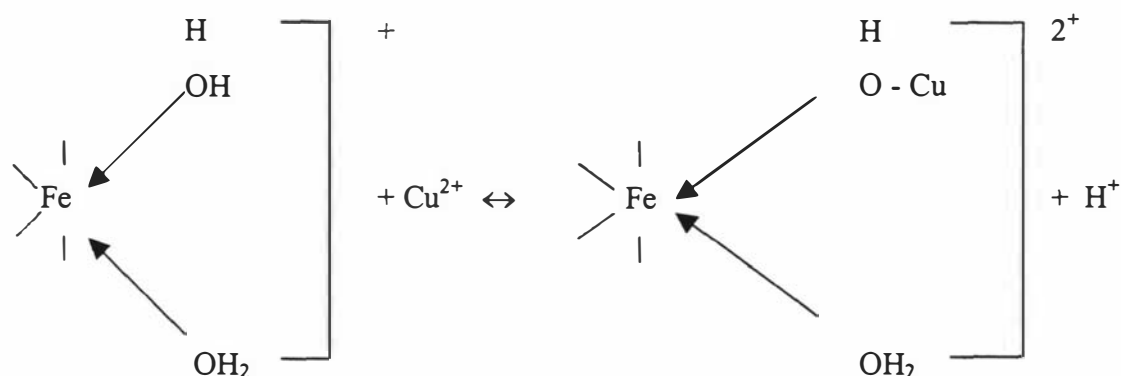
2.4.1.2.2 Sorption by hydrous oxides

According to Jenne (1968) hydrous oxides play a significant role in the control of Cu concentrations in soil solution. Reports are readily available of Cu retention by hydrous Fe oxide (Okazaki *et al.*, 1986), hydrous Mn oxides (Kabata-Pendias, 1980) and hydrous Al oxides (Shuman, 1977; Barrow, 1986).

Retention of Cu ions by oxide surfaces is inversely dependent on the degree of crystallinity (Okazaki *et al.*, 1986) and, since the oxides have variable charge, the extent to which retention occurs is dependent on the pH of the solution (Barrow, 1987; Bolan *et al.*, 1999). Predictably, surface conditions strongly influence Cu sorption by hydrous oxides. Cavallaro and McBride (1984) found that treatment of clays for the removal of organics tended to either enhance or have little effect on sorption and fixation of Zn and Cu. They suggested that the oxide component of soil clay was more significant than the organic component in metal sorption and fixation, and that oxide forms in general and organic coatings have an affinity for metals (Lion *et al.*, 1982). In studying the effect of phosphate on the sorption of Cu by Al hydroxide, McBride (1985) observed decreased sorption of Cu, when phosphate was present. Using electron spin resonance, he demonstrated that the sorbed phosphate blocked the co-ordination of Cu^{2+} to the surface AlOH group. McBride *et al.* (1998) conducted adsorption at two pH ranges (5 to 5.5 and 6.5 to 7.0) using a suspension of natural organic matter and iron oxide (FeOOH) that were equilibrated with a wide range of concentrations of added Cu (50 to 5000 mg kg^{-1}). Both FeOOH and organic matter adsorbed >98% of the added Cu up to an addition level of 1000 mg kg^{-1} . At higher Cu additions, adsorption efficiency of the oxide decreased markedly, when the pH was 5.5 or lower. Whereas the organic matter still adsorbed >98% of the Cu. Across the range of pH and Cu loading studied, the Cu^{2+} activity in the organic matter was generally one to two orders of magnitude lower than with the oxide, even after long equilibration periods. It appears that under slightly acidic conditions in soils, organic matter is more likely to limit Cu^{2+} activity and phytotoxicity than Fe oxides.

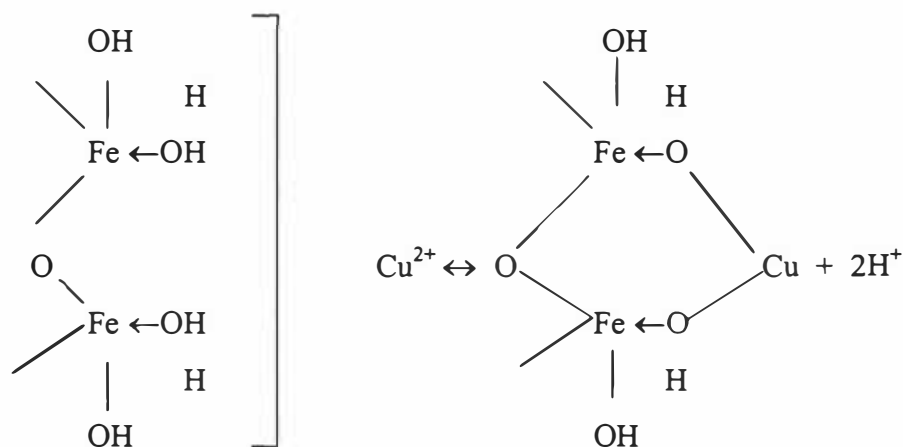
Two mechanisms for the adsorption of Cu on goethite were proposed by Padmanabham (1983).

(1) Specific adsorption on positive surface sites



Adsorption of Cu by this mechanism involves low bonding energy, and over time, adsorbed Cu may isomorphously substitute into the oxide lattice. At low pH a higher proportion of Cu is present as Cu^{2+} . In this valence state Cu is similar in size to Fe(III) and is able to be easily fixed or occluded in the oxide lattice.

(2) Specific adsorption on neutral sites



Hydrolysis occurring at the oxides surface results in the adsorption of Cu^{2+} and the release of two protons.

2.4.1.2.3 Sorption by silicate clay minerals

Cu sorption by silicate clay minerals has been shown to be an important retention site for this ion (Kabata-Pendias, 1980). Shuman (1980) pointed out that clays are capable of sorbing both Cu and Zn in excess of CEC. Wakatsuki *et al.* (1975) found that Cu was

sorbed by kaolinite clays at solution Cu activities lower than required for oxide / hydroxide precipitation. They interpreted this to indicate that Cu is retained at specific sorption sites on clays. While Kabata-Pendias and Pendias (1992) maintain that the ability of soil minerals to remove Cu from solution is dependent on the mineral surface charge, the relationship between CEC and Cu is not always straight forward. Okazaki *et al.* (1989) reported a direct relationship between the SiO₂ / Al₂O₃ ratio of synthetic aluminosilicates and Cu adsorption. Jenne (1968) argued that retention is more a function of hydrous Mn and Fe oxides on clay surfaces than on the properties of the surfaces themselves.

2.4.2 *Desorption of Cu*

Presently it is generally accepted that soil solution concentrations of micronutrients are regulated by sorption-desorption reactions at the surfaces of soil colloidal material (Swift and McLaren, 1991). Hogg *et al.* (1993) reported the availability of soil Cu to plants is dependent on desorption in the soil solution of Cu from the surfaces of soil colloidal materials. They found that the proportion of added Cu desorbed was reduced substantially by increasing the contact period to 12 weeks before desorption. The longer the contact period the greater the amount of Cu that was irreversibly sorbed. The nature of the slow reactions that result in a decrease in the desorption of added Cu remains open to conjecture. With increased time of sorption, there is a slow redistribution of Cu ions to more strongly binding or less accessible sites, possibly involving diffusion into extremely small pores and inter particle spaces. Desorption of native soil Cu was increased by increasing the temperature at which desorption was carried out. They concluded that the amount of Cu that can desorb readily from soil depends not only on the total amount of labile Cu in the soil, but also on soil pH, temperature, and in the case of added Cu, on to the length of time since the addition of the Cu to the soil. Soil acidification enhances Cu desorption from the solid phase, thereby increasing its concentration in the soil solution (Basta and Tabatabai, 1992). This enhanced desorption may intensify the bioavailability of Cu. Cu uptake by plants is a function of the activity of the soil solution Cu²⁺, which is controlled by the concentration of total soluble Cu, the inorganic and organic ligands and by soil pH. A decrease in pH enhances Cu²⁺ absorption by plants, because of the increased solution activity of Cu²⁺ associated with the dissolution of soil minerals, the decrease in organic complexing and solid phase

adsorption of Cu (Baker and Senft, 1995). McLaren *et al.* (1990) found that initially less than 10% of the Cu adsorbed by the soil was desorbed and after three months of soil contact, only a negligible amount (<1%) of the adsorbed Cu could be desorbed.

Temminghoff *et al.* (1994) studied the effect of pH on Cu desorption from a sandy soil, and complex formation by dissolved organic fractions. They observed Cu desorption and its pH dependency could be modelled with two species of the Freundlich equation. As the soil pH decreased, the amount of Cu desorbed from the soil increased. The amount of Cu bound to solid organic carbon was almost equal to Cu bound by dissolved organic carbon.

2.4.3 *Cu chelate equilibria in aerobic and anaerobic soils*

Copper is a strongly chelated metal, as indicated by the low value of free Cu compared with chelated forms. Diethylenetrinitrilopentaacetic acid (DTPA), especially, and N-(2-Hydroxyethyl)ethylenedinitrilotriacetic acid (HEDTA), ethylenedinitrilotetraacetic acid (EDTA) and trans-1,2-Cyclohexylenedinitrilotetraacetic acid (CDTA) are relatively effective chelators for Cu in alkaline soils, in which Ca is the main competing cation. Experimental studies using DTPA and EDTA in soils provide support for the prediction of Cu (Norvell and Lindsay, 1972). Competition from Fe restricts the chelation of Cu at low pH, but HEDTA has the potential to chelate Cu effectively under moderately acidic conditions. Ethylenediiminobis(2-hydroxyphenyl)acetic acid (EDDHA) appears to have some limited potential as a chelator for Cu under alkaline conditions. However, experimental results indicate that Cu²⁺ solubility in soils is generally too low to allow effective chelation, when Cu-EDDHA itself is added (Aboulroos, 1981). N-(2-Hydroxyethyl)iminodiacetic acid (HIDA) and nitrilotriacetic acid (NTA) have the greatest ability to chelate Cu under moderately acidic conditions. At low pH, none of the ligands is particularly effective, and soluble organic materials in the soil solution may provide similar levels of complex formation as noted by Norvell (1972).

The experimental studies using EDTA and DTPA show that competition from Fe²⁺ and Mn²⁺ can limit the stability of chelated Cu and Zn in soils under reducing conditions (Reddy and Patrick, 1977). The relative effectiveness of many ligands is not altered by changing redox conditions. For example, HEDTA is the dominant chelator for several

metals at all redox levels. The most prominent among these ligands is EDDHA, which becomes a much better chelating ligand for other metals as the Fe^{3+} concentration declines with increased reduction. This effect is most obvious for Cu^{2+} because Cu-EDDHA chelates are also reasonably stable. EDDHA is one of the most effective chelators for Cu^{2+} under reducing conditions, but it is one of the least effective chelators under strong oxidising conditions.

2.5 PLANT AVAILABILITY OF COPPER IN SOILS

The availability of Cu to plants is commonly measured by two methods:

- Plant uptake
- Chemical extraction from soils.

Plants are grown in the field or more commonly in a glasshouse, where environmental conditions can be monitored if not controlled. In order to obtain a measurement of Cu uptake, which can be usefully compared by chemical assessment, all limitations to plant growth must be removed. This includes adequate fertilisation with all nutrients (except Cu), and control of the moisture content and temperature of the soil. Soil Cu can be measured by chemical extraction, and then related to Cu uptake, by the Cu content of the experimentally grown plants.

2.5.1 *Availability of native Cu*

Copper in soil is strongly held on inorganic and organic exchange sites and in complexes with organic matter. For this reason a large proportion of the total Cu content of soils is not available for plant uptake. The proportion of the total Cu taken up by plants has been found to be greater in mineral soils than in peats (Kabata-Pendias, 1963). Deficiencies in crops may be due to an inherently low total Cu content of a soil, or to only a small amount being in an available form. Although Cu held on exchange sites is not readily available to plants, cation exchange for Cu^{2+} and CuOH^+ can take place and is best affected by H^+ (Mengel and Kirkby, 1978).

Some organic forms of Cu in soils are thought to be more available than others. It has been shown that in organic soils the availability of Cu depends not only on the concentration in the soil solution, but also on the form in which the Cu is present

(Mercer and Richmond, 1970). Kubota *et al.* (1963) reported that the Cu concentration increased in the soil solution with increasing wetness in two soils from New Hampshire, but decreased with increasing soil moisture in two soils from Nevada. It has been observed in Britain that Cu deficiency is more severe in dry, sunny years than when dull, moist conditions occur (Caldwell, 1971).

The application of Cu-free fertilisers to soils may lead to Cu deficiency in crops or aggravate it. It was observed in several published reports that nitrogenous and phosphatic fertiliser compounds lead to an increase in Cu deficiency in cereal crops (Caldwell, 1971; Touchton *et al.*, 1980; Robson and Reuter, 1981). Kubota (1983) reported that low Cu levels in grass is likely to result in Cu deficiency in ruminant animals, especially where the soil has an elevated Mo level.

Brennan (1993) reported that for either previously or currently applied Cu, the application of nitrogenous fertilisers decreased the Cu concentration (lsd ($p=0.05$) = 0.25) in yellow brown earth soils, except on the plot currently receiving Cu. This is mainly attributed to increased plant growth, diluting the Cu concentration in the plants.

2.5.2 *Availability of fertiliser Cu*

Copper deficiencies can be corrected by applying Cu to plant foliage, or to the soil by either band or broadcast application procedures. Use of soil application procedures for the correction of Cu deficiency is usually justified on the basis of a relatively high residual value for soil-incorporated Cu (Gartrell, 1980). Copper added to soil is rapidly immobilised, and plant utilisation is mainly dependent on plant roots being in the vicinity of the added Cu. Generally finely ground powders are more rapidly effective than coarser materials, which have a greater residual effect. Placement of fertiliser may have an influence on the effectiveness of the fertiliser. McLaren and Williams (1981) found that surface applied Cu had little influence on the yield of ryegrass and red clover, when compared to soil incorporated fertilisers. Cu added to the soil as chelates have been found to be more effective than CuSO_4 , due to a greater solubility and mobility in the soil (Gartrell, 1981; McLaren and Williams, 1981). Wallace and Mueller (1973) showed that chelated Cu increased the concentration of Cu in bush beans (*Phaseolus vulgaris*) compared with the equivalent rate of CuSO_4 fertiliser.

The general use of CuSO_4 as a Cu source reflects its high water solubility, and wide availability (Alloway and Tills, 1984; Karamanos *et al.*, 1986). The same rate of CuSO_4 and CuO were recommended for correcting Cu deficiency by either band or broadcast Cu application (Mengel, 1980; Vitosh *et al.*, 1981). The suitability of CuO when broadcast has been found to depend on particle size. CuO broadcast on to the soil surface at 5 kg Cu ha^{-1} , and worked into the soil, did not correct Cu deficiencies in canola, barley, or wheat during the year of application, but corrected the deficiencies in the following years (Karamanos *et al.*, 1986). The lack of Cu response to CuO was mainly due to the low water solubility of the coarse, granular CuO, which ranged in particle diameter from <0.2 to 3.0 mm . Coarse Cu carriers are also ineffective where inadequate contact occurs between the roots and the applied Cu (Gartrell, 1981). Seed treatment is recognised as being less effective than either soil application or foliar sprays (Gartrell, 1981).

2.5.3 *Cu uptake and translocation*

2.5.3.1 *Cu absorption by plant roots*

Copper in biological systems is predominantly present as Cu^{2+} , which has great significance in terms of Cu absorption by plant roots from the soil solution, because most organic chelates bind Cu^{2+} strongly (Graham, 1981). In the soil solution, up to 98% of the Cu is complexed to low molecular weight organic compounds (Hodgson *et al.*, 1966). The Cu^{2+} ion has a high affinity for peptide N and S, and binds quite strongly to proteins, especially proteins high in cysteine residues. Also due to its affinity for carbonyl, carboxyl, sulfhydryl, and phenolic groups, all of which are found in cell walls, there is a strong and specific adsorption of Cu to cell walls that is not easily desorbed (Harrison *et al.*, 1979). It should also be noted that because Cu^{2+} can be reduced to Cu^+ in a range of physiological redox potentials, the significance of the absorption of Cu^+ by roots should not be ignored (Graham, 1981). There is considerable evidence that the free Cu^{2+} ion is the absorbed species. A number of researchers have demonstrated that Cu is absorbed more rapidly from Cu^{2+} solutions than from solutions of Cu complexed to synthetic chelators such as DTPA or EDTA (Harrison *et al.*, 1979; Wallace, 1980). Also, Goodman and Linehan (1979), using electron paramagnetic resonance spectrometry to study root absorption, presented evidence consistent with the dissociation of Cu^{2+} from EDTA during absorption from a Cu^{2+} -EDTA solution.

Graham (1981) has suggested that Cu^{2+} uptake could be facilitated by a carrier type protein that can bind Cu^{2+} on either side of the plasma membrane. This would have a greater probability of releasing Cu inside the cell due to the lower electrochemical potential for Cu in this compartment. Thus Cu^{2+} uptake could occur by a passively driven, carrier type membrane protein.

Loneragan (1981) concluded that the absorption of Cu^{2+} and Zn^{2+} is a metabolically active process at least at normal soil levels. This conclusion is supported by the finding that absorption is reduced by metabolic inhibitors. Since higher activities of either Zn^{2+} or Cu^{2+} in soil or solution cultures are antagonistic to plant uptake of other ions, it is usually recognised that both ions are absorbed in the same way. Other cations such as Ca^+ , K^+ and NH_4^+ reduce the Cu absorption. However, it is likely that these ions affect the uptake of Cu^{2+} via differential complexing, and other surface effects, which determine the soil solution activity of Cu^{2+} and membrane permeability.

2.5.3.2 *Translocation of Cu in plants*

Gilkes (1981) reported that the rates of absorption of Cu is the lowest of the essential elements, and there are large genetic differences among plant species and cultivars within species. Baker and Low (1970) concluded that the Cu in the environment of the rhizosphere is almost all organically complexed by root exudates and soil humus. But uptake and translocation are functions of the activity of Cu^{2+} in true solution at the active absorption sites. According to published literature (Gupta, 1979; Baker and Low, 1970; Dragun and Baker, 1982), while root absorption of Cu includes specific adsorption onto cell walls of the root free space, the limiting step of transport across the plasmalemma involves an electrochemical gradient relating the activity of Cu^{2+} in solution outside the root to that of the cytoplasm of the cortical cells. Because of the plant component, the absorption process is controlled by the plant species and cultivars, it becomes obvious that Cu uptake is a function of the activity Cu^{2+} at the absorption site outside the plasmalemma.

The translocation of Cu^{2+} within plants occurs in both the xylem and phloem, where the metal is bound by organic nitrogen compounds such as amino acids. Loneragan (1981) reported that the concentrations of 1.5 to 2.0 μM Cu^{2+} in the xylem and 3-140 μM in the

phloem. Much of the Cu^{2+} associated with plant roots may not be translocated into the shoot even when a deficiency occurs in an aerial part. In the shoot, N metabolism appears to control the binding and transport of Cu^{2+} . Cu is a relatively immobile element in plants. Green leaves may accumulate high concentrations of Cu^{2+} , and subsequently do not release it to younger leaves and other tissues, such as inflorescences, despite their deficiency. Cu concentration in young leaves of wheat is important in diagnosing Cu deficiency (Snowball and Robson, 1984), and is now being used to provide fertiliser recommendations for farmers (Gartrell *et al.*, 1979). If Cu deficiency is diagnosed before flowering, a foliar application of Cu can be made without much yield loss (Graham, 1975).

2.5.3.3 *Interaction with other ions and nutrients*

The uptake, metabolism and accumulation of Cu by organisms can be affected by the action of other ions (Pellegrini *et al.*, 1993; Shuttleworth and Unz, 1993). A number of variables must be considered in studies of the role of Cu in ecological and physiological functions. For example, metal ions may be competing for binding sites on proteins such as metallothionein (Witkowska *et al.*, 1991). The complexity of metal to metal interactions has an important implication for bioassays and the toxicity testing of mixtures since multiple elements and compounds often co-occur in industrial and/or agricultural residues (Mullick and Konar, 1991). The important interactions of Cu with other elements include copper-iron, copper-molybdenum and copper-zinc and with copper-N and P fertilisers.

Copper-iron

Copper-iron antagonism is indicated as Cu chlorosis. High levels of Cu in plants decreases the Fe content in the chloroplast (Reilly and Reilly, 1973). Fe, on the other hand, reduces Cu absorption from soil solutions, especially in peat soils. The optimal Cu/Fe ratio varies for different plant species. The toxic effect of Cu can be decreased by the addition of Fe. However a synergistic effect of Cu on Fe absorption by rice seedlings was reported by Lidon and Henriques (1993).

Copper-cadmium

Copper-cadmium interactions reported by some authors as both antagonistic and synergistic in the elements uptake by roots. Synergism may be a secondary effect of damage to membranes due to the imbalance in proportion of the metals. Khan *et al.*

(1996) reported that increasing the rate of Cu fertiliser application had no significant effect on Cd concentration in a mixed pasture.

Copper-Manganese

Studies by Lidon and Henriques (1993) using rice seedlings have shown a decrease in net uptake by the roots and translocation to the shoots of several elements, including Zn, Fe and Mn, with an increase in the external concentration of Cu. An increased level of external Cu has been reported to affect the Zn, Fe and Mn metabolism in plants (Bowen, 1981; Alva and Chen, 1995).

Copper-Molybdenum

Copper-Molybdenum interactions are closely related to N metabolism. Cu interferes with the role of Mo in the enzymatic reduction of NO₃. The mutual antagonism existing between these elements is highly dependent on plant species, and the type of N nutrition. Copper aggravates Mo deficiency in plants, especially those using N from NO₃.

Copper-Zinc

A number of researchers have observed mutually competitive interactions between Cu and Zn (Bowen, 1979; Luo and Rimmer, 1995). Bowen (1979) found that Cu and Zn competitively inhibit the uptake of the other, and proposed that both ions were absorbed by the same transport system. High Zn concentrations in the soil accentuate Cu deficiency (Halder and Mandal, 1981). The effect is not primarily due to dilution effects or reduced translocation of Cu from roots to tops. Since Cu and Zn seem to be absorbed through the same mechanism, each competitively inhibits uptake of the other (Giordano *et al.*, 1974). Studies of Lidon and Henriques (1992b and 1993) found a decrease in net uptake of Cu by the roots due to an increase in the translocation to the shoots of Cu, and other elements, including Zn, Fe and Mn, with an increase in the external concentration of Cu in rice (*Oryza sativa* L.) seedlings. In contrast, Beckett and Davis (1978) working with nutrient solutions found that Cu had little effect on the amount of Zn uptake in barley or vice versa. Kabata-Pendias and Pendias (1992) described that the Zn-Cu interaction as an antagonism effect and also observed that addition of large amounts of Zn decreased the uptake of Cu in the plant top.

Copper-Nitrogen and Phosphorus

Several published works reported that nitrogenous and phosphatic fertiliser compounds induce a Cu deficiency in cereal crops (Caldwell, 1971; Touchton *et al.*, 1980). In citrus, highly concentrated long-term applications of P fertilisers have resulted in, and

intensified Cu deficiencies (Olsen, 1972). Copper deficiency on initially infertile soils was enhanced after the soil N supply was elevated by leguminous pastures (Gartrell, 1981). Brennan (1993) reported that for either previously or currently applied Cu, an application of nitrogenous fertilisers decreased the Cu concentration in yellow brown earth soils, except on the plot currently receiving Cu. Kumar *et al.* (1990) reported that N and Cu were found to have a mutually antagonistic effect on each other's concentration in the wheat plant. The antagonism was greater with NH_4^+ sources than NO_3^- compounds.

Soil P also reduces the concentration of plant Cu. This may be due to plant dilution effects as a result of added P increasing plant growth without a consequent increase in Cu uptake (Robson and Reuter, 1981). The P absorbed or precipitated the metals to form insoluble complexes, and therefore make both the Cu and P unavailable to plants (Lindsay, 1979; Ross, 1994). In subterranean clover, the interaction between P and Cu was indirect and positive on its effect on growth, and when the P supply was increased from a marginally deficient level to an adequate level, there was depressed Cu absorption and an accentuated Cu deficiency (Reuter *et al.*, 1981b).

Varvel (1984) found that applied Cu competed with both the uptake of P by wheat plants and also the translocation of P from root to shoot. Plants given no Cu application contained 6.5 g P kg⁻¹ DM, addition at Cu rates of 3.4 to 21.5 kg Cu ha⁻¹ decreased the content of P to an average of 3.4 g P kg⁻¹ DM. Availability of P in the presence of Cu, and vice versa, was thought to be decreased due to the formation of insoluble phosphate compounds in the soil (Halder and Mandal, 1982).

2.5.4 Effect of Cu fertiliser on DM yield and Cu concentration

Copper concentrations in forage and pasture crops depend on the soil availability of Cu, plant species, stage of growth, time of year, and lime and fertiliser applications. Legumes tend to take up larger amounts of Cu than grasses. In some cases, non-crop species or weeds may also contribute to the increased dietary intake of Cu by grazing livestock. Kubota (1983) found that mixed pasture herbage rarely contains more than 20 mg Cu kg⁻¹ and usually less than 10 mg Cu kg⁻¹. However, grazing animals may ingest up to 10 times more Cu in the form of soil than in the herbage. Soil ingestion commonly

ranges from 1-10% of the dry matter intake of grazing cattle and up to 30% in sheep (Thornton, 1979).

Wells (1957) recorded that the pasture Cu concentration ranged from 3.5 to 18 mg Cu kg⁻¹. He also found that pasture species had marked differences in Cu concentration. The Cu concentration in white clover, red clover and ryegrass was 10.6 mg kg⁻¹, 19.1 mg kg⁻¹ and 4.0 mg kg⁻¹, respectively, when these plants were grown on the same soil. Reuter *et al.* (1981a) recorded that the Cu concentration in subterranean clover declined from 3.9 mg kg⁻¹ at 26 days after sowing to 1.6 mg kg⁻¹ at 98 days.

Sherrell and Rawnsley (1982) found that an application of 2 to 4 kg Cu ha⁻¹ as copper sulphate increases herbage Cu concentration from 5 to 12 mg kg⁻¹ within 4 weeks and then it decreases markedly to 8 mg kg⁻¹ over the next 9 to 10 months. He also observed that the clovers tended to take up larger amounts of Cu. Willimott (1995) found that an application of 5, 10 kg Cu ha⁻¹ as copper sulphate increased the herbage Cu concentration up to three months after the Cu application, and then decreased back to the initial levels nine months after application.

Gilkes and Lim-Nunez (1979) observed that increasing levels of Cu promoted Cu uptake in wheat. Jarvis (1978) found that with increasing concentrations of Cu in solution, the Cu concentration in the roots increased much more rapidly than in the shoots of ryegrass. He also found that in most species Cu concentrations seldom exceeded 30 mg Cu kg⁻¹ even when very large additions of Cu (953 mg Cu kg⁻¹ of soil or 10 mg Cu L⁻¹ of solution) were made to either soils or solution cultures. Rasheed and Seeley (1966) found that the Cu concentration in legume shoots increases more rapidly than in grasses. Davis and Beckett (1978) estimated that the upper critical tissue concentration of Cu in rye grass was 21 mg kg⁻¹. Similarly Davis and Carlton-Smith (1984) recorded that the upper critical foliar concentration of Cu in ryegrass was 22 mg Cu kg⁻¹.

Reuter *et al.* (1981a) noted that with increasing rates of Cu application (ranging from 0 to 533 mg Cu kg⁻¹), the Cu concentration increased in both plant tops and roots. Plenderleith and Bell (1984) found that with increasing additions of Cu (4 to 600 mg Cu kg⁻¹ soil) the Cu concentration in tropical grasses increased from 17 to 27 mg kg⁻¹. Alva

and Chen (1995) observed that the concentration of Cu in shoots increased linearly both in *Cleopatra mandarin* and *Swingle citrumelo* citrus rootstock seedlings with an increase in external Cu concentration.

2.5.5 *Residual effectiveness of applied Cu*

The residual effects of Cu application have been recorded in a number of publications (Forbes, 1978; Brennan *et al.*, 1980; Gartrell, 1980; Sherrell, 1989; Lévesque and Mathur, 1986). Bélanger *et al.* (1986) observed that residual Cu had a positive effect on the Cu content of an oat crop grown in the soil four years later. Cox (1992) reported that the residual effect of Cu fertilisation with time varied between soils. An application of 4.48 kg Cu ha⁻¹ increased the extractable Cu above the initial concentration for 9 to 27 years, the average time for the five soils tested was about 16 years. Gartrell (1980) observed that Cu applied four years earlier increased the yield of wheat up to three times more than current applications banded with the seed. Copper applied 12 years earlier was still more effective at increasing the Cu uptake of wheat crops than from current fertiliser applications. Soil analysis revealed that all applied Cu remained in the top 10cm up to four years after the initial application. Brennan *et al.* (1980) observed in an incubation study that when Cu fertiliser was thoroughly mixed with soil, slow reactions between added Cu and the soil decreased the residual effectiveness of the Cu fertiliser. Although the residual effectiveness of soil applied Cu has been clearly demonstrated, there is little information available relating to the change in the form and distribution of soil Cu with time. Sherrell (1989) reported that the effect of CuSO₄ applied to an established stand of lucerne (*Medicago sativa* L.) on a yellow brown pumice soil was measured over a 4 year period. Dry matter yield was not affected, but plant and soil Cu concentrations were increased by the Cu application. Extractable soil Cu indicated that the residual effect of a Cu application might last for a considerable time.

2.5.6 *Biochemical functions of Cu*

Extensive studies have been made on the form and behaviour of Cu in plants. All the findings described in a number of publications can be summarised as follows:

- Copper is mainly complexed with organic compounds of low molecular weight and with proteins.

- Copper occurs in compounds with no known functions as well as in enzymes having vital functions in plant metabolism.
- Copper plays a significant role in several physiological processes:- photosynthesis, respiration, carbohydrate distribution, N reduction and fixation, protein metabolism, and cell wall metabolism.
- Copper influences water permeability of xylem vessels and thus controls water relationships.
- Copper controls the production of DNA and RNA, and its deficiency greatly inhibits the reproduction of plants (reduced seed production and pollen sterility).
- Copper is involved in the mechanisms of disease resistance. The resistance of plants to fungal diseases is likely to be related to an adequate Cu supply. There is also evidence that plants with enriched Cu concentrations are susceptible to some diseases. These phenomena may indicate that the role of Cu in disease resistance is an indirect one.

2.5.7 *Deficiency and toxicity of Cu in plants*

Gartrell (1981) reported that peats and mucks have commonly produced Cu deficient crops throughout Northern and Western Europe, some Midwestern and Eastern states of the United States, New Zealand and Australia. Barnes and Cox (1973) reported that in general, soils most commonly found to be deficient in Cu are poorly drained mineral soils, and mineral soils that are high in organic matter. Lucas and Knezek (1972) indicated that the total Cu in the soil should exceed 4 to 6 mg kg⁻¹ in a mineral soil and 20 to 30 mg kg⁻¹ in organic soils to sustain maximum yield of Cu responsive crops. Robson and Reuter (1981) reported that in most plant species, Cu deficiency is characterised by chlorosis, necrosis, leaf distortion, and terminal dieback, with symptoms occurring first in young shoot tissues. Once absorbed, Cu is poorly translocated in plants. Hence, the terminal growth, of most plants, is the first to be affected. Specific symptoms often depend on plant genotypes and the stage of the deficiency.

Copper is required in trace amounts for various metabolic processes in plants, at higher concentrations Cu is toxic to plant growth. Published reports on Cu toxicity to plants include the effects on photosynthesis (Lidon and Henriques, 1991) and nitrogen

metabolism (Weber *et al.*, 1991). Copper toxicity most frequently occurs where Cu containing fungicides have long been in use (Lepp *et al.*, 1984). Plants affected by Cu toxicity are slow growing and frequently exhibit symptoms typical of Fe deficiency. Heavy metals such as Cu tend to accumulate in the roots and in turn, affect the growth of the whole plant. Lidon and Henriques (1992a and 1993) observed that the Cu concentration in roots increased linearly with increasing external Cu concentrations. Decreased translocation of Cu to the above ground parts from the roots has been suggested as a mechanism to withstand Cu toxicity. Beckett and Davis (1977) found that at high levels, Cu depressed plant growth in barley. Alva and Chen (1995) observed that with an increase in external Cu concentration, the shoot and root dry weight of citrus plants decreased significantly. Luo and Rimmer (1995) observed Cu and Zn interactions on barley plant growth, and concluded that the growth of barley was controlled principally by the amounts of both added Zn and Cu. The effect of the added Cu was to increase the toxicity of the added Zn. Plenderleith and Bell (1984) conducted an experiment to evaluate the growth response of 12 sub-tropical grasses with additions of Cu and Zn. They observed that depending on the species, the Cu and Zn concentration associated with a 50% yield reduction ranged from 17 to 27 mg kg⁻¹ and 475 to 1925 mg kg⁻¹, respectively.

Characteristic symptoms of Cu toxicity include Cu induced chlorosis and root malformation, which are the most common symptoms. The growth depression of sensitive plants was observed at 15 to 20 mg kg⁻¹ Cu in tissues (Kloke *et al.*, 1984) and 10 % yield decrease is most likely at 10 to 30 mg kg⁻¹ Cu (Macnicol and Beckett, 1985).

2.5.8 Copper and animal health

There are very limited data available to relate soil extractable Cu levels to animal health. Peverill *et al.* (1988) suggested that EDTA extractable soil Cu levels between 0.5 and 1.0 mg kg⁻¹ are considered low for animal health, but noted that critical levels could vary with pasture species grown on soil types with varying soil properties. A poor relationship between soil extractable Cu and Cu levels in grazing animals has often been observed (McDonald and Mahoney, 1982).

There are several reasons for this poor relationship. The Cu concentrations in pasture plant species vary considerably with soil type (Gartrell, 1981; Sherrell and Rawnsley, 1982) and with seasonal climatic conditions (Gartrell, 1981; Reay and Waugh, 1983; McFarlane *et al.*, 1997). Generally, a Cu concentration of 5.0 mg kg⁻¹ of dry matter appears adequate for the Cu nutrition of animals (Underwood, 1977). However, if Cu deficiency is diagnosed in animals, plant analysis is required to identify the cause of the deficiency. The cause of the deficiency could be low Cu concentrations in the plants as a result of low soil Cu, or with interactions in the animal involving Mo or S in the plants. Where there are high concentrations of Mo relative to Cu in the pasture plants, Cu is less available to the animal and Cu deficiency may occur. McFarlane *et al.*, (1997) suggest that if Mo levels in pasture are high (>10 mg kg⁻¹ dry matter) a direct Cu supplement in the diet is required. Only the relationships between soil extractable Cu and Cu concentration in the plant can be defined by soil analysis.

2.5.9 *Soil and plant tests for Cu*

Common chemical extractions used to predict plant available Cu extract from one or more of three main pools: -

- Water soluble Cu.
- Exchangeable Cu.
- Complexed, chelated or adsorbed Cu.

Water and neutral salts have often been used as extractants to simulate the ability of plant roots to absorb Cu from the soil solution. The amount of Cu extracted by these extractants is small and does not give any indication of the capacity of the soil to supply further Cu to the soil solution. Research shows that accurate analysis of low Cu concentrations was difficult and contamination hard to avoid. Therefore extractants, like strong acids, were chosen, which extracted more Cu than normally required by plants.

Dilute acid solutions usually remove micronutrients from the soil solution and from exchange sites on clays and organic matter. Strong acids should be avoided because they generally extract micronutrients from non-labile solid phases (primary minerals), which are unavailable to the growing plants. However the suitability of dilute acid extractants is confined to acidic soils, because they generally are not sufficiently buffered to extract meaningful levels of micronutrients from calcareous soil. An

extensive database relating acid extractable levels of most micronutrients to crop response for USA soils exists, because of their extensive field and laboratory studies. The most commonly used dilute acids are Mehlich 1 and 0.1 M HCl.

Of all the extractants used, chelating agents show the most promise. This is due to the fact that Cu extracted by chelating agents is a combination of solution exchangeable, and also specifically adsorbed forms of soil Cu. Thus, chelating agents give a good indication of the labile pool of Cu.

2.5.9.1 Water soluble Cu

The concentration of water soluble Cu in soils is very small. Gupta and Mackay (1966) found that water-soluble Cu ranged from 0.09 to 0.46 mg Cu kg⁻¹ in some mineral soils. Miller *et al.* (1986) found less than 0.1 mg Cu kg⁻¹ soil water soluble Cu in all but one of the soils used in the study. Fiskell and Leonard (1967) grew citrus on a severely Cu deficient soil. Levels of water soluble Cu were highly correlated ($r=0.67$, $p<0.01$) with root Cu concentration. Additions of Cu to the soil in the form of CuSO₄ and CuO sprays probably increased the pool of water soluble Cu, thus accounting for the good correlation.

2.5.9.2 Exchangeable Cu

As with water soluble Cu, exchangeable Cu was found to represent only a small fraction of the total Cu. Hooda *et al.* (1997) observed that the Cu content of plant tissue was significantly correlated to soil Cu concentrations extracted by 1.0 M NH₄NO₃ for spinach, and 0.05 M CaCl₂ for wheat plants. Chlopecka (1997) reported that Cu concentrations in maize shoots and 1.0 M CaCl₂ extractable soil exchangeable Cu, correlated very well ($r=1.00$, $p>0.01$). The correlation between wheat and barley plant Cu concentrations and concentrations of Cu in the soil extracted with 1.0 M NH₄NO₃ was found to be significant, indicating that a weaker extractant gave a better assessment of plant available Cu in soils with variable properties (Arnesen and Singh, 1998). Ammonium acetate is the other main reagent used to extract exchangeable Cu from soils. Several workers have found ammonium acetate extractable Cu correlated to plant uptake (Chand and Singh, 1981; Selvarajah *et al.*, 1982; Sedberry *et al.*, 1988). Ma and

Uren (1998) observed that the addition of CaCO_3 increased the soil pH from 7.08 to 7.68 and decreased the exchangeable Cu concentration.

2.5.9.3 *Complexed, chelated and adsorbed Cu*

Copper that is complexed, chelated or adsorbed by iron and aluminium oxides, organic matter and clay minerals can be extracted by different types of reagents. Single chemical extractions have been extensively evaluated over the years, as a method for the estimation of the amount of plant available micronutrients in soils (Cox and Kamprath, 1972; De Abreu *et al.*, 1996). They have been used with varying degrees of success for diagnosing micronutrient deficiency and toxicity in soils. In neutral and alkaline soils, TEA-DTPA [0.005 M DTPA in 0.01 M CaCl_2 solution buffered by 0.1M TEA (HOCH_2CH_2)₃N at pH 7.3] has been used to extract available Zn, Fe, Mn and Cu (Lindsay and Norvell, 1978). Chelated based extractants are usually well buffered near neutrality (pH=7.3) to avoid dissolution of any carbonate minerals that could release occluded and unavailable micronutrients. The ionic strength of the extracting solution is maintained near 0.01 M to promote flocculation, and to regulate chelate activity. Direct transfer of an extractant that is buffered at a pH appropriate for calcareous soils to acidic soils is inappropriate, and can succeed only if the pH and buffering capacity of the reagents are modified.

The Mehlich (M1; 0.05 N HCl and 0.025 N H_2SO_4) method was employed to measure soil micronutrients by a number of Soil Testing Laboratories in the United States (Hanlon and DeVore, 1989). Because M1 was found unsuitable for neutral and alkaline soils, the method was modified as M2 and then as M3 (0.2 M glacial acetic acid, 0.25 M NH_4NO_3 , 0.015 M NH_4F , 0.013 M HNO_3 and 0.001 M EDTA) so that it could be used over a wide range of pH conditions to measure both macro and micronutrients (Mehlich, 1984).

The use of extractions in routine soil analysis, such as M1, M3, 0.1N HCl, 0.01 M $\text{Ca}(\text{NO}_3)_2$, 0.01 M CaCl_2 , 0.1 M NaNO_3 , 1 M NH_4NO_3 , TEA-DTPA and ammonium acetate salt solution, could be a fast and simple way to evaluate availability of macro and micronutrients to plants. The TEA-DTPA extraction method, developed by Lindsay and Norvell (1978), seems to be the most appropriate method for the extraction of

available Cu in soils (Shuman, 1986; Agrawal, 1992; Singh *et al.*, 1994), but other multinutrient extractants such as Morgan, M1, M3, and ammonium bicarbonate (AB) DTPA and ammonium acetate procedures (Raij, 1994) have been preferred for use with the ICP-AES technique in routine analysis.

Very few studies have been done comparing the extraction of Cu from soil, and the actual absorption of Cu by plants. Reed *et al.* (1993) evaluated the Cu availability for corn using the M3 extracting reagent, and found this procedure to be promising to detect the deficiency and toxicity levels of Cu in soils. On the other hand, Walworth *et al.* (1992) observed that neither the DTPA nor the M3 procedures were effective in detecting Cu availability for broccoli and potatoes cultivated in soils from Alaska. Similar results were also observed by Makarim and Cox (1983) for corn, wheat and soybeans. Such information indicates that the best procedure for determining Cu availability in all soils has yet to be determined. Minnich *et al.* (1987) measured Cu^{2+} activity on saturated soil extracts, and related it to the accumulation of Cu in young snap beans (*Phaseolus Vulgaris* L.). They found that the Cu concentration in both shoots and roots increased with measured Cu^{2+} activity.

The efficiency of extractants for the dissolution of soil Cu has been well researched. The choice of extractant is dependent upon several factors:

- Pool of Cu to be extracted.
- Soil pH.
- Presence of free calcium carbonate.
- Organic matter.

Many reagents remove far greater amounts of Cu from the soil than the plant is able to. However, of all the extractants tested, the chelating agents such as DTPA and EDTA are the most promising for use in non-calcareous soils. Good correlations have been obtained between EDTA and TEA-DTPA extractable soil Cu and plant Cu under a variety of conditions. These types of extractants have largely superseded the dilute salt and acid extractants, which do not appear to give reliable estimations of plant available Cu.

2.6 ENVIRONMENTAL FACTORS AFFECTING REACTIONS AND THE AVAILABILITY OF COPPER IN SOILS

This section will review the specific effects of environmental factors on the solubility of Cu in soils. In addition, it will focus on the effects of temperature, light, and soil moisture on the uptake, translocation, and metabolism of Cu. The seasonal pattern of pasture Cu concentration may vary with location, soil type, climate, pasture species and management practices.

2.6.1 *Soil moisture content and redox reaction*

Ponnamperuma (1972) reviewed the chemistry of a submerged soil. After submersion, the aerobic soil becomes anaerobic and undergoes changes in soil pH, organic matter decomposition and microbial activity. Other important changes also occur, but the aforementioned effects are relevant to Cu availability. The effects on soil Cu mobility in anaerobic soils result from the following:

- In acid and alkaline soils pH tends to stabilise around pH 7.0.
- Iron and manganese oxides are reduced to release Cu.
- Decomposition of organic matter is slower than in aerobic soils, but results in a large number of strongly complexing organic compounds.

Ponnamperuma (1972) in his review suggested that the overall effect of soil chemical reduction is to increase the mobility of Cu in the soil. However, it appears that acid soils with higher amounts of organic matter undergoing soil reduction, will have less 'plant-available' Cu. Complexing of Cu by soluble organic matter will increase, firstly as a function of soil pH, and secondly due to the decomposition of organic matter. Beckwith *et al.* (1975) grew rice plants in both flooded and well drained soils. There was very little change in yield between treatments. They found that, although EDTA-extractable Cu increased with flooding, the uptake of Cu by rice shoots was decreased. This trend persisted from sowing to harvest maturity, so did not appear to be related to the stabilisation of soil reduction changes. Soil solution Cu levels were too low to be accurately measured, so no speculation could be made about the influence of soluble organic complexing.

Williams and McLaren (1982) incubated dry and moist soils, containing 5 mg Cu kg⁻¹ soil, for a total of 43 weeks. The amount of EDTA extractable Cu decreased with time in all soils. Recovery of added Cu by EDTA ranged from 87% to 67% for dry soils and 67% to 45% for moist soils. The decrease in extractability being greatest for the moist soils. This was found to be correlated to the manganese and iron oxide content of the soils. Ageing of amorphous oxides, which are considered to be attacked by EDTA, results in crystallisation of the oxide structure. Hence EDTA extractable Cu decreases with time. Under moist conditions, diffusion of Cu to sites that are not attacked by EDTA is faster. Sims and Patrick (1978) observed that a large proportion of Cu solubilised by soil reduction would not remain in the water soluble fraction, but would be complexed by organic material and become unavailable to plants. Nambiar (1977) concluded that ryegrass (*Lolium multiflorum* Lam.) absorbed significant amounts of Cu from nearly air-dry soil, when the roots had access to subsoil water. Reddy *et al.* (1981) stated that the moisture content of a soil did not influence the concentration of Cu in the soil solution or affect its availability to plants. In both cases, aerobic soils were studied and these are expected to behave differently to anaerobic soils. Williams (1981) suggested that the concentration of soil solution Cu is independent of the soil moisture content if both solid and solution phase Cu are in true equilibrium. In conclusion, it appears that the influence of soil redox conditions on the availability of Cu to plants is complex. This is mainly because a number of different soil chemical properties change when a soil becomes reduced, and it becomes difficult to separate the effect of a single property.

2.6.2 Seasonal variation and soil temperature

It was reported that the variation in season affects Cu concentration in plants. Hemmingway (1962) obtained a higher concentration of Cu in clover and grasses in the autumn, but Reddy *et al.* (1981) obtained higher pasture Cu concentrations in winter both in glass house and field experiments. Whereas Metson *et al.* (1979) observed no seasonal influences on Cu concentration in pasture species. Several scientists (Hogg and Moore, 1976; Reddy *et al.*, 1981; Merry *et al.*, 1986) observed that increasing soil temperature caused an increase in the availability of Cu to plants. Reddy *et al.* (1981) grew subterranean clover in a lateritic podzol and a calcareous sandy soil. They imposed two soil temperature (12 °C and 22 °C) and moisture levels. They obtained the higher dry matter yield under the higher temperature and moisture regime. The Cu

concentration in clover was higher at 22 °C compared to 12 °C. This was irrespective of the soil moisture content. The greater availability of Cu to clover plants at 22 °C was related to the higher amount of Cu extracted by calcium chloride at 22 °C.

Brennan *et al.* (1980, 1984) found in their incubation studies that increasing the soil temperature up to, but not more than 35 °C decreased the relative effectiveness of Cu fertilisers on plants. Incubation periods were maintained for up to 120 days, and it was suggested that incubation of warm soils promoted diffusion reactions of Cu into the soil colloids. It appears that seasonal variations in the Cu concentration of pasture are related to soil moisture content, although laboratory studies could not substantiate this.

Copper availability seems to be at a peak in the autumn and in the winter seasons with transient changes in soil temperature having little effect. When different temperatures are imposed over longer periods of time several effects are thought to occur:

- Increased rate of mineralisation of organic matter with increasing temperature.
- Adsorption of Cu by soil colloids and diffusion into the soil materials may be influenced by temperature.
- Equilibrium between solid and solution phases may be established more rapidly under higher soil temperatures (Beckwith, 1963).

2.6.3 *Radiation*

Light does not seem to have any major effects on the frequency of Cu deficiency under field conditions. However, Graves and Sutcliffe (1974) showed that Cu deficiency slowed down the rate of flower initiation and development in chrysanthemum (*Chrysanthemum morifolium* Ramat.), which required a short photoperiod for flowerbud initiation. The severity of Cu deficiency, unlike that of Zn, in subterranean clover (*Trifolium subterranean* L.) was unaffected by a reduction in light intensity under green house conditions (Millikan, 1953).

The effects of radiation on Cu uptake by plants are mediated through its effect on plant growth. The effects, of temperature on crop development and solar radiation on biomass accumulation, combine to impose well-defined limits on potential crop yields under various environmental conditions. The frequency of solar radiation defines the

maximal limit on crop yield, because intercepted solar radiation provides the energy for photosynthetic fixation of CO₂. By assuming an upper limit to the efficiency of CO₂ fixation (or crop biomass accumulation) per unit of intercepted solar radiation, an estimate can be calculated for the limit on crop yield, based on the amount of solar radiation during the growing season. Spaeth *et al.* (1987) were able to account for much of the yield variability between seasons, as a consequence of differences in temperature and incident solar radiation. The radiation use efficiency (RUE) and the harvest index were held constant under all conditions in their calculation. Both the pattern of leaf area development (as determined by temperature) and incident solar radiation in these environments influenced the amount of intercepted radiation, which in turn accounted for differences in yield between seasons in nonstressed soybeans grown in Japan. Yields of nonstressed spring wheat in Israel were examined in terms of environmental variation in temperature and solar radiation (Amir and Sinclair, 1991). Woledge and Dennis (1982) observed that the photosynthetic rate of leaves was twice as high at 15°C than at 5°C for perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium pratense* L.) grown at different temperatures. Ryegrass and clover had similar photosynthetic rates, which responded similarly to temperature. Reay and Waugh (1983) observed Cu concentration fluctuated monthly in seasonal variation trials. They also observed that the Cu concentration, which fluctuated without seasonal trends in ryegrass leafblades, correlated with the organic-N concentration.

2.6.4 *Plant and other organisms*

There is considerable evidence to include the plant as an external factor acting on the soil. The influences the plant exerts on the availability of Cu have been reviewed by Wilkinson (1972) and Marschner (1986). These effects are outlined as below:

- Absorption of ions from the soil solution by plant roots results in a change in the concentration gradient in the rhizosphere.
- During plant respiration, oxygen is absorbed and carbon dioxide is evolved into the rhizosphere. There is a subsequent decrease in pH resulting in a greater proportion of Cu²⁺ available for plant uptake.
- Roots are able to exude various substances, which include: H⁺, OH⁻, HCO₃⁻ and CO₂. These are all capable of changing soil pH. Organic and amino acids are also released

and may complex solution Cu. Organic exudates are thought to be involved in the release of exchangeable and specifically adsorbed Cu from the solid phase of the soil.

- The growth of bacterial and fungal populations are stimulated in the rhizosphere and are believed to accelerate the release of Cu from the solid phase of soils.

Nielsen (1976) obtained samples of soil solution by suction from a calcareous peat soil in which barley plants were growing. As expected, the levels of Cu in the soil solution remained constant, when no plants were grown. In the presence of barley plants, Cu in the soil solution increased. Williams (1981) suggested that some portion of the organic material solubilised in the presence of plants would be due to root exudates. Linehan *et al.* (1985) displaced the soil solution by centrifugation, from pots containing barley plants. The concentration of Cu in the rhizosphere was found to be greatest during early plant development and decreased up to harvest. Microbial activity was considered to be involved. The pattern of change of the Cu concentration in the rhizosphere with time was similar to that of a changing soil biomass. It was suggested that enhanced Cu levels in the rhizosphere could also be related to soil pH changes. Stevenson and Fitch (1981) found that the decomposition of crop residues and organic wastes by microorganisms may lead to the release of significant quantities of Cu. Complex formation may reduce the concentration of Cu^{2+} to a non-toxic level, when excess Cu^{2+} is present.

2.6.4.1 *Mycorrhizae*

Mycorrhizae are naturally occurring associations between certain soil fungi and plant roots in which the fungi colonise the root tissue. But also act as extensions of the plant root system, in which hyphae external to the root absorb and transport water and various nutrients back to the root zone. Kothari *et al.* (1991) reported that mycorrhizal plants normally contain significantly higher levels of P, and various micronutrients, most notably Zn and Cu. This effect may partly be due to the enhanced vigour of mycorrhizal plants, but mycorrhizae have also been shown to take up and transport metals to the roots where they are absorbed and translocated to the plant shoot. Timmer and Leyden (1980) concluded that the reduction of plant Cu concentration at high P levels resulted from a reduced exploitation of the soil by mycorrhizae.

Baker and Senft (1995) concluded that the activity of Cu^{2+} involved in uptake by plant roots is a function of the soil solution activity of Cu^{2+} as modified by mycorrhizal effects. The uptake of Cu, Zn and P is enhanced by fungi associated with roots, known as vesicular-arbuscular mycorrhizae, whose hyphae penetrate the root at one end with the other end extending several centimetres into the soil. It is not known whether the beneficial effects of mycorrhizae result from an increased activity of ions or to a greater effective root surface area.

While the contribution of mycorrhizal fungi to plant element uptake were quantified in detail for several plant species under a controlled environmental conditions (George *et al.*, 1995), the quantification of the mycorrhizal contribution to plant growth under field conditions is difficult, because control plants cannot be maintained with mycorrhiza-free root systems. Indirect evidence, however, indicates benefits due to mycorrhizal infection on low-P soils are large enough to be measurable, albeit to varying magnitudes (George *et al.*, 1994; Abbott *et al.*, 1995).

CHAPTER 3 ADSORPTION AND DESORPTION OF COPPER IN PASTURE SOILS

3.1 INTRODUCTION

3.1.1 *Cu adsorption*

The chemical behaviour of cations and anions in the soil is primarily governed by retention and release reactions of solute within the soil matrix. The retention and release reactions in soils include precipitation/dissolution, ion exchange, and adsorption / desorption reactions (Amacher *et al.*, 1986). Retention may be due to precipitation and /or adsorption, and depends on factors such as the nature of the ion, the nature of the mineral and organic constituents of the soil, and the composition of the soil solution. For adsorption, cations are held either through electrostatic attraction, thus giving rise to ion exchange with the surrounding ions, or by specific adsorption by surface bonding with organic and mineral substrates. Surface sorption is considered the most important mechanism that regulates the concentrations of Zn, Cu and Cd in the soil solution (Boekhold *et al.*, 1991; Guadalix and Pardo, 1995). Several studies of heavy metal adsorption by individual soil components such as organic matter (humic and fulvic acids), silicate minerals (montmorillonite, kaolinite, illite etc) and sesquioxides (iron, aluminium or manganese oxide) have indicated relatively strong bonding and high capacities of various materials for adsorption (Barrow *et al.*, 1981; Fu *et al.*, 1991; Oden *et al.*, 1993). Bivalent transition metal cations exhibit a similar pH dependent sorption behaviour; many authors have reported that the amount of sorbed metal ions increases with the raising of solution pH within a certain range (Elliott *et. al.*, 1986; Guadalix and Pardo, 1995).

Soil is complicated by the heterogenous nature of its components. Thus interaction among different soil components is likely. In soils, Cu is adsorbed on the surface of clay minerals, Fe and Mn oxides, and organic matter; it can precipitate with sulphide, carbonate, and hydroxide ions and complex with soluble organic molecules (McBride, 1981; Baker, 1990). Although organic matter and oxides are important in adsorption reactions, differences exist in their relative importance. The Cu adsorption ability of

soils is affected by pH, organic matter, iron and aluminium oxides, and clay minerals (McLaren *et al.*, 1983b; Stevenson *et al.*, 1993; McBride, 1994; Luo and Chistie, 1996). pH is a major factor controlling the sorption of Cu, and many authors confirm that the sorption of Cu onto soils increases with an increase in soil pH (Cavallaro and McBride, 1978; Kuo and Baker, 1980; Harter, 1983; King, 1988).

Whatever the processes may be, retention in soils greatly determines the mobility and the bioavailability of Cu, and it is therefore essential to improve our knowledge of these processes in soils, which vary in their chemical characteristics. The measurement of adsorption and mobility of Cu in soils with different compositions is required for determining the loading capacity of Cu in a given soil.

3.1.2 *Cu desorption*

To predict the fate and mobility of Cu in soils and to develop effective remediation strategies to overcome Cu deficiency, and toxicity in pasture soils and orchards, which receive frequent applications of Cu fertilisers and pesticides, information on desorption is required. Surface application of high levels of Cu to pasture soils through fertilisers and effluents with high concentrations of Cu may result in Cu toxicity in the grazing animal. Although rare, Cu phytotoxicity has been encountered in soils receiving long-term additions of sewage sludge, industrial wastes and repeated applications of Cu fertiliser and pesticides (Tisdale *et al.*, 1993). It is generally accepted that low Cu concentrations typically found in the soil solution are regulated by adsorption and desorption phenomena associated with various soil colloidal materials.

Soil acidification enhances Cu desorption from the solid phase, thereby increasing its concentration in the soil solution (Basta and Tabatabai, 1992). This enhanced desorption may intensify the bioavailability of Cu. Copper uptake by plants and animals is a function of the activity of soil solution Cu^{2+} , which is controlled by the concentration of total soluble Cu, inorganic and organic ligands and by soil pH. A decrease in pH enhances Cu^{2+} absorption by plants, because of the increased solution activity of Cu^{2+} associated with the dissolution of soil minerals, decreased organic bonding and in solid phase adsorption of Cu (Baker, 1990). The adsorption of Cu by soils and its relationship with plant availability have been examined by many authors. In contrast, very little

information is available on desorption of Cu. Such information is required in order to improve our ability to predict the release of both native Cu from soils, and the release of Cu added to soils as fertiliser or as a pollutant. In terms of plant and ultimately animal nutrition, it is the supply of Cu from the solid phase into solution, which determines the availability of Cu for plant uptake.

Objectives:

The objectives of the experiments reported in this chapter are:

- To determine time-dependent Cu adsorption by five soils.
- To determine the adsorption capacity of soils with widely differing properties.
- To examine the effect of pH on Cu adsorption.
- To quantify the contribution of different soil components to Cu adsorption.
- To examine the effect of pH and incubation period lengths on Cu desorption in the various soils.

3.2 MATERIALS AND METHODS

3.2.1 *Soils used*

Five different soils (Manawatu, Tokomaru, Ramiha, Ngamoka and Mangamahu) were used for the adsorption and desorption study. The soils used for different adsorption and desorption experiments, and the experimental conditions are given in Table 3.1.

Table 3.1 The soils and experimental conditions used in the various adsorption and desorption experiments.

Sl No	Experiments	Soils	Experimental condition
1	Time-dependent experiment on Cu sorption	Manawatu, Tokomaru, Ramiha, Ngamoka and Mangamahu	Sorption at 10 mg Cu L ⁻¹ shaken for 30-120 minutes
2	pH dependent Cu sorption isotherm for soil.	Manawatu, Tokomaru, Ramiha and Ngamoka	Sorption at 5-50 mg Cu L ⁻¹ shaken for 2 hrs.
3	Cu sorption isotherm for soil and soil components	Manawatu, Tokomaru, Ramiha, Ngamoka and Mangamahu	Sorption at 5-50 mg Cu L ⁻¹ shaken for 2 hrs.
4	Extractable soil Cu	Manawatu and Ngamoka	Extraction was done with M1, M3, DTPA, 0.1M HCl and 0.04M EDTA.
5	Desorption of native Cu	Manawatu and Ngamoka	Desorption of native Cu equilibrated for 2 hrs.
6	Desorption of added Cu	Manawatu and Ngamoka	Desorption in 50 mg Cu kg ⁻¹ soil equilibrated for 2 and 24 hrs.
7	Effects of contact times on desorption of added Cu and native Cu.	Manawatu and Ngamoka	Soils incubated with Ca(NO ₃) ₂ and 50 mg Cu kg ⁻¹ for different periods. Desorption for both were equilibrated for 2 and 24 hrs contact periods.
8	pH dependents desorption of soil.	Manawatu, Tokomaru, Ramiha and Ngamoka	Desorption at 30 and 50 mg Cu L ⁻¹ equilibrated for 2 and 24 hrs contact periods at pH 5 and 8.

3.2.2 Soil physical and chemical analysis

Soil particle size (sand, silt and clay) analysis was completed after oxidising the organic matter in the soil samples with aliquots of 30% hydrogen peroxide. Soil pH (1:2.5 H₂O), cation exchange capacity (by 1M NH₄OAc (pH 7) extraction), Olsen P (by 0.5 M NaHCO₃ extraction), sulphate (by 0.04 M Ca(H₂PO₄)₂ extraction), organic carbon (by 1N K₂Cr₂O₇ extraction) were measured according to Black (1965). Acid oxalate-extractable Fe and Al, and exchangeable K, Ca, Mg and Na were determined for the initial soil samples by the standard methods (Blackmore *et al.*, 1987).

3.2.3 Time-dependent experiment on Cu sorption

Duplicate soil samples of 1.0g were weighed into 50 ml centrifuge tubes. Twenty ml of 10 mg Cu L⁻¹ [as Cu(NO₃)₂] solution was added and the tubes were shaken in an end-over-end shaker for 30, 60, 90, 120 and 180 minutes at 20°C. The samples were then centrifuged at 7719 g and filtered through Whatman No. 44. The concentration of Cu in

the filtrate was measured by flame atomic absorption spectrophotometry (F-AAS). The Cu sorbed was calculated as the difference between the amount added and that remaining in the equilibrium solution.

3.2.4 *pH dependent copper sorption isotherm for soils*

Copper sorption isotherms at pH 5.0, 6.0, 7.0 and 8.0 were obtained for each soil as follows: 1.0 g of each soil was suspended in 20 ml of 0.01 M $\text{Ca}(\text{NO}_3)_2$ solution. The above pH values were achieved by adding 0.01M HCl or sodium hydroxide (NaOH) and allowed to equilibrate for 48 hrs. Various concentrations (0, 5, 10, 20, 30, and 50 mg Cu L^{-1}) of Cu were added as $\text{Cu}(\text{NO}_3)_2$. The soil suspension was equilibrated for 24 hrs before centrifuging and filtering. The Cu in the filtrate was determined by F-AAS. The Cu sorbed was calculated as the difference between the amount added and that remaining in the equilibrium solution.

3.2.5 *Copper sorption isotherm for soil components*

Copper adsorption isotherms were obtained for the original soil samples together with subsamples of the same soils treated to remove the organic matter, iron and aluminium oxides. Organic matter was removed by using 0.7M sodium hypochlorite at pH 8.5 and iron and aluminium oxides were removed by using a mixture of 0.2 M ammonium oxalate, 0.2 M oxalic acid and 0.1 M ascorbic acid. The measurement of adsorption after the removal of various soil components provides useful quantitative information on the relative importance of each different soil component on Cu sorption. Samples of the original soils and their subsamples, after the removal of the different components (organic matter removed and organic matter plus oxides removed), were equilibrated with a 10 ml solution containing known amounts of Cu as $\text{Cu}(\text{NO}_3)_2$; 0.01 M $\text{Ca}(\text{NO}_3)_2$ was used as the background electrolyte and a range of Cu concentrations (0, 5, 10, 20, 30, 40 and 50 mg Cu L^{-1}) were used. Equilibration was carried out in centrifuge tubes on an end-over-end shaker for 24 hrs at 20°C. The time dependent experiment indicated that more than 90% of Cu adsorption occurred within a 2 hrs period. The samples were then centrifuged at 7719 g and filtered through Whatman No. 44 and the Cu was determined in the filtrate. Cu in the soil filtrate was analysed using F-AAS. Cu adsorbed was calculated from the initial and the final solution Cu concentrations.

3.2.6 *Fractionation of soil Cu*

Fractionation of soil Cu was carried out according to the sequential extraction method described by McLaren and Ritchie (1993). The soil Cu is fractionated into four forms: (i) exchangeable Cu; (ii) organically bound Cu; (iii) Cu associated with iron oxide; and (iv) residual Cu (Cu remaining after the removal of the first three fractions) (Table 3.2). Hydrochloric acid was used instead of hydrofluoric acid for the measurement of the residual fraction.

Step A (Exchangeable Cu): A known weight (5.0 g) of the finely ground soil samples were placed into the 50 ml polyethylene centrifuge tubes with 35 ml 0.01M $\text{Ca}(\text{NO}_3)_2$. The suspension was shaken in an end-over-end shaker for 24 hrs at 20°C and then centrifuged for five minutes at 7719 g in the Sorvall RC 5C automatic superspeed refrigerated centrifuge and the supernatant was filtered through Whatman No. 42.

Step B (Organic matter bound Cu): A known weight (10.0 g) of the finely ground soil samples were placed into the 250 ml polyethylene centrifuge bottles with 20 ml 0.7M sodium hypochlorite (NaOCl); NaOCl was adjusted to pH=8.5 immediately before using. The suspension was then boiled in a water bath for 30 minutes and stirred occasionally. After the organic matter extraction and water wash, the soil was air dried, crushed and passed through 400 μm sieve. The water washed NaOCl solution was condensed in a water bath and filtered through Whatman No. 42.

Step C (Cu associated with iron oxide): A known weight (1.0 g) of the finely ground soil samples from step B was placed into the 250 ml polyethylene centrifuge bottles and mixed with 25 ml of a mixture containing 0.2M ammonium oxalate [$(\text{NH}_4)_2\text{C}_2\text{O}_4 \cdot \text{H}_2\text{O}$], 0.2M oxalic acid [$\text{H}_2\text{C}_2\text{O}_4$], and 0.1M ascorbic acid [$\text{C}_6\text{H}_8\text{O}_6$]. The suspension was then boiled in a water bath for 30 minutes and stirred occasionally. After the iron oxide Cu extraction and water wash, the soil was air dried and crushed. The water washed solution was condensed in a water bath and filtered through Whatman No. 42.

Step D (Residual Cu): The soil remaining after the removal of iron oxide Cu (step C) was used to determine residual Cu by wet digestion with a mixture of HNO_3 , HClO_4 and HCl at the ratio (by volume) of 5:5:7. The Cu in the filtrate was measured by F-AAS.

Table 3.2 Sequential fractionation scheme for Cu in soil.

Step	Fraction	Extractant	Soil g	Solution ml	Conditions
1.	Exchangeable Cu	0.01M calcium nitrate	5	35	Shaken 24 hrs
2.	Organic bound Cu	0.7M sodium hypochlorite (pH=8.5)	10 ^A	20	Boiling in a water bath 30 min, stirred occasionally
3.	Copper associated with iron oxides	0.2 M ammonium oxalate 0.2 M oxalic acid 0.1 M ascorbic acid	1 ^B	25	Boiling in a water bath 30 min, stirred occasionally
4.	Residual Cu	Concentrated nitric, perchloric and hydrochloric acid	1 ^C	25	Digested at 160 ^o C

^ASeparate sample from step 1. ^B1 g from step 2 after drying and grinding. ^CResidue from step 3.

3.2.7 Desorption of native and added Cu

Desorption of Cu from the soil samples was measured repeatedly using 1.0 g duplicate soil samples, which were pre-equilibrated for 2 hrs and 24 hrs with a 20 ml solution containing known amounts of Cu [as Cu(NO₃)₂ in 0.01 M Ca(NO₃)₂]. Equilibration for the desorption step was carried out in centrifuge tubes on an end-over-end shaker for 2 hrs and 24 hrs at 20^oC. After each 2 hrs and 24 hrs equilibration period, the samples were centrifuged, and the supernatant decanted and filtrated through Whatman No. 42 filter paper. The soil was resuspended with fresh 0.01 M Ca(NO₃)₂, and the procedure repeated as necessary. The pH of the desorption solution was measured at the end of each 2 hrs and 24 hrs desorption period. Cu in the soil extractant was analysed using F-AAS. Cumulative Cu desorption was calculated from the Cu concentrations in each successive equilibration solution.

3.2.8 Desorption study for incubated soil

Two soils (Manawatu and Ngamoka), which represent similar pH values but varying organic matter content, were chosen for the desorption study. The soil was mixed with Cu fertiliser to give 50 mg Cu kg⁻¹ soil and incubated for different periods (control, 0, 8 and 28 days). The desorption of Cu was measured following the method described in 3.2.7.

3.2.9 Desorption study at different pH levels

The soil that received 30 and 50 mg Cu L⁻¹ during the sorption experiment at pH 5.0 and 8.0 were subsequently used in the desorption study. In the earlier study in which desorption at low levels of Cu (5-10 mg Cu L⁻¹) was examined, measurement of low levels of Cu in the desorption solution becomes difficult, especially in soils with a high organic matter content. So in this experiment, high Cu addition rates were selected to minimise the difficulties and uncertainties in the measurement of low Cu levels in the solution by F-AAS. The centrifuged soils from the sorption experiment (Section 3.2.4) were resuspended in 20 ml of 0.01 M Ca(NO₃)₂ at pH 5.0 or 8.0, and were equilibrated on an end-over-end shaker for two hours. Subsequently, the soil suspension was centrifuged, filtered and the Cu in the supernatant was determined by F-AAS. The desorption procedure was repeated 10 times for each samples.

3.2.10 Extractable soil Cu

The amount of Cu extracted by various soil test reagents was measured; so a comparison could be made with the amounts removed during the desorption experiment.

3.2.10.1 0.04M EDTA extracting solution

0.5 g duplicate soil samples (Manawatu and Ngamoka) were equilibrated with a 35 ml solution containing 0.04 M EDTA. Equilibration was carried out in centrifuge tubes on an end-over-end shaker for 2 hrs and 24 hrs at 20^o C; after each equilibration period, the samples were centrifuged, and the supernatant solutions decanted and filtered through Whatman No. 42 filter paper. Copper in the extractant was measured by F-AAS.

3.2.10.2 Mehlich-1 extracting solution

The Mehlich-1 solution was prepared to contain 0.05 N HCl and 0.025 N H₂SO₄ (Mehlich, 1953). For Mehlich-1 extraction, 20 ml of the extractant was added to 5 g of soil in a 50 ml centrifuge tube, shaken for ten minutes and filtered through a Whatman No. 40 filter paper.

3.2.10.3 *Mehlich-3 extracting solution*

The Mehlich-3 solution was prepared to contain 0.2 M glacial acetic acid, 0.25 M NH_4NO_3 , 0.015 M NH_4F , 0.013 M HNO_3 and 0.001 M EDTA (Mehlich, 1984). For Mehlich-3 extractions, 25 ml of the extractant was added to 2.5 g of soil in a 50 ml centrifuge tube, shaken for ten minutes and filtered through a Whatman No. 40 filter paper.

3.2.10.4 *0.1M HCl extractant*

A known weight (8.0 g) of the finely ground soil was placed into 50 ml polyethylene centrifuge tube with 20 ml 0.1M HCl (Haynes and Swift, 1985). The suspension was then shaken in an end-over-end shaker for 2 hrs at 20⁰ C. Then centrifuged for three minutes at 3015 g in the Sorvall RC 5C automatic superspeed refrigerated centrifuge and the supernatant was filtered through Whatman No. 42.

3.2.10.5 *DTPA extracting solution*

The DTPA extracting solution was prepared to contain 0.005 M DTPA, 0.01M CaCl_2 , and 0.1M TEA and adjusted to pH 7.3 (Lindsay and Norvell, 1978). Ten grams of air dried soil was placed in a 125 ml conical flask, and 20 ml of the DTPA extracting solution was added. Each flask was covered with stretchable Parafilm and secured upright on a horizontal shaker with a stroke of 8.0 cm with a speed of 120 cycles/min. After two hours of shaking, the suspensions were filtered by gravity through Whatman No. 42 filter paper. The filtrates were analysed for Cu using F-AAS with the appropriate standards.

3.3 RESULTS AND DISCUSSION

3.3.1 *General description of soils*

Manawatu silt loam: This soil sample was collected from the Massey University Fruit Crop unit. The Manawatu soil is a dark brown fine sandy loam, alluvium, slightly plastic, and weathered fluvial recent soil, quartzo-feldspathic, mixed greywacke and argillite. These soils are characterised by dark greyish brown friable fine sandy loam or

silt loam with moderately developed nut structured topsoils overlying olive brown firm fine sandy loam with weakly developed nut structured subsoils.

Tokomaru silt loam: This soil sample was collected from the Massey University Dairy Farm unit 4. It is formed on fairly thick deposits of loess of fine sandy loam texture. It is classified as a Central Yellow Grey Earth. It is a dark greyish brown silt loam with weakly to moderately developed nut structure and some fine brown mottles at topsoils. Tokomaru silt loam is a poorly drained soil, which experiences wet conditions in winter, and relatively dry conditions in summer.

Ramiha silt loam: This soil sample was collected from the Massey University Farm (Tuapaka). It is a strongly leached Yellow Brown Earth formed from loess and solifluction material and slope deposits. It is a dark greyish brown friable silt loam with strongly developed nut structured topsoils. Angular greywacke stones may occur through the profile, and slightly weathered greywacke occurs at variable depths ranging from 25 cm to over 90 cm but on average at about 60 cm.

Ngamoka silt loam (Ballantrae High Fertility Soil): The soil sample was collected from the Ballantrae hill area and related steepland. It is classified as Central Yellow Brown Earth or steepland intergrades to Yellow Grey Earth formed from a mixture of parent materials, including sandy siltstones, silty sandstones and silty mudstones. These soils are characterised by deep well structured silt loam topsoils overlying yellowish brown friable to slightly firm subsoils. Ngamoka soils are strongly leached and the topsoil is strongly to moderately acid, and the temperature regime is mesic or frigid. Ngamoka soil is highly weathered, rich in illite, vermiculite and montmorillonite, with dark-coloured topsoils and low (<60%) base saturation.

Mangamahu steepland soil (Ballantrae Low Fertility soil): This soil was obtained from Ballantrae hill country area and is classified as a steepland soil associated with Central Yellow Brown Earth and intergrades to Yellow Grey Earth. Dark brown fine sandy loam topsoil with moderately developed fine nutty structure. Parent material is silty sandstone. Subsoil contains some pale yellowish brown mottles. Mangamahu soils occur on the steep faces. Profiles are in general shallow with a fine sandy loam.

3.3.2 *Soil properties*

The highest and the lowest pH values were recorded for the Manawatu soil (pH 6.0) and the Mangamahu (pH 4.8) soil, respectively (Table 3.3). The Tokomaru and the

Ngamoka soils have the same pH (5.6) value. The data presented in Table 3.3 indicates that the Manawatu soil contained the highest amount of Olsen P (51.9 mg kg⁻¹) and the lowest amount (10.5 mg kg⁻¹) was in the Mangamahu soil. The Ngamoka soil contained the highest amount (5.85%) of organic carbon and the lowest amount (2.91%) was in the Manawatu soil. The CEC of the Ngamoka soil was twice as high (26 cmol kg⁻¹) as that of the Mangamahu soil (13 cmol kg⁻¹). The Manawatu and the Mangamahu soils contained higher amounts of exchangeable Cu than the other soils. Clay content was significant in all soils, ranging from 178 g kg⁻¹ in the Manawatu and 255 g kg⁻¹ in Ngamoka soil. The Ramiha soil contained the highest amount of both Al and Fe. The Manawatu and the Tokomaru soils contained the lowest amounts of Al and Fe.

Table 3.3 Initial soil properties

Parameters	Manawatu	Tokomaru	Ramiha	Ngamoka	Mangamahu
Soil classification	Typic Udifluent	Typic Fragiaqualf	Andic Haplumbrept	Umbric Dystrochept	Typic Distrochepts
Particle size (g kg ⁻¹)					
Sand	203	197	129	197	207
Silt	606	575	597	548	497
Clay	178	210	256	256	262
Organic carbon%	2.9	3.4	5.6	5.9	4.9
pH	6.0	5.6	5.3	5.6	4.8
Olsen P (mg kg ⁻¹)	51.9	16.8	19.5	24.3	10.5
SO ₄ (mg kg ⁻¹)	5.0	6.0	14.8	13.3	6.8
CEC (cmol kg ⁻¹)	17.0	13.0	23.0	26.0	20.0
K (cmol kg ⁻¹)	0.92	0.24	0.84	1.56	0.31
Ca (cmol kg ⁻¹)	9.5	4.4	9.1	7.4	3.5
Mg (cmol kg ⁻¹)	1.41	1.18	1.67	1.64	1.4
Na (cmol kg ⁻¹)	0.09	0.16	0.17	0.09	0.20
Acid oxalate					
Al (%)	0.09	0.12	1.9	0.42	0.23
Fe (%)	0.31	0.28	1.2	0.38	0.28
Exchangeable Cu (mg kg ⁻¹)	0.54	0.31	0.51	0.33	0.47
Total Cu (mg kg ⁻¹)	33.39	14.86	26.75	23.38	17.69

3.3.3 Adsorption

3.3.3.1 Copper sorption as a function of time

Copper sorption by the soils was rapid in the beginning, this fast rate of sorption generally took place within a few minutes. Later on the rate of sorption slowed down, this might be due to the diffusion of Cu into the soil aggregates. In all soils, sorption of Cu increased with time (Figure 3.1). The effect of time on sorption varied among the

soils. The sorption percentage ranged from 86% in the Ngamoka soil to 95% in the Ramiha soil within 30 minutes of equilibration at 200 mg Cu L⁻¹. In the Ngamoka soil the sorption increased from 86% to 92% within 3 hrs (Figure 3.1). Initial Cu (10 mg Cu L⁻¹) concentration (and loading rates) in this study was affected by sorption. Hue *et al.* (1997) reported that at a lower loading rate of 30 mg Cu L⁻¹, between 98% and 99.9% of the added Cu was sorbed within one hour. At a lower rate more than 99% of the added Cu was sorbed within 2 hrs. The time required for the maximum sorption was relatively consistent in all soils. The results indicated that a quasistationary state was achieved within half an hour of shaking in all soils. However, the Mitscherlich function indicated that more than 90% of sorption occurred within 2 hours, and hence in order to give sufficient contact time to ensure an equilibrium system, a 2 hours shaking time was adopted for all subsequent adsorption studies.

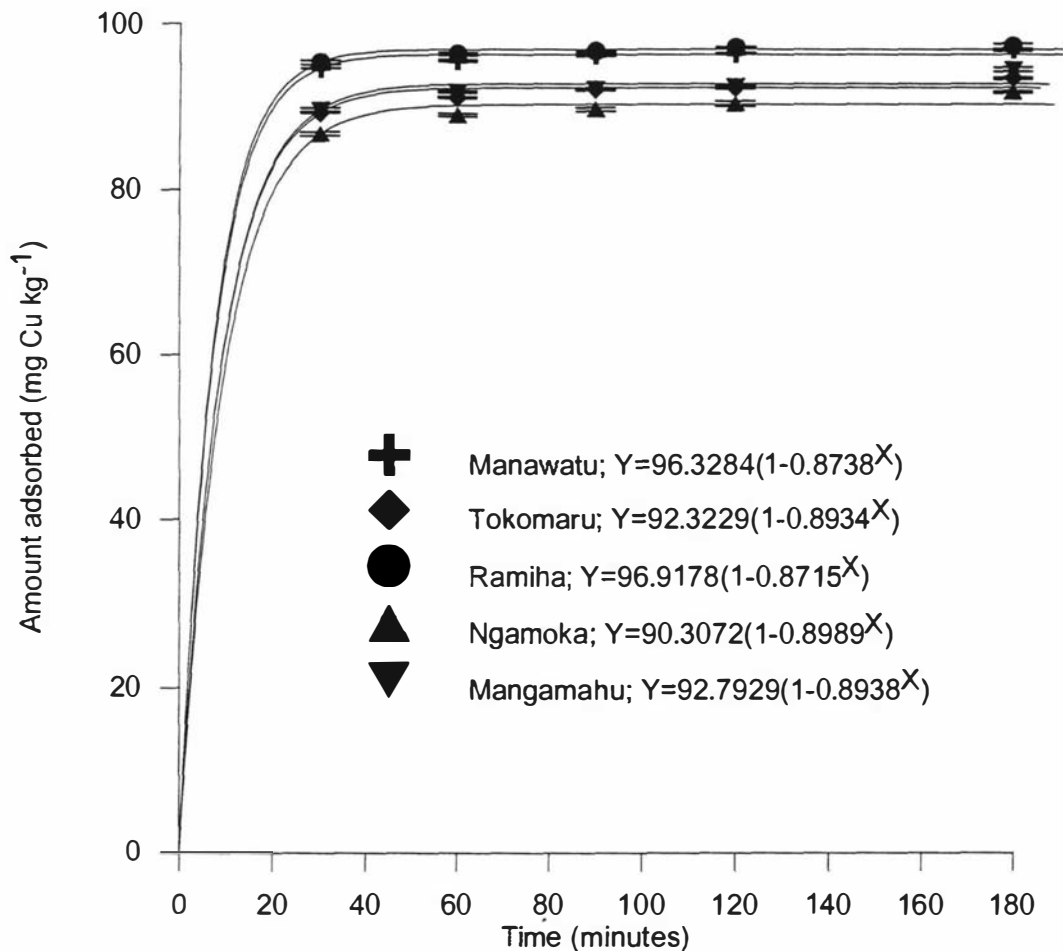


Figure 3.1 Time dependent Cu adsorption isotherms. The data were fitted to Mitscherlich growth function, $Y = A(1 - B^X)$, where Y = amount sorbed, X = time, A and B are constants.

3.3.3.2 Adsorption isotherms for the original soils

Copper adsorption isotherms for the original soils are shown in Figure 3.2. The solution Cu concentrations used in determining the isotherms were much higher than would exist in the soil solution under normal field conditions. These high concentrations were used mainly because the measurement of Cu at low concentration was difficult, especially in pasture soils with high organic matter. In some recently developed orchard soils the Cu concentration in the soil solution are found to be very high.

The data were fitted to the Freundlich equation:

$$Y = KC^N \quad (\text{Eq. 3.1})$$

where, Y is the amount of Cu sorbed (mg Cu kg^{-1} soil), C is the concentration of Cu (mg L^{-1}), and K and N are constants. K is termed the Freundlich unit-capacity coefficient, and N is a joint measure of both the relative magnitude and diversity of energies associated with a particular sorption process. Both K and N are used to characterise Cu sorption by soils.

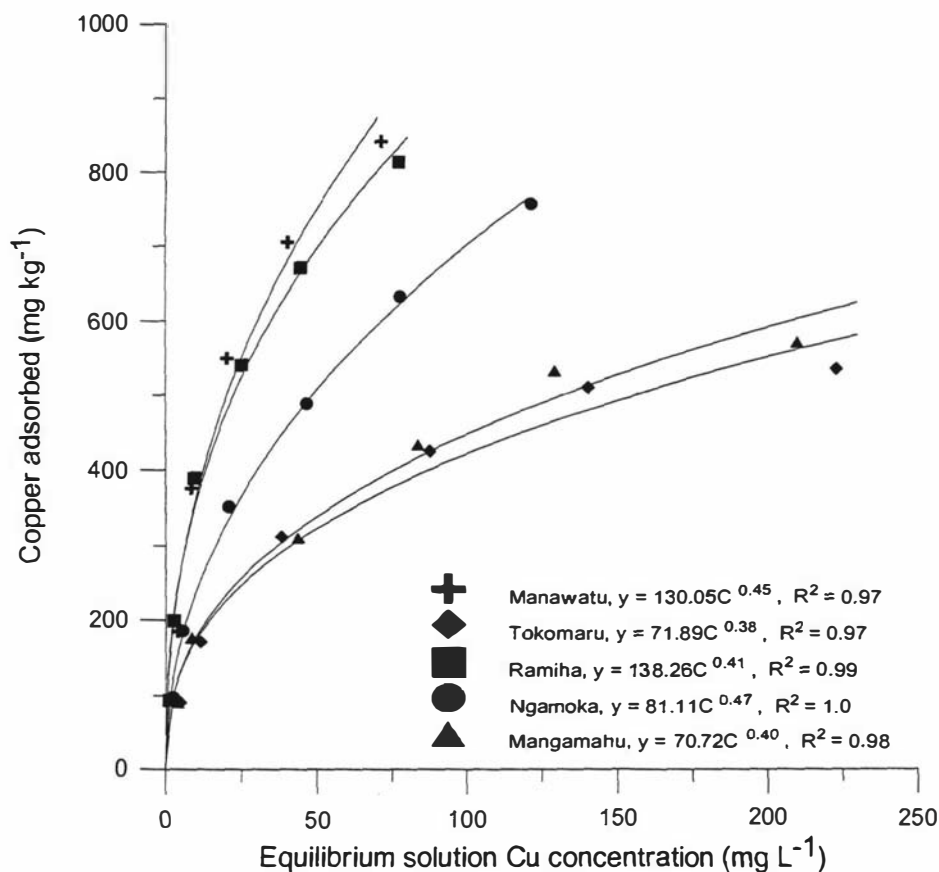


Figure 3.2 Copper adsorption isotherms for the different soils.

3.3.3.3 *Copper sorption affected by soil properties*

The sorption of Cu followed: Ramiha > Manawatu > Ngamoka > Tokomaru soil > Mangamahu. The Ngamoka and Ramiha soils contained higher amounts of organic matter and the Manawatu soil contained the highest percentage of silt. The difference in sorption between the soils is attributed to the difference in the chemical characteristics of the original soils. Higher pH values, organic matter, silt and clay content of the Ramiha and Manawatu soils contributed to the higher sorption capacity of these soils. The Manawatu soil had the highest pH value of all the soils and sorbed the higher amount of Cu. Elzinga *et al.* (1999) reviewed the batch sorption data for Cu from the literature with a wide variety of soils, experimental conditions, and metal concentrations ranging over five orders of magnitude. They used multiple linear regressions and reported that the inorganic and organic complex formation increases with organic matter, soil pH, and CEC. In this study it was observed that Cu sorption as measured by the sorption constant K, correlated with soil properties, such as silt content ($r=0.60$), organic carbon ($r=0.25$) and soil pH ($r=0.53$). Soil textures, particularly the clay and silt fractions, have often been found to be positively correlated with the Cu sorption capacities of soils (Dhillon *et al.*, 1981).

A correlation coefficient of >0.73 was observed between adsorption of Cu and soil pH for the pH dependent Cu sorption study. Welp and Brümmer (1999) conducted a correlation analysis and observed that pH was the main factor controlling the partitioning of the metals between the solid and the liquid phase for 8 of 10 metals. They also reported that sorption capacity decreases with decreasing soil pH. The Tokomaru soil has a lower K value, indicating less affinity for Cu sorption than the other soils. This may be due to the very low CEC value of this soil. In this study, insignificant correlation between adsorption of Cu and CEC of the soil was observed. The influence of CEC on Cu sorption has also been established by others (Kuo and Baker, 1980; Zachara *et al.*, 1992; Elzinga *et al.*, 1999; Wu *et al.*, 1999). The very low sorption capacity shown by the Tokomaru soil may be attributed to a low net surface negative charge density. Copper sorption increased as the organic carbon content of the soils increased, except for the Manawatu soil, which had the highest pH. Organic carbon has been reported to increase Cu sorption, often so strongly that Cu deficiency is common in organic soils (Harter, 1991; Stevenson, 1991). This retention of Cu by

organic matter appears to be the predominant mechanism involved, confirming the results described by Ritchie and Jarvis (1986). The effect of pH and organic matter on Cu sorption can be explained by the changes in charge characteristics on soil surfaces (Elliott *et al.*, 1986).

3.3.3.4 *Soil components and Cu sorption*

To investigate the sorption of Cu by various soil components, the extraction scheme described earlier (Section 3.2.6) was used to remove the various soil components. Copper sorption was measured for the whole soil (Step 1). The whole soil was extracted with NaOCl to remove organic matter and the Cu sorption for these sub samples was measured (Step 2). The remaining soil was extracted to remove the oxide components, and the Cu sorption for these subsamples, after the removal of organic matter and oxides was measured (Step 3).

The data for Cu sorption isotherms for the whole soil and the sub samples after the removal of organic matter and iron and aluminium oxides were fitted to the Freundlich equation. From this equations the sorption at different equilibrium concentrations were estimated for the soil samples. The sorption values for the organic matter component are estimated by subtracting the Cu sorption values of step 2 from those of step 1. Similarly the sorption values for the oxide component are estimated by subtracting the Cu sorption values of step 3 from those of step 2. The sorption values for step 3 give the sorption for the residue component.

Copper sorption isotherms for the soil components of the different soils are presented in Figures 3.3-3.7. The K value calculated from the Freundlich equation for the different components are presented in Table 3.4, and show that the organic matter alone is responsible for most of the retention of Cu in all the soils. Oxide components have a significant contribution for retention of Cu only in the Ramiha, Ngamoka and Mangamahu soils. Copper is adsorbed by various soil constituents, including organic matter and iron oxide (Sims, 1986; Lindsay, 1991). Analysis of the relative importance of mineral and organic components of multiphase soil systems suggests that organic matter has an intrinsically stronger affinity for Co, Cu and Cd than the common silicate or oxide minerals (McLaren *et al.*, 1983b; Zachara *et al.*, 1992).

Table 3.4 Freundlich equation describing the adsorption of Cu in different soil components

Name of soils	Freundlich equation for Cu adsorption		
	Whole soil	Organic	Oxide
Manawatu	$Y = 130.05 C^{0.45}; R^2 = 0.97$	$Y = 79.35 C^{0.45}; R^2 = 0.97$	$Y = 1.23 C^{0.75}; R^2 = 0.99$
Tokomaru	$Y = 71.89 C^{0.38}; R^2 = 0.97$	$Y = 23.51 C^{0.47}; R^2 = 0.91$	$Y = 0.43 C^{0.92}; R^2 = 0.99$
Ramiha	$Y = 138.26 C^{0.41}; R^2 = 0.99$	$Y = 120.32 C^{0.30}; R^2 = 0.95$	$Y = 5.58 C^{0.75}; R^2 = 0.99$
Ngamoka	$Y = 81.11 C^{0.47}; R^2 = 0.99$	$Y = 68.07 C^{0.25}; R^2 = 0.94$	$Y = 5.84 C^{0.72}; R^2 = 0.99$
Mangamahu	$Y = 70.72 C^{0.40}; R^2 = 0.98$	$Y = 22.87 C^{0.43}; R^2 = 0.96$	$Y = 5.63 C^{0.61}; R^2 = 0.95$

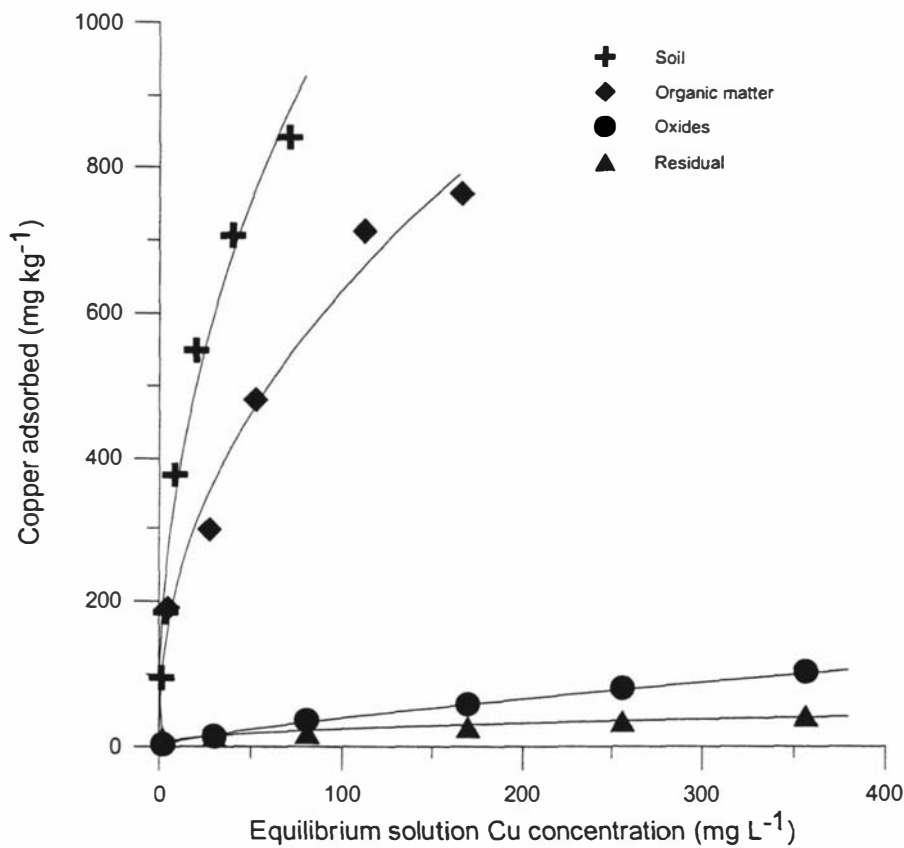


Figure 3.3 Copper adsorption isotherm for different soil components of the Manawatu soil.

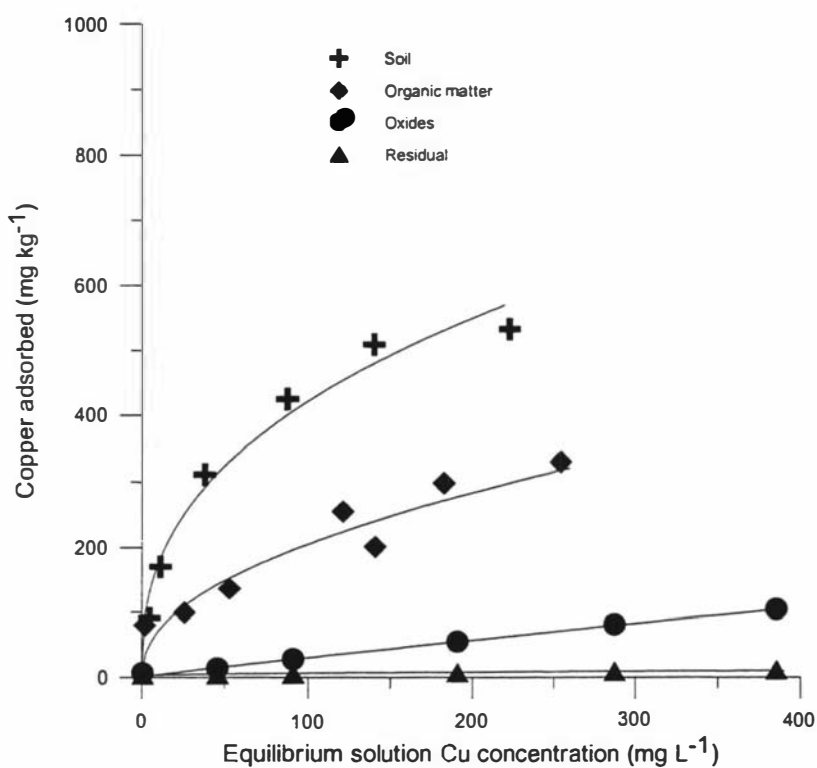


Figure 3.4 Copper adsorption isotherm for different soil components of the Tokomaru soil.

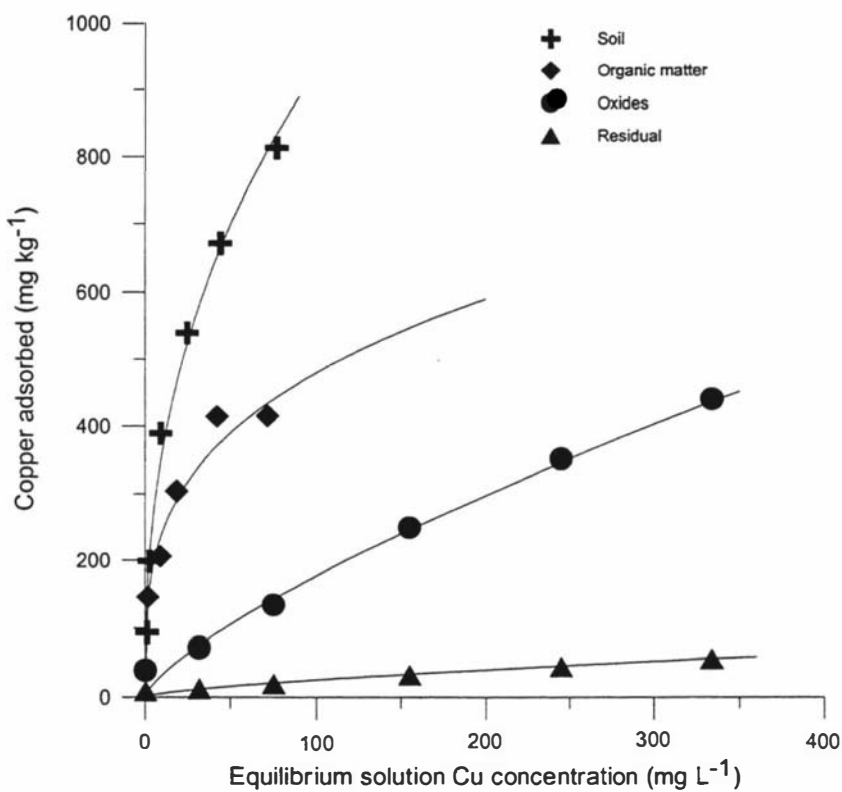


Figure 3.5 Copper adsorption isotherm for different soil components of the Ramiha soil.

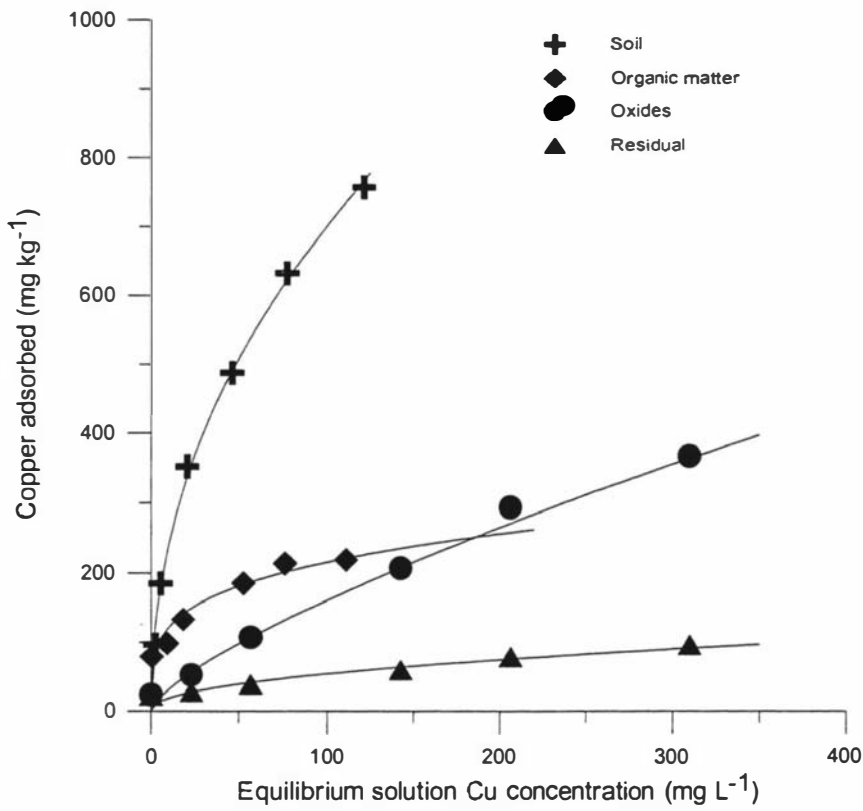


Figure 3.6 Copper adsorption isotherm for different soil components of the Ngamoka soil.

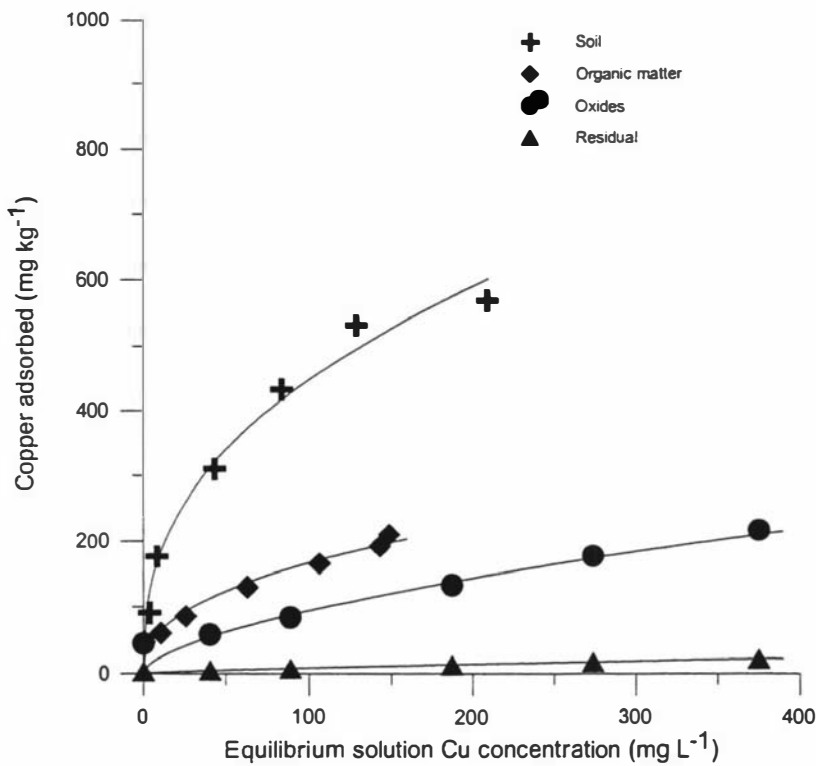


Figure 3.7 Copper adsorption isotherm for different soil components of the Mangmahu soil.

The effect of organic matter on Cu adsorption increased with increasing solution Cu concentration. With increasing solution Cu concentration the relative importance of organic matter on Cu adsorption decreases, but that of oxides increases (Table 3.5). In all the soils, organic matter was the principal component of Cu retention. Wu *et al.* (1999) reported that Cu was preferentially sorbed on to organic matter associated with the coarse clay fraction. In their study after the removal of organic matter, the fine clay exhibited higher Cu retention than did the coarse and medium clays. Narwal and Singh (1995) studied adsorption on four soils and reported that Cu adsorption followed: alum shale > peat soil > organic soil > sandy loam soil. In the alum shale soil almost all of the added Cu was retained and this was the case for the peat soil and the organic soil, but only 49% of the added Cu was adsorbed in the sandy loam soil.

Copper retention by the Ramiha, Ngamoka and Mangamahu soils is also dominated by both the organic matter and oxide components. These three soils contain relatively higher amounts of Al and Fe, which may contribute to sorption by the oxide component, in comparison with the Manawatu and Tokomaru soils. The organic fractions seem to be a source of sites for specific Cu sorption (McLaren and Crawford, 1973a), perhaps because the Cu ion is unique in its ability to form inner-sphere complexes at a wide range of pH levels (McBride, 1981). Conversely, organic complex formation could lead to increased mobility in the soil, since Cu is known to form stable soluble complexes with fulvic acid (Kabata-Pendias and Pendias, 1992). Sorption onto organic matter, clay minerals and metal oxides in soils is the main mechanism of heavy metal removal from the soil solution (McBride, 1989; Scheidegger and Sparks, 1996). Reports are readily available on Cu retention by hydrous iron oxide (Okazaki *et al.*, 1986), hydrous manganese oxides (Kabata-Pendias, 1980) and hydrous aluminium oxides (Shuman, 1977; Barrow, 1986). Retention of Cu ions by oxide surfaces is inversely dependent on the degree of crystallinity (Barrow *et al.*, 1981; Okazaki *et al.*, 1986). Since the oxides have variable charge, the extent to which retention occurs is dependent on the pH of the solution (Barrow, 1987; Bolan *et al.*, 1999). Cavallaro and McBride (1984) found that treatment of the clays for the removal of organics tended to either enhance or have little effect on sorption and fixation of Zn and Cu. They suggested that oxide components of soil clay are more significant than the organic component in metal sorption and fixation. Wu *et al.* (1999) suggested that Cu sorption may involve the formation of Cu-O-Al bonds for sites on the lateral edges at 2:1 phyllosilicates. This chemisorption process is

likely to be controlled by the nature and quantity of surface hydroxyl groups. However, the results of their study indicate that chemisorption on variable charge sites cannot account for all of the observed immobilisation of Cu on the inorganic soil clay component. To date, however little is known about the relative contribution of the soil clay component to Cu sorption.

Table 3.5 Percent contribution of the organic matter and oxide components to Cu adsorption at two initial concentration levels.

Soils	At 10 mg L ⁻¹ initial solution concentration		At 50 mg L ⁻¹ initial solution concentration	
	Organic	Oxides	Organic	Oxides
Manawatu	88	6	86	8
Tokomaru	83	12	74	22
Ramiha	71	24	55	40
Ngamoka	55	30	48	38
Mangamahu	46	42	44	48

3.3.3.5 pH dependent adsorption isotherms for different soils

Copper sorption isotherms measured at different pH levels for the soils are presented in Figure 3.8-11. In all soils, Cu sorption increased with increasing pH. Freundlich constant (K) values increased with increasing pH in all soils (Table 3.6). Except for the Ramiha soil, large variations in K values were obtained within the pH range. There is no doubt that changes in soil pH by several units will also change the sorption capacity (Figures 3.8-11) of the soil for trace metals as has been shown in many studies for minerals and soils (Boekhold *et al.*, 1991; Duker *et al.*, 1995; Yuan and Lavkulich, 1997). The greatest increase in sorption occurred at pH 8.0 in all soils, perhaps because for most components with variable charge, such as organic molecules and oxide minerals, charge reversal from positive to neutral to negative occurs at this pH; and the retention of Cu, mostly as Cu²⁺, is much stronger by negatively charged surfaces than positively charged ones (Hue *et al.*, 1997). A practical implication of this effect is that Cu concentrations in the soil solution, can be regulated by changing soil pH; raising soil pH to 7.0 or slightly above increases Cu sorption and thus reduces its potential toxicity to crops. In higher pH regions, Cu²⁺ may precipitate as Cu(OH)₂. Harter (1983) reported that the amount of Cu(OH)₂ at pH 6 accounted for only 2% of Cu²⁺ but at pH 8 for 92%

of Cu^{2+} . In this study, it was difficult to distinguish between Cu^{2+} adsorbed and Cu^{2+} precipitated after soil suspensions reached the equilibrium state. The amount of Cu sorbed tends to be low if the indigenous Cu is high and organic C is low. The pH dependent characteristics of metal sorption by soils and soil materials has been reported by Barrow (1986), Naidu *et al.* (1994), and Pardo (1997).

Table 3.6 Freundlich equation describing the adsorption of Cu at various pH levels in different soils.

Soil pH	Freundlich equation for Cu adsorption			
	Manawatu	Tokomaru	Ramiha	Ngamoka
5.0	$Y = 36.00 C^{0.65}$ $R^2 = 0.99$	$Y = 5.29 C^{0.94}$ $R^2 = 0.97$	$Y = 69.17 C^{0.49}$ $R^2 = 0.94$	$Y = 10.27 C^{0.78}$ $R^2 = 0.93$
6.0	$Y = 80.04 C^{0.55}$ $R^2 = 0.98$	$Y = 8.94 C^{0.93}$ $R^2 = 0.95$	$Y = 81.50 C^{0.56}$ $R^2 = 0.99$	$Y = 49.31 C^{0.56}$ $R^2 = 0.95$
7.0	$Y = 94.64 C^{0.73}$ $R^2 = 0.97$	$Y = 66.49 C^{0.57}$ $R^2 = 0.99$	$Y = 81.77 C^{0.62}$ $R^2 = 0.96$	$Y = 59.78 C^{0.58}$ $R^2 = 0.92$
8.0	$Y = 113.13 C^{0.84}$ $R^2 = 0.99$	$Y = 95.29 C^{0.56}$ $R^2 = 0.84$	$Y = 89.58 C^{0.63}$ $R^2 = 0.87$	$Y = 127.84 C^{0.43}$ $R^2 = 0.87$

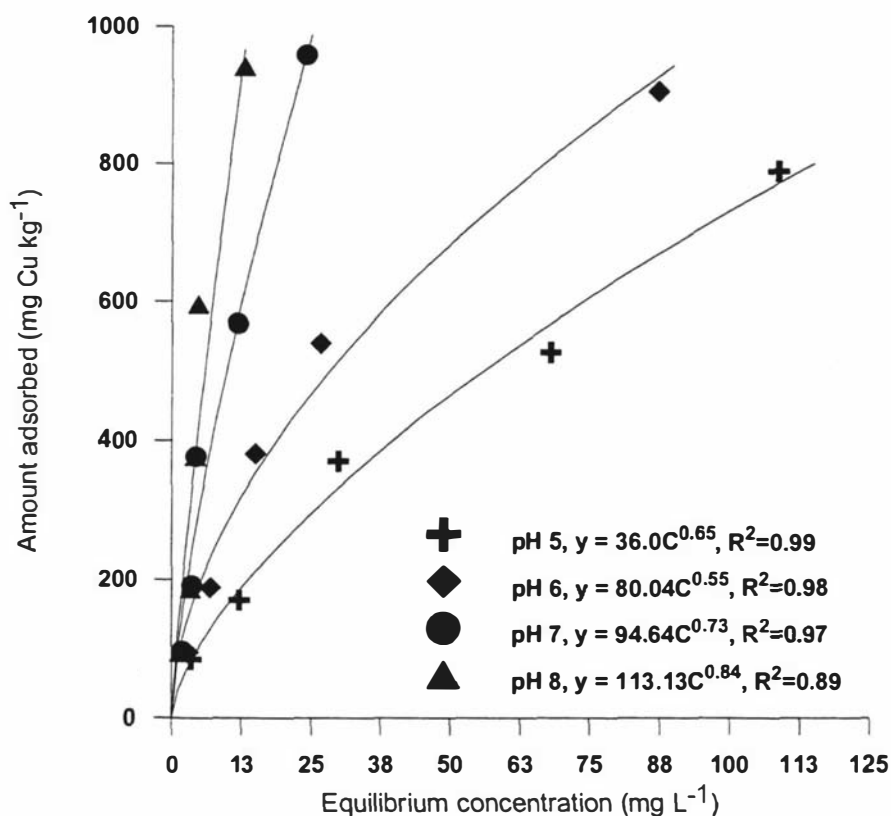


Figure 3.8 Effect of soil pH level on Cu sorption of the Manawatu soil.

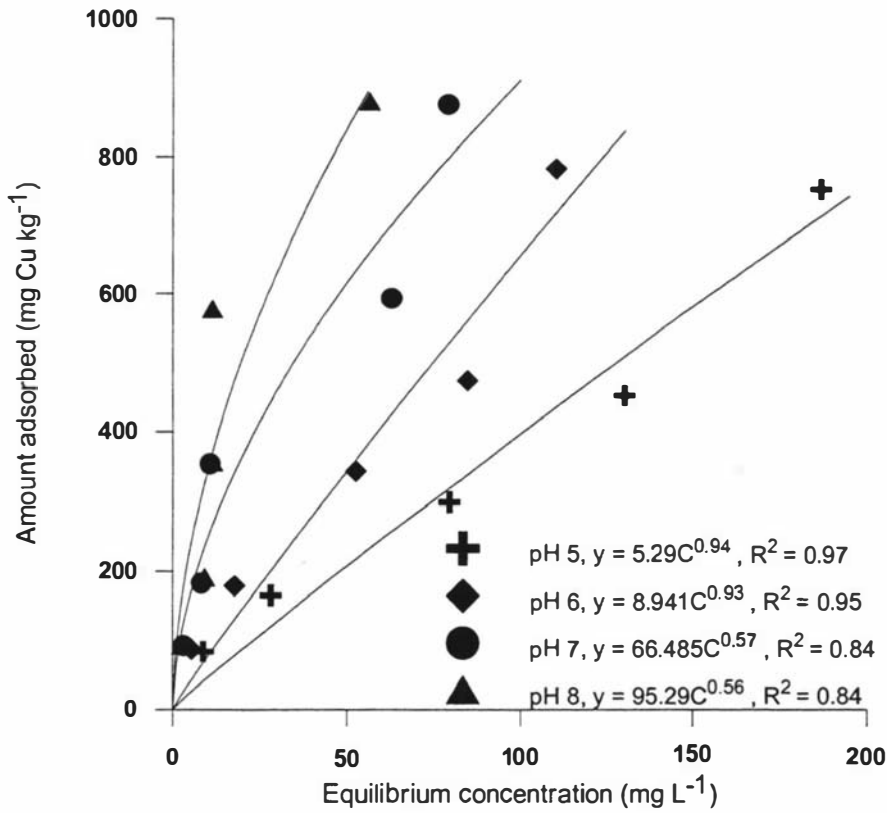


Figure 3.9 Effect of soil pH level on Cu sorption of the Tokomaru soil.

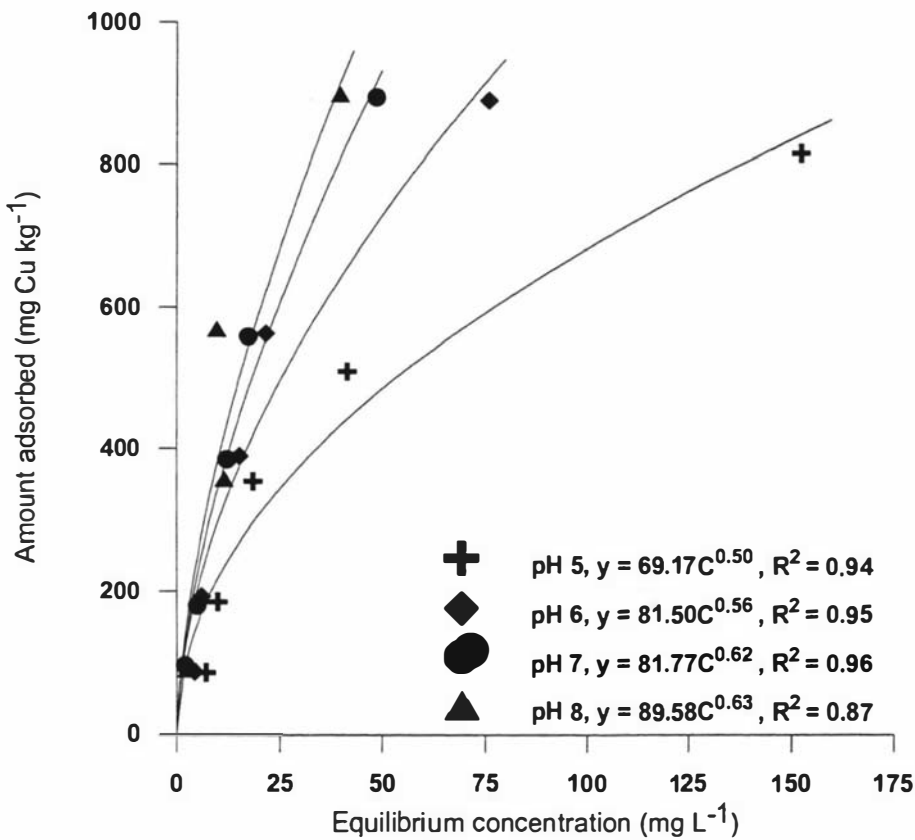


Figure 3.10 Effect of soil pH level on Cu sorption of the Ramiha soil.

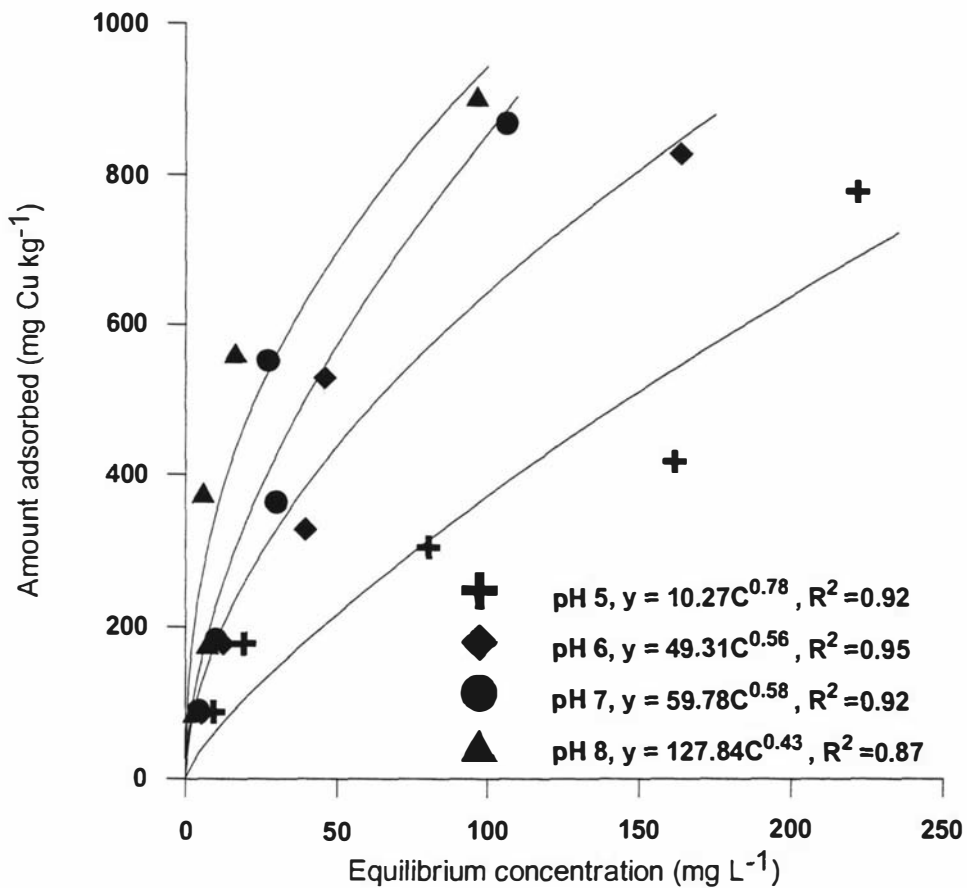


Figure 3.11 Effect of soil pH level on Cu sorption of the Ngamoka soil.

The solution Cu concentration decreased with increasing soil pH (Figure 3.12). The Tokomaru soil showed the highest solution Cu, which may be due to the low organic matter content and CEC of that soil. Cu is adsorbed by soil colloids predominantly as the Cu^{2+} ion, it is clear that the presence of organic complexes substantially decrease solution Cu levels below the levels that would be maintained by the equilibrium between the solution and adsorbed Cu^{2+} ions alone. McLaren *et al.* (1990) reported that the proportion of solution Cu present as Cu^{2+} decreased significantly with an increase in soil pH.

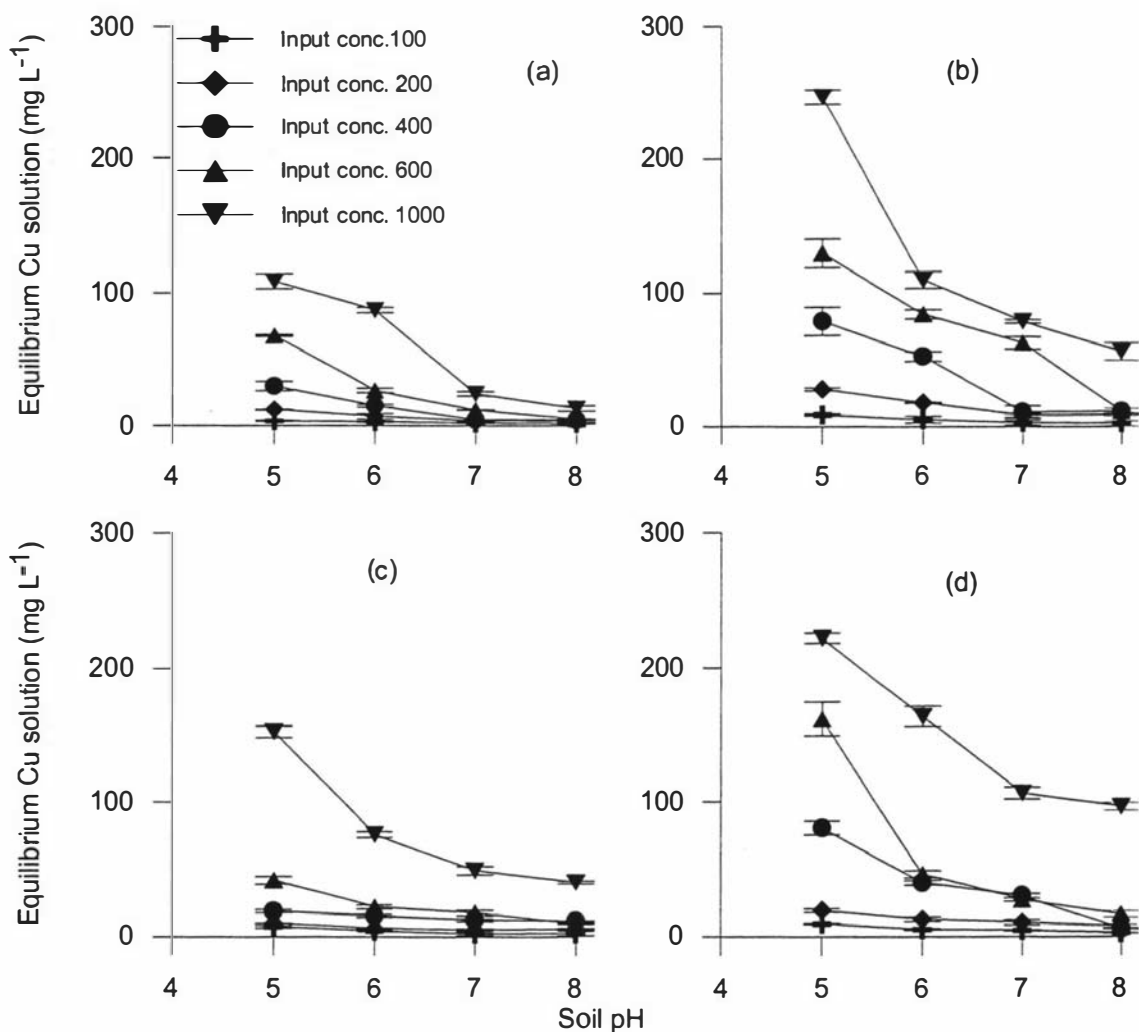


Figure 3.12 Effect of pH levels on equilibrium solution Cu concentrations at various input concentrations (mg L^{-1}) (a) Manawatu, (b) Tokomaru, (c) Ramiha and (d) Ngamoka soils. Data are means \pm SE, $n=2$.

Adsorption constant (K) strongly correlated with soil pH (Figure 3.13) and the r values were 0.93, 0.91, 0.89 and 0.92 for the Manawatu, Tokomaru, Ramiha and Ngamoka soils, respectively.

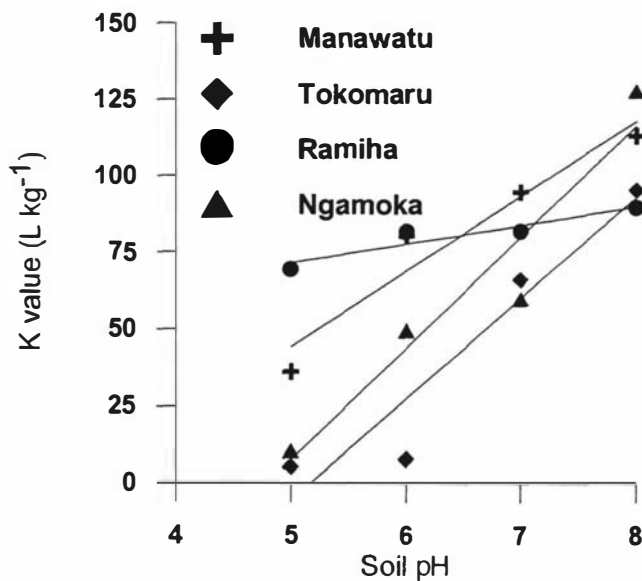


Figure 3.13 Freundlich constant (K) for Cu adsorption by different soils at various pH values.

3.3.4 *Distribution coefficients*

3.3.4.1 *Distribution coefficients for the original soils*

Distribution coefficients (K_d) were calculated from the amount of Cu adsorbed per unit Cu concentration in soil solution at various equilibrium concentrations and it ranged from 12 to 109, 2.5 to 27, 10 to 121, 6 to 49 and 2.8 to 31 $L\ kg^{-1}$ for the Manawatu, Tokomaru, Ramiha, Ngamoka and Mangamahu soils, respectively. The Freundlich isotherms for Cu sorption showed that the shape of the curve was dependent on the concentration of the equilibrium solution and the soil types. The K_d values decreased with increasing solution concentration (Figure 3.14). Zehetner and Wenzel (2000) observed that distribution coefficients (K_d) ranged from 0.8 to 1544 $L\ kg^{-1}$ for Cu and that K_d values decreased with increasing metal concentrations applied. Adsorption constant (K) values correlated well with soil pH ($r=0.53$) and organic carbon ($r=0.25$) for the original soils.

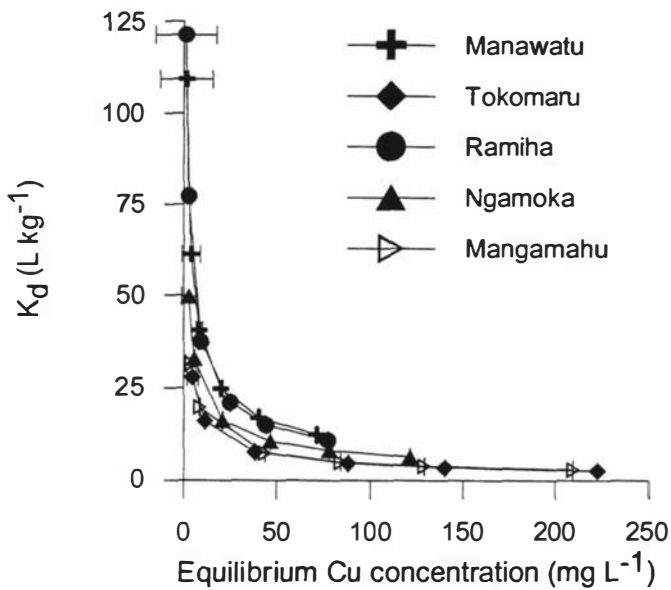


Figure 3.14 Distribution coefficient (K_d) for Cu adsorption by different soils at various equilibrium concentration levels. Data are means \pm SE, $n=2$.

3.3.4.2 *Distribution coefficients from the pH dependent experiment*

Distribution coefficient (K_d) values increased with increasing pH (Figure 3.15), indicating stronger sorption of Cu at higher pH levels. This is consistent with the results of Basta and Tabatabai (1992) and may be attributed to the affinity of Cu for highly selective sites (specific sorption) at low concentrations and to sorption on less selective sites (nonspecific sorption) at high concentrations. Buchter *et al.* (1989) found that the distribution coefficient (K_d) for Cu retention by 11 soils increased with cation exchange capacity (CEC) and pH value. A large K_d value represents high soil affinity for Cu and a low mobility; whereas a low K_d value indicates a high mobility and low retention in soils.

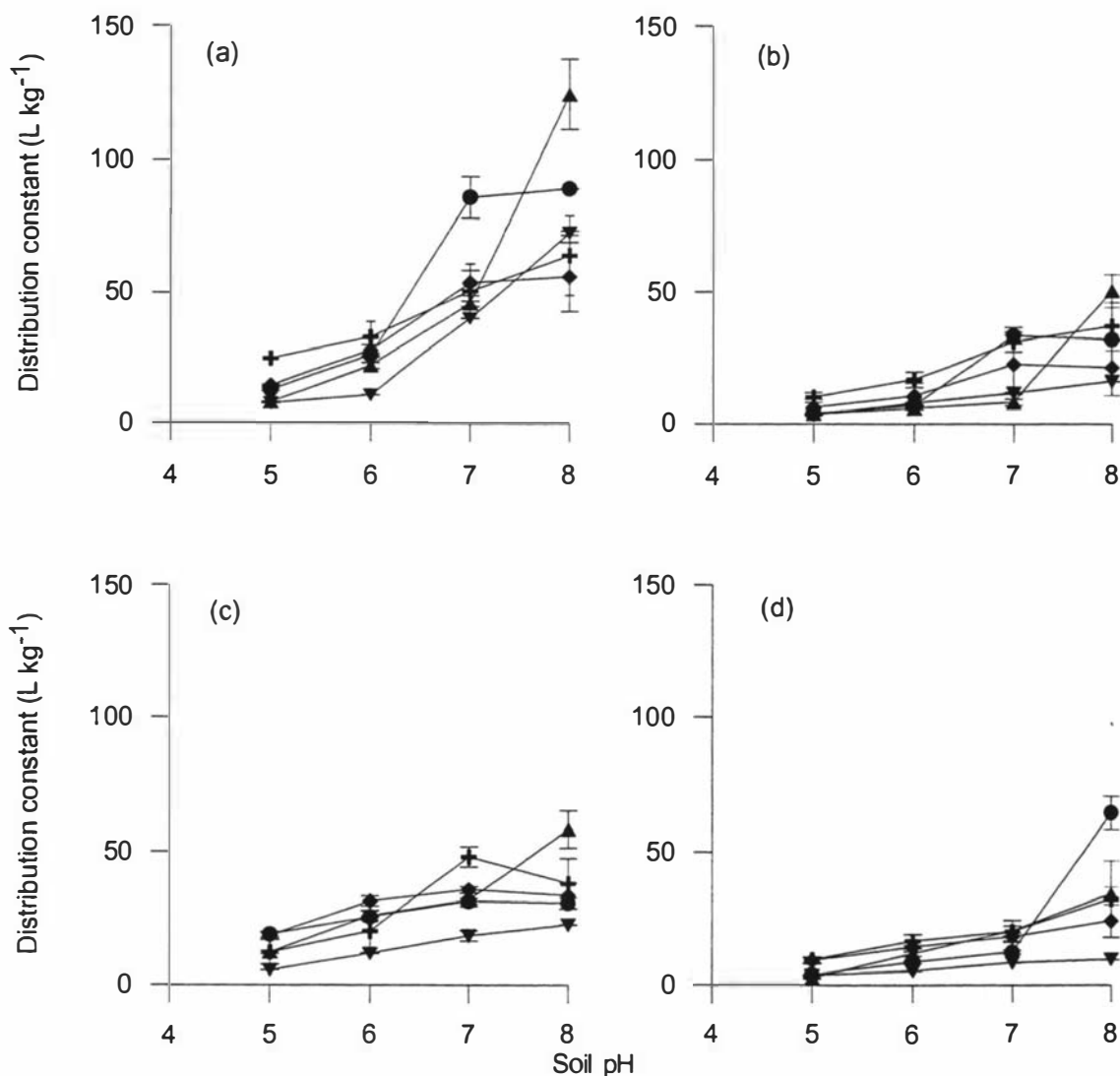


Figure 3.15 Distribution coefficient (K_d) of Cu sorption at different input concentrations by (a) Manawatu, (b) Tokomaru, (c) Ramiha and (d) Ngamoka soils at different pH levels. [Input concentrations (mg Cu L^{-1}): + 100; \blacklozenge 200; \bullet 400; \blacktriangle 600; \blacktriangledown 1000]. Data are means \pm SE, $n=2$.

3.3.5 Desorption

3.3.5.1 Desorption of native Cu

The reversibility of the sorption reaction, was determined in terms of desorption, induced in response to a reduction of the Cu solution concentration and showed clear differences between soils (Figure 3.16). The cumulative amounts of native soil Cu desorbed from the two soils during 10 successive 2 hrs desorption periods are presented in Figure 3.16. As with the forward sorption reaction, quantitative differences observed

in the desorbability of the sorbed Cu between the soils were a result of differences in the physico-chemical characteristics of the soil matrix. The amounts and patterns of desorption differed between the soils. The cumulative amounts of Cu desorbed after 10 equilibrations with $\text{Ca}(\text{NO}_3)_2$ were compared with the amounts of Cu extracted from the same soils with different soil test extractants (M1, M3, TEA-DTPA, 0.1M HCl, and 0.04M EDTA). The reagents are used widely to estimate the plant available Cu in soils, and may be considered to give an estimate of total labile soil Cu. The cumulative amount of Cu desorbed is expressed as a percentage of different soil test reagent extractable Cu for both soils (Table 3.7). The percentage of extractable Cu desorbed in $\text{Ca}(\text{NO}_3)_2$ solution varied between the extractants and the soils. It was observed that 0.1M HCl (>17% Cu desorbed from both soils) and 0.04M EDTA (4.7% and 18% Cu desorbed from the Manawatu and Ngamoka soils) extractable Cu produced higher amounts of desorbable Cu than other soil test extractants after 10 desorption equilibration in both soils. These two extractants probably remove different proportions of ions from the labile pool. For instance, 0.04M EDTA may preferentially remove some ions from organic matter, whereas 0.1M HCl may remove the same ions preferentially from mineral surfaces. Both the 0.1M HCl (Haynes, 1997) and 0.04M EDTA extractants (Sims and Johnson, 1991) have been shown to be successful in predicting soil Cu availability to plants. The desorption data would however suggest that these reagents overestimate the amounts of readily plant available Cu in soils. DTPA and EDTA reagents extract Cu predominantly from the organic fraction of the soil, much of which is probably not directly available to plants. Desorption of Cu into a dilute $\text{Ca}(\text{NO}_3)_2$ solution will depend not only the total amount of labile Cu in the soil but also other factors such as the relative proportion of soil components responsible for the retention of Cu in the soil, and soil pH.

The Manawatu and Ngamoka soils desorbed 0.86 and 0.33 mg kg^{-1} cumulative native Cu, respectively during 10 successive 2 hrs desorption periods (Table 3.7). These amounts are much less than the total native Cu in these soils. In soil systems, it is difficult to attribute the loss of exchangeability of Cu to any single mechanism. The sorption of Cu on oxides is an inner-sphere complex that does not obey the reversible mass action relationship predicted by simple cation exchange (McBride, 1989). In mineral soil with regards to the organic matter, it has been stated that in soils the retaining ability for Cu is predominantly controlled through CEC, rather than the

chelating ability (Cavallaro and McBride, 1984). The Ngamoka soil contains a higher CEC and more organic matter than the Manawatu soil, and this should represent an important contribution to the total number of Cu sorption sites. Thus, the sorption of Cu onto the organic matter may explain the more plant available form of Cu, that complexed with this soil component.

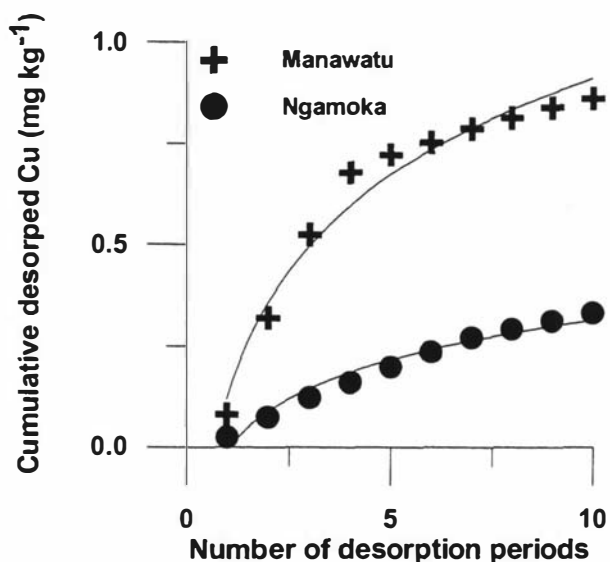


Figure 3.16 Desorption of native Cu from two soils (Manawatu and Ngamoka).

Table 3.7 Cumulative desorbed native soil Cu and different extractable Cu concentrations.

Soil	Desorbed Cu mg kg ⁻¹	Soil test extractable Cu mg kg ⁻¹					Desorbed as a percentage of soil test extractable Cu (%)				
		M1	M3	TEA- DTPA	0.1M HCl	0.04M EDTA	M1	M3	TEA- DTPA	0.1M HCl	0.04M EDTA
Manawatu	0.86	17.4	19.3	16.7	4.8	18.4	4.9	4.4	5.2	17.9	4.7
Ngamoka	0.33	3.6	9.6	6.2	1.8	1.8	9.1	3.5	5.4	18.2	18.3

In both soils, a significant portion of the sorbed Cu was apparently strongly bonded to the soil. Nevertheless, whether the surface reaction is genuinely irreversible or simply very slow in the backward direction is unclear (Sparks, 1989). Pardo (2000) carried out a laboratory experiment to evaluate the sorption and desorption by three soils with contrasting characteristics. The soil with a high native pH of 6.4 and CEC sorbed more

Cu than the other two soils. He observed that the differences in the reversibility of the sorption reaction, in response to a reduction of the solution Cu concentration resulted from the differences in physico-chemical characteristics of the soil matrix. In the present study it was observed that Cu desorption values in the Ngamoka soil were low compared to the Manawatu soil, indicating that Cu is strongly bound to organic matter and oxides in the former soil. The quantitative differences observed in the extractability of sorbed Cu between the soils indicated that soil properties (organic matter, oxides and soil pH), which enhanced Cu sorption, contributed at the same time to the slowing down of the backward reaction. Temminghoff *et al.* (1994) studied the effect of pH on Cu desorption from a sandy soil and on complex formation by dissolved organic fractions. They observed that as the soil pH decreased, the amount of Cu desorbed from the soil increased. The amount of Cu bound to solid organic carbon was almost equal to Cu bound by dissolved organic carbon. Cavallaro and McBride (1984) have also shown that desorption of Cu from soil clays decreases with an increase in soil pH. The fact that the desorption of Cu from the soil labile pool is influenced by pH has an important implication for the uptake of soil Cu by plants. Soil organic matter and the oxide components play an important role in the retention of Cu. Desorption data will not necessarily provide an effective measure of soil Cu availability. Many other factors, such as physico-chemical properties of the soil (soil pH, organic matter, oxides and clay), the plant itself and its influence on the root environment also affect Cu uptake.

3.3.5.2 *Desorption of added Cu*

Two contrasting soils were used for this study. The soil samples were incubated for 0, 8 and 28 days with 50 mg Cu kg⁻¹ soil. Subsequent successive desorptions from the 0 day incubated soil resulted in large increases in the amounts of Cu desorbed, compared with samples where only native Cu was present (Figure 3.17). The Manawatu soil desorbed more Cu than the Ngamoka soil after 2 hrs and 24 hrs desorption periods. It suggests that soil organic matter complexes Cu with time. The proportion of added Cu desorbed during 10 desorption periods were extremely low, ranging from 2.5% in 24 hrs to 6% in 2 hrs cumulative desorption periods (Figure 3.17). The increase in soil pH (Table 3.8) during desorption periods decrease the cumulative desorption. The low proportion of added Cu desorbed from the soils agrees with the results for native Cu in these two soils. The sorption study (Section 3.3.3.4) revealed that the soil organic matter and

oxides were the major components involved in Cu sorption in three soils (Ramiha, Ngamoka and Mangamahu). McLaren *et al.* (1983b) demonstrated that the amount of Cu desorbed from the soil component (humic acid and soil oxide) was very small. Hogg *et al.* (1993) provided evidence for the existence of slow reactions between the added Cu and the soil that reduce the ability of the Cu to desorb back into the soil solution. Narwal and Singh (1995) studied adsorption on four soils and reported that Cu adsorption among the soils followed: alum shale > peat soil > organic soil > sandy loam soil. Desorption of Cu by 0.005M CaCl₂ was <1% in the alum shale, peat soil and organic soil but in the sandy loam soil the maximum amount desorbed was 14%. This suggests that a near complete irreversibility of adsorbed Cu in all soils with the exception of sandy soils.

Wu *et al.* (1999) studied Cu desorption on various clay fractions and reported that 39% of adsorbed Cu was desorbed from the fine clay after five desorption cycles, while 27% and 25% of the adsorbed Cu was desorbed from the medium and coarse clays, respectively. They also observed organic matter associated with coarse clay had a strong Cu retention affinity. Copper retained on permanent charge sites of 2:1 phyllosilicates by electrostatic attraction should be readily desorbed by mass action. Therefore, the irreversible sorption of Cu after the removal of organic matter in fine and medium clay samples suggests that ion exchange is not a major mechanism for retention of Cu on smectite in the 0.01M CaCl₂, pH 6.0 system. Sorption of Cu by a range of soil components has been shown to be largely irreversible, or only slowly reversible (McLaren *et al.*, 1983b; McBride *et al.*, 1984). The irreversibility or reversibility behaviour of Cu sorption varies with different factors. McBride (1991) reported that activation energies for desorption may be much larger than those for sorption, and rates of sorption at ambient temperature are likely to be much faster than desorption rates. Thus the low proportions of added Cu desorbed in this and other studies may reflect, in part, non-equilibrium conditions caused by slow desorption rather than true irreversibility. Barrow (1985) has also mentioned that initial sorption reactions may be followed by slower reactions that would render a proportion of the sorbed Cu unavailable for immediate equilibrium with the soil solution. It is uncertain, however, whether such reactions are likely to be of importance for a relatively short desorption period of 24 hrs. The proportion of added Cu desorbed is higher in 2 hrs cumulative desorption periods than 24 hrs periods due to higher sorption at 24 hrs desorption

periods. Although such small proportions of the added Cu were desorbed during 10 desorption equilibrations, it should be noted that at the end of this period, Cu was still being desorbed. It might be expected that, with time, greater amounts of the added Cu could be desorbed back into solution and therefore be considered to remain available for plant uptake.

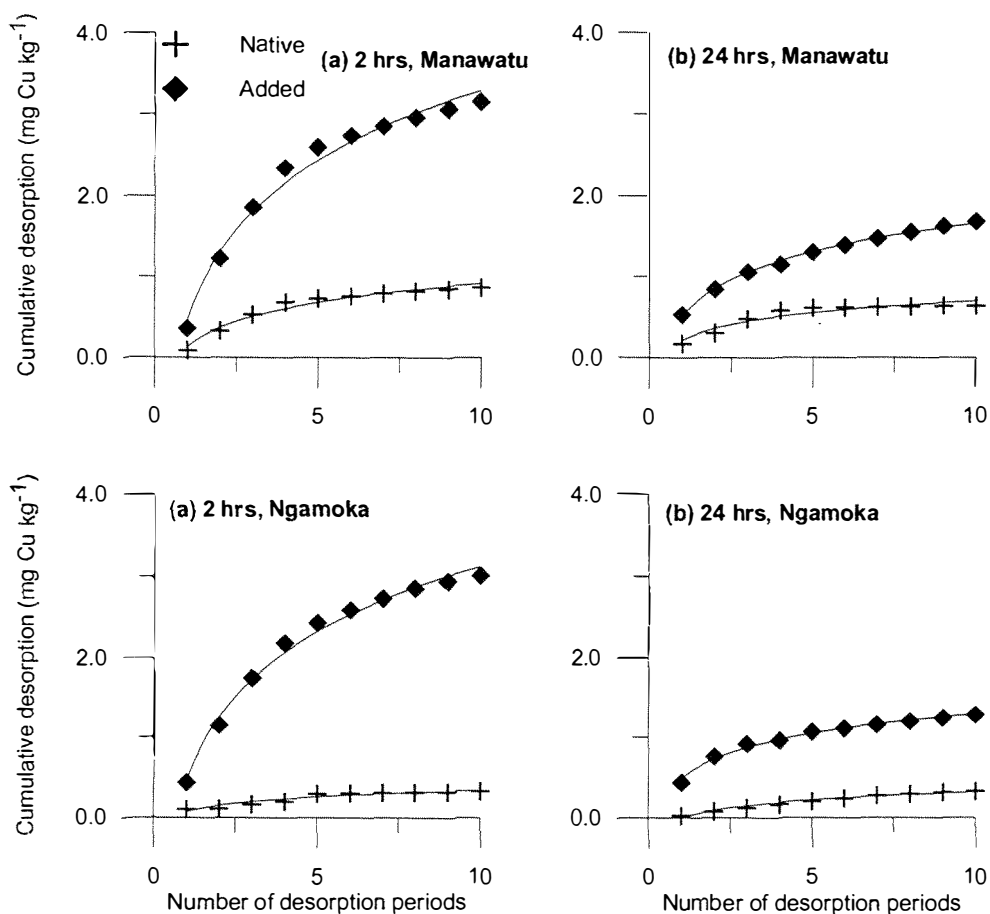


Figure 3.17 Desorption of native and added Cu at (a) 2 hrs and (b) 24 hrs desorption period in Manawatu and Ngamoka soil.

Table 3.8 Soil pH at various intervals of desorption.

Incubation periods	Manawatu soil		Ngamoka soil	
	Contact time of desorption		Contact time of desorption	
	2 hrs	24 hrs	2hrs	24 hrs
Control	4.92	5.01	4.31	4.36
0 day	4.61	4.75	4.35	4.39
8 day	4.60	4.79	4.36	4.39
28 day	4.58	4.90	4.34	4.51

3.3.5.3 *Effect of contact time on desorption of added Cu*

An experiment was carried out with the Manawatu and Ngamoka soils treated with 50 mg Cu kg⁻¹ soil and incubated for 0, 8 and 28 days. Desorption was carried out on the incubated soil after 2 hrs and 24 hrs initial contact periods. Desorption of Cu decreased with increasing contact times between the added Cu and the soil during the sorption period (Figure 3.18). The effect of the contact period was more pronounced in the Ngamoka soil, which contains higher levels of organic matter and more oxides. These results are in general agreement with the trends reported for Cu desorption from soils by Lehmann and Harter (1984). In both soils, the longer the incubation period the greater the amount of Cu that was irreversibly sorbed. The nature of the slow reactions that result in this decrease in desorption of added Cu remains open to conjecture. It would appear more likely that, with increased time of sorption, there is a slow redistribution of Cu ions to more strongly binding or less accessible sites, possibly involving diffusion into extremely small pores and inter particle spaces. Although the actual amounts of Cu involved in these slow reactions appear to be small compared with the total amounts of Cu sorbed, they do represent a considerable proportion of the Cu that can initially be desorbed from the soil. Therefore, the corresponding effects on plant availability of added Cu could also be significant.

The control soil samples (without Cu addition) were also incubated with Ca(NO₃)₂ for 0, 8 and 28 days before Cu desorption was measured. The results from the control samples are also shown in Figure 3.18. Somewhat unexpectedly the native soil Cu also showed a decrease in desorption with increasing time of initial equilibration. An increase in soil pH during the initial contact time might result in a decrease in desorption. It might also be thought that native soil Cu would be in equilibrium states and continuing reaction with soil components was unlikely. In common with other nutrients, Cu will be continually cycled in the soil through decaying organic materials and even through mineralisation, a pool of Cu ions will be maintained that has not had time to react irreversibly with the soil. It seems likely, however, that the nature of the equilibration process in Ca(NO₃)₂ at a wide solution/soil ratio creates conditions favourable for the slow reactions to take place. The rate at which such reactions occur in soils at normal soil moisture content is probably much slower than those observed in soil suspensions in the laboratory. Williams and McLaren (1982) have obtained evidence that slow

reactions between Cu and soil do also occur in field moist soils. They showed that during a period of 44 weeks, EDTA extractability of both native and added Cu declined in soils incubated under field moist conditions. Cox (1992), in field experiments with a range of soils, has also shown a decrease with time in the extractability of Cu applied as a fertiliser.

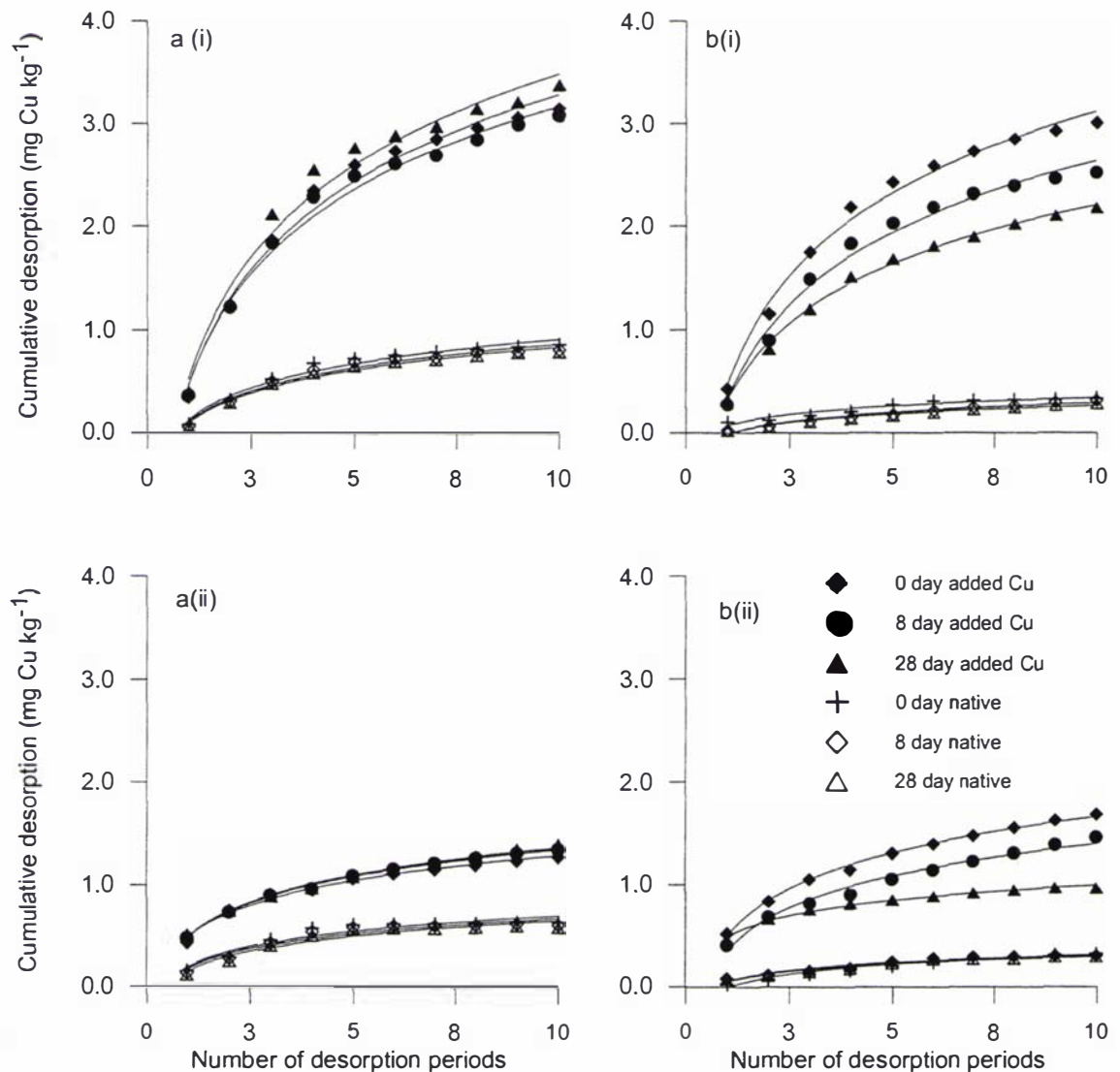


Figure 3.18 Cumulative desorption of native and added Cu from the (a) Manawatu soil and (b) Ngamoka soil incubated with added Cu for different periods. Desorption was carried out using two desorption periods [(i) 2 and (ii) 24 hours].

3.3.5.4 *pH dependent desorption for different soils*

Copper desorption in various soils (Manawatu, Tokomaru, Ramiha and Ngamoka) at two pH levels is illustrated in Figure 3.19. Only a small portion of Cu was desorbed,

even after 10 consecutive extractions at a 1:20 soil-to-solution ratio. At 30 mg Cu L⁻¹ addition, the desorption decreased from 35%, 29%, 18% and 10% at pH 5 to 13%, 7%, 3% and 5% at pH 8 for the Tokomaru, Ngamoka, Manawatu and Ramiha soils, respectively. Increasing sorption of Cu by soil as pH increased results in a decreased desorption (Figure 3.19). A similar result was reported by Hue *et al.* (1997) and they obtained relatively higher desorption (30%) at pH 5.0 than at pH 8.0 (10%). They also observed that more Cu was sorbed in soils with high organic carbon and low indigenous Cu. Atanassova and Okazaki (1997) examined the Cu adsorption and desorption under acid conditions by soil clay fractions in 0.01M Ca(NO₃)₂ and found a considerable amount of sorbed Cu could be solubilised by decreasing pH values to 4; 39% was desorbed in the Planosol clay and 45% was desorbed in the Gleyic Arcisol clay.

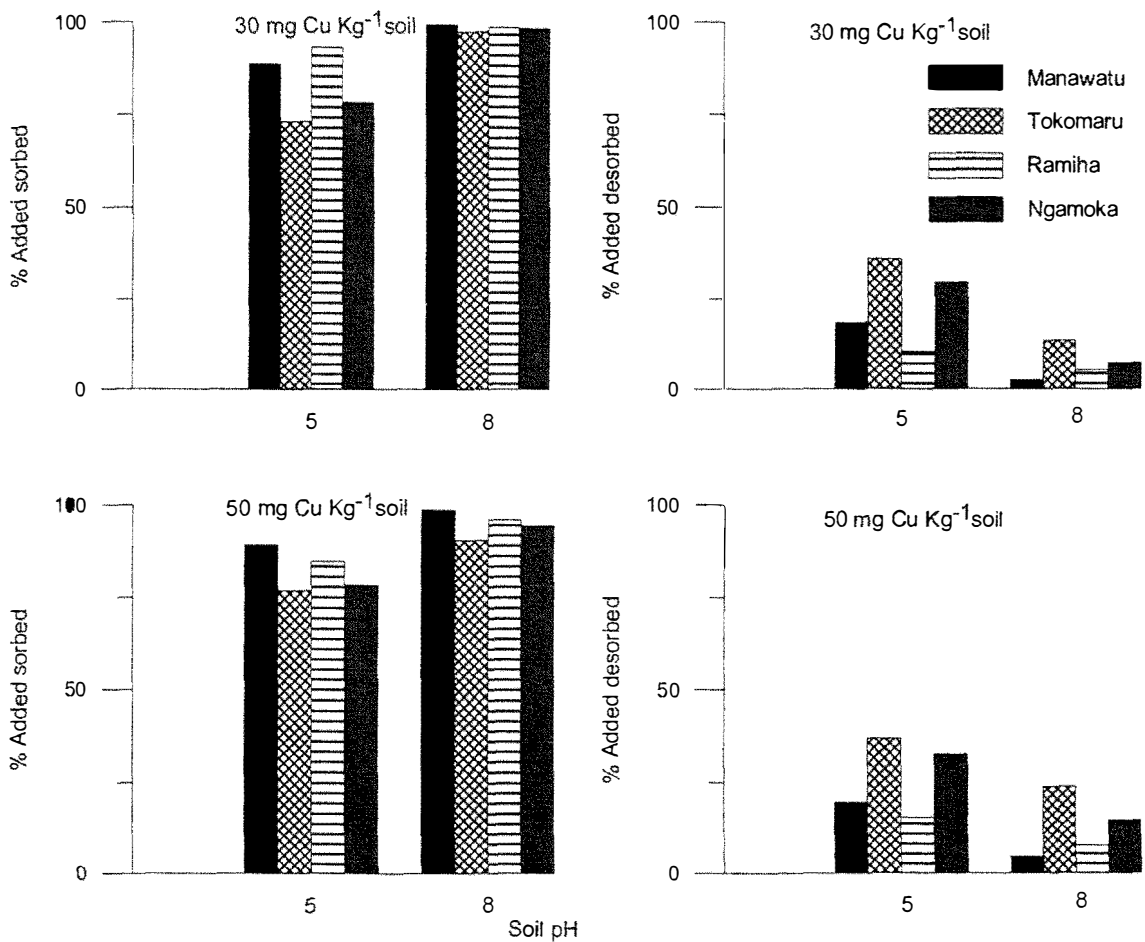


Figure 3.19 Effect of pH on Cu adsorption and desorption.

The effect of pH and the level of sorbed Cu on the desorption of Cu by the four soils is illustrated in Figure 3.20. Desorption increased with increasing levels of added Cu in

the soil (Figure 3.20). The Tokomaru soil contains lesser amounts of soil organic matter, oxides, and CEC, resulting in largest amounts of desorption of added Cu at both pH levels. The desorption of Cu decreased with increasing soil pH (Figure 3.20). The Ramiha soil contains higher amounts of organic matter, oxides, and CEC, and has the highest K_d value at pH 5.0, resulting in the lowest desorption of Cu, but at the high pH level the Manawatu soil showed the lowest desorption. The Ngamoka soil resulted in a higher desorption of Cu at both pH levels, which may be related to the disruption and reformation of organo-mineral associations induced by wetting and re-drying in the incubation process, and also due to the low base saturation of this soil.

The differences of desorption behaviour in these soils was due to the speciation of Cu at two levels of pH in the different soils. Cavallaro and McBride (1984) have also shown that desorption of Cu from soil clays decreases with an increase in soil pH. A number of studies have shown that Cu^{2+} in soil solution, especially at higher pH, exists primarily in a form complexed with soluble organic matter (Hodgson *et al.*, 1966; Fotovat and Naidu, 1997; Wu *et al.*, 2000). The amount of organically complexed Cu in solution generally increases above pH 7.0 because of a greater solubility of soil organic matter at higher pH (McBride and Blasiak, 1979), while the concentration of free ionic Cu^{2+} at higher pH is much lower, usually in the range of 10^{-9} to 10^{-8} M (McBride and Blasiak, 1979). It has been estimated that hydrolysis products of Cu [CuOH^+ and $\text{Cu}_2(\text{OH})_2^{2+}$] are the most significant species below pH 7, while above pH 8 anionic hydroxy complexes of Cu become important. Solubility of metals in soils and mineral oxide surfaces as a function of pH is often dictated by the presence of organic and inorganic ligands (McBride, 1989; Sparks, 1995; Harter and Naidu, 1995). In this study, it was observed that the irreversible retention of Cu might be the result of complex formation of Cu at high pH.

Quantification of the plant availability of Cu in soil suspensions requires the determination of labile Cu (ions in the solid and solution phases in equilibrium with free Cu forms in the soil solution) and the activity of Cu^{2+} in the soil solution of acid soils and $\text{Cu}(\text{OH})_2$ in neutral or alkaline soils. It suggests that the desorption of Cu from the soil labile pool is affected by pH, which has an important implication for the uptake of soil Cu by plants.

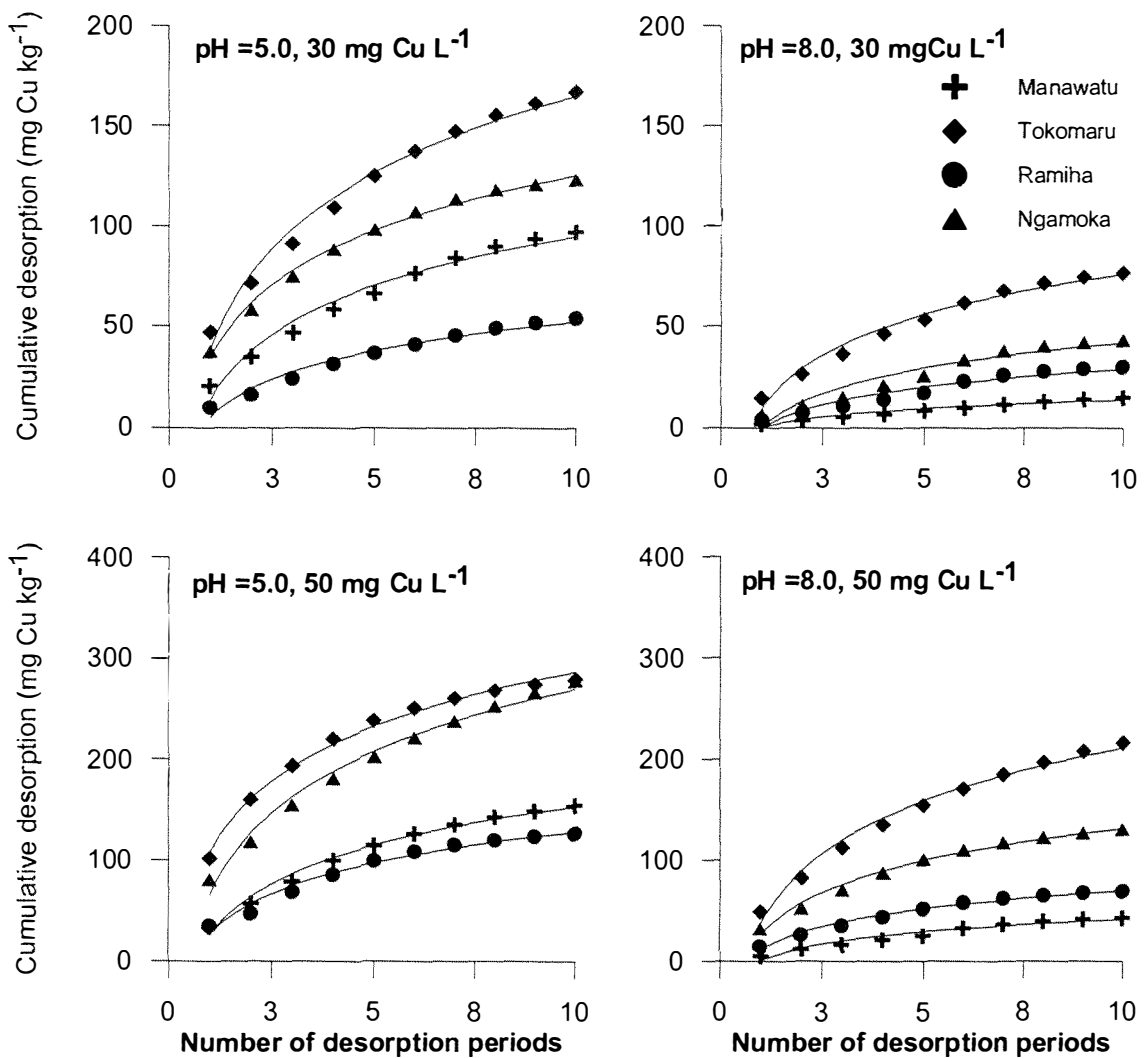


Figure 3.20 Cumulative desorption of Cu at two pH (5 and 8) and two sorption levels (30 and 50 mg L⁻¹) in different soils.

3.4 CONCLUSION AND FURTHER STUDY

- Sorption capacity increased with time and reached the maximum level within 2 hours in all soils.
- Adsorption of Cu varied amongst the five soils, which vary in their physico-chemical properties.
- The Freundlich equation adequately described the Cu adsorption data.
- The highest value of adsorption constant (K) for Cu was observed in the Ramiha soil and the lowest in the Mangamahu soil.
- The order of Cu adsorption followed: Ramiha > Manawatu > Ngamoka > Tokomaru > Mangamahu.

- Copper retention by the Ramiha, Ngamoka and the Mangamahu soils is mostly determined by both the organic matter and oxide components.
- Organic matter is the principal component for Cu retention in all soils.
- In all soils, Cu concentration in solution decreased and Cu sorption increased with an increasing pH level.
- Desorption of native Cu from all soils was generally low, but increased with an increasing concentration of added Cu in solution.
- The cumulative desorption of added Cu from the soils decreased with increasing pH.
- In the Manawatu soil, 4.7 % of 0.04M EDTA extractable Cu could be desorbed after 10 desorption equilibrations with $\text{Ca}(\text{NO}_3)_2$, while for the Ngamoka soil 18% of the extractable Cu could be desorbed.
- The proportion of added Cu desorbed during 10 desorption periods was extremely low, ranging from 2.5% in 24 hrs to 6% in 2 hrs.
- Desorption was 24% (at pH 5.0) and 10% (at pH 8.0) of the sorbed Cu for both soils.

Adsorption and desorption reactions are likely to be the major factors controlling the availability of Cu to plants. In this chapter, a basic knowledge of the type of surfaces that will adsorb Cu and the relative amounts of Cu retained under given conditions has been acquired. The availability of Cu from different Cu source as fertilisers in different pasture soils will be discussed in the next chapter.

CHAPTER 4 PLANT AVAILABILITY OF COPPER FROM DIFFERENT COPPER FERTILISERS IN PASTURE SOILS

4.1 INTRODUCTION

Copper is essential for both plants and animals. Copper is involved in the functioning of a wide range of enzymes, and is required in very small amounts by both plants and animals. Copper levels are low in some New Zealand soils (Wells, 1957; Haynes and Swift, 1983; McLaren *et al.*, 1984). This can limit pasture growth (During, 1972) and the Cu supply to the grazing animal (Goold and Smith, 1975). Deficiency of Cu occurs when the Cu concentration is $< 4 \text{ mg kg}^{-1}$ in pasture and $< 0.3 \text{ mg L}^{-1}$ in animal blood. In animal nutrition, Cu deficiency is almost entirely confined to grazing animals due to low levels of Cu in the herbage/forage, or because of the normal to low levels of Cu accompanied by elevated intakes of Mo, S and Fe that are sufficient to limit Cu absorption and retention. Willimott (1995) reported that Cu deficiency in grazing ruminants is widespread and occurs on the Yellow Brown Pumice soils and Yellow Brown Earths of the Wairoa district, resulting in problems with brittle bones in lambs. Copper fertiliser is the second most frequently used trace element in Northland, New Zealand. Many of the Yellow Brown Earths, sand and peat soils have a requirement for Cu either to aid clover vigour, as is the case on some of the West Coast sands or to address low animal Cu levels (Johnson, 1995). Korte *et al.* (1996) reported widespread Cu deficiency in grazing animals in New Zealand costing the farming industry annually several million dollars in animal remediation. Good correlation between herbage Cu concentration and liver Cu concentration of grazing Romney sheep has often been observed (Grace *et al.*, 1998; Knowles *et al.*, 1998).

There are conflicting opinions to the best means of correcting Cu deficiency in livestock. Copper deficiency in grazing animal is corrected either through application of Cu fertilisers or by the use of Cu bullets. Copper sulphate is the most commonly used Cu fertiliser in New Zealand. A wide range of Cu sources have been examined for their effectiveness as fertilisers, when applied to soils or foliage (Gilkes, 1981). Pasture topdressing with CuSO_4 has been the major measure to prevent the primary Cu

deficiency (Grace *et al.*, 1998). The effectiveness of pasture topdressing with Cu ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) depends on the nature of the pasture and soil properties (Sherrell and Rawnsley, 1982). The residual effects of Cu topdressing also differ between soils and the types of Cu fertilisers. There is a need to define the rates of change in the effectiveness of Cu fertiliser with time over the range of soils and climatic conditions encountered. This information is required so that the needs of crops, pastures and the grazing animal can be met and agricultural production maintained.

Copper in the soil solution occurs mainly as copper-organic matter complexes. Little is known about Cu availability and the rates of absorption by plants from different Cu organic complexes. Therefore it is difficult to predict changes in the supply of plant available Cu in field situations from the existing knowledge of the chemistry of Cu in soils.

Objectives:

The objectives of the experiment reported in this chapter are as follows:

- To examine the effectiveness of a range of Cu fertilisers for plant growth and raising pasture Cu concentration.
- To monitor the transformation of Cu in soils.

4.2 MATERIALS AND METHODS

4.2.1 *Soil collection and preparation*

Soil samples were collected from different locations (Chapter 3; Section 3.3.1) and air dried. The soil samples were ground and passed through 6.0 mm sieve to remove the weeds and stones. One kg (dry weight basis) of soil was mixed with the required amount of fertiliser and was placed in pots. The soil in the pots was maintained at 80% field capacity for equilibration.

4.2.2 *Copper fertilisers used*

The different Cu sources used as fertiliser in this experiment are given in Table 4.1. The CuSO_4 and CuO are granular fertilisers and $\text{Cu}(\text{OH})_2$ is a powder. To maintain the uniformity of particle size, the fertilisers were ground to a 150-400 μm particle size range and used in the experiment. The solubility data indicates that CuSO_4 is more

readily soluble than the other two fertilisers (Table 4.1). The fertiliser rates used were: 0, 50, 100 and 200 mg Cu kg⁻¹ soil. Assuming a bulk density of 1.0 tonne m⁻³ and a depth of incorporation of 1 cm, the rates are equivalent to 0, 5, 10, 20 kg Cu ha⁻¹.

Table 4.1 Sources of Cu used as fertilisers in the plant growth experiment.

Fertiliser types	Copper content (g kg ⁻¹)	Solubility in water (g m ⁻³)	Supplied by
Copper sulphate	250	100	Ravensdown Fertilisers, New Zealand.
Copper hydroxide	308	0.04	Mankind Trading Co, New Zealand.
Copper oxide	250	0.10	BOP Fertilisers Ltd, New Zealand.

4.2.3 *Plant growth experiment*

A modified split plot design was used in this glasshouse trial. The treatments were five soils (Manawatu, Tokomaru, Ramiha, Ngamoka and Mangamahu) as main plot and three Cu sources [CuSO₄, Cu(OH)₂ and CuO] at four levels (0, 50, 100 and 200 mg Cu kg⁻¹ soil) as sub-plot (Plate 4.1). The pots were placed randomly on each trolley (block) by using a random number table (Plate 4.2). The treatments were replicated four times. There were 240 (60 × 4) pots in this experiment. Two weeks after fertiliser application, fifty seeds of ryegrass (*Lolium perenne* cv. Super Nui) were sown in each pot. The pots were covered with brown paper until germination. The first Cu-free nutrient solution was given 21 days after sowing time. The Cu-free nutrient solution was given twice a week.



Plate 4.1 Plant growth experiment with different levels of CuSO_4 fertiliser (Ballantrae high fertility and Ballantrae low fertility known as Ngamoka and Mangamahu soil, respectively).



Plate 4.2 Effect of soil types on plant growth experiment (Ballantrae low fertility known as Mangamahu soil).

4.2.4 Grass and soil sample preparation

The grass was harvested at 68, 96, 125, 153 and 181 days after sowing. The plants were cut to a height of approximately 3.0 cm above the surface of the soil. The samples were dried at 70 °C in a forced air oven. The dry weights of the grass samples were recorded and the samples were then ground using a coffee grinder and kept in airtight polyethylene bags for chemical analysis.

A soil sample was collected from each pot using a soil corer at 0, 8, 28, 106, 163 and 191 days intervals. The soil samples were air dried, ground and passed through a 2.00 mm sieve. The soil samples were analysed for exchangeable, organic, oxide and residually bound Cu. The results of Cu transformation in the soils are presented in Chapter 5.

4.2.5 Ryegrass samples for Cu analysis

A known weight (0.4 g) of the finely ground grass samples was placed in 50 ml acid-washed conical flasks. Approximately 10 ml of Aristar grade HNO₃ (69%) was added to each conical flask. A reflux funnel was placed on top of the conical flasks and left for 16 hrs in a fumehood. The samples were then digested for at least 4 hrs at 150 °C (until the brown fuming stopped). The reflux funnels were removed and the temperature was increased slowly to 250 °C. The samples were boiled until just dry and then allowed to cool. The volume of the digest was made up to 10 ml with 1% HNO₃. The digest aliquots were then stored for chemical analysis.

4.2.6 Chemical Analysis

Copper in the plant digests and in the soil extracts was analysed using flame atomic absorption Spectrophotometry (F-AAS). All glassware was acid washed (2M HCl), rinsed with deionised water, and oven dried before use.

To check the reproducibility of the analytical procedure, three blanks were included in each batch of 50 samples of grass and of soil. Certified herbage and soil samples were also analysed in parallel with the unknown samples. All results are expressed on an oven-dry weight basis.

4.2.7 *Statistical analysis*

Statistical analysis of the data was carried out by the Statistical Analysis System (SAS). Significant differences between treatment means were evaluated using analysis of variance (ANOVA), performed by using the SAS GLM procedure (SAS Institute, 1996).

4.3 RESULTS AND DISCUSSION

4.3.1 *Initial soil characteristics*

The initial soil characteristics are discussed in chapter 3 (Section 3.3.2). The soils varied in their Olsen P, organic matter content, CEC, and pH values. In general all five soils used in this experiment were slightly acidic and are optimum in soil Cu.

4.3.2 *Dry matter yield*

4.3.2.1 *Effect of the soil types*

The dry matter (DM) yields were significantly different among the soils at all harvests (Figure 4.1). The highest dry matter yield was obtained from the Manawatu silt loam and the lowest from the Tokomaru silt loam at harvest 1. The low DM yield of the Tokomaru soil was due to the low fertility of this soil as indicated by the low Olsen P, organic matter, soil pH and CEC. The high dry matter yield of the Manawatu soil was due to a high fertility status resulting from the recent alluvial origin of the soil. A good correlation between DM yield at harvest 1 and Olsen P ($r=0.90$), soil pH ($r=0.54$), clay ($r=-0.31$) and silt ($r=0.34$) was obtained. At harvest 2 correlations were obtained between DM and CEC ($r=0.46$) and Olsen P ($r=0.39$), and for harvest 5 with CEC ($r=0.29$) (Table 4.2). All significant correlations (Table 4.2) are in accord with the suggestion that the yields were influenced by the physical and chemical status of the soils. The DM yields increased in the Tokomaru soil up to harvest 3 and then decreased. The DM yields decreased at harvests 4 and 5 due to insect infestation, and to high temperatures in the glasshouse. At harvest 4, the Mangamahu soil produced the highest DM yield, which was statistically similar to the Manawatu and Ramiha soils, the Tokomaru soil produced the lowest DM yield, which was statistically similar to the

Ngamoka soil. At harvest 5, the Ramiha soil produced the highest DM yield, which was significantly more than all the other soils. The Tokomaru soil produced the lowest DM yield, which was also significantly different from the others. In this study, there was no response in DM yield with Cu fertiliser additions. King (1974) and Gartrell (1981) obtained a good response to the application of Cu to sand plain soils and sodic soils for wheat crops in Western and Southern Australia. In Australia, inherently the soil Cu is very low, which may be the reason for a Cu response in dry matter yield.

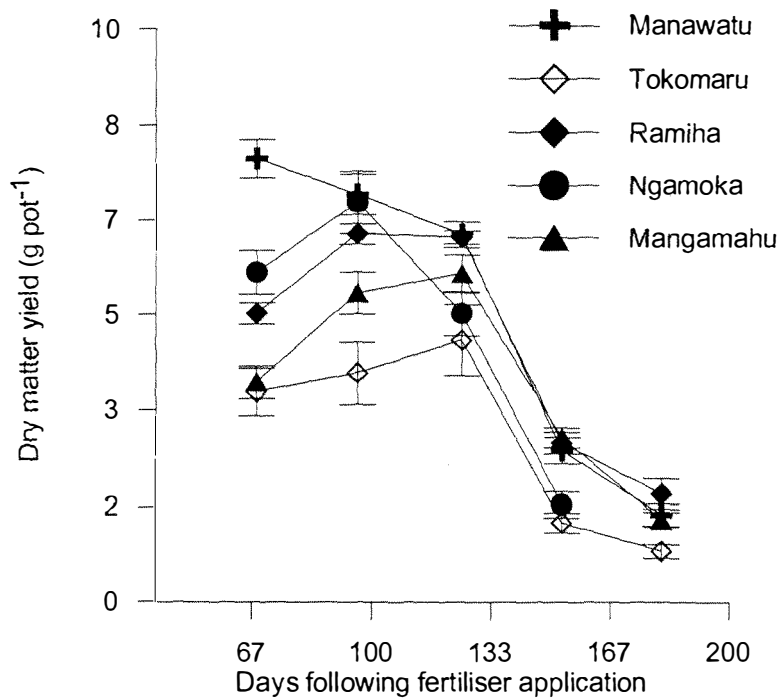


Figure 4.1 Effect of different soils on dry matter yield (g pot^{-1}) of rye grass at different harvests. Data are means \pm SE, $n=48$.

Table 4.2 Correlations of dry matter yield with soil properties.

Soil properties	Harvest 1	Harvest 2	Harvest 3	Harvest 4	Harvest 5
Olsen P	0.90**	0.39**	0.22*	0.02	0.03
pH	0.54**	0.12	0.003	0.15	0.04
Organic carbon	0.07	0.08	0.001	0.012	0.16
CEC	0.03	0.46**	0.05	0.05	0.29*
Silt	0.34*	0.08	0.12	0.004	0.05
Clay	-0.31*	0.004	0.01	0.03	0.08

** and * denote significance at 99% and 95% level, respectively.

4.3.2.2 Effect of types of Cu fertiliser

There was no significant yield response observed with the addition of Cu fertilisers when compared to control. The fertiliser type caused a different effect on DM yield in harvests 1, 2 and 4 (Figure 4.2). At harvest 1, CuSO_4 produced the lowest DM yield, which was significantly different to the other two fertilisers. CuSO_4 is a readily soluble fertiliser. Thus, the decrease in yield might be due to a quick release of Cu from CuSO_4 causing yield depression at higher levels of Cu. At harvest 2, $\text{Cu}(\text{OH})_2$ produced the highest DM yield, which was similar to CuO , but higher than the CuSO_4 fertiliser. $\text{Cu}(\text{OH})_2$ produced the highest DM yields at each harvest, this may be due to the liming effect of the $\text{Cu}(\text{OH})_2$ fertiliser. It contains 30% lime as an inert material.

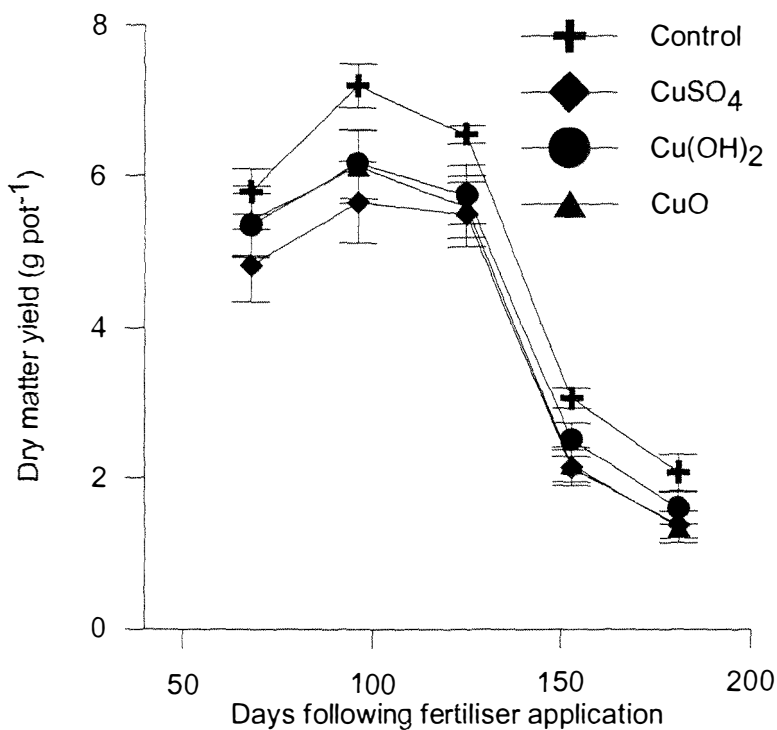


Figure 4.2 Effect of fertiliser type on dry matter yield at different harvests. Data are means \pm SE, $n=80$; control $n=20$.

4.3.2.3 Effect of fertiliser rates

Increasing fertiliser rates resulted in a significant decrease in DM yield in all harvests (Table 4.3). At harvest 1, the Cu level of 50 mg Cu kg⁻¹ produced the highest DM yield, but it was statistically similar to the other treatments except the 200 mg Cu kg⁻¹

treatment level. The Cu level of 200 mg Cu kg⁻¹ produced the lowest DM yield and it was significantly different to the other treatments. A similar trend was also observed at all other harvests. During (1972) reported that as little as 11 kg CuSO₄ ha⁻¹ can depress pasture growth. Carter and Day (1977) found that at a rate of 2 kg Cu ha⁻¹ (8 kg CuSO₄ ha⁻¹) pasture yield was depressed in field trials in South Australia. Khan *et al.* (1996) in an earlier field trial showed that ryegrass yields did not respond to Cu application, indicating that ryegrass is less sensitive to Cu than legumes. Copper concentration in legumes increased with increasing levels of Cu up to 5 kg Cu ha⁻¹, but grasses did not show any response with increasing levels of Cu. This may reflect the requirement of Cu by rhizobia for nitrogen fixation (van der Elst *et al.*, 1961). Yield responses to Cu occur only where the Cu concentration in plants is below 4 mg kg⁻¹ (Sherrell and Rawnsley, 1982). Forbes (1978), in a survey of lucerne on Yellow Brown Pumice soils found very few stands with <4 mg kg⁻¹ Cu, indicating widespread DM yield response to Cu is unlikely to occur in these soils.

Copper sulphate fertiliser at 200 mg Cu kg⁻¹ soil produced the lowest DM yield in all harvests, and it was significantly different from the other treatments (Table 4.3). The treatment at 200 mg Cu kg⁻¹ soil for the Cu(OH)₂ and CuO fertilisers also produced significantly lower DM yields than other treatment levels. This indicates that the higher Cu levels might have caused toxicity, and thereby depressed the growth of rye grass. The effect of Cu levels on yield depression was less pronounced for the Cu(OH)₂ and CuO fertilisers than for the CuSO₄ fertiliser. Beckett and Davis (1978) found that the high levels of Cu depressed plant growth in barley. Alva and Chen (1995) observed that with an increase in external Cu concentrations, the shoot and root dry weights of citrus plants decreased significantly. Plenderleith and Bell (1984) conducted an experiment to evaluate the growth response of 12 sub-tropical grasses with additions of Cu and Zn. They observed that depending on the species the Cu and Zn concentrations in the plants was associated with a 50% yield reduction ranging from 17 to 27 mg kg⁻¹ and 475 to 1925 mg kg⁻¹, respectively. Heavy metals such as Cu tend to accumulate in the roots and in turn, affect the growth of the whole plant. Lidon and Henriques (1992b and 1993) observed that the Cu concentration in roots increased linearly with increasing external Cu concentrations. Decreased translocation of Cu to the above ground parts from the roots has been suggested as a mechanism to withstand Cu toxicity.

Table 4.3 Effect of fertiliser rates on dry matter yield (g pot⁻¹) of ryegrass at different harvests.

Fertilisers	Treatments (mg Cu kg ⁻¹ soil)	Harvest 1	Harvest 2	Harvest 3	Harvest 4	Harvest 5
Control	Control	5.79a	7.20a	6.55a	3.05a	2.07a
CuSO ₄	50	5.81a	6.64a	5.99a	2.56abc	1.43bc
	100	5.35a	6.18a	6.18a	1.70de	1.27bcd
	200	2.32c	2.59c	3.23c	1.22e	0.75d
Cu(OH) ₂	50	6.11a	6.92a	6.43a	2.78ab	1.42bc
	100	5.43a	6.30a	5.76a	2.44bc	1.56ab
	200	4.09b	4.22b	4.27b	1.74d	1.36bcd
CuO	50	5.90a	6.78a	6.00a	2.27bc	1.09bcd
	100	5.48a	6.19a	5.83a	2.17cd	1.33bcd
	200	4.47b	4.29b	3.98bc	1.21e	0.91cd

* Treatment means followed by the same letter within a column are not significantly different at the 5% level.

4.3.3 Copper concentration in ryegrass

4.3.3.1 Effect of the soil types

The Cu concentration in ryegrass was significantly different among soils (Table 4.4). The ryegrass grown in the Manawatu silt loam soil contained the highest Cu concentration at all harvests. The Ramiha soil contained the lowest amount of Cu at harvest 1. At harvest 2, the Tokomaru and Ngamoka soils ryegrass contained the lowest concentration of Cu and was significantly different to the others. The ryegrass of the Ngamoka soil contained the lowest concentration of Cu and was significantly different to the others at harvest 3. There was a general trend of decrease in the Cu concentration in all soils after the first harvest (Table 4.4), but the Cu concentration increased at harvests 4 and 5. The decrease in Cu concentration in ryegrass with time is due to a decreasing Cu concentration in the soil resulting from changes in soil pH in the rhizosphere (Linehan *et al.*, 1985), and reactions of Cu with soil constituents (Brennan *et al.*, 1980, 1983). A positive correlation between Cu concentration in plants and soil pH ($r=0.18$); a positive correlation between Cu concentration in plants and Olsen P ($r=0.42$); and a negative correlation between Cu concentration in plants and organic matter ($r= -0.54$) and clay content ($r= -0.49$) were observed for all harvests. A positive

correlation between the Cu concentration in plants and Olsen P was obtained at each harvest. The availability of Cu to plants is largely dependent on soil pH, organic matter and the clay content of the soil. A positive relationship between the Cu concentration and soil pH was observed up to harvest 3 (Table 4.5). This may be attributed to the effects of pH on the mobility of the Cu in soils and its absorption by roots. There is evidence to suggest that the mechanisms, for the absorption of Cu by roots, are affected by changes in soil pH. However, in the previous chapter 3 (Section 3.3.3.5), as pH is increased towards neutrality, the mobility of Cu in solution is reduced as the result of increased sorption. There are marked increases in the gradients of sorption isotherms as the pH of the soil is raised. At low concentrations of Cu, the gradients of the sorption isotherms are highly correlated with soil pH, but not with some of the other soil properties thought to be important in controlling mobility (Jarvis, 1981a). Copper availability is reduced at a soil pH above 7 and is most readily available below pH 6 (Lucas and Knezek, 1972; Locascio, 1978).

The role of organic matter in the incidence of Cu deficiency, complex formation, and sorption of Cu have been reported by many authors (McLaren and Crawford, 1973b; Harter, 1983; Shorrocks and Alloway, 1987; McBride, 1991; Wu *et al.*, 2000). The relative sorption capacity of different soil components for Cu (Chapter 3) indicates that, among soil constituents, organic matter and oxides are the most important for the sorption of Cu. Nielsen (1976) obtained samples of soil solution by suction from a calcareous peat soil in which barley plants were grown. As expected, the levels of Cu in the soil solution remained constant when no plants were grown. In the presence of barley plants, Cu in the soil solution increased. Williams (1981) suggested that some portion of the organic material solubilised in the presence of plants would be due to root exudates. Linehan *et al.* (1985) displaced the soil solution by centrifugation in the presence of the barley plants. The concentration of Cu in the root rhizosphere was found to be maximum during early plant development and decreased up to harvest. Microbial activity was considered to be involved in the transformation of Cu in soil. The pattern of change of Cu concentration in the rhizosphere with time was similar to that of the changing soil biomass. It was suggested that enhanced Cu levels in the rhizosphere could also be related to soil pH changes.

The increase in plant Cu concentration in all soils except the Ramiha soil at harvests 4 and 5 was due to a lower growth rate and high temperatures. The grass grown in the Ramiha soil contained the lowest concentration of Cu at harvests 4 and 5 and was significantly different to the others. The Ngamoka and Ramiha soils are rich in organic matter and oxides, Cu complex formation occurs more rapidly in these two soils, resulting in Cu being unavailable for plant uptake. Ryegrass grown in the soil with the lowest organic matter (Manawatu) contained the highest Cu concentration at each harvest, while ryegrass grown in the Ramiha and Ngamoka soils with the higher amounts of organic matter contained the lowest concentration of Cu at later harvests. This is consistent with the more rapid immobilisation of Cu into the organic fraction in the Ramiha and Ngamoka soils. Cu in soil is strongly held on inorganic and organic exchange sites and in complexes with organic matter. For this reason a large proportion of the total Cu content of soils is not available for uptake by plants. The proportion of the total Cu taken up by plants has been found to be greater in mineral soils than in peats (Kabata-Pendias, 1963).

Since most of the Cu added to pasture soils is associated with organic matter, a large proportion of the Cu taken up by plants must originate from soluble organic complexes. The dissociation of Cu^{2+} from the organic ligand apparently must occur prior to plant uptake (Goodman and Linehan, 1979). The ease with which the dissociation and subsequent absorption of Cu by roots takes place will therefore depend upon the complexing ligand and this, in turn, will depend on the particular soil in which the plant is growing, as well as conditions within that soil. Stevenson and Fitch (1981) contended that organic colloids and clay minerals play a major role in Cu retention by soil. In most mineral soils, Cu may be bound as clay-metal-organic complexes, since in these soils, organic matter is intimately bound to clay. A significant negative correlation between Cu concentration and soil clay was obtained at each harvests (Table 4.5). As well as reactions with organic matter, applied Cu may have been rendered unavailable to plants by reactions with clays (McBride, 1981), and sesquioxides (Grimme, 1968). Dragun and Baker (1982) conducted a glasshouse experiment to determine the physical and chemical properties of soils, which control Cu availability to corn seedlings using 16 Northeastern United States soils and obtained a good relationship between Cu availability and soil pH, organic matter, CEC and percent clay.

Table 4.4 Effect of soil types on Cu concentration (mg kg^{-1}) in ryegrass at different harvests.

Soils	Harvest 1	Harvest 2	Harvest 3	Harvest 4	Harvest 5
Manawatu	33.43a	31.62a	20.46a	25.68a	33.34a
Tokomaru	27.77ab	22.47c	18.65b	22.16b	23.25b
Ramiha	25.30b	24.92b	12.74d	12.96c	12.08c
Ngamoka	28.63ab	22.81c	11.62e	22.23b	21.20bc
Mangamahu	29.57ab	25.21b	15.30c	22.44b	26.38b

*Treatment means followed by the same letter within a column are not significantly different at the 5% level.

Table 4.5 Correlations of Cu concentration in ryegrass with soil properties.

Soil properties	Harvest 1	Harvest 2	Harvest 3	Harvest 4	Harvest 5
Olsen P	0.55**	0.70**	0.28*	0.21*	0.34*
pH	0.20*	0.18*	0.22*	0.14	0.15
Organic carbon	-0.42**	-0.31*	-0.98**	-0.38**	-0.63**
CEC	0.09	0.04	-0.80**	-0.16	-0.28*
Silt	0.001	0.18*	0.12	0.04	0.01
Clay	-0.45**	-0.42**	-0.81**	-0.32*	-0.45**

** and * denote significance at the 99% and 95% levels, respectively.

4.3.3.2 Effect of types of Cu fertiliser

The fertiliser types resulted in a significant effect on Cu concentration in ryegrass (Table 4.6). The application of CuSO_4 fertiliser resulted in the highest concentration of Cu at harvest 1 and it was significantly different from CuO. This might be attributed to the quick releasing characteristics of CuSO_4 (Gilkes, 1981). At harvest 2, the application of $\text{Cu}(\text{OH})_2$ resulted in the highest concentration of Cu in plants, and was significantly higher than CuO. At harvests 3 and 4, the highest Cu concentration was observed in CuO treated ryegrass, and it was significantly higher than the $\text{Cu}(\text{OH})_2$ and CuSO_4 fertilisers (Table 4.6). The higher residual effect of $\text{Cu}(\text{OH})_2$ and CuO fertilisers might be due to the slow release characteristics of these two insoluble fertilisers. The suitability of CuO for broadcasting depends on particle size. CuO broadcast on to the soil surface at 5 kg Cu ha^{-1} , and worked into the soil, did not correct Cu deficiencies in canola, barley, or wheat during the year of application, but corrected these deficiencies in following years (Karamanos *et al.*, 1986). The lack of a Cu response to CuO was

mainly due to the low water solubility of the coarse, granular CuO, which ranged in particle diameter from <0.2 (powder) to 3.0 mm. Coarse Cu carriers are also ineffective, where inadequate contact occurs between the plant root and the applied Cu (Gartrell, 1981). The Cu concentration decreased with time from the first harvest and again it increased at harvests 4 and 5 resulting in low dry matter yields. Willimott (1995) found that application of 5 and 10 kg Cu ha⁻¹ as copper sulphate increased the herbage Cu concentration up to three months after the Cu application, and then decreased back to the initial levels nine months after application.

Table 4.6 Effect of fertiliser type on Cu concentration (mg kg⁻¹) of ryegrass at different harvests.

Fertilisers	Harvest 1	Harvest 2	Harvest 3	Harvest 4	Harvest 5
CuSO ₄	33.23a	25.65ab	15.10b	21.36b	26.29a
Cu(OH) ₂	29.32a	26.19a	15.68b	18.04c	20.45b
CuO	24.26b	24.39b	16.48a	23.88a	24.55a

* Treatment means followed by the same letter within a column are not significantly different at the 5% level.

4.3.3.3 *Effect of fertiliser rates*

The increasing levels of Cu increased the Cu concentration in ryegrass (Table 4.7). CuSO₄ at the 200 mg Cu kg⁻¹ soil level resulted in the highest Cu concentration at harvest 1, which was significantly different from all other treatments. Cu(OH)₂ fertiliser at the 100 and 200 mg Cu kg⁻¹ soil levels and the CuO fertiliser at the 200 mg Cu kg⁻¹ soil level contained statistically similar amounts of Cu but significantly different from the others at harvest 1. Cu(OH)₂ and CuO fertilisers at the 200 mg Cu kg⁻¹ soil level contained the highest concentration of Cu in ryegrass, which was significantly higher than the other treatment at harvest 2. The CuO fertiliser at the 200 mg Cu kg⁻¹ soil level contained the highest amount of Cu, which was significantly higher than the other treatments at harvests 3, 4 and 5. This reflected the low solubility characteristics of the CuO fertiliser. The treatment levels of 100 and 200 mg Cu kg⁻¹ of soil resulted in statistically similar concentrations of Cu in Cu(OH)₂ at harvest 1. Reuter *et al.* (1981a) noted that the increasing rates (ranged from 0 to 533 mg Cu kg⁻¹) of Cu application increased the Cu concentration in plant tops and roots. Plenderleith and Bell (1984)

found that with increasing additions of Cu (4 to 600 mg Cu kg⁻¹ soil) the Cu concentration in tropical grasses increased from 17 to 27 mg kg⁻¹. Reuter *et al.* (1981a) recorded that the Cu concentration in subterranean clover declined from 3.9 mg kg⁻¹ at 26 days after sowing to 1.6 mg kg⁻¹ at 98 days. Sherrell and Rawnsley (1982) found that applications of 2 to 4 kg Cu ha⁻¹ as copper sulphate increased herbage Cu concentrations from 5 to 12 mg kg⁻¹ within 4 weeks and then it decreased markedly to 8 mg kg⁻¹ over the next 9 to 10 months. They also observed that clover tended to take up larger amounts of Cu and its persistence in maintaining higher Cu concentration that was greater than grasses. Khan *et al.* (1996) observed that applications of 2, 5 and 10 kg Cu ha⁻¹ as CuSO₄ increased the pasture Cu concentrations ranging from 9.98 to 15.89 mg kg⁻¹ and 6.51 to 6.69 mg kg⁻¹ at 73 and 187 days after fertiliser application, respectively.

Table 4.7 Effect of fertiliser rates on Cu concentration (mg kg⁻¹) of ryegrass at different harvests.

Name of fertilisers	Treatments (mg Cu kg ⁻¹ soil)	Harvest 1	Harvest 2	Harvest 3	Harvest 4	Harvest 5
Control	Control	12.48d	12.52h	6.41f	6.15g	6.65f
CuSO ₄	50	23.52c	23.47fg	12.38e	12.36f	13.46e
	100	41.94b	32.08bc	18.04d	29.39d	39.43c
	200	54.98a	34.52ab	23.57c	37.55b	45.63ab
Cu(OH) ₂	50	23.78c	26.20ef	12.80e	13.03f	15.28e
	100	38.48b	29.44cd	17.35d	20.12e	19.15de
	200	42.56b	36.58a	26.15b	32.85c	40.71bc
CuO	50	21.97c	22.04g	13.80e	13.80f	16.50e
	100	25.80c	26.62de	17.08d	28.96d	24.68d
	200	36.80b	36.36a	28.62a	46.60a	50.38a

* Treatment means followed by the same letter within a column are not significantly different at the 5% level.

4.3.4 Copper uptake

4.3.4.1 Effect of the soil types

There was a general trend of decreasing Cu uptake with increasing time after fertiliser application from the 96 days harvest (Figure 4.3). The Manawatu soil showed a greater rate of decline in Cu uptake in comparison to the other soils. The differences in the rate

of decline may be due to differences in soil pH, Olsen P, organic matter, oxides and clay minerals of these soils. The decrease in Cu uptake is due to adsorption/desorption processes, which control the amount and rate of release of Cu for plant uptake. This applies both to native soil Cu and Cu applied as fertiliser. Results obtained for Cu sorption by soil components (Section 3.3.3.4) and the pH dependent adsorption-desorption study in Chapter 3 (Section 3.3.3.5 and 3.3.5.4) explain the reasons for the reduction in Cu uptake. Reduction in Cu uptake was due to the stronger adsorption of Cu to organic ligands or mineral surfaces. McLaren *et al.* (1990) reported that the adsorption of Cu is strongly influenced by the amount of added Cu and soil pH. They found that initially less than 10% of the Cu adsorbed by the soil was desorbed, and after three months of soil contact, only a negligible amount (<1%) of the adsorbed Cu could be desorbed. Tiwari and Kumar (1982) conducted an experiment with rice plants in soils where the pH values ranged from 3 to 8, and they observed that Cu in the rice seedlings was negatively correlated with soil pH ($r = -0.83$, $p < 0.01$). It was suggested that the decrease in Cu uptake with increasing pH is related to the formation of insoluble inorganic Cu compounds, especially carbonates and hydroxides. Adsorption measurements in Chapter 3 (Section 3.3.3.5) showed similar trends. A positive correlation between Cu uptake and soil pH ($r = 0.44$), a positive correlation between Cu uptake and Olsen P ($r = 0.93$), and negative correlations between Cu uptake and organic carbon ($r = -0.20$) and clay content ($r = -0.42$) were obtained for harvest 1 (Table 4.8). Significant positive correlations existed between Cu uptake and Olsen P at each harvest. Increasing levels of P increased the Cu uptake (Section 7.4.3; Figure 7.2c). P status (Olsen P) of the soil is directly related to dry matter yield, causing a lift in Cu uptake. There was a good correlation between the time after fertiliser application and Cu uptake in different soils. The linear model equations relating negative Cu uptake with time after fertiliser application explained 95%, 91%, 82%, 88% and 87% of the variation in Cu uptake in the Manawatu, Tokomaru, Ramiha, Ngamoka and Mangamahu soils, respectively.

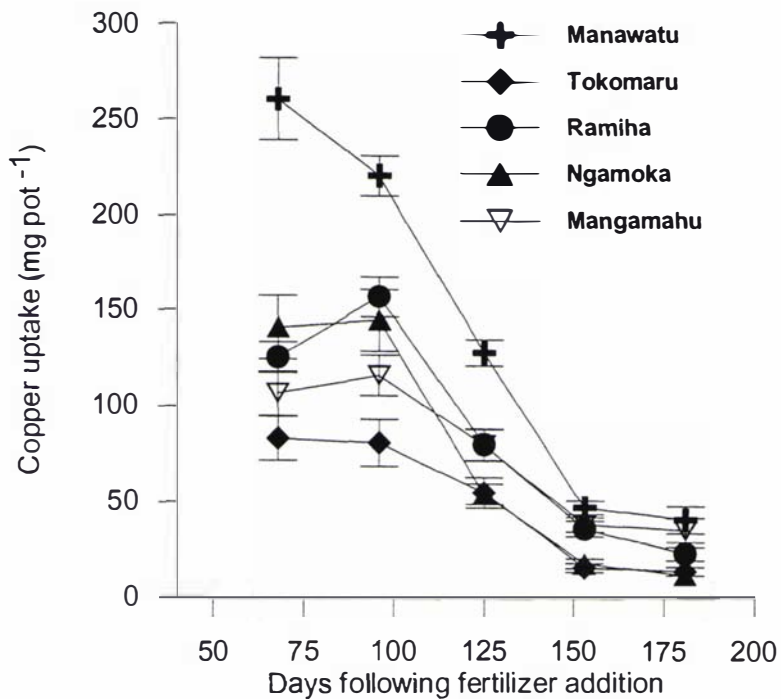


Figure 4.3 Effect of soil types on Cu uptake after fertiliser addition. Data are means \pm SE, $n=48$.

Table 4.8 Correlations of copper uptake with soil properties.

Soil properties	Harvest 1	Harvest 2	Harvest 3	Harvest 4	Harvest 5
Olsen P	0.93**	0.76**	0.62**	0.25*	0.23*
pH	0.44**	0.28*	0.13	0.001	0.002
Organic carbon	-0.20*	0.03	-0.28*	0.07	-0.19*
Silt	0.27*	0.29*	0.17	0.03	0.009
Clay	-0.42**	-0.18*	-0.36**	0.06	0.12

** and * denote significance at the 99% and 95% level, respectively.

4.3.4.2 Effect of types of Cu fertiliser

Figure 4.4 shows a general trend of decreasing Cu uptake of all fertilisers from 96 days after fertiliser addition. There was a greater decline in Cu uptake between harvests 1 and 2 for the CuSO_4 than for the other two fertilisers. For each type of fertiliser, increasing the time of contact between the soil and Cu source led to a decline in the Cu uptake in all soils. The r values relating Cu uptake and time after fertiliser application are 0.94, 0.90 and 0.87 for CuSO_4 , $\text{Cu}(\text{OH})_2$, and CuO , respectively. The reasons for this decline

may be related to the changes in the distribution of Cu in the soil (See chapter 5), and to the decrease in dry weight of the shoots during each growth period. On average, $\text{Cu}(\text{OH})_2$ and CuO showed the highest and the lowest Cu uptake, at each harvest. This low response to CuO is mainly related to the slow release characteristics and low solubility of this source.

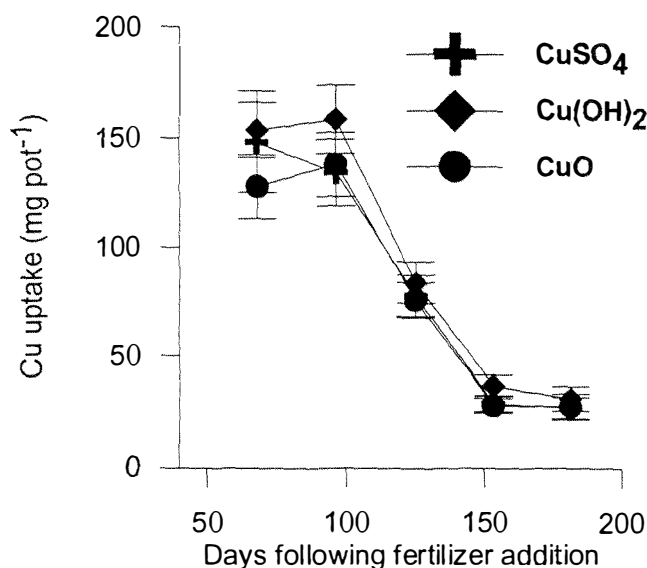


Figure 4.4 Effect of sources of Cu on Cu uptake following fertiliser addition. Data are means \pm SE, $n=80$.

4.3.4.3 Effect of fertiliser rates

Increasing levels of fertiliser up to $100 \text{ mg Cu kg}^{-1}$ increased the Cu uptake at all harvests (Table 4.9). Cu uptake was the highest at the $100 \text{ mg Cu kg}^{-1}$ level for both CuSO_4 and $\text{Cu}(\text{OH})_2$ but not for CuO . Gilkes and Lim-Nunez (1979) observed that increasing levels of Cu promoted Cu uptake in wheat. A Cu level of $200 \text{ mg Cu kg}^{-1}$ with CuSO_4 fertiliser showed a greater decrease in Cu uptake at each harvest, it might be due to a low growth rate of ryegrass at this level. Minnich *et al.* (1987) conducted a glasshouse trial in a Mardin silt loam amended with either CuSO_4 ($75\text{-}300 \text{ mg Cu kg}^{-1}$) or sludge (120 g kg^{-1}). They found a good correlation between soil solution Cu^{2+} activity and Cu accumulation in young snapbean plants.

Table 4.9 Effect of fertilisers and treatment levels on Cu uptake by ryegrass.

Fertilisers	Treatments	Harvest 1	Harvest 2	Harvest 3	Harvest 4	Harvest 5
Control	Control	75.1c	94.3d	43.0e	20.7c	13.7d
CuSO ₄	50	136.8b	147.9c	74.9d	33.8b	16.2dc
	100	254.7a	199.3a	113.8a	32.0b	45.2ab
	200	126.1b	94.7d	79.6d	28.1bc	31.1bc
Cu(OH) ₂	50	143.1b	172.1abc	82.6dc	34.6b	23.7dc
	100	233.9a	193.7ab	106.8ab	44.3a	27.4dc
	200	162.3b	173.2abc	103.0ab	46.9a	57.9a
CuO	50	123.8b	143.8c	77.9d	28.3bc	17.8dc
	100	140.5b	165.6bc	96.3bc	34.1b	26.4dc
	200	171.1b	147.5c	85.8dc	27.1bc	50.6a

* Treatment means followed by the same letter within a column are not significantly different at the 5% level.

4.3.5 Recovery of Cu fertilisers

The apparent percentage recovery of applied Cu fertiliser (R_i) was calculated for each fertiliser Cu source:

$$R_i = \frac{(C_t - C_c)}{C_a} \times 100 \quad (\text{Eq 4.1})$$

Where C_t is the cumulative shoot Cu uptake from a Cu fertiliser treatment with different forms of Cu; C_c is the cumulative shoot Cu uptake from a nil Cu fertiliser; C_a is the rate of Cu applied per pot.

The results indicated the apparent average recovery of Cu is very low, ranging from 0.26 to 1.23 for CuSO₄, from 0.44 to 1.36 for Cu(OH)₂ and from 0.48 to 0.97 for CuO at different Cu levels in harvest 1. The apparent recovery of Cu at the 50 mg Cu kg⁻¹ rate was higher than the 200 mg Cu kg⁻¹ rate of fertiliser application (Figure 4.5). This might be related to the higher DM yield of ryegrass at 50 mg Cu kg⁻¹ level. The Cu(OH)₂ fertiliser showed a higher recovery at 50 and 200 mg Cu kg⁻¹ rates than the other two fertilisers. At 100 mg Cu kg⁻¹ soil level CuSO₄ showed the highest recovery of Cu. The CuO fertiliser resulted in the lowest recovery rate, except at the 200 mg Cu kg⁻¹ soil level, which is due to the lower rate of dissolution. All fertilisers showed a decline in the rate of recovery with time after fertiliser application.

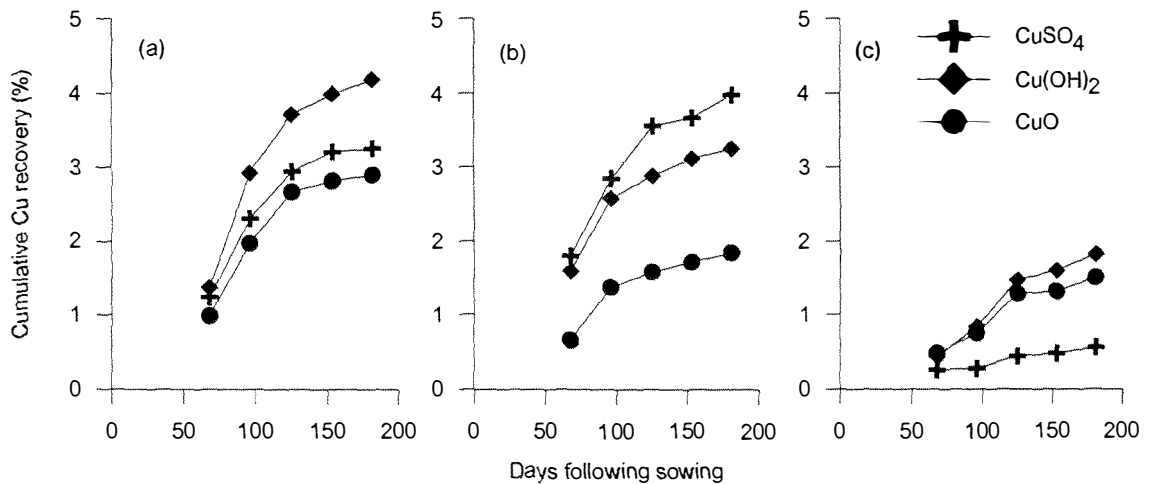


Figure 4.5 Cumulative recovery of Cu at (a) 50 mg Cu kg⁻¹ soil, (b) 100 mg Cu kg⁻¹ soil and (c) 200 mg Cu kg⁻¹ soil of different Cu fertilisers.

The concentration of Cu in plant shoots, as well as absorption by roots, is influenced by soil properties. The transport of Cu from root to shoot, specially in Gramineae is restricted (Jarvis, 1978). This is an important phenomenon resulting in large proportions of the absorbed Cu being immobilised in roots even when plants are suffering from acute Cu deficiency (Jarvis and Robson, 1982). Such restricted transport may be partially responsible for the low recovery in the shoots of Cu added to soils as fertilisers (Gilkes and Sadleir, 1978). When ryegrass was grown under controlled environmental conditions in 21 soils with wide ranging properties, from 49 to 79 percent of the total Cu absorbed was retained in the roots at the last of six harvests of the shoots (Jarvis and Whitehead, 1981). Variation of Cu concentration in the shoots of plants grown in a particular soil between harvests was almost as great as that between plants grown in all soils (Jarvis and Whitehead, 1981). A similar variation of Cu concentration at various harvests was observed in this study. Other studies (Chapter 7) show that the change in Cu concentration has been related to the supply of N or P from the soil. The soil pH and organic matter influenced the variation in Cu concentration in plants with time after fertiliser application, but the major variation in Cu concentration in shoots could often be accounted for by the variation of plant growth and the N and P content of the soil.

4.4 CONCLUSION AND FURTHER STUDY

- Soils have a significant effect on DM yield and on Cu concentration at all harvests.
- High Cu level (200 mg Cu kg⁻¹ soil) caused a significant decrease in dry matter yield, but at levels of 0, 50 and 100 mg Cu kg⁻¹ soil produced similar yields.
- Increasing levels of Cu increased the Cu concentration at all harvests.
- Source of Cu in fertilisers has a significant negative effect on DM yield and a positive effect on Cu concentration at all harvests.
- Copper sulphate showed significantly lower DM yields and significantly higher Cu concentration than the other two fertilisers at harvest 1.
- Copper hydroxide showed significantly higher DM yields, lower Cu concentration and higher Cu uptake than the other two fertilisers.
- Except for harvests 1 and 2, copper oxide resulted in significantly higher Cu concentration at all other harvests.
- Copper concentration and uptake are correlated with soil pH, organic matter and clay content of the soil.
- Copper uptake in ryegrass decreased with time from the first harvest.
- Copper uptake is directly related to DM yield of ryegrass.
- Recovery of Cu was highest at lower levels of fertiliser applied.
- Copper hydroxide demonstrated the highest recovery of Cu than other two fertilisers

Relationships between soil properties and plant Cu concentration will be difficult to formulate until more is known about the fraction of Cu in the soil solution and the extent to which they may be utilised by plants. The results of transformation of Cu and its relationship with soil properties and plant uptake are presented in chapter 5.

CHAPTER 5 **TRANSFORMATION AND PLANT UPTAKE OF COPPER IN SOILS**

5.1 INTRODUCTION

It has been demonstrated in the previous glasshouse trial (Chapter 4), using different sources of Cu as fertilisers, that the availability of Cu to plants declines with increasing time of contact between the soil and Cu fertilisers. The rate of decline in the availability of Cu depends on the soil organic matter, iron and aluminium oxides, which control the availability of Cu in soils. A similar decline in the availability was also reported from a long term Cu fertiliser field experiment (Brennan *et al.*, 1986). This observed decline in Cu availability has been interpreted as resulting from slow reactions, which convert a proportion of applied Cu into forms with lower availability. Knowledge of the Cu distribution in different fractions and the relative availability of these fractions for plant use is fundamental to an understanding of the transformation of Cu in soil. The distribution of nutrient forms in soils is not always experimentally clear, fractionation provides insight into their forms and their availability. The proportions of the different Cu fractions in soils vary considerably, depending on the soil characteristics and the fractionation technique used. The contribution of various soil Cu fractions to plant available Cu in different soils is not well understood.

In this chapter a chemical fractionation scheme have used to examine the different forms of Cu present in soils obtained from the previous glasshouse (Chapter 4) experiment.

Objectives:

The main objectives are as follows:

- To determine the transformation of Cu fertilisers with time, which might account for the changes in Cu availability to plants.
- To evaluate the plant availability of the various fractions of Cu.

In this chapter the distribution of native Cu and Cu added through fertiliser addition is discussed. The effect of Cu sources on the distribution of Cu fractions is also discussed.

5.2 MATERIALS AND METHODS

5.2.1 *Soil sampling from pots*

The soil samples were collected from the previous glasshouse trial pots by using a minicorer at 0, 8, 28, 106 163 and 191 days intervals. The treatments for the glasshouse trial included three Cu sources [CuSO₄, Cu(OH)₂ and CuO], five soils (Manawatu, Tokomaru, Ramiha, Ngamoka and Mangamahu) and four Cu levels (0, 50, 100 and 200 mg kg⁻¹ soil). The soil samples were air dried, ground and passed through 2.00 mm sieve. The soil was analysed for total Cu and various fractions of Cu that included exchangeable Cu, organically bound Cu, oxide bound Cu and residual Cu (Table 3.2).

5.2.2 *0.005 M EDTA-extractable Cu*

Soil samples (10 g) in triplicate were extracted for 16 hrs on an end-over-end shaker with 25 ml 0.005 M EDTA (disodium salt of ethylene diaminetetraacetic acid) in 0.01 M Ca(NO₃)₂. The samples were then centrifuged at 7719 g for 10 minutes and filtered through Whatman No. 42. The Cu concentration in the filtrate was measured by flame atomic absorption spectrophotometry (F-AAS).

5.2.3 *Total soil Cu*

Soil samples (1.0g) in triplicate were used for the determination of total Cu. The original soil samples and residual fractions remaining after the removal of oxide bound Cu (step C; Table 5.1) were used to determine total Cu by wet digestion with a mixture of HNO₃, HClO₄ and HCl at the ratio (by volume) of 5:5:7 (Bolan and Hedley, 1987). The Cu in the filtrate was measured by F-AAS.

5.2.4 *Fractionation of soil Cu*

Fractionation of soil Cu was carried out according to the sequential extraction method described by McLaren and Ritchie (1993). Hydrochloric acid was used instead of hydrofluoric acid on the residual fraction. Details of the methods are described in Section 3.2.6.

5.2.5 *Total Cu of particle size fractions*

The particle size fractionation of the bulk soil was carried out as described in Section 3.2.2. The individual fractions (sand, silt and clay) were digested by wet digestion with a mixture of HNO₃, HClO₄ and HCl at the ratio (by volume) of 5:5:7 (Bolan and Hedley, 1987). The Cu in the filtrate was measured by F-AAS.

5.2.6 *Chemical analysis*

Copper in the soil extracts was analysed using flame atomic absorption spectrophotometry (F-AAS). Details of this technique and other precautions are discussed in section 4.2.6.

5.2.7 *Statistical analysis*

Statistical analysis of the data was carried out by the Statistical Analysis System (SAS). As discussed in section 4.2.7.

5.3 RESULTS AND DISCUSSION

5.3.1 *Recovery of Cu by fractionation*

5.3.1.1 *Recovery of native Cu*

To examine the robustness of the fractionation procedure, the sum of the individual fractions was plotted against the total soil Cu, as measured by tri acid digestion (Figure 5.1). Five soils (Manawatu, Tokomaru, Ramiha, Ngamoka and Mangamahu) were used for the measurement of total Cu and the various fractions of Cu. Results show a satisfactory agreement ($r^2=0.98$) between the total soil Cu values and the sum of the Cu extracted from the individual fractions. The sum of the individual fractions resulted in the recovery of more than 80% total Cu. The soil with a high amount of total Cu ($>30 \text{ mg kg}^{-1}$) resulted in a lesser recovery of Cu in the individual fractions. This may be due to losses during each step of the extraction procedure and / or errors, which occurred during the measurement of the high concentrations of Cu in the F-AAS procedure.

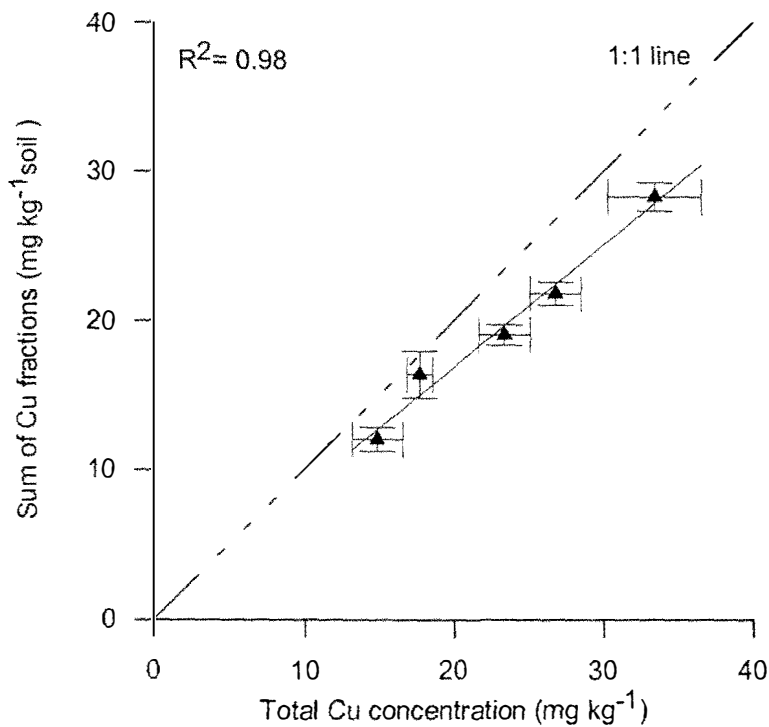


Figure 5.1 Comparison of total native Cu with the sum of individual fractions for each soil. Data are means \pm SE, $n=3$.

5.3.1.2 Recovery of applied Cu during fractionation

The soils treated with Cu from the glasshouse trial (Chapter 4) were used for this fractionation study. Soil samples were collected at 0, 8, 28, 106, 163 and 191 day intervals after fertiliser application. The measured independent total Cu versus sum of Cu recovered from the different fractions is presented in Figure 5.2. A good relationship ($r^2=0.99$) was obtained between the measured independent Cu values and the sum of Cu extracted from the individual fractions. The percentage of total Cu extracted by the individual fractions was more than 80% (Figure 5.2).

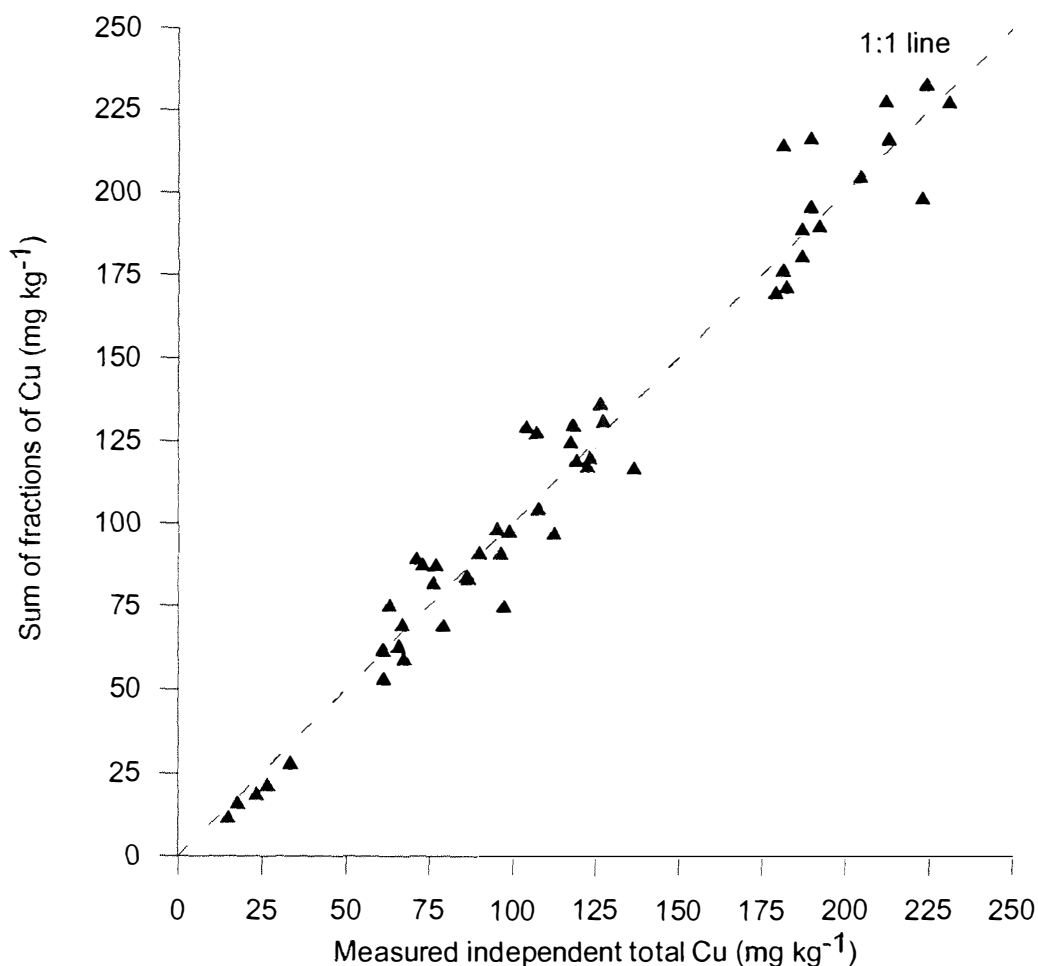


Figure 5.2 Comparison of total applied Cu determined by tri-acid extraction with the sum of individual fractions 28 days after application.

5.3.2 *Distribution of native Cu*

5.3.2.1 *Fractionation of control soil*

The fractionation data for the native Cu, 0.005 M EDTA extractable Cu and the total Cu in the whole soil are presented in Table 5.1. The Manawatu and the Tokomaru soils contained the highest and the lowest concentrations of total native Cu, respectively. The Manawatu and the Mangamahū soils contained the highest and the lowest amounts of 0.005M EDTA extractable Cu, respectively. I observed that the native Cu was present mainly in the residual (43.2%), organic bound (28.5%) and oxide bound (26.0%) fractions (Table 5.1). Only a small proportion of the total Cu was present in the exchangeable fraction (2.2%).

Table 5.1 Fractions of native Cu (mg kg⁻¹) in the whole soil.

Soils name	Exchangeable	Organic	Oxide	Residual	Sum of total	Total	0.005 M EDTA
Manawatu	0.54	5.07	8.44	14.2	28.25	33.39	9.21
Tokomaru	0.31	3.02	2.98	5.68	11.99	14.86	1.64
Ramiha	0.51	7.86	4.97	8.41	21.75	26.75	2.28
Ngamoka	0.33	6.52	5.31	6.85	19.01	23.38	1.38
Mangamahu	0.47	5.27	3.68	6.94	16.36	17.69	1.12

5.3.2.2 *Total Cu in particle size fractions*

The total Cu concentrations in the whole soil and in the particle size fractions of the soils are presented in Table 5.2. Sands contributed the lowest amount of Cu in all soils compared to the silt and clay fractions. Most soils contained higher amounts of Cu in the clay fractions. Exception being the Manawatu and Ramiha soils. Silt contains the highest concentration of Cu in the Manawatu and Ramiha soils. Silt and clay contributed most of the Cu in these five soils. The humus-clay complexes have large specific surface areas and surface charges to which Cu is attracted. The higher contents of Cu in the fine particle fractions compared to those of the bulk soil were due to the larger specific area and surface charge of the fine particles. Shuman (1985) reported that Cu was found mainly in the silt, clay and crystalline Fe oxide fractions of 16 acid topsoils. According to the sum of Cu concentration in the particle size fractions, the Manawatu and the Tokomaru soils resulted in the highest and the lowest amounts of total Cu, respectively. The trend in total Cu concentration in soils is similar to the sequential fractionation of native Cu (Section 5.3.2.1). Stevenson and Fitch (1981) concluded that organic colloids and clays play a major role in Cu retention by soils. In most mineral soils, Cu may be bound as clay-metal-organic complexes, since in these soils, organic matter is intimately bound to clay.

Table 5.2 Concentration of total native Cu in whole soil and particle size fractions.

Soils name	Particle size fractions	% Particle size	Copper concentration (mg kg ⁻¹)	Copper content (mg) in different particle size	Contribution of particle size (%)
Manawatu	Sand	20.34	31.58	6.42	13.20
	Silt	60.64	56.99	34.56	71.03
	Clay	17.82	43.04	7.67	15.76
	Whole soil	98.80	48.65		
Tokomaru	Sand	19.72	8.38	1.65	8.79
	Silt	57.45	14.11	8.11	43.10
	Clay	21.02	43.04	9.05	48.11
	Whole soil	98.19	18.81		
Ramiha	Sand	12.93	16.7	2.16	6.58
	Silt	59.67	29.87	17.82	54.35
	Clay	25.62	50.01	12.81	39.07
	Whole soil	98.22	32.80		
Ngamoka	Sand	19.68	49.7	9.78	23.05
	Silt	54.83	27.42	15.03	35.43
	Clay	25.57	68.9	17.62	41.52
	Whole soil	100.0	42.43		
Mangamahu	Sand	20.37	16.4	3.34	9.70
	Silt	49.7	24.9	12.38	35.94
	Clay	26.23	71.36	18.72	54.36
	Whole soil	96.30	34.43		

5.3.3 *Distribution of applied Cu*

Copper applied to soils reacts with inorganic and organic compounds within the soils (McBride 1981; James and Barrow, 1981). The effect of these reactions on the availability of Cu to plants is not well understood. The effectiveness of a Cu fertiliser applied for promoting growth and supplying Cu to plants is strongly affected by the period of contact between the soil and the Cu fertiliser (Brennan *et al.*, 1980). The mean concentrations of individual fractions in the samples from the pots at each application rate are described in the following sections. The mean recoveries of the applied Cu from the various fractions showed that the oxide and organic fractions are the major fractions holding Cu.

5.3.3.1 *Exchangeable Cu*

The Tokomaru and the Ramiha soils contained the highest and the lowest concentrations of exchangeable Cu, respectively, at the various sampling times and were significantly different from the other soils. This difference in exchangeable Cu is related to the variation in soil organic matter among the soils. The Tokomaru and the Ramiha soils

contained the lowest and the highest organic matter, respectively. The exchangeable Cu increased from fertiliser application till 106 days after fertiliser addition and then decreased gradually in the Ramiha and the Ngamoka soils (Figure 5.3). Generally most incubation studies showed that the concentration of exchangeable Cu in soils decreased with increasing time after fertiliser application. But in this study, the large increases in exchangeable Cu at 106 days might be attributed to high temperature ($\geq 30^{\circ}\text{C}$) in the glasshouse, root development and higher microbial activity in soil. McGrath *et al.* (1988) examined the effect of soil organic matter on the concentrations of Mn, Zn and Cu in soil solution and their extractability from soil. They observed that 0.01M CaCl_2 extractable exchangeable Cu was the smallest fraction, and it decreased with increasing levels of soil organic matter.

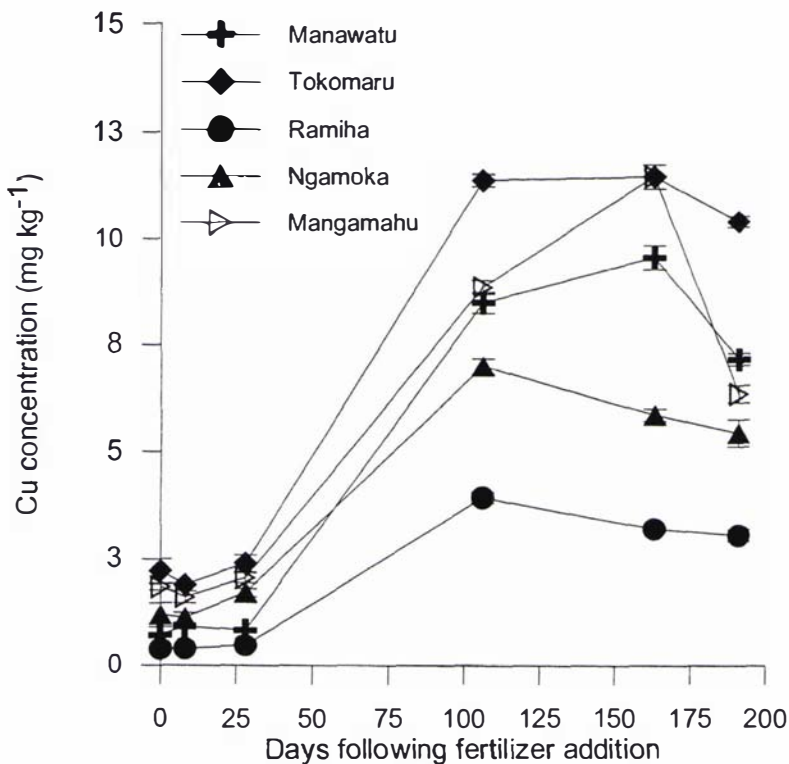


Figure 5.3 Effect of sampling periods on exchangeable Cu concentration (mg kg^{-1}). Data are means \pm SE, $n=12$.

Fertiliser Cu sources have a significant effect on exchangeable Cu measured for 0, 8, 28 and 163 days soil samplings. CuSO_4 fertiliser resulted in the highest exchangeable Cu at the beginning of the experiment due to higher solubility characteristics. CuO fertiliser resulted in the highest concentration of exchangeable Cu for soil samples collected at 106, 163 and 191 days after fertiliser application. The $\text{Cu}(\text{OH})_2$ resulted in the lowest

concentration of exchangeable Cu in soil samples collected at all sampling times. This is due to the lower solubility of $\text{Cu}(\text{OH})_2$. The results presented in Figure 5.4 show that the amount of exchangeable Cu for CuSO_4 , $\text{Cu}(\text{OH})_2$ and CuO fertilisers were 8.1, 6.8 and 8.8 mg kg^{-1} , respectively and constituted 5.4, 6.1 and 5.3% of total Cu in soils at 106 days after fertiliser application.

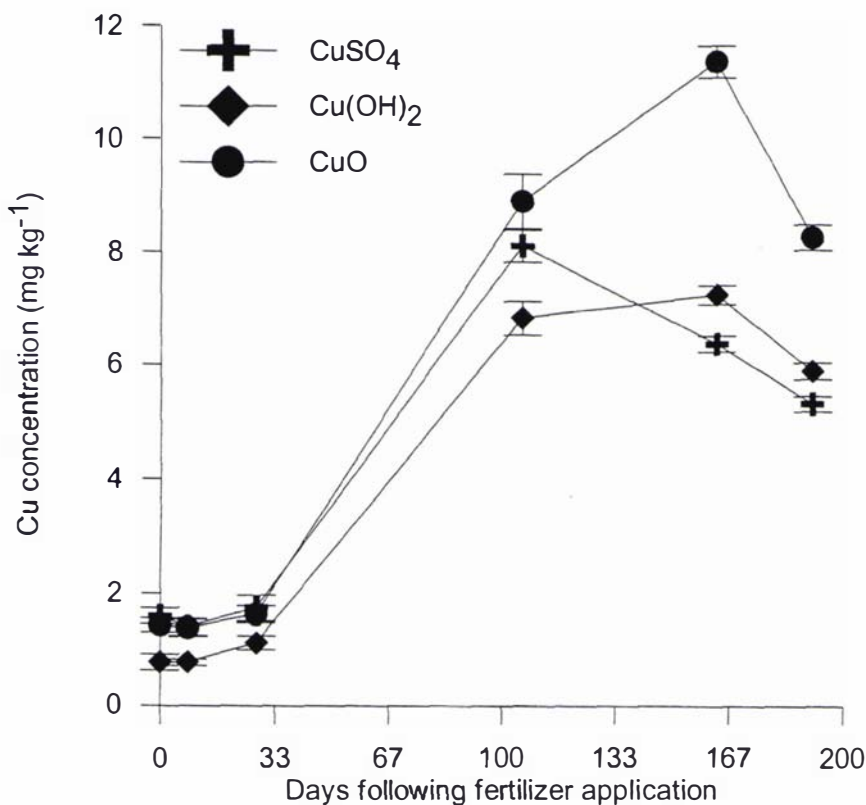


Figure 5.4 Effect of fertilisers on exchangeable Cu concentration (mg kg^{-1}) at various sampling periods. Data are means \pm SE, $n=20$.

The treatment levels had significant effect on exchangeable Cu. High Cu levels ($200 \text{ mg Cu kg}^{-1}$ soil) caused a significant increase in the exchangeable Cu (Figure 5.5). At 106 days, the exchangeable Cu increased sharply due to high temperatures ($\geq 30^\circ\text{C}$) in the glasshouse. The amount of exchangeable Cu for 50, 100, and $200 \text{ mg Cu kg}^{-1}$ soil levels were 2.6, 7.8 and 13.3 mg kg^{-1} , respectively, and constituted 3.6, 5.9 and 6.3% of total Cu in soils at 106 day after fertiliser application. Reddy *et al.* (1981) observed that the concentration of CaCl_2 extractable Cu increased as soil temperature increased from 12 to 22°C . Williams and McLaren (1982) reported that both extractable Cu and soluble organic matter increased when the temperature was raised from 10 to 30°C . They suggested that this extra Cu originated from the organic matter.

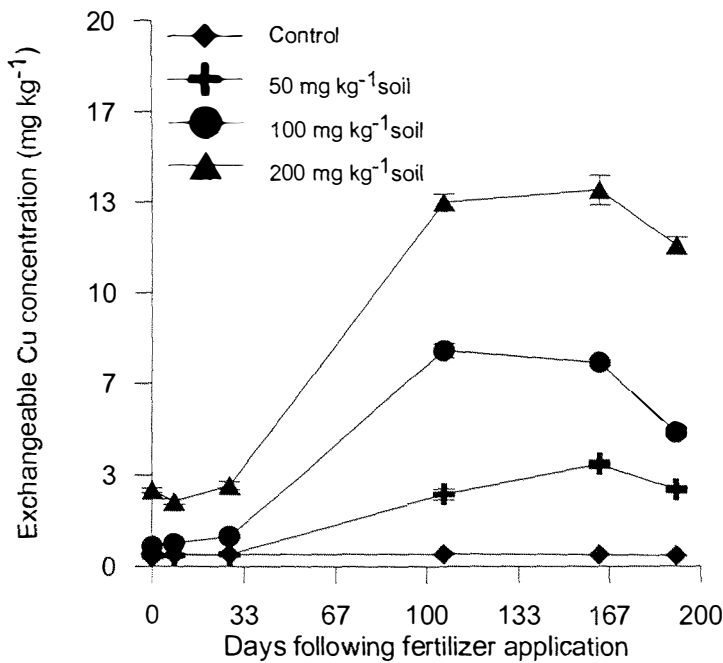


Figure 5.5 Effect of treatment levels on exchangeable Cu concentration (mg kg^{-1}) at various sampling periods. Data are means \pm SE, $n=15$.

5.3.3.2 *Organic bound Cu*

The Ramiha and the Tokomaru soils contained the highest and the lowest concentration of the organically bound Cu fractions, respectively. The organically bound fraction Cu decreased with time after fertiliser application (Table 5.3). The organically bound Cu fraction was often found to be higher in organic soils compared with mineral soils. Since Cu is mostly associated with the organic fraction. It is therefore important that Cu complexing by organic ligands and subsequent release by microbes should be emphasised in chemical fractionation studies (Sims, 1988). Luo and Christie (1996) observed that the addition of Cu to soils significantly increased Cu in the adsorbed fractions and decreased it in the organic fractions. The Ramiha and Ngamoka soils are rich in organic matter compared to the other soils. Cu added to organic soils, or added in sewage sludge or manures will revert to the more insoluble and plant unavailable fractions with time. Cu that is added to peat soils changed to more and more non-reactive forms, causing less replacement of weakly held metals (Mathur and Lévesque, 1988). Cu has a strong ability to bind to organic matter. Chang *et al.* (1984) observed that in untreated soil, Cu was highest in the sulphide residual fraction, but with sewage sludge addition, the Cu level in the carbonate and organic fractions increased more than

in the other fractions. Liang *et al.* (1991) observed that most of the added Cu accumulated in forms strongly bound to sesquioxides, organic matter and clay minerals in twenty-seven Saskatchewan soils. McLaren and Ritchie (1993) reported that organically bound and iron oxide bound Cu fractions accounted for most of the applied Cu, irrespective of the rate of application.

Table 5.3 Effect of soil types on organically bound Cu concentration (mg kg^{-1}) at various sampling periods.

Soils	0 days	8 days	28 days	106 days	163 days	191days
Manawatu	52.49bc	85.06b	76.57a	50.18b	12.88b	6.05b
Tokomaru	40.26c	64.92c	51.61b	20.58c	19.46b	9.22b
Ramiha	77.99a	106.7a	96.76a	81.13a	66.03a	55.11a
Ngamoka	67.3ab	85.66b	95.96a	74.05ab	66.16a	44.12a
Mangamahu	61.59b	81.52bc	88.21a	53.61ab	48.42a	22.70a

* Treatment means followed by the same letter within a column are not significantly different at the 5% level.

Fertiliser caused a significant effect on the organically bound Cu fraction in soil samples collected at different times after fertiliser application (Table 5.4). All the fertilisers increased the organically bound Cu up to 8 days except $\text{Cu}(\text{OH})_2$, then decreased with time after fertiliser application. The results presented in Table 5.4 show that the Cu concentration in the organic fraction for CuSO_4 , $\text{Cu}(\text{OH})_2$ and CuO fertilisers were 58.7, 42.7 and 66.2 mg kg^{-1} , respectively, and constituted 39.4%, 38.0% and 40.1% of the total Cu in the soils, at 106 days after fertiliser application. Stevenson and Fitch (1981) reported that, based on a commonly used soil Cu fractionation scheme, organically bound Cu accounts for 20% to 50% of the total soil Cu. Luo and Christie (1996) observed that the added Cu was present mainly in the adsorbed state (45%), iron-manganese oxide bound (35%), organic matter bound (15%) and residual fraction (10%).

Table 5.4 Effect of fertilisers on organically bound Cu concentration (mg kg^{-1}) at various sampling periods.

Fertilisers	0 days	8 days	28 days	106 days	163 days	191days
CuSO_4	62.05a	84.28b	73.66b	58.77ab	38.01	25.66
Cu(OH)_2	47.28b	64.85c	76.00b	42.76b	39.76	28.10
CuO	70.47a	105.19a	95.80a	66.19a	43.85	34.71

* Treatment means followed by the same letter within a column are not significantly different at the 5% level.

Increasing levels of Cu increased the Cu concentration in the organic fraction of all the soils. At the highest application rate, the added Cu appeared to be distributed evenly between the organic matter and oxide fractions (Figure 5.6). Ma and Uren (1998) reported that the proportions of recently added Cu in extractable fractions decreased markedly with time.

Miller *et al.* (1987) observed that when Cu-enriched manure was added to soils the Cu initially existed in specifically adsorbed forms, but after 12 days, some was found in the Mn and amorphous Fe oxides, indicating a shift away from plant availability. Schalscha *et al.* (1999) reported that added Cu increased exchangeable Cu in soils only slightly, however, significant increases in the carbonate, reducible (associated with oxide form) and oxidisable (bound to organic matter) fractions were observed.

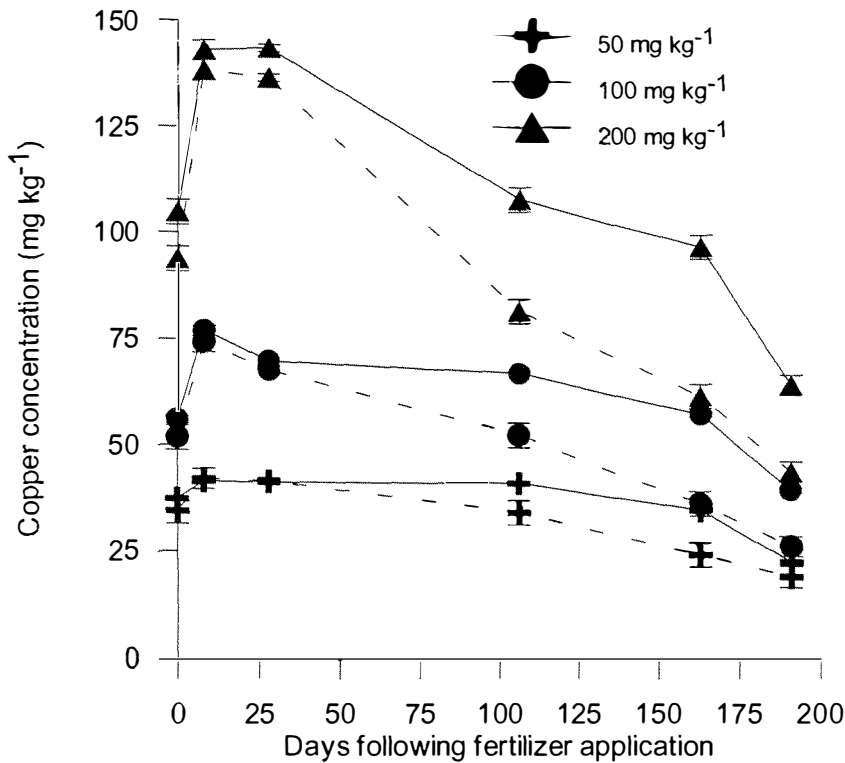


Figure 5.6 Effect of treatment levels on organic bound Cu (---) and oxide bound Cu (—) concentration (mg kg⁻¹) at various sampling periods. Data are means \pm SE, n=15.

5.3.3.3 Oxide bound Cu

The Ramiha and the Tokomaru soils contained the highest and the lowest concentrations of the oxide bound Cu fraction, respectively (Table 5.5). In most of the soils, oxide bound Cu initially increased and then decreased with time after fertiliser application. The observed changes with time in the distribution of applied Cu between fractions are thus most probably a result of a redistribution of Cu from organic sites to sites associated with strongly crystalline iron oxide materials. The organically bound and the oxide bound Cu fraction values were often related to soil organic matter content. An increase in the organic Cu and a decrease in the oxide fraction with organic matter additions have been observed, indicating a possible increase in bioavailability (McGrath *et al.*, 1988). McLaren *et al.* (1983b) were able to induce some redistribution of Cu between components of a humic acid/soil oxide/ montmorillonite system in a laboratory study. The impetus for redistribution of Cu is most likely to be the slow movement of Cu into solid oxide materials. Such movement has been observed by several researchers, and is known to exhibit extremely limited reversibility (Swift and McLaren, 1991).

Such movement is due to diffusion into lattice structures, or by penetration of extremely small pores and inter-particle spaces. The net result is a reduction in the concentration of Cu adsorbed at the surface of the oxide. This, in turn, would lead to the establishment of new equilibria between the soil solution and surface adsorbed forms of Cu including the Cu adsorbed by soil organic matter (McBride, 1991). Increases in the oxidation of organic matter generally resulted in the redistribution of Cu from the exchangeable and organic fractions into the Fe oxide fractions, reducing plant availability (Sims and Patrick, 1978). Cu concentration is usually high in the carbonate fraction of alkaline soils and in the Fe-oxide fraction of acid soils, probably due to occlusion and strong adsorption (Sposito *et al.*, 1982; Kuo *et al.*, 1983; Sims, 1986).

Table 5.5 Effect of soil types on oxide bound Cu concentration (mg kg^{-1}) at various sampling periods.

Soils	0 days	8 days	28 days	106 days	163 days	191days
Manawatu	64.19ab	94.78ab	86.14a	78.82a	54.91bc	35.59b
Tokomaru	54.62b	75.68c	57.2b	43.67b	46.52c	18.03b
Ramiha	76.22a	99.41a	94.2a	85.91a	70.8ab	59.63a
Ngamoka	70.86a	85.51abc	97.99a	86.46a	80.57a	58.77a
Mangamahu	63.97ab	80.02bc	88.35a	63.42ab	60.87bc	37.01b

*Treatment means followed by the same letter within a column are not significantly different at the 5% level.

Fertiliser Cu sources have a significant effect on the oxide bound fraction of Cu. CuO resulted in the highest oxide bound Cu fraction of all the various time samples (Figure 5.7). The amounts of oxide bound Cu for CuSO_4 , $\text{Cu}(\text{OH})_2$ and CuO fertilisers were 75.7, 56.3 and 82.9 mg kg^{-1} , respectively and constituted 50.7%, 50.1% and 50.3% of total Cu in the soils, respectively, at 106 days after fertiliser application. This indicated that the majority of total Cu is held within the oxide mineral structure.

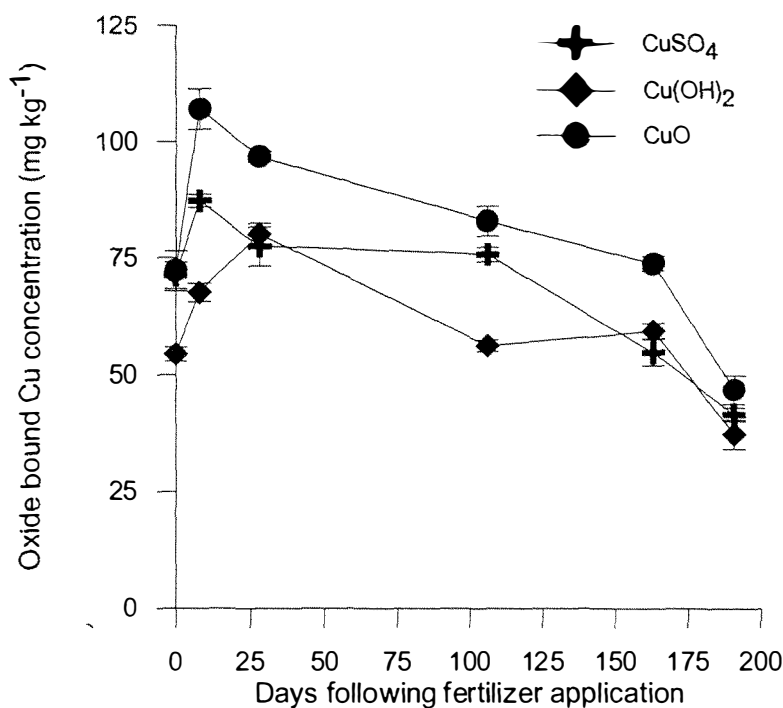


Figure 5.7 Effect of forms of Cu fertilisers on oxide bound Cu concentration (mg kg⁻¹) at various sampling periods. Data are means \pm SE, n=20.

Increasing levels of Cu significantly increased the oxide bound Cu concentration (Figure 5.8). Oxide fractions of Cu increased initially, and then decreased with time after fertiliser application, and the effect was more pronounced at the higher level than at lower level of Cu fertiliser. The adsorption study (Section 3.3.3.4) in the Ramiha, Ngamoka and Mangamahu soils showed that oxide components are also responsible for sorption. Studies of Cu adsorption after selective extraction show that amorphous Fe oxides are more important than organic matter in adsorption especially in mineral soils (Cavallaro and McBride, 1984) and that oxide forms in general and organic coatings have an affinity for metals (Lion *et al.*, 1982).

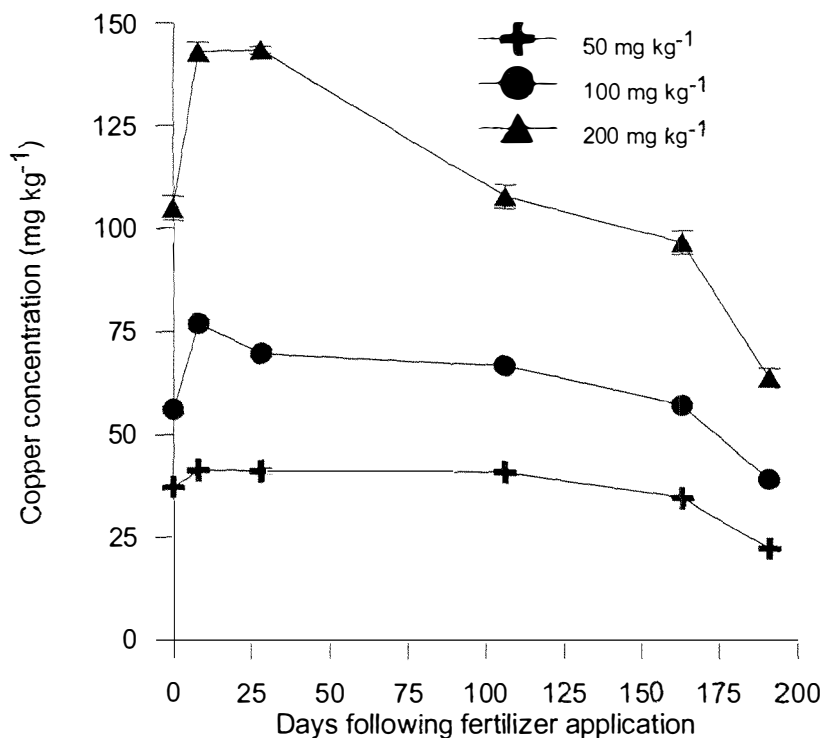


Figure 5.8 Effect of treatment levels on oxide bound Cu concentration (mg kg^{-1}) at various times of soil sampling following fertiliser application. Data are means \pm SE, $n=15$.

5.3.3.4 Residual Cu

Results presented in Table 5.6 indicate that the soils have a significant effect on the residual Cu concentration. The Ramiha and the Tokomaru soils contained the highest and the lowest residual Cu concentrations, respectively. Although it is not immediately available for plant uptake, the residual Cu is a potentially available source for plants. Residual Cu in clay mineral structures can be weathered, thus releasing Cu slowly as a more readily available form during the growing season, resulting in increases in exchangeable Cu.

Table 5.6 Effect of soil types on residual Cu concentration (mg kg^{-1}) at various sampling periods.

Soils	0 days	8 days	28 days	106 days	163 days	191days
Manawatu	6.10ab	5.69cd	6.10	5.12cd	5.34d	6.45c
Tokomaru	2.02c	3.93d	8.32	3.32d	3.24e	3.29d
Ramiha	8.83a	12.69a	8.32	11.25a	11.56a	10.52a
Ngamoka	3.94bc	6.42bc	6.51	6.41bc	6.91c	6.46c
Mangamahu	5.44abc	8.35b	8.30	7.85b	8.41b	9.06b

* Treatment means followed by the same letter within a column are not significantly different at the 5% level.

Copper oxide fertiliser resulted in the highest residual Cu concentration, which was significantly different from the other two fertilisers at 0, 8, and 28 days soil samplings (Figure 5.9). This may be due to the low solubility of CuO. The amounts of residual Cu for CuSO_4 , $\text{Cu}(\text{OH})_2$ and CuO fertilisers were 6.8, 6.6, and 5.2 mg kg^{-1} , respectively, and constituted 4.6, 5.9 and 4.2% of total Cu in the soils, respectively, at 106 days after fertiliser application.

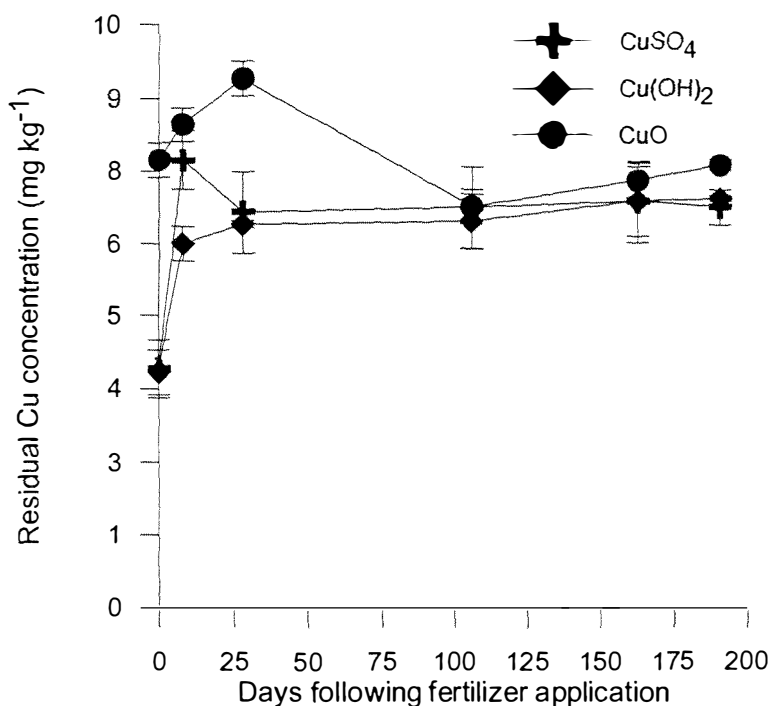


Figure 5.9 Effect of forms of Cu fertilisers on residual Cu concentration (mg kg^{-1}) at various sampling periods. Data are means \pm SE, n=20.

Increasing Cu levels have resulted in a significant increase in the residual Cu concentration (Figure 5.10). The amounts in the residual fraction changed with time after fertiliser application. Since there has been little loss of Cu from the soil during the period of the trial, the implication is that these changes have occurred in the way in which the applied Cu is associated with the various soil constituents. Initially, Cu added to the soil at the two lower rates of application appeared to be mainly organically bound, whereas at the highest application rate, the added Cu distributed evenly between the organic and iron oxide fractions. McLaren and Ritchie (1993) reported that at the lowest Cu application rate, the proportion of applied Cu present in the organically bound fraction decreased markedly during the period of the trial and was mirrored by an increase in the residual Cu fraction.

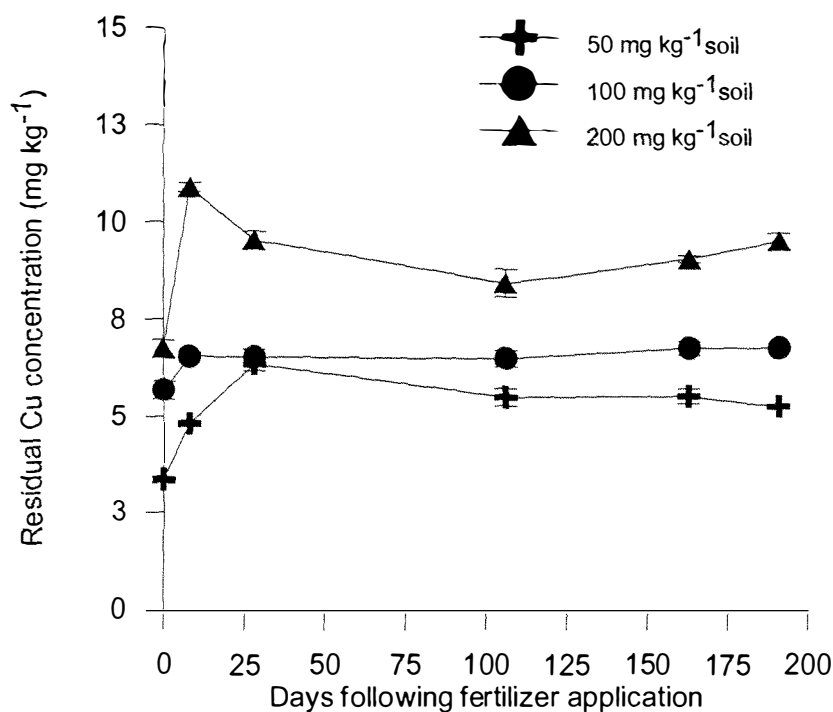


Figure 5.10 Effect of treatment levels on residual Cu concentration (mg kg⁻¹) at various sampling periods following fertiliser application. Data are means \pm SE, n=15.

5.3.4 Total sum of Cu fractions

It was observed that the sum of the fractions of Cu decreased after fertiliser application in the Manawatu and the Tokomaru soil at each level of Cu applied (Figure 5.11), indicating the formation of another pool of tightly bound Cu. This fraction might not

have been extracted by triacid digestion. It is interesting to note that in the native soil, a high proportion (43%) of Cu is associated with the residual fraction (Section 5.3.2.1). The rate of decrease in the sum of fractions varied among the soils, indicating that the build up in the fractions not extracted by triacid digestion is affected by reactions with soil constituents (organic matter and oxides component).

The earlier chapter (Section 4.3.4.1) showed that the availability of Cu from different sources of fertiliser declined with time, which might be due to build up of the residual Cu fractions after fertiliser application.

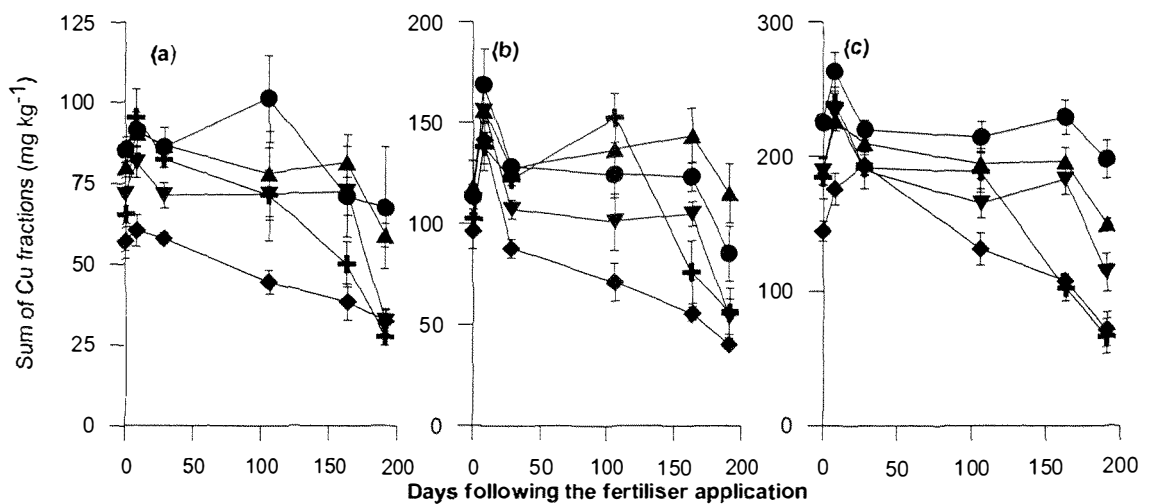


Figure 5.11 Sum of fractions of Cu (mg Cu kg^{-1}) at different levels (a) 50 mg Cu kg^{-1} , (b) $100 \text{ mg Cu kg}^{-1}$, (c) $200 \text{ mg Cu kg}^{-1}$ following the fertiliser application. [(+Manawatu; ◆Tokomaru; ●Ramiha; ▲Ngamoka; and ▼Mangamahu)]. Data are means \pm SE, $n=3$.

5.3.5 Effect of soil properties on fractionation

The mean concentrations of soil Cu present in the different fractions decreased in the order: oxide bound > organic > residual > exchangeable. Limura (1993) examined the distribution of Cu in non-contaminated and contaminated paddy soils irrigated with the river water from mines. He reported an accumulation of Cu in fractions, which reacted with the soil solution (i.e. exchangeable, inorganically and organically bound fractions). Mullins *et al.* (1982a) observed that most of the added Cu was accumulated as organically bound and sesquioxide occluded Cu fractions. In most of the soils in the

present study, exchangeable Cu, organically bound Cu and oxide bound Cu increased initially and then decreased with increasing time after fertiliser application. The amount of organically bound Cu is dependent on the soil organic matter and the maximum amount was obtained in soils with high organic matter levels (Ramiha and Ngamoka). A good correlation between the Cu concentration in the different fractions and soil organic matter, CEC, clay content and soil pH was obtained (Table 5.7). Soil pH showed a negative relationship with organically bound Cu ($r = -0.05$) and residual Cu ($r = -0.21$). An increase in pH has often been shown to cause a redistribution of Cu from the exchangeable and organic fractions to the Mn oxide and Fe oxide fractions (Sims and Patrick, 1978). Thus Cu lost from the exchangeable fractions change into the less soluble and less bio-available fractions. The effects of pH on Cu transformation have been reported by McLaren and Crawford (1973a) and Jarvis (1981b). These workers and our previous study (Section 3.3.3.5) showed that Cu adsorption was related to soil pH, which was primarily attributed to a greater association with soil organic matter and oxides.

Table 5.7 Correlations between soil properties and Cu concentration in the different fractions.

Soil properties	Copper fractions			
	Exchangeable	Organic	Oxide	Residual
pH	0.07	-0.05	0.0004	-0.21*
Organic carbon	-0.07	0.30**	0.17	0.30**
CEC	-0.11	0.33**	0.28*	0.26*
Clay	-0.03	0.20*	0.07	0.27*

** and * denote significance at the 99% and 95% levels, respectively.

Soil organic matter shows a negative relationship with exchangeable Cu ($r = -0.07$) and a significant positive relationship with the organically bound Cu ($r = 0.30$), oxide bound ($r = 0.17$) and residual Cu ($r = 0.30$) fractions (Figure 5.12). Since Cu is associated mostly with organic matter, it could be expected that organic matter additions would cause a redistribution of Cu among the fractions, especially to increase the organic fraction of Cu. However this has not always been found by others (Shuman, 1988b). Elsokkary and Lag (1978) reported that the organic and oxide fractions were related to soil organic matter. A decrease in both the organic and oxide bound Cu fractions found by the

present study indicates a possible decrease in bio-availability of Cu. Iwasaki *et al.* (1997) investigated the depth wise distribution of Cu in selected agricultural soils and in a natural soil, near an abandoned Cu mine. They also observed Cu was mainly adsorbed onto organic matter and / or occluded by oxides and hydroxides of Fe and Mn.

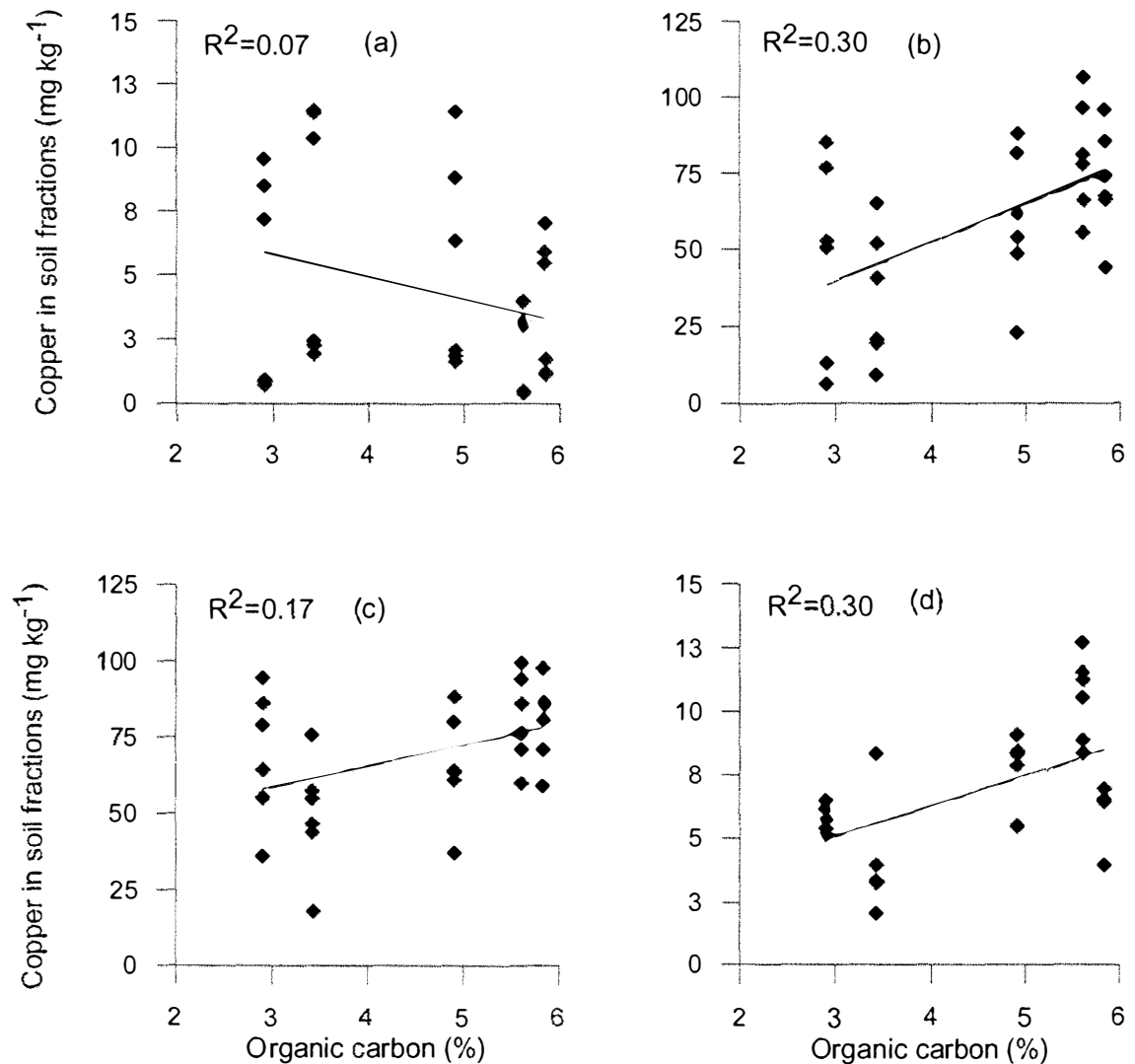


Figure 5.12 Relationship between soil organic carbon and different fractions of Cu: (a) exchangeable Cu; (b) organically bound Cu; (c) oxide bound Cu; and (d) residual Cu.

Clay and CEC correlated with different fractions of soil Cu (Table 5.7). Although clay and CEC had a negative relationship with the exchangeable Cu, these were not significant, but a positive relationship with organically bound Cu, oxide bound Cu and residual Cu (Figure 5.13-14). Clays are the key soil fraction affecting soil Cu

availability in mineral soils. Shuman (1985) observed a correlation coefficient between fractions of Cu and soil properties such as pH, CEC, organic matter, and clay. Shuman (1980) has pointed out that clays are capable of sorbing Cu in excess of CEC. Cu is translocated with clays and tends to be most abundant when the clay content is high (Fagbami *et al.*, 1985). Kabata-Pendias and Pendias (1992) reported that the ability of soil minerals to remove Cu from solution is dependent on the mineral surface charge. The large surface area and the associated high surface charge in the organic-mineral complexes is one of the reasons why the majority of Cu is adsorbed on to the surfaces of humic clay complexes. Iron and manganese oxides have high capacities for specific adsorption of Cu in soils (Jenne, 1968).

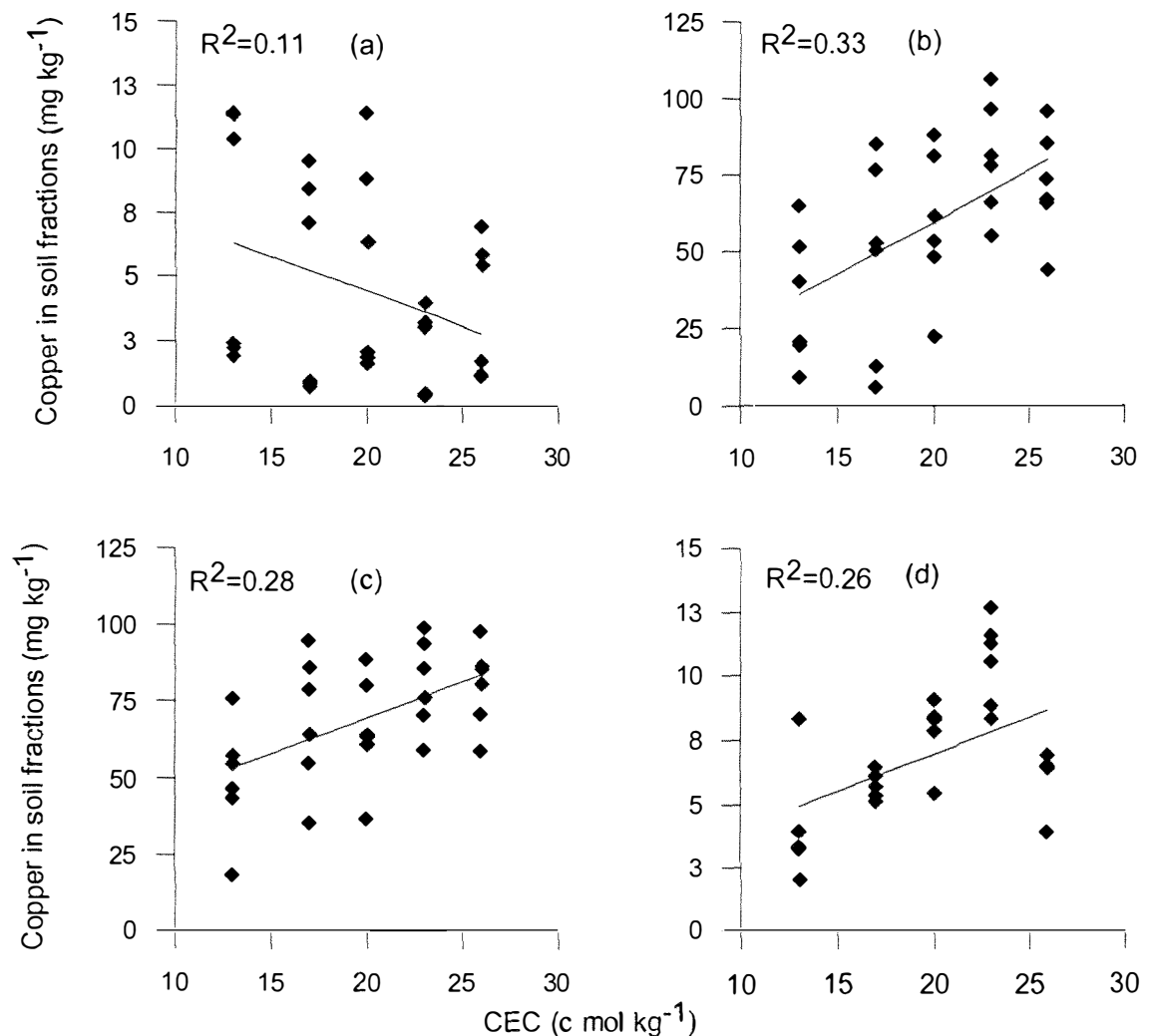


Figure 5.13 Relationship between CEC and different fractions of Cu: (a) exchangeable Cu; (b) organically bound Cu; (c) oxide bound Cu; and (d) residual Cu.

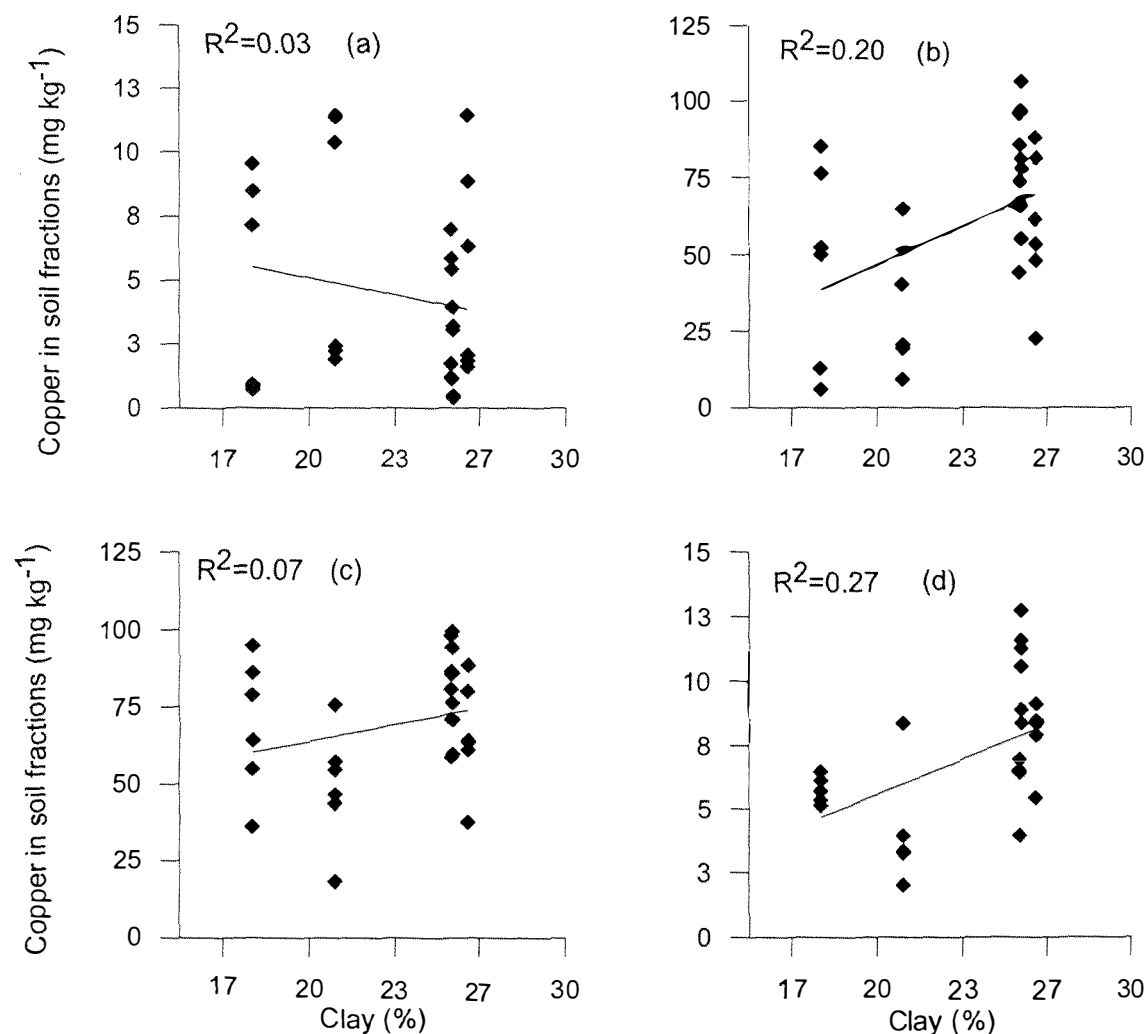


Figure 5.14 Relationship between clay content and different fractions of Cu: (a) exchangeable Cu; (b) organically bound Cu; (c) oxide bound Cu; and (d) residual Cu.

It was not surprising that a large proportion of Cu was present in the oxide fraction with less Cu in the organic matter fraction. This could be explained by humic substances in the soil being dissolved with NaOCl used during the recovery of the organically bound Cu fraction and most were again extracted in the oxide fraction by ammonium oxalate, oxalic and ascorbic acids. This might account for the yellowish brown colour that was observed in the oxide bound fraction extracts. Thus a substantial proportion of the organically complexed forms could well have been included in the oxide fractions. The greatest concentration of Cu was associated in the oxide bound and organically bound fractions, while the lowest concentration of Cu is in the exchangeable form. This suggests that a large proportion of native Cu in the soils is in forms likely to be

unavailable for plant uptake. There is evidence to suggest that the soil test extractants commonly used to determine plant available Cu concentrations also extracted soil exchangeable Cu.

The Cu adsorption study (Chapter 3) appears to support the results of the fractionation studies. Adsorption of Cu appeared to be dominated by organic matter in all the soils and both the organic matter and oxide components contributed to the retention of Cu in the Ramiha, Ngamoka and Mangamahu soils.

5.3.6 *Plant availability to Cu fractions*

Applications of Cu significantly increased Cu concentration in ryegrass. However the effect varied between different soils. The ryegrass grown in the Manawatu silt loam soil resulted in the highest Cu uptake at all harvests, but the grass grown in the Ramiha soil showed the lowest amount of Cu uptake (Chapter 4; Figure 4.3). The difference between these two soils is attributed to the difference in soil pH, organic matter content and the transformation of Cu. The fertiliser types caused a significant effect on Cu uptake in ryegrass. The application of CuSO_4 fertiliser resulted in the lowest Cu uptake (except at harvest 1) (Chapter 4; Figure 4.4). The application of $\text{Cu}(\text{OH})_2$ resulted in the highest Cu uptake by plants. The CuO fertiliser showed the highest amount of organically bound Cu from the 106 days soil sample in the fractionation study but initially the CuSO_4 fertiliser resulted in the highest amount of organically bound Cu. The Cu concentration in plants and plant uptake increased significantly with increasing levels of Cu fertiliser additions in all the soils.

The correlations between the amounts of Cu in the individual fractions of the different soils and the plant Cu uptake are presented in Figure 5.15. Both the organic and oxide bound Cu were correlated with plant Cu uptake. Cu uptake has often been found to be significantly correlated with organic Cu and oxide bound Cu, as well as with exchangeable Cu (Sims, 1986). The role of the oxide bound Cu has generally been discounted in assessing Cu availability (Viets, 1962; McLaren and Crawford, 1973a), but the data from the present study indicate that in these soils it may be a source of available Cu.

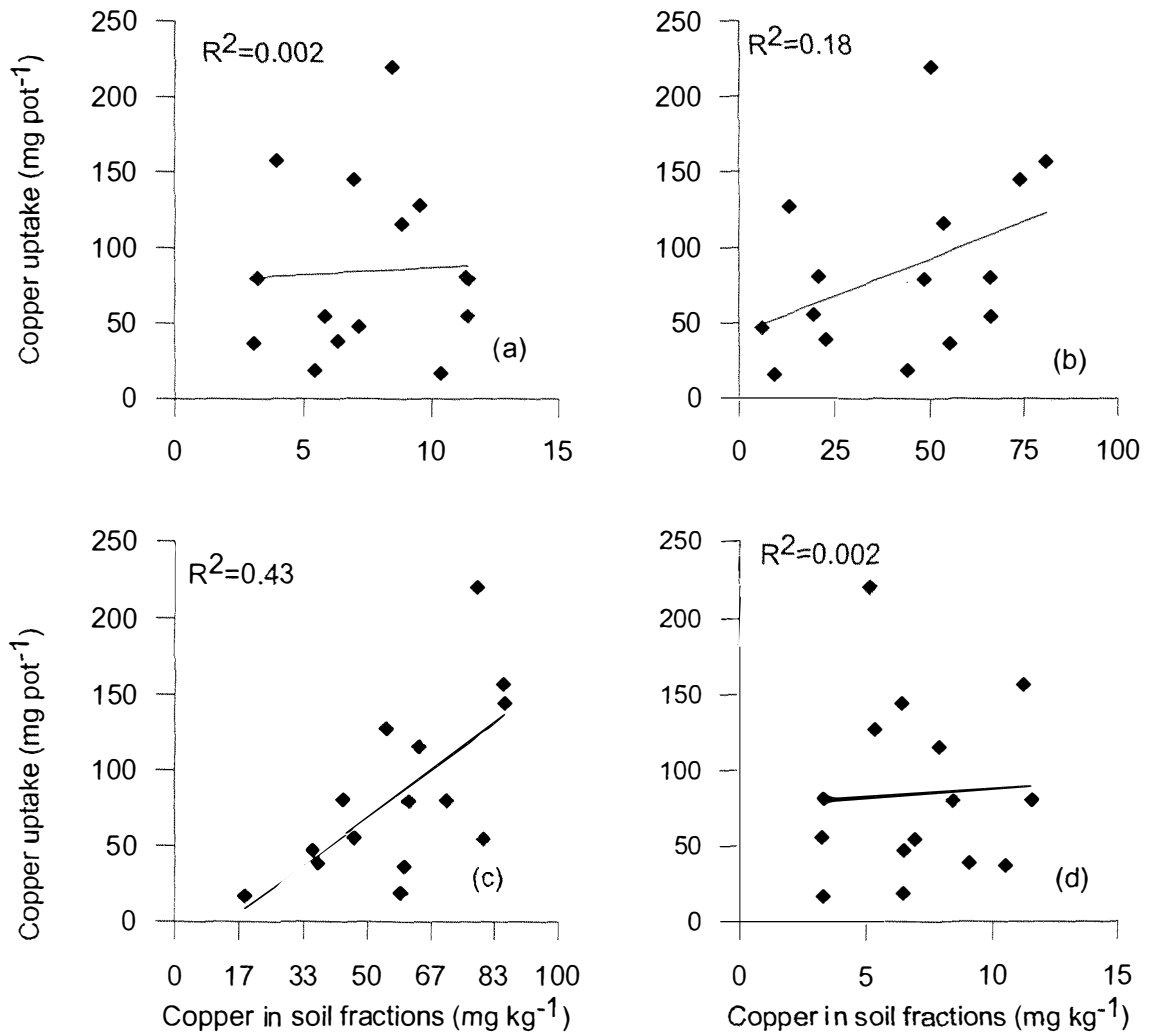


Figure 5.15 Correlation of (a) soil exchangeable Cu, (b) organically bound Cu, (c) oxide bound Cu and (d) residual Cu on plant uptake.

The Cu concentration and Cu uptake by ryegrass varied with the amounts of Cu in the fractions (i.e. organic, oxides, total, EDTA extractable and clay). A significant relationship between EDTA-extractable Cu and plant Cu concentration ($r=0.20$) and Cu uptake ($r=0.18$) was obtained. The sum of the fractions of Cu strongly correlated with the measured total Cu. This indicates that the distribution of Cu in the individual fractions in the soils studied was dependent on the total Cu content of the particular soil. Copper associated with oxides and organic matter is more important to Cu availability than Cu in the other fractions. The changes to applied Cu observed in this study confirmed when compared with, the results of plant Cu uptake carried out using the same soils in chapter 4. In this experiment, the availability of applied Cu for uptake by ryegrass declined with time of contact between the soil and the applied Cu was

observed. Brennan *et al.* (1986) reported that the decline in plant Cu uptake by wheat plants with applied Cu resulted from slow reactions between Cu and the soil in the organic and oxide fractions.

5.4 CONCLUSION AND FURTHER STUDY

- The mean concentrations of Cu present in the different fractions decreased in the order: oxide bound > organic > residual > exchangeable.
- The organically bound Cu fraction was highest in soils with high levels of organic matter.
- The Ramiha and Tokomaru soils contained the highest and the lowest concentrations of organically bound Cu, respectively.
- Both the organically bound and oxide bound Cu fractions decreased with time after fertiliser application.
- The sum of the Cu fractions declined with time indicating the formation of a tightly bound fraction not extractable with triacid digestion.
- Both the organic and oxide bound Cu were strongly correlated with plant Cu uptake.
- The availability of applied Cu for uptake by ryegrass declined with time of contact between the soil and the applied Cu.

The most important application of the fractionation technique is to examine Cu redistribution among the fractions caused by changes in soil properties. Cu tends to shift from less to more soluble fractions in response to decreasing soil pH and organic matter content. Selective sequential extraction techniques for determining Cu forms in soils have not been used extensively. The proportion of Cu that can be extracted by soil test extractants varied with the soil matrix. Soil testing for micronutrients is, in general, a well-established practice, based on fundamental principles of soil chemistry, and verified by field and glasshouse research. The plant responses to Cu are rare and occur in quite specific situations (high organic matter soils). As a result, Cu soil tests have not received much attention, and extractable Cu is measured by the same procedures used for other cations. An attempt was made to identify the various forms of Cu extracted by different soil test reagents. This could help to identify the plant available forms of Cu and to examine the efficiency of various extractants to predict the availability of Cu in pasture soils; these are presented in chapter 6.

CHAPTER 6 SOIL TEST TO PREDICT THE AVAILABILITY OF COPPER

6.1 INTRODUCTION

The use of single chemical extractions in routine soil analysis can be a fast and simple way to evaluate the plant availability of soil nutrients to plants. These have been used, with varying degrees of success, for diagnosing micronutrient deficiency and toxicity in soils. Some of the common extractants used for cations include: Mehlich-1, Mehlich-3, 0.1M HCl, 0.01 M $\text{Ca}(\text{NO}_3)_2$, TEA-DTPA, 0.02 M SrCl_2 , 0.01 M CaCl_2 , 0.1 M NaNO_3 and 1 M NH_4NO_3 salt solution. The TEA-DTPA extraction method, developed by Lindsay and Norvell (1978), seems to be the most appropriate method for extraction of available Cu from soils (Shuman, 1986; Agrawal, 1992; Singh *et al.*, 1994), but other multinutrient extractants such as Morgan, Mehlich-1, Mehlich-3, and AB-DTPA and ammonium acetate (Raij, 1994) have been preferred for use with an Inductively Coupled Plasma-Atomic Emission Spectrophotometry (ICP-AES) technique in routine analysis.

Very few studies have compared the extraction of Cu from soils by soil test extractants, with the actual absorption of Cu by the plants. The Mehlich-1 (M1; 0.05 N HCl and 0.025 N H_2SO_4) method was employed to measure soil micronutrients at a number of Soil Testing Laboratories in the United States (Hanlon and DeVore, 1989). Because M1 was found unsuitable for neutral and alkaline soils, the method was modified as M2 and then as M3 (0.2 M glacial acetic acid, 0.25 M NH_4NO_3 , 0.015 M NH_4F , 0.013 M HNO_3 and 0.001 M EDTA) so that it could be used over a wide range of soils to measure both macro and micronutrients (Mehlich, 1984). Alva (1992) reported a good correlation between the concentrations of Zn, Fe, Mn and Cu extractable by neutral NH_4OAc , M1, M3 and AB-DTPA in soils from citrus groves in Florida. Reed *et al.* (1993) evaluated the Cu availability for corn using the Mehlich-3 reagent and found this procedure very promising in detecting the deficiency and toxicity levels of Cu in soils. On the other hand, Walworth *et al.* (1992) observed that neither the DTPA nor the Mehlich-3 procedures were effective in detecting Cu availability for broccoli and potatoes cultivated in soils from Alaska. Similar results were also observed by Makarim and Cox

(1983) for corn, wheat and soybeans. These studies indicate that the suitability of a chemical extractant to predict the availability of Cu depends on both the soil and the plant species.

As plant available Cu in soils has not been routinely determined in the past, there is no standard soil test for Cu in New Zealand. The extraction procedures are standardised or undergoing standardisation in several European countries. 0.01 M CaCl₂ solution has been recommended as an extractant in The Netherlands (Houba *et al.*, 1990), 0.1 M NaNO₃ solution in Switzerland (VSBo, 1986) and Germany (DIN, 1995) and 1M NH₄NO₃ solution in Germany (DIN, 1995). Correlations between the quantities of metal extracted by these extractants and the metal absorbed by plants have been used to justify the use of these extraction methods (Lebourgh *et al.*, 1996).

The absorption of trace metals by plants depends on their concentration, chemical speciation, and particularly on their activity in the soil solution (Brümmer *et al.*, 1986). Micronutrients in soils generally exist in several forms, including free ions in the soil solution, exchangeable, organic, precipitated and residual. Various sequential extraction procedures have been developed to estimate quantities of micronutrients in each of these forms (Sposito *et al.*, 1983; Shuman, 1985). The forms and proportion of micronutrients that can be extracted by a soil test reagent depend on the type of extractant used. Elucidation of the forms of micronutrients extracted by different soil test reagents could help to identify the plant available form of Cu.

In this study a sequential extraction procedure, described by McLaren and Ritchie (1993), was used to partition Cu into the various fractions (exchangeable, organic, oxide and residual) in two soils, which varied in organic matter content. The concentration of these fractions was also measured, after removal by the three most commonly used soil test extractants (M1, M3 and TEA-DTPA). This chapter will discuss the application of sequential extraction to measure the forms of Cu extracted by various soil tests, these tests are used to predict the plant availability of Cu in soils.

Objectives:

The main objectives are as follows:

- To identify the forms of Cu extracted by different soil tests.

- To evaluate the efficiency of different extraction reagents for the determination of Cu availability in pasture soils.

6.2 MATERIALS AND METHODS

6.2.1 *Soils and soil analysis*

The soil samples were collected immediately after the termination of the glasshouse experiment (Chapter 4), in which the effect of a slow (CuO) and a fast release (CuSO₄) Cu sources, applied at four Cu levels (0, 50, 100 and 200 mg kg⁻¹) to two contrasting soils (Manawatu and Ngamoka) on Cu uptake by ryegrass was examined. The soil samples were air dried, ground and passed through 2 mm sieve. The soil was extracted with various extractants for the measurement of Cu.

6.2.2 *Single soil test extractants*

6.2.2.1 *Methods for M1, M3, 0.1M HCl, and TEA-DTPA extractants*

Soil samples were extracted either by Mehlich-1 (0.05 N HCl and 0.025 N H₂SO₄; Mehlich, 1953), Mehlich-3 (0.2 M glacial acetic acid, 0.25 M NH₄NO₃, 0.015 M NH₄F, 0.013 M HNO₃ and 0.001 M EDTA; Mehlich, 1984), 0.1M HCl (Haynes and Swift, 1985) or TEA-DTPA [0.005 M DTPA, 0.01M CaCl₂ and 0.1M (HOCH₂CH₂)₃N (TEA); adjusted to pH 7.3; Lindsay and Norvell, 1978] reagents. The detail methods for Mehlich-1, Mehlich-3, 0.1M HCl, and DTPA extracting solution are described in Chapter 3 (Sections 3.2.10.2-5). The methods for the other extraction reagents used in this study are presented below.

For each extraction, the soil suspension was shaken for the times as shown in Table 6.1, centrifuged, and the supernatant solution filtered using Whatman filter papers. All extractions were done in duplicate.

Table 6.1 Comparison of methods used in determination of extractable Cu

Sl No	Extractants	Soil weight (g)	Volume ml	Shaker	Shaking time	Centrifuge time /rcf (g)	Whatman No.
1	Mehlich-1	5	20	End-over-end	10 min	3min/3015	40
2	Mehlich-3	2.5	25	End-over-end	10 min	3min/3015	40
3	0.01 M HCl	8	20	End-over-end	2 hrs	3min/3015	42
4	0.01 M Ca(NO ₃) ₂	5	35	End-over-end	24 hrs	5min/7719	42
5	0.005M TEA DTPA	10	20	Horizontal	2 hrs	120 cycles /min	42
6	0.02M SrCl ₂	3	35	Horizontal	30 min	120 cycles /min	42
7	0.01 M CaCl ₂	5	35	End-over-end	2 hrs	5min/3015	42
8	0.1M NaNO ₃	5	35	End-over-end	2 hrs	5min/3015	42
9	1 M NH ₄ NO ₃	5	35	End-over-end	2 hrs	5min/3015	42

6.2.2.2 0.01 M Ca(NO₃)₂ extractant

Known weights (5.0 g) of the finely ground soil were placed into the 50 ml polyethylene centrifuge tubes with 35 ml 0.01M Ca(NO₃)₂. The suspension was then shaken in an end-over-end shaker for 24 hrs at 20⁰ C and then centrifuged for five minutes at 7719 g in the Sorvall RC 5C automatic superspeed refrigerated centrifuge and the supernatant was filtered through Whatman No. 42.

6.2.2.3 0.01 M CaCl₂ extractant

Known weights (5.0 g) of the finely ground soil were placed into the 50 ml polyethylene centrifuge tubes with 35 ml 0.01M CaCl₂. The suspension was then shaken in an end-over-end shaker for 2 hrs at 20⁰ C and then centrifuged for five minutes at 3015 g in the Sorvall RC 5C automatic superspeed refrigerated centrifuge and the supernatant was filtered through Whatman No. 42.

6.2.2.4 *0.1 M NaNO₃ extractant*

Known weights (5.0 g) of the finely ground soil were placed into the 50 ml polyethylene centrifuge tubes with 35 ml 0.1M NaNO₃. The suspension was then shaken in an end-over-end shaker for 2 hrs at 20⁰ C and then centrifuged for five minutes at 3015 g in the Sorvall RC 5C automatic superspeed refrigerated centrifuge and the supernatant was filtered through Whatman No. 42.

6.2.2.5 *1 M NH₄NO₃ extractant*

Known weights (5.0 g) of the finely ground soil were placed into the 50 ml polyethylene centrifuge tubes with 35 ml 1M NH₄NO₃. The suspension was then shaken in an end-over-end shaker for 2 hrs at 20⁰ C and then centrifuged for five minutes at 3015 g in the Sorvall RC 5C automatic superspeed refrigerated centrifuge and the supernatant was filtered through Whatman No. 42.

6.2.2.6 *0.02 M SrCl₂ extractant*

The SrCl₂ extracting solution was prepared to contain 0.02 M SrCl₂ and 0.05M citric acid. Three grams of air dried soil was placed in a 125 ml conical flask, and 35 ml of the SrCl₂ extracting solution was added. Each flask was covered with stretchable Parafilm and secured upright on a horizontal shaker with stroke of 8.0 cm with a speed of 120 cycles/min. After thirty minutes of shaking, the suspensions were filtered by gravity through Whatman No. 42 filter paper. The filtrates were analysed for Cu using atomic absorption spectrophotometry with appropriate standards.

6.2.3 *Fractionation of Cu*

Fractionation of soil Cu was carried out according to the sequential extraction method described by McLaren and Ritchie (1993). Details of the methods are described in section 3.2.6.

6.2.4 *Chemical form study*

The fractionation of Cu was measured for the original soil samples after the termination of the glasshouse study. To investigate the forms of Cu extracted by the single soil test reagents, the sequential extraction scheme described earlier was used to remove the various forms of Cu in four steps. First the whole soil was extracted with the different reagents (step 1). A sample of the original soil was extracted to remove the exchangeable Cu and part of this soil was treated with the 3-soil test extractants (step 2). A sample of the original soil was extracted to remove both exchangeable and organically bound Cu. A part of this sample was extracted with the 3 soil test reagents (step 3). A sample of the original soil was extracted to remove the exchangeable, organically bound and oxide bound Cu fractions. This soil sample was extracted with the 3 soil test reagents (step 4).

The difference in Cu concentration in the soil test extractants between steps 1 and 2 represents exchangeable Cu, between steps 2 and 3 the organic form, between steps 3 and 4 the oxide form and step 4 represents the residual form of Cu in the soil test extractants. All extractions were done in duplicate. The relationship between the concentration of the individual forms of Cu extracted by the sequential extraction procedure, and that extracted by the soil test extractants was examined using the SAS program.

6.2.5 *Speciation of Cu*

The concentrations of both the total Cu and the free ionic Cu were measured in the soil test extractants, and in the various chemical fractions of Cu. The difference between the total and ionic Cu was considered as complexed Cu. The total Cu was measured using AAS and the ionic Cu was measured by using an Orion 9629 cupric ion selective electrode (ISE). The ion selective electrode was calibrated each day with activity buffers. The electrode is known to measure free Cu^{2+} activity reasonably accurately in solutions of chelating organic ligands, although unstable results were observed with highly stable Cu complexes (eg. EDTA, DTPA) (Neshkova and Sheytanov, 1985).

6.2.6 *Chemical analysis*

Copper in the soil extracts was analysed using flame atomic absorption Spectrophotometry (F-AAS). Details of the other procedures are discussed in section 4.2.6.

6.2.7 *Statistical analysis*

Statistical analysis of the data was carried out by the Statistical Analysis System (SAS). Details are discussed in section 4.2.7.

6.3 RESULTS AND DISCUSSION

6.3.1 *Soil characteristics*

Both the soils are slightly acidic with the Ngamoka soil (5.9% organic carbon) containing a two fold higher amount of organic carbon than the Manawatu soil (2.9% organic carbon). The Ngamoka soil also contained a higher amount of Olsen P (124 mg kg⁻¹) than the Manawatu soil (52 mg kg⁻¹). The Manawatu soil is a recent soil, weakly weathered, alluvium, quartzo-feldspathic, mixed greywacke and argillite; the Ngamoka soil is highly weathered with a mineralogy rich in illite, vermiculite and montmorillonite, and a low (<60%) base saturation. The initial soil properties were discussed in Chapter 3 (Section 3.3.2).

6.3.2 *Fractionation of Cu*

The concentration of Cu in individual fractions and the measured total Cu from the soils are presented in the Table 6.2. For both soils, Cu concentration was lower in the exchangeable fraction and higher in the oxide bound fraction. The mean concentrations of Cu present in the different fractions (Table 6.2) decreased in the order: oxide bound > organic > residual > exchangeable. The present study revealed that a substantial proportion (>80%) of the Cu is present in the oxide and organic fractions and only a small amount is present in the exchangeable and residual fractions. Similar results were reported by Liang *et al.* (1991), who observed that most of the added Cu accumulated in forms strongly bound to sesquioxides (26-54%), organic matter (10-36%) and clay

minerals (15-36%) in seven Saskatchewan soils. McLaren and Ritchie (1993) reported that the organically bound and iron oxide bound Cu fractions accounted for most of the applied Cu, irrespective of the rate of application.

The sequential fractionation of the whole soil showed a significant difference in the exchangeable and organic fractions between the soils. Increasing levels of fertiliser addition resulted in a significant increase in the Cu concentration in all fractions. The effect was more pronounced for the exchangeable, organically bound and oxide bound Cu fractions. CuO fertiliser resulted in a higher extraction of exchangeable Cu (Table 6.2) at 295 days after fertiliser application, which was significantly different to the CuSO₄ fertiliser. This may be due to the slow releasing characteristic of CuO fertiliser.

Table 6.2 Effect of treatment combinations on various forms of Cu by sequential fractionation procedure at 295 days after fertiliser application.

Soil	Fertilisers	Levels (mg Cu kg ⁻¹)	<i>Fractions</i>				Sum of Cu fractions (mg kg ⁻¹)	Total Cu (mg kg ⁻¹)
			<i>Exchangeable</i> (mg kg ⁻¹)	<i>Organic</i> (mg kg ⁻¹)	<i>Oxide</i> (mg kg ⁻¹)	<i>Residual</i> (mg kg ⁻¹)		
Manawatu	CuSO ₄	0	0.5	5.1	8.4	14.5	28.5	33.4
		50	2.6	26.6	26.4	6.1	61.8	62.9
		100	4.6	51.2	34.4	6.7	97.0	118.0
		200	6.8	89.9	57.2	6.6	160.5	187.1
	CuO	50	3.3	31.7	37.2	6.1	78.4	86.3
		100	10.6	53.7	45.4	8.2	118.0	146.6
200		23.3	103.4	77.4	8.8	213.0	204.4	
Ngamoka	CuSO ₄	0	0.3	6.5	5.4	6.9	19.1	23.4
		50	1.7	25.4	30.6	3.9	61.6	76.9
		100	2.3	51.6	45.1	4.8	103.8	103.9
		200	6.3	73.7	94.8	7.6	182.5	189.6
	CuO	50	2.3	10.4	38.1	3.2	54.1	60.9
		100	4.4	28.0	72.4	8.2	113.0	107.5
200		18.8	71.6	86.0	11.9	188.4	211.6	

6.3.3 *Soil test extractants (M1, M3 and TEA-DTPA)*

Higher amounts of Cu were extracted by the soil test extractants from the Manawatu soil than the Ngamoka soil (Table 6.3). This may be due to the lower level of organic matter in the former soil. In pasture soils, regular application of Cu fertiliser would result in an increase in extractable Cu. Among the three soil test reagents used, M3 and TEA-DTPA extracted significantly greater amounts of Cu than M1 (Table 6.3). The quantity of Cu extracted by TEA-DTPA, M3 and M1 accounted for 45%, 44%, 39% of the total Cu, respectively, as measured by tri-acid digestion. Zhu and Alva (1993) reported that the amount of Cu extracted by AB-DTPA, M3 and M1 accounted for 38 to 61%, 54 to 66% and 0 to 47% of the total Cu, respectively, for a range of soils. According to Sims (1989), Mehlich-3 extracted 50% more Cu than Mehlich-1.

Garcia *et al.* (1997) compared TEA-DTPA, AB-DTPA, EDTA and M3 in the extraction of Cu, Zn, Mn and Fe; M3 yielded the highest extraction for the four micronutrients. It is possible to infer that the acid condition of M3 could cause the dissolution of these cations from their solid phase increasing their activity in the soil solution. Thus, the formation of metal-chelates is also increased. It was also reported that M3 without EDTA extracted only 36% of the Cu removed by M3+EDTA, illustrating the importance of EDTA (Mehlich, 1984). Results presented in Table 6.3 indicate that TEA-DTPA extracted a higher amount of Cu than the M1 and M3 soil test extractants. The soil with a high level of organic matter (Ngamoka) released significantly less Cu than the soil with a low level of organic matter (Manawatu) to the TEA-DTPA and M3 extractants. TEA-DTPA extracted the highest amounts of both native Cu and Cu added through fertiliser application. Increasing levels of Cu addition resulted in a significant increase in Cu concentration in all soil test extractions. CuO fertiliser accounted for a higher release of Cu than CuSO₄ with all soil test extractants. This may be due to the longer residual effect of this fertiliser.

Table 6.3 Copper concentration in soil test extractants.

Soils	Fertilisers	Treatment levels (mg Cu kg ⁻¹)	Copper concentration in soil tests (mg Cu kg ⁻¹)		
			Mehlich-1	Mehlich-3	TEA-DTPA
Manawatu	CuSO ₄	0	17.7	20.2	21.5
		50	20.2	29.0	26.9
		100	46.5	53.9	59.2
		200	88.9	98.2	107
	CuO	50	30.8	37.3	38.2
		100	79.3	68.5	72.6
200		95.1	108	117	
Ngamoka	CuSO ₄	0	4.50	10.4	8.10
		50	27.3	39.3	42.0
		100	59.7	45.1	44.1
		200	67.9	84.6	90.9
	CuO	50	28.3	20.0	20.0
		100	56.8	59.5	55.3
200		95.7	91.9	95.7	

6.3.4 Extraction of Cu by soil test reagents

6.3.4.1 Effect of soil type on Cu concentration

Results presented in Table 6.4 show that higher amounts of Cu were extracted from the Manawatu soil than the Ngamoka soil. The difference in the amount of Cu extracted between the soils was significant for 0.1M HCl, 0.02M SrCl₂, and 0.1M NaNO₃ solutions. The Manawatu soil contained a lesser amount of organic matter than the Ngamoka soil. With extracting reagents of an acidic nature produced a higher amount of Cu in the Manawatu soil. As a practical test to assess the bioavailability of the metal in soils, extraction with an unbuffered electrolyte solution has been recommended (Novozamsky *et al.*, 1993; Lebourgh *et al.*, 1998). M3 extracted the highest amount of Cu. The mean concentrations of Cu in the different extractants decreased in the order: Mehlich-3 > TEA-DTPA > Mehlich-1 > 0.02M SrCl₂ > 0.1M HCl > 1.0M NH₄NO₃ > 0.01M CaCl₂ > 0.1M NaNO₃ > 0.01M Ca(NO₃)₂.

Table 6.4 Effect of soils and types of fertiliser on extractable soil Cu (mg kg⁻¹).

Extractants	Soils		Fertilisers	
	Manawatu	Ngamoka	CuSO ₄	CuO
Mehlich-1	45.8	32.5	37.5	40.8
Mehlich-3	52.2	41.3	44.0	49.5
0.1M HCl	18.3a	5.9b	11.1	13.1
0.01M Ca(NO ₃) ₂	2.2	2.2	2.1	2.3
TEA-DTPA	48.8	37.1	41.2	44.6
0.02M SrCl ₂	37.1 a	21.9b	27.5	31.5
0.01M CaCl ₂	2.4	2.5	2.5	2.4
0.1M NaNO ₃	2.6a	2.1b	2.3	2.4
1 M NH ₄ NO ₃	4.1	3.9	3.7	4.3

* Treatment means followed by the same letter within a row are not significantly different at the 5% level.

6.3.4.2 *Effect of types of fertiliser*

Copper oxide showed a higher amount of extractable Cu in all extractants except the 0.01M CaCl₂ solutions (Table 6.4). However the difference in the concentration of extractable Cu was not significant. The extraction was carried out after the termination of the glasshouse trial (Chapter 4), the Cu was incorporated into the soil 295 days before starting this study. Higher amounts of extractable Cu at 295 days after fertiliser application reflected the low solubility characteristics of CuO fertiliser.

6.3.4.3 *Effect of level of residual Cu*

Increasing the rate of residual Cu in the soil increased the extractable Cu in all extractants. TEA-DTPA extracted most Cu. The mean concentrations of Cu present in the different extractants followed: TEA-DTPA > Mehlich-3 > Mehlich-1 > 0.02M SrCl₂ > 0.1M HCl > 1.0M NH₄NO₃ > 0.01M CaCl₂ > 0.1M NaNO₃ > 0.01M Ca(NO₃)₂ (Table 6.5). The use of single extraction reagents has some limitations. The results presented in the Table 6.5 show that the extraction reagents 0.02M SrCl₂ and 0.1M HCl also have extracted significant quantities of total Cu as measured by tri acid digestion.

Table 6.5 Effect of Cu levels on extractable soil Cu (mg kg⁻¹).

Treatments (mg kg ⁻¹)	M1	M3	0.1M HCl	0.01M Ca(NO ₃) ₂	TEA-DTPA	0.02M SrCl ₂	0.01M CaCl ₂	0.1M NaNO ₃	1 M NH ₄ NO ₃
0	11.10c	15.30d	2.80b	0.58c	14.80d	7.07c	0.67d	0.84d	0.89d
50	26.65c	31.40c	5.73b	1.18c	31.70c	14.82c	1.41c	1.49c	2.30c
100	60.57b	56.70b	13.64b	2.43b	57.80b	30.23b	2.97b	2.74b	4.36b
200	86.90a	95.80a	26.23a	4.55a	102.70a	65.76a	4.70a	4.28a	8.34a

* Treatment means followed by the same letter within a column are not significantly different at the 5% level.

6.3.5 Speciation of Cu

In this section the speciation of Cu with only for three soil test extractants (M1, M3 and TEA-DTPA) is discussed. In both soils, more than 98% of the Cu extracted by the three soil test extractants was in a complexed form and only a small amount of Cu remained as the ionic form. The Manawatu soil gave a significantly higher concentration of free Cu²⁺ than the Ngamoka soil. Brümmer *et al.* (1986) and McBride (1989) considered that at pH values close to 7, more than 99% of the Cu in solution is complexed by organic matter. Fotovat and Naidu (1997) in their speciation studies of Cu reported that <5 % of the total Cu is present as free Cu in the soil extract. Among the soil test extractants, TEA-DTPA extracted the highest level of complexed Cu. TEA-DTPA being a strong complexing agent not only enhanced the solubility of the complexed Cu, but also reduced the resorption of Cu during extraction due to surface coordination.

Among the soil test extractants, TEA-DTPA produced a significantly lower amount of free Cu²⁺ (Table 6.6). All extractants showed a significant increase in free Cu²⁺ with increasing levels of fertiliser addition. The acid condition of M1 and M3 could cause the dissolution of Cu²⁺ from the solid phase enhancing the concentration in the soil solution. According to Baker and Senft (1995), in acid conditions, the main form is free Cu²⁺, and CuOH⁺, CuCO₃ and Cu(OH)₂ occur in alkaline soil solutions.

Wu *et al.* (2000) observed that the activity of the Cu²⁺ ion in soils fell in the range of 10.9×10⁻¹¹ to 4.0×10⁻⁹ M, which accounted for 0.1-7.8% of the dissolved Cu. In saturated soil extracts, the concentration of dissolved Cu varied from 4.78×10⁻⁸ to 11.4×10⁻⁸ M, which constituted 0.002 to 0.008% of total soil Cu. The dominant species

of Cu in soil solution are the Cu^{2+} and CuOH^+ ions, which are about 10 times more common than CuHCO_3^+ . They found that the activity of Cu^{2+} was governed significantly by the factors of soil pH, Ca^{2+} concentration, CEC and DOC. The highly significant correlation coefficient between any two of Cu^{2+} activity, pH, and DOC, implying that the concentration of free Cu^{2+} ions in soil system was strongly pH dependent. This is due to the strong complexing of Cu by organic matter, and also due to the high pH (7.3) value of the solution. McBride *et al.* (1998) concluded that EDTA, while dissolving and dispersing substantial amounts of high molecular weight organic matter, also displaces most of the bound Cu^{2+} from organic complexes by chelating the metal. The high efficiency of Cu extraction by EDTA must be attributed in part to the disruption of organic aggregation structures by the chelating agent, thereby making surface bound and occluded Cu accessible to extraction. The use of extraction with chelating agents may overestimate the bioavailability of trace elements in soils, where organic matter limits the activity of trace metals.

There was no significant difference in free Cu^{2+} measured in the extractant of the exchangeable and organically bound Cu fractions. Among the chemical fractions of Cu, the exchangeable Cu fraction maintained a higher concentration of free ionic Cu^{2+} than the other chemical fractions (Table 6.7). This is expected because the Ca^{2+} competes more strongly with the exchangeable fraction [extracted with 0.01M $\text{Ca}(\text{NO}_3)_2$] and it represents the ionic species of Cu adsorbed to cation exchange sites (Temminghoff *et al.*, 1994).

The Manawatu soil showed a higher amount of free Cu^{2+} both in the M1 and M3 extractants, but the Ngamoka soil contained a higher amount of free Cu^{2+} in the TEA-DTPA extractant for the organic fractions of Cu. A higher concentration of free Cu^{2+} was measured in the M3 extractant in soils with or without the addition of Cu. Increasing levels of Cu addition resulted in a significant increase in free Cu^{2+} in all the soil test extractants for exchangeable and organically bound Cu fractions.

Table 6.6 Copper species in soil test extractants.

Soils	Fertilisers	Treatment levels (mg Cu kg ⁻¹ soil)	Copper concentration in soil tests					
			Mehlich-1		Mehlich-3		TEA-DTPA	
			Free Cu (mg L ⁻¹)	Complexed Cu (%)	Free Cu (mg L ⁻¹)	Complexed Cu (%)	Free Cu (mg L ⁻¹)	Complexed Cu (%)
Manawatu	CuSO ₄	0	0.02	99.8	0.08	99.5	0.01	99.9
		50	0.03	99.8	0.10	99.6	0.02	99.9
		100	0.03	99.9	0.13	99.7	0.02	99.9
		200	0.04	99.9	0.15	99.8	0.03	99.9
	CuO	50	0.03	99.9	0.11	99.6	0.02	99.9
		100	0.03	99.9	0.13	99.8	0.02	99.9
200		0.04	99.9	0.16	99.8	0.03	99.9	
Ngamoka	CuSO ₄	0	0.01	99.7	0.04	99.5	0.01	99.8
		50	0.02	99.9	0.08	99.7	0.01	99.9
		100	0.02	99.9	0.11	99.6	0.02	99.9
		200	0.03	99.9	0.13	99.8	0.02	99.9
	CuO	50	0.02	99.9	0.09	99.5	0.01	99.9
		100	0.02	99.9	0.12	99.7	0.01	99.9
200		0.03	99.9	0.15	99.8	0.02	99.9	

Table 6.7 Copper species in chemical fractions.

Soils	Fertiliser	Copper concentration in soil fractions								
		Exchangeable		Organic bound Cu						
		Free Cu (mg L ⁻¹)	Complexed Cu (%)	M1		M3		TEA-DTPA		
				Free Cu (mg L ⁻¹)	Complexed Cu (%)	Free Cu (mg L ⁻¹)	Complexed Cu (%)	Free Cu (mg L ⁻¹)	Complexed Cu (%)	
Manawatu	CuSO ₄	0.02	99.1	0.02	99.9	0.01	99.9	0.02	99.9	
		0.04	98.4	0.02	99.9	0.02	99.9	0.03	99.8	
		0.07	98.4	0.02	99.9	0.03	99.9	0.04	99.9	
		0.11	98.4	0.02	99.9	0.04	99.9	0.04	99.9	
	CuO	0.03	99.1	0.02	99.9	0.02	99.9	0.04	99.8	
		0.08	99.2	0.02	99.9	0.02	99.9	0.05	99.9	
0.10		99.5	0.03	99.9	0.04	99.9	0.05	99.9		
Ngamoka	CuSO ₄	0.02	98.5	0.02	99.7	0.01	99.8	0.02	99.6	
		0.03	98.1	0.02	99.9	0.01	99.9	0.03	99.8	
		0.06	97.3	0.02	99.9	0.02	99.9	0.05	99.9	
		0.10	98.4	0.02	99.9	0.04	99.9	0.05	99.9	
	CuO	0.03	98.6	0.02	99.8	0.02	99.8	0.04	99.6	
		0.08	98.2	0.02	99.9	0.02	99.9	0.05	99.8	
0.10		99.4	0.03	99.9	0.04	99.9	0.06	99.9		

6.3.6 *Ratios of fractions in the soil test extractants*

The amounts of Cu extracted by M1, M3 and TEA-DTPA from the whole soil and the percentage of different Cu fractions in these soil test extractants are presented in Table 6.8. M3 extracted a higher amount of exchangeable Cu than M1 and TEA-DTPA. The M3 and TEA-DTPA removed higher amounts of organically bound Cu than M1. Again M1 extracted a higher amount of oxide bound Cu than M3 and TEA-DTPA. TEA-DTPA extracted higher amounts of residual Cu. The ratios of exchangeable, organic, oxide bound and residual Cu fractions in the different extractants were 1:16:12:4, 1:11:6:2 and 1:55:31:13 for M1, M3 and TEA-DTPA, respectively. It clearly indicates that TEA-DTPA extracted more of the organic form of Cu than M1 and M3. Cu treatment levels have no significant effect on the ratios of the Cu fractions removed by the soil test extractants.

The ratios of exchangeable, organic, oxide bound and residual forms of Cu in M1, M3 and TEA-DTPA for the Manawatu soil were 1:20:25:4, 1:15:8:2 and 1:56:35:8, respectively, and the values for the Ngamoka soil were 1:14:6:4, 1:9:5:2 and 1:55:26:17, respectively.

The ratios of exchangeable, organic, oxide bound and residual forms of Cu in M1, M3 and TEA-DTPA are 1:12:9:2, 1:12:5:2 and 1:60:28:10, respectively for CuSO₄ and 1:15:12:4, 1:8:6:0.16 and 1:51:33:15, respectively, for the CuO fertiliser. The ratio of the different fractions for M3 was closer to that for M1. Again TEA-DTPA showed a higher amount of Cu in the organic fractions compared with M1 and M3. This can be explained by the fact that the solution contains a complexing agent that extracts the Cu complexed with organic matter, an important pool of soil Cu. CuSO₄ showed more organic form Cu than the CuO fertiliser, which may be due to quicker releasing and complex formation of CuSO₄ with organic matter.

The ratios of different forms of Cu strongly suggest that the Cu is residing in soils mainly in the organic form and decreased in order: organic > oxide > residual > exchangeable. This result strongly supports the adsorption study (Section 3.3.3.4), where Cu retained on organic components in all soils and on both the organic and oxide components for Ramiha, Ngamoka and Mangamahu soils.

Table 6.8 Copper fractions in the soil test extractants.

Soils	Fertilisers	Treatment levels (mg Cu kg ⁻¹ soil)	Mehlich 1					Mehlich 3					TEA-DTPA				
			Whole soil (mg kg ⁻¹)	Different fractions (%)				Whole soil (mg kg ⁻¹)	Different fractions (%)				Whole soil (mg kg ⁻¹)	Different fractions (%)			
				Exchan-geable	Organic	Oxide	Residual		Exchan-geable	Organic	Oxide	Residual		Exchan-geable	Organic	Oxide	Residual
Manawatu	CuSO ₄	0	17.7	1.86	28.8	52.8	16.6	20.2	4.55	57.9	25.7	11.8	21.5	0.98	49.7	35.3	13.9
		50	20.2	2.82	36.1	54.4	6.53	29.0	4.14	69.6	20.0	6.17	26.9	1.08	52.4	38.2	8.5
		100	46.5	2.54	47.5	45.1	4.90	53.9	4.19	64.7	27.6	3.36	59.2	0.91	63.0	32.0	4.0
		200	88.9	2.68	58.2	34.0	4.98	98.2	5.24	68.4	22.6	3.76	107.2	1.76	66.3	26.2	5.7
	CuO	50	30.8	2.05	31.4	54.5	11.8	37.3	3.57	57.9	29.7	8.74	38.2	0.73	61.2	31.1	6.96
		100	79.3	1.90	41.6	52.5	3.93	68.5	4.45	36.0	55.3	4.19	72.6	0.95	49.1	45.3	4.59
200		95.1	2.31	49.1	45.7	3.32	108.5	3.99	56.4	36.4	3.19	117	1.47	54.2	42.1	2.11	
Ngamoka	CuSO ₄	0	4.5	9.78	57.7	4.44	27.1	10.4	8.65	16.3	65.9	9.0	8.1	2.35	57.2	24.8	15.4
		50	27.3	3.74	58.2	33.7	4.3	39.3	5.11	74.8	5.9	14.1	42.0	0.83	69.2	25.0	4.8
		100	59.7	2.48	60.6	24.6	12.1	45.1	5.10	77.8	3.0	14.0	44.1	1.54	60.7	14.7	23.1
		200	67.9	3.64	50.0	40.6	5.66	84.6	5.84	65.1	24.5	4.4	90.9	1.24	60.8	31.6	6.1
	CuO	50	28.3	1.91	72.7	6.8	18.5	20.0	5.25	65.5	6.2	23.3	20.0	1.10	40.5	22.4	36.0
		100	56.8	2.94	39.9	36.0	21.1	59.5	5.90	49.9	26.8	17.1	55.3	1.46	47.7	24.4	26.4
200		95.7	3.07	44.6	43.3	8.9	91.9	6.43	53.1	33.3	7.1	95.7	1.49	49.5	38.5	10.4	

6.3.7 *Chemical forms in the soil test extractants*

The quantities of exchangeable, organic, oxide and residual forms of Cu extracted by M1, M3 and TEA-DTPA were measured at each fractionation step. The soil test extraction of the whole soil showed significant differences in the organic fractions between soils. The results indicated that the organically bound Cu fractions are the major form extracted by the three soil test extractants. M1, M3 and TEA-DTPA extracted 54%, 66% and 69% of the organic bound Cu. The corresponding values for oxide bound are 41%, 32% and 38% and for exchangeable Cu are 22%, 45% and 26%, respectively. A substantial amount of exchangeable Cu is removed by plants, as measured by the soil test extractants. This is due to high temperatures during the growth periods in the glasshouse. This suggests that residual Cu in clay mineral structures can be weathered and released slowly as more readily available forms of Cu during the growing season, resulting in increases in exchangeable Cu. It also suggests that as the amount of Cu loading in soils is increased, more Cu is associated with non-residual fractions, which increases the potential for mobility and bioavailability of Cu.

All the three soil test extractants removed significantly higher amounts of oxide bound Cu from the Manawatu soil than the Ngamoka soil. It was also observed that TEA-DTPA extracted the highest amount of native Cu. The extraction capacities of the reagents tested are in agreement with the extraction mechanism proposed for each method, and with the chemical forms of Cu in the soil. Dilute acids, such as those employed by the M1 procedure, apparently induce only a partial solubilisation of the Cu containing solids. However chelating agents, such as DTPA or EDTA, reduce the Cu activity in the solution by forming complexes, thus promoting further dissolution of the solid labile forms of Cu. As organic matter may keep up to 45% of the total Cu complexed in the soil (McLaren and Crawford, 1973a; Haynes and Swift, 1984), it is reasonable to assume that the methods which show a preference for extracting organically bound Cu present a greater extraction capacity than the dilute acids (M1). Increasing levels of Cu through fertiliser application significantly increased the concentration of Cu in all forms (except the residual form) extracted by the soil test extractants (Figure 6.1).

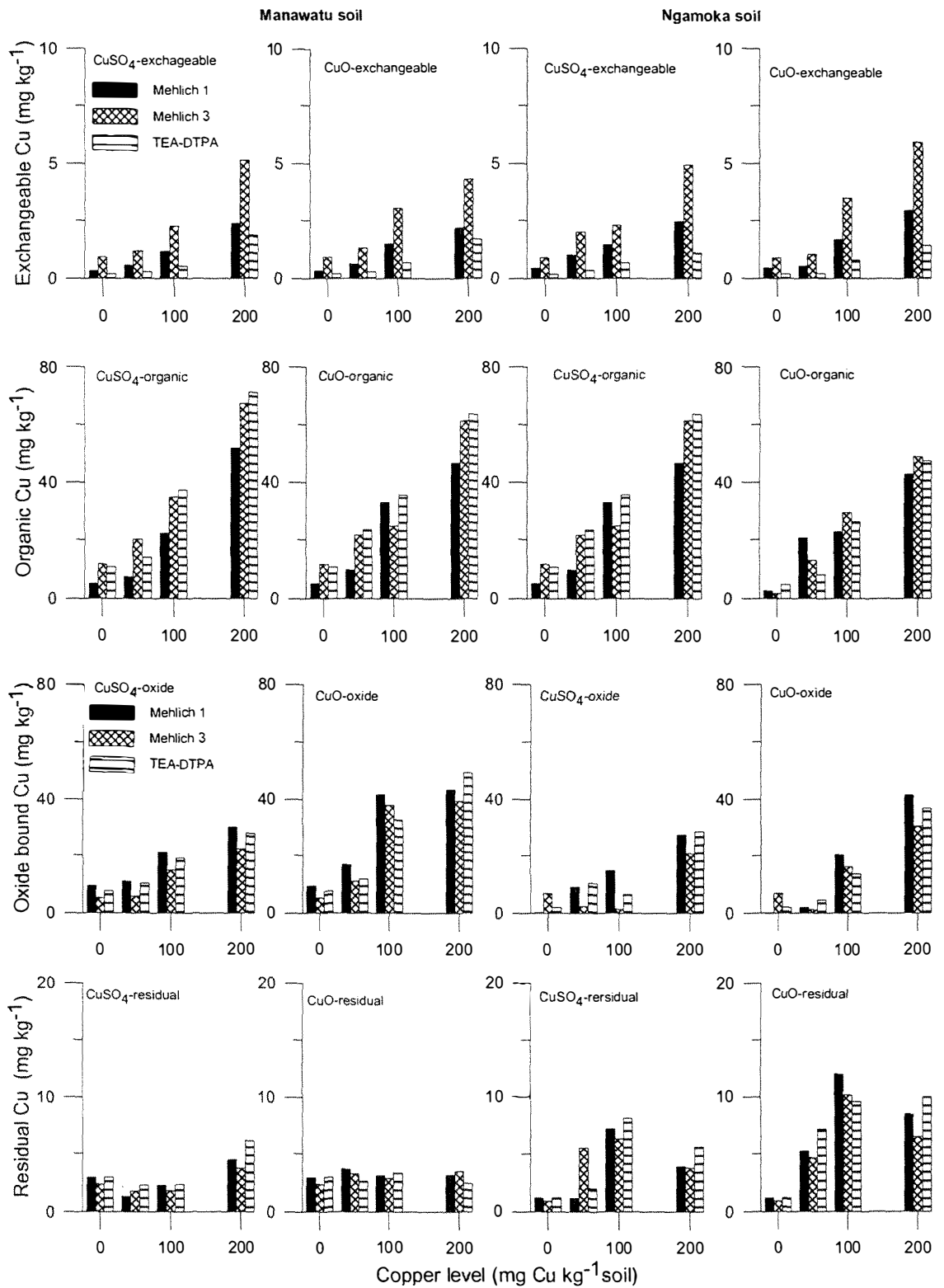


Figure 6.1 Concentration of different fractions of Cu extracted by soil tests at different levels of Cu application.

CuO fertiliser resulted in significantly higher concentrations of oxide bound Cu extracted by the soil test extractants than the CuSO₄ fertiliser. The effect of fertiliser

type on Cu concentration was more pronounced for the organic bound Cu than the other chemical forms.

As expected a low percentage of organically bound Cu was extracted by the soil test reagents from the soil with a low level of organic matter (Manawatu) than from the soil with a high level of organic matter (Ngamoka). The M1, M3 and DTPA extracted 44%, 56% and 65% of organically bound Cu from the Manawatu soil as compared to 63%, 77% and 72%, respectively, from the Ngamoka soil (Figure 6.2).

The major form of Cu extracted by these three soil test extractants was the organic form, followed by the oxide and residual forms for the Manawatu soil, but the Ngamoka soil resulted in the residual form followed by the organic and oxide forms (Figure 6.2). Among the three soil test extractants, > 45% of the exchangeable Cu was extracted by M3, while an average of 24% was extracted by TEA-DTPA and M1. M3 and TEA-DTPA extractants removed more of the organic form of Cu than M1. An average of > 67% of the total organic form of Cu was extracted by M3 and TEA-DTPA and the corresponding value for M1 was 54%. More than 32% of the oxide form of Cu was extracted by M3, while an average 40% of the oxide form Cu was extracted by TEA-DTPA and M1 (Figure 6.2). A considerable amount (>63%) of the residual form of Cu was also extracted by M1 and M3, which was less than that extracted by TEA-DTPA (72%). Similar results were reported by Zhu and Alva (1993) who found 62% and 33% of the total organically bound Cu was extracted by M3 and M1. Both extractants removed >30% of the precipitated form of Cu and 62% of the residual form of Cu.

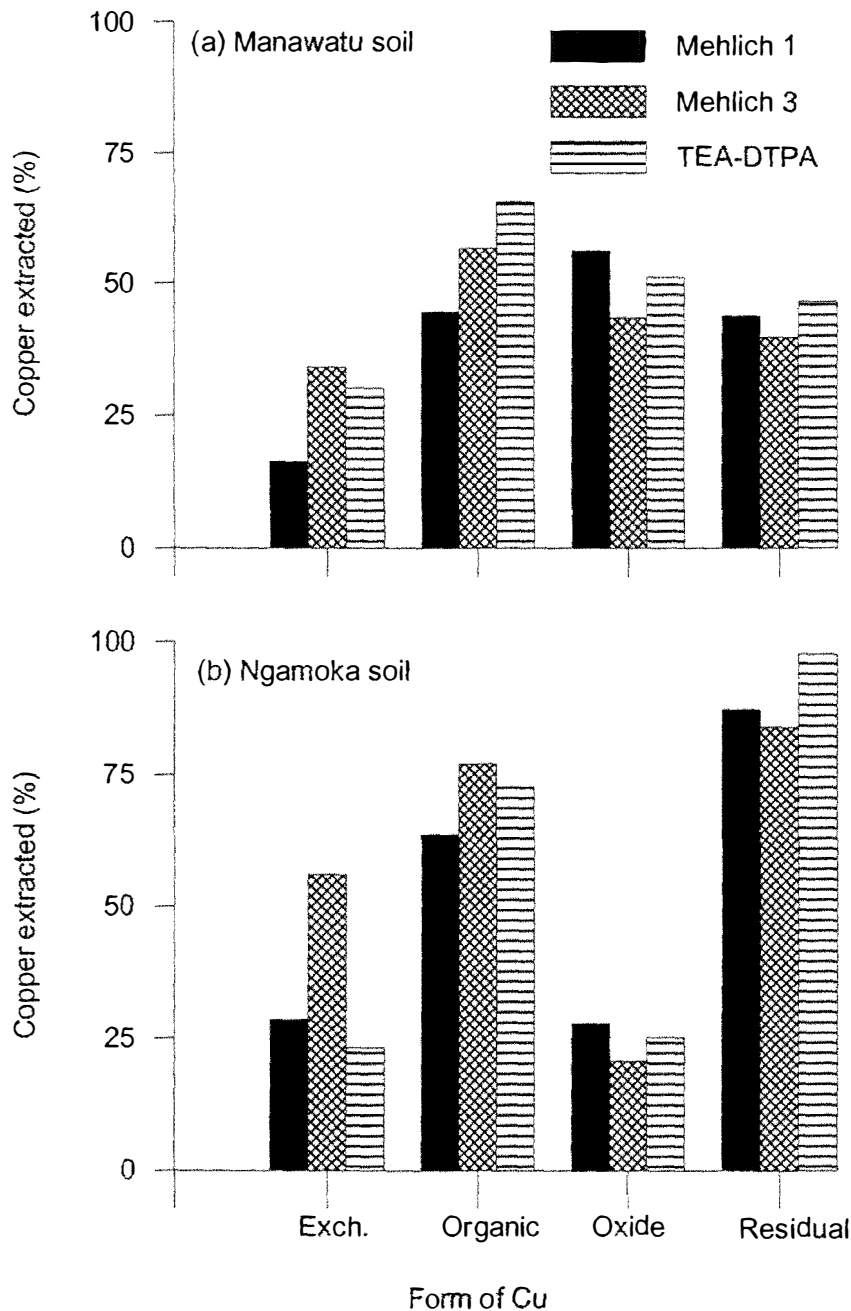


Figure 6.2 Percentage of different fractions of Cu (a) Manawatu soil and (b) Ngamoka soil extracted by the soil test extractants.

According to the regression analysis presented in Figure 6.3, Cu extracted by TEA-DTPA, M3 and M1 was strongly correlated with the exchangeable, organic and oxide fractions of the sequential extraction. Zhu and Alva (1993) reported significant linear correlation between Cu extractable by sequential fractions (exchangeable, organic and precipitated) and Cu extracted by M1, M3 and AB-DTPA. Exchangeable Cu and organically bound Cu have been suggested as the plant available forms (McLaren and Crawford, 1973a).

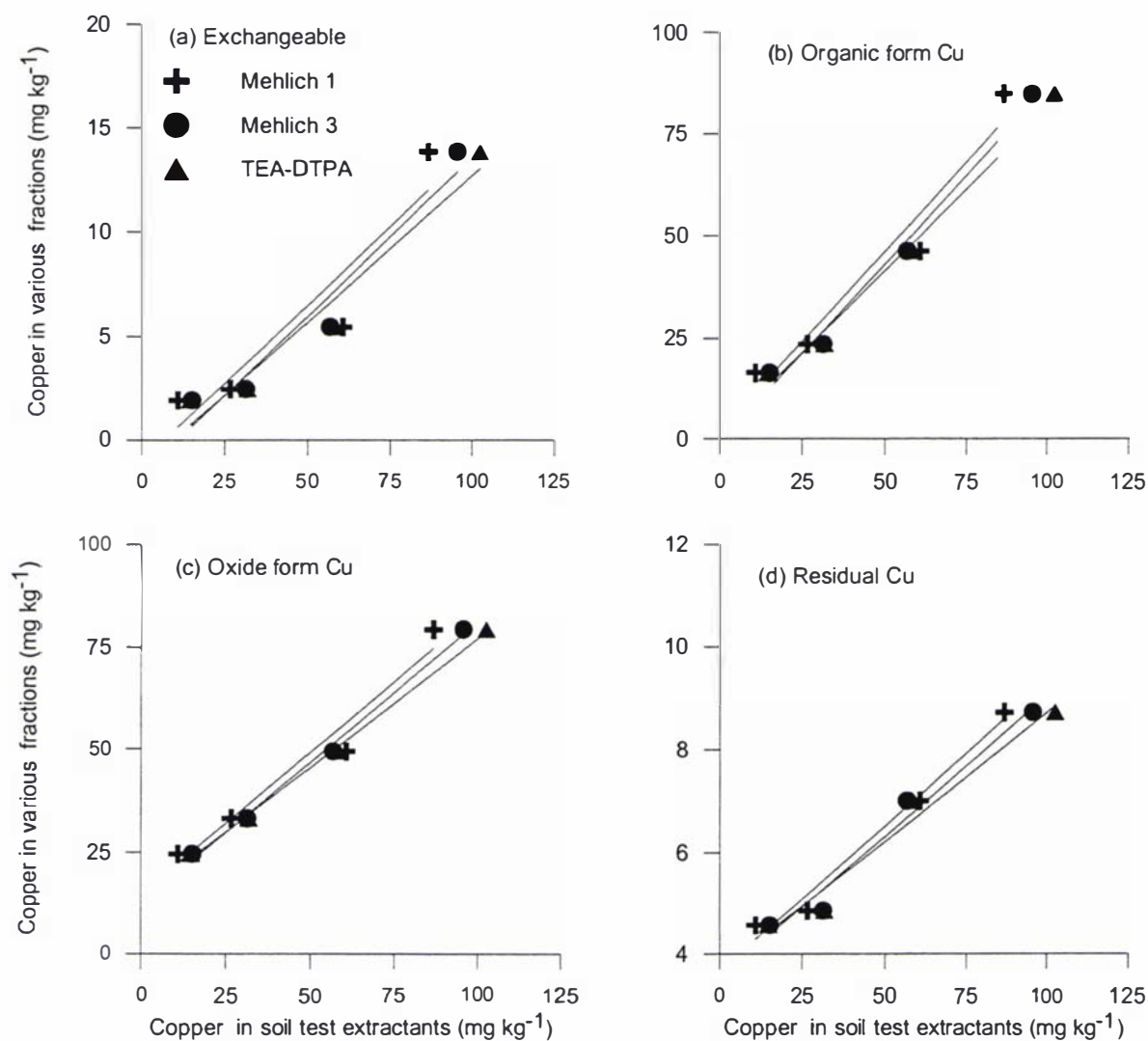


Figure 6.3 Correlation between Cu in soil test extractants and (a) exchangeable (b) organic bound Cu (c) oxide and (d) residual Cu fractions.

6.3.8 Relationships between the extractants

A highly significant linear relationship was found between the concentrations of Cu extractable by M1 and M3 ($r^2=0.91$), M1 and TEA-DTPA ($r^2=0.89$), and M3 and TEA-DTPA ($r^2=0.99$). The slope of the regression equation was close to 1 (Figure 6.4) indicating each reagent extracted from the same pool, but by different mechanisms. In contrast Zhu and Alva (1993) showed that the amount of Cu extractable by M3 was greater than that extractable by M1. More than 50% of the total Cu in the soils used by Zhu and Alva (1993) was in the organically bound form. In the current study, Cu was added as inorganic CuSO_4 or CuO . In the M3 extraction solution, EDTA as a chelating agent to remove organically bound forms of Cu (Mehlich, 1984; Alva, 1992). This

explains why Cu extractable by M3 was much greater than that extracted by M1 in the study by Zhu and Alva (1993).

The M3 and TEA-DTPA extractants appear to be very similar with respect to their capacity to extract soil Cu, probably this close relationship is due to the fact that most of the soils used in this study have a low pH. Walworth *et al.* (1992) compared M3 and TEA-DTPA in the extraction of Cu, Zn, Mn, and Fe. They observed r values between TEA-DTPA and M3 of 0.85, 0.97 and 0.95 for Cu, Zn, and Mn, respectively. Mozaffari *et al.* (1996) also reported a highly significant relationship ($r^2=0.97$) between the concentrations of Cu extractable by M3 with that by AB-DTPA from several soils representing the major soil series used for citrus production. The DTPA soil test, developed for near neutral and calcareous soils by Lindsay and Norvell (1978), illustrates the evolution of a soil test extractant from theoretical principles derived from soil chemistry to verification through green house and field calibration studies. The DTPA extractant was selected because it offered the most favourable combination of stability constants necessary to simultaneously extract four micronutrient cations (Fe, Mn, Cu and Zn). The buffered pH and presence of soluble Ca^{2+} prevents excessive dissolution of CaCO_3 , avoiding the release of unavailable micronutrients occluded by this solid phase. It should be noted that at pH 7.3, 70 to 80% of the buffering capacity provided by TEA has been consumed. Therefore, using the DTPA extractant on acidic soils, is likely to result in neutralisation of the remaining buffer capacity, resulting in an unpredictable extraction pH (Norvell, 1984).

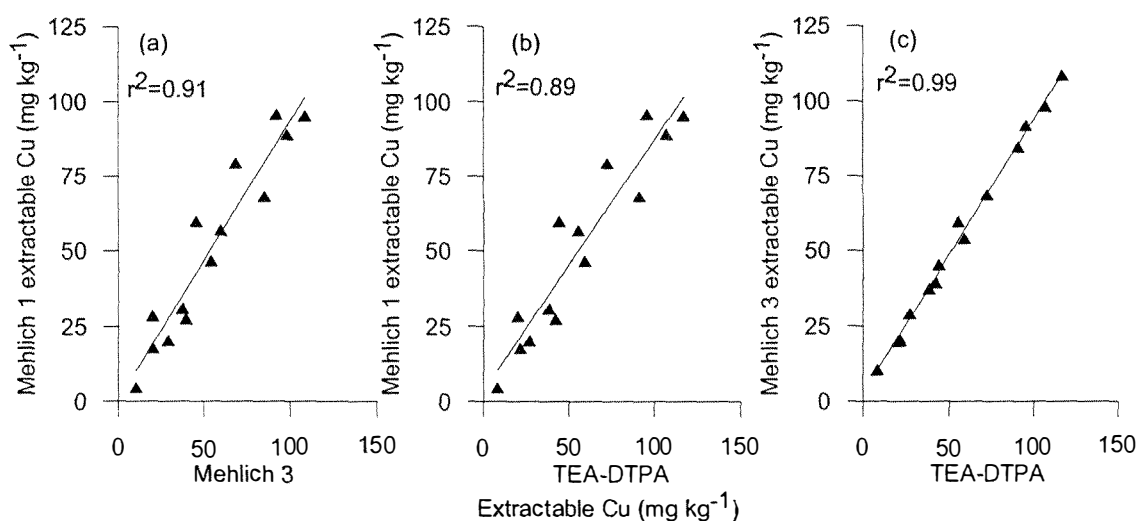


Figure 6.4 The relationship between the concentrations of soil Cu extractable by (a) M1 and M3, (b) M1 and TEA-DTPA (c) and M3 and TEA-DTPA extractants from the two soils that received 0 to 200 mg Cu kg⁻¹ soil from two different Cu fertilisers.

6.3.9 Relationships between the extractants and plant Cu concentration

Considering that the Cu concentration in the shoot of the plant should be an adequate indicator of the availability of this element in the soil, it was of interest to perform a regression analysis comparing the plant Cu concentration with the amount of extractable Cu from the soil for each extraction reagent (Table 6.9). Nearly similar correlation coefficient (r) values were obtained between Cu extracted by the different soil test extractant reagents and the Cu concentration in the plants. This similarity can be explained by the fact that M3 and TEA-DTPA solutions contain the complexing agents, which extract the Cu complexed by organic matter, an important soil pool of Cu. Similar results were observed by Walworth *et al.* (1992), who obtained a correlation coefficient (r) of 0.73 for Cu extracted by both the M3 and TEA-DTPA reagents. Haddad and Evans (1993), comparing extractants using clover as the test plant, found TEA-DTPA and AB-DTPA were comparable and better than M3 in predicting Cu concentration in the clover plant. Gimenez *et al.* (1992) reported that DTPA and HCl extractable soil Cu were found to give higher correlations with coffee seedlings for Cu concentrations in both sandy and clay soils.

All the extractants explained > 90 % variation found in the Cu concentration in the shoot (Figure 6.5). It can also be noted from this figure, that high correlation coefficients were found, indicating that the soil analysis for evaluation of the Cu availability to plants is feasible. Singh *et al.* (1994) observed an *r* value of 0.994 between the Cu concentration in plants and the Cu extracted by the TEA-DTPA procedure for two gramineae plant species grown in polluted soils. De Abreu *et al.* (1996) observed that the extraction of available Cu from a soil decreased in the order: M3 > TEA-DTPA > M1 and strongly correlated this with the Cu concentration in the aerial part of the wheat plants grown; the *r* values between plant Cu concentration and soil test Cu were 0.80, 0.74 and 0.45 for M3, TEA-DTPA and M1, respectively.

Grundon and Best (1982) evaluated DTPA as a soil test for Cu on 35 soils in Australia, and noted responses by wheat either as grain yield or increased grain Cu, where DTPA extractable Cu was <0.4mg kg⁻¹ for grey or grey brown soils. Karamanos *et al.* (1986) studied the response of wheat, flax, barley and canola to Cu application for 2 years at six locations in the sandy transitional grassland soils of Saskatchewan, Canada. DTPA extractable Cu was found to be more effective than plant analysis in the diagnosis of Cu deficiency. These results indicate that in soils that have received recent Cu additions, TEA-DTPA, M3 and M1 extractants are effective for extracting Cu from the soil. Thus, the results indicate that TEA-DTPA gave the highest soil Cu extraction capacity, followed by the M3 and M1 extraction reagents. Therefore, TEA-DTPA, M3 and M1 reagents were found to be equally efficient extractants for the evaluation of Cu availability in soils.

Table 6.9 Correlation coefficient of different extractants with plant available Cu.

	Mehlich-1	Mehlich-3	0.1N HCl	0.01M Ca(NO ₃) ₂	TEA- DTPA	0.02M SrCl ₂	0.01M CaCl ₂	0.01M NaNO ₃	1 M NH ₄ NO ₃
Correlation coefficient	0.91	0.90	0.87	0.88	0.90	0.85	0.91	0.91	0.89

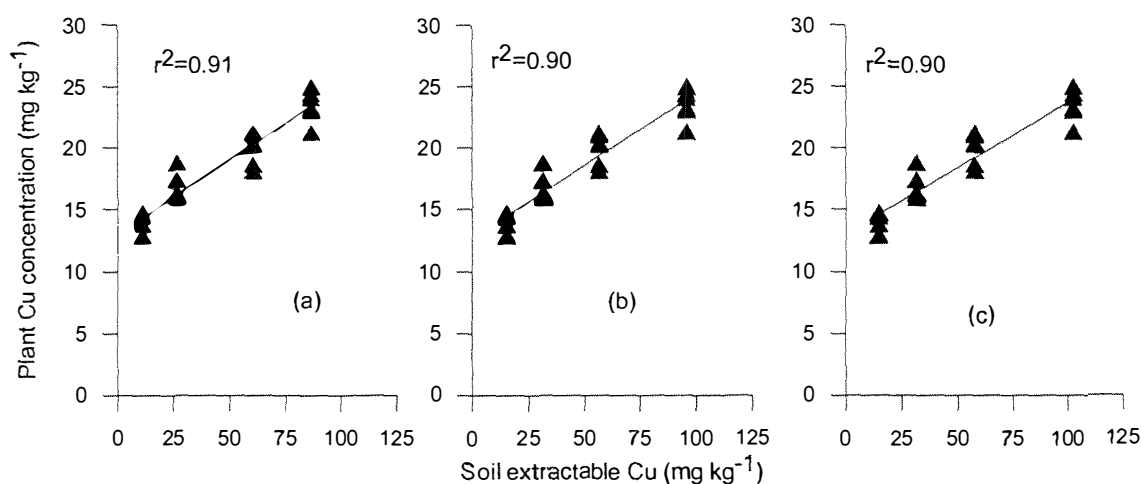


Figure 6.5 Linear correlations of Cu in the ryegrass with the amounts of extractable Cu determined by using the (a) M1, (b) M3 and (c) TEA-DTPA extracting procedures.

6.4 CONCLUSION AND FURTHER STUDY

The result of the present study demonstrated that the major forms of Cu that can be extracted by M1, M3 or TEA-DTPA soil test extractants are the organically bound and oxide bound Cu. Increasing levels of Cu application significantly increased the exchangeable, organic and oxide bound forms of Cu in each extractant. The chemical forms of Cu extractable by M1, M3 or TEA-DTPA from pasture soils varied with the organic matter. As expected that the soil test extractants extracted higher levels of organically bound Cu for soils with high levels of organic matter than soils with low level of organic matter. Soil test extractants removed higher amounts of oxide bound Cu from soils treated with CuO than those treated with CuSO₄. The ratios of the different fractions for M3 was closer to that for M1. M3 and TEA-DTPA were more effective than M1 in extracting the organically bound form of Cu. The TEA-DTPA and M1 extractants were equally effective in extracting the oxide bound form of Cu. Again TEA-DTPA and M1 were more efficient than M3 in extracting the residual form of Cu. Further, the M3 showed a higher amount of free Cu than TEA-DTPA and M1. M3 demonstrated a greater increase of Cu from the exchangeable form and organic complexes due to the dual activity of EDTA and acids for the different fractions, and it is best suited for predicting the available Cu in pasture soils. The ratios of the different forms of Cu in the soil test extractants strongly suggest that the Cu is residing in the soil

mainly in the organic form and decreased in the order: organic > oxide > residual > exchangeable.

TEA-DTPA yields a higher concentration of Cu than the other test extractants. By means of the correlation equations, the amounts extracted using one method may be compared with the results obtained by means of any of the other tests having a larger data base or calibration curves for ryegrass. TEA-DTPA, M3, and M1 extracted large amounts of Cu, when compared with the total Cu content measured by tri-acid digestion. The soils that have received recent Cu additions, TEA-DTPA, M3 and M1 extractants are effective for extracting Cu. The efficiency in extracting the Cu from the soil, followed: $0.01\text{M Ca(NO}_3)_2 < 0.1\text{M NaNO}_3 < 0.01\text{M CaCl}_2 < 1.0\text{M NH}_4\text{NO}_3 < 0.1\text{M HCl} < 0.02\text{M SrCl}_2 < \text{Mehlich-1} < \text{Mehlich-3} < \text{TEA-DTPA}$.

That Cu in soils is not available for plant uptake as it is strongly complexed with organic matter is shown in this study. The chemical forms of Cu extractable by soil test extractants from pasture varied considerably with organic matter. An attempt has been made to examine the effect of soil amendments (N and P fertilisers, lime and EDTA) on the uptake of both native and residual Cu. These results will be discussed in the next chapters.

CHAPTER 7 EFFECT OF NITROGEN AND PHOSPHORUS FERTILISER ON THE AVAILABILITY OF NATIVE COPPER

7.1 INTRODUCTION

Copper in soils is strongly held on inorganic and organic exchange sites, and it complexes with organic matter. For these reasons a large proportion of the total Cu content of a soil is not available for uptake by plants. The proportion of the total copper taken up by plants has been found to be greater in mineral soils than in organic soils. Copper deficiencies in crops may be due to either an inherently low total Cu content of the soil or to only small amounts being in an available form. In New Zealand, the pastoral system is legume-based and nitrogen fertiliser is used in pastures for encouraging out of season pasture growth. Phosphate fertilisers are also regularly used in legume based pasture systems. Copper requirements have tended to increase with increases in soil fertility, due to the growing of legumes, regular applications of superphosphate and the use of nitrogenous fertilisers (Gartrell, 1981).

It has been observed in several published reports that the application of N and P fertiliser compounds leads to increases in the Cu deficiency in cereal crops (Caldwell, 1971; Touchton *et al.*, 1980). In citrus, highly concentrated long term applications of P fertilisers have resulted in and intensified Cu deficiencies (Olsen, 1972). Application of a high N supply may cause rapid plant growth and accentuate Cu deficiency by exhausting the Cu supply in the soil solution (Robson and Reuter, 1981; Alloway and Tills, 1984). Copper deficiency in infertile soils was enhanced after the soil N supply was elevated by the use of leguminous pasture species (Gartrell, 1981). Brennan (1993) reported high levels of N in soils diluted the Cu concentration in wheat plants.

In soils with a sufficient Cu supply, Cu levels in plants have been shown to increase with increasing N levels. Gladstones *et al.* (1975) observed that the Cu concentration in shoots of a number of cereals and pasture grasses increased linearly with N concentration. Kumar *et al.* (1990) reported that N and Cu were found to have a

mutually antagonistic effect on each other's concentration in the wheat plant. The antagonism was greater with NH_4^+ sources than with NO_3^- compounds.

Increasing levels of soil P also tend to reduce the concentration of plant Cu. This may be due to a plant dilution effect as a result of added P increasing plant growth without a consequent increase in Cu uptake (Robson and Reuter, 1981). The P added induces the absorption and precipitation of metal ions to form insoluble complexes and therefore makes both the Cu and P unavailable to plants (Lindsay, 1979; Ross, 1994). In subterranean clover, the interaction between P and Cu on growth was indirect and positive, and when the P supply increased from a marginally deficient level to an adequate level, there was depressed Cu absorption and an accentuated Cu deficiency (Reuter *et al.*, 1981b).

The present study aims to evaluate the effects of N and P fertilisers on the uptake of native Cu by ryegrass in two contrasting soils.

7.2 MATERIALS AND METHODS

7.2.1 *Soils*

Two contrasting soils (Manawatu and Ramiha) were used in this experiment. The soil samples were ground and passed through 6.0 mm sieve. One kg of soil was mixed with the required amount of N and P fertilisers and placed in pots. The soil in the pots was maintained at 80% field capacity for equilibration.

7.2.2 *Fertilisers*

There were four levels of N added as urea ($\text{NH}_2\text{-CO-NH}_2$) and five levels of P added as monocalcium phosphate [$\text{Ca}(\text{H}_2\text{PO}_4)\cdot\text{H}_2\text{O}$]. Potassium and sulphur fertilisers were applied as a basal dose.

7.2.3 *Plant growth experiment*

A completely randomised factorial design was used in this glasshouse trial. The treatments included two soils (Manawatu and Ramiha), four levels of nitrogen (0, 50,

100 and 200 mg kg⁻¹ soil) and five levels of phosphate fertiliser (0, 50, 100, 150 and 200 mg kg⁻¹ soil). The treatments were replicated three times. There were 120 (2 × 4 × 5 × 3) pots in this experiment. The treatments were placed randomly in each tray by using a random number table. After equilibration with water, forty seeds of ryegrass were sown in each pot. The pots were covered by brown paper until germination. The first Cu free nutrient solution was given after 25 days from sowing. The nutrient solution was applied twice a week.

7.2.4 Grass sample preparation

The ryegrass was harvested at 90 days after sowing. The plants were cut to a height of approximately 2.5-3.0 cm above the surface of the soil. The samples were dried at 70 °C in a forced air oven. The dry weights of the ryegrass samples were recorded and the samples were then ground using a coffee grinder and kept in airtight polyethylene bags for chemical analysis.

7.2.5 Ryegrass samples for Cu analysis

The method of the preparation of the ryegrass samples for chemical analysis is described in section 4.2.5.

7.2.6 Chemical Analysis

Copper in the plant digests and the soil extracts was analysed following the methods described in section 4.2.6.

7.2.7 Statistical analysis

Statistical analysis of the data was carried out by the Statistical Analysis System (SAS). Details of which are discussed in section 4.2.7.

7.3 RESULTS AND DISCUSSION

7.3.1 *Initial soil*

The initial soil properties discussed in Chapter 3 (Section 3.3.2) indicate that the Ramiha soil contained higher amounts (5.62%) of organic carbon than the Manawatu soil (2.91%).

7.3.2 *Effect of soil types on dry matter yield and Cu concentration*

Results presented in Table 7.1 show significant differences in dry matter yield and also Cu uptake between the two soils. The Ramiha soil shows a higher amount of dry matter yield, a lower Cu concentration, but a higher Cu uptake. The variation in shoot Cu concentrations indicated that soil Cu was less available from the high organic matter content Ramiha soil; the low plant Cu concentration in this soil may also be due to a dilution effect as a result of greater dry matter production. A similar observation was made by Soon *et al.* (1997) and demonstrated that the Cu concentration is less in a Black Solodic soil rich in organic matter. There was a sharp difference in Cu uptake between the two soils.

Table 7.1 Effect of soil types on DM yield, Cu concentration and uptake.

Soils	Dry matter yield (g pot ⁻¹)	Cu concentration (mg kg ⁻¹)	Cu uptake (mg pot ⁻¹)
Manawatu	1.08b	8.10 ns	8.85b
Ramiha	1.91a	7.26	11.74a

* Treatment means followed by the same letter within a column are not significantly different at the 5% level and ns means not significant.

7.3.3 *Effect of N on dry matter yield, Cu concentration and Cu uptake*

The dry matter (DM) yield increased with increasing levels of N (Figure 7.1a). At the highest level of N there was a slight decrease in yield, but it was not significantly different from the other N levels. Increasing levels of N increased the Cu concentration (Figure 7.1b) and Cu uptake (Figure 7.1c) in both soils. Gladstones *et al.* (1975)

observed that the Cu concentration in shoots of a number of cereals and pasture grasses increased linearly with N concentration. Filipek-Mazur (1991) found N application increased Cu content in herbage. Buttigieg *et al.* (1989) reported those N rates of 250 and 500 kg N ha⁻¹ year⁻¹ significantly increased the Cu concentration pasture. A number of reasons could be attributed to an increase in Cu concentration and Cu uptake with increasing levels of N application (Hill *et al.*, 1978; Robson and Reuter, 1981): (i) An increase in root growth due to N is likely to cause greater exploitation of the soil volume resulting in increased uptake of Cu; this is known as the priming effect; (ii) an increase in N uptake increases the amino acid concentration in roots leading to greater translocation of Cu from root to shoot. Bineev *et al.* (1985) confirmed that free amino acids in soils form chelates with Cu and thereby facilitate the migration of the trace element in the soil plant system. More recently, it was found that free nicotianamine and histidine are the major Cu transporters in tomato and chicory xylem sap (Pich and Scholz, 1996; Liao *et al.*, 2000). High levels of N however, markedly reduce the rate of translocation of Cu from older leaves to meristems. The coupling of Cu movement to leaves to N movement may account for relatively high critical concentration of Cu in the tops of plants found at high fertiliser rates (Loneragan *et al.*, 1980). Soon *et al.* (1997) conducted a study to quantify the variation in Cu and P uptake among some common Canadian wheat cultivars due to N fertiliser and the environment. Nitrogen fertiliser produced four times as much variability in Cu concentration and uptake as did the genotype. There was a strong correlation between N and shoot Cu concentration ($r=0.70$, $P=0.01$).

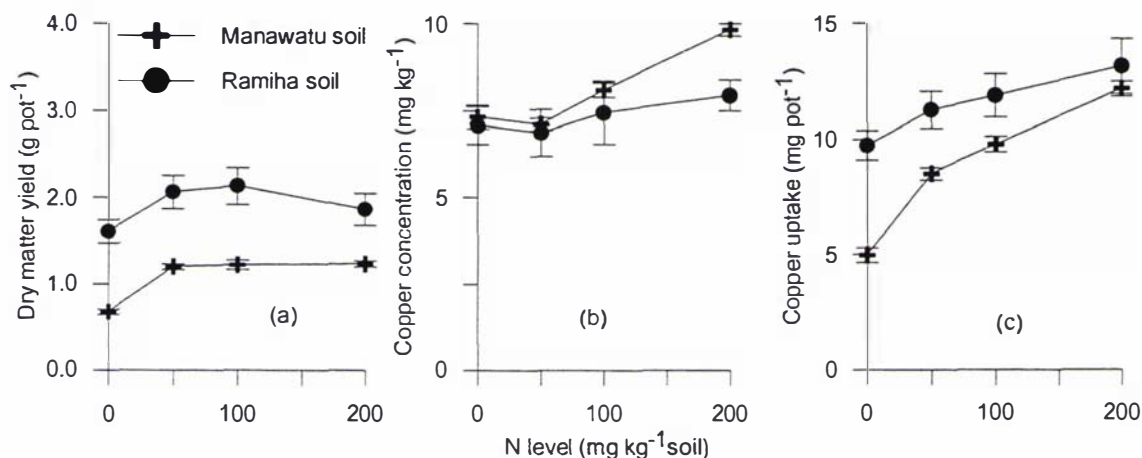


Figure 7.1 Effect of N levels on (a) dry matter yield, (b) Cu concentration and (c) Cu uptake. Data are means \pm SE, $n=3$.

7.3.4 *Effect of P on dry matter yield, Cu concentration and Cu uptake*

Increasing levels of P significantly increased the DM yield in the Ramiha soil (Figure 7.2a) due to the low Olsen P. Increasing rates of P fertilisers increased the available P status (Olsen P) of the soil and thereby increased the dry matter yield. Morton *et al.* (1995) and Morton and Roberts (1999) found a good relationship between soil Olsen P and relative pasture production for volcanic ash soils.

The addition of P fertiliser decreased the concentration of Cu in plants (Figure 7.2b), but there was no significant difference in the Cu concentration between different P levels. This effect was more pronounced in the Ramiha soil than the Manawatu soil. This may be due to the dilution of Cu in plant tissue through the promotion of plant growth by phosphate fertilisers (Loneragan *et al.*, 1979) and the complexing of $\text{CuH}_2\text{PO}_4^+$ at a pH range of 5.8 to 6.8 (Lindsay, 1979). Wallace *et al.* (1978) showed that at high pH increasing solution P decreased the uptake of Zn, Cu, and Mn by soybean and bush bean, whereas at low pH it resulted in an increase in their uptake. Ahumada and Schalscha (1995) observed that the addition of P decreased sorption of Cu in soils due to complex formation with H_2PO_4^- .

Increasing levels of P increased the Cu uptake (Figure 7.2c). Maciejewska (1992) observed that P application increased the Cu uptake by perennial ryegrass (*Lolium perenne*). Both transport to shoot and uptake rate of Cu increased with the increase in P supply. The proportion of Cu retained in roots (65%) did not vary with differential Cu uptake at the various P levels. Cu and P concentrations in roots were positively correlated (Gill *et al.*, 1992). Timmer and Leyden (1980) concluded that the reduction in plant Cu concentration at high P levels resulted from a reduced exploitation of the soil by mycorrhizae. De Iorio *et al.* (1996) demonstrated that the addition of P has a positive interaction in lettuce with high levels of Cu.

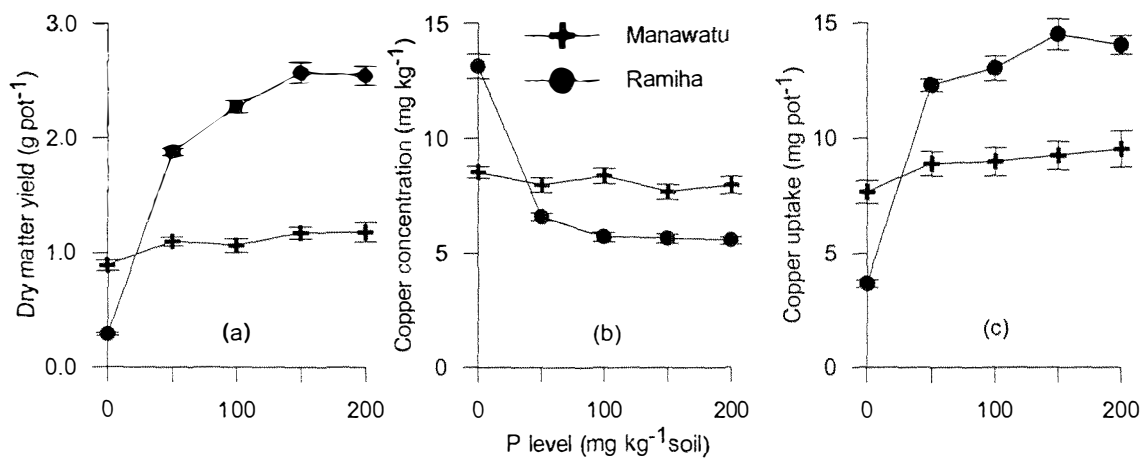


Figure 7.2 Effect of P levels on (a) dry matter yield, (b) Cu concentration and (c) Cu uptake. Data are means \pm SE, n=3.

7.4 CONCLUSION AND FURTHER STUDY

- Soils have a significant effect on DM yield and also on Cu concentration.
- Increasing levels of N increased DM yield and Cu concentration.
- Increasing levels of P increased the DM yield, but decreased Cu concentration.
- Copper uptake was affected by the growth of ryegrass through the addition of both N and P fertilisers.

The effect of lime, nitrogen fertiliser and EDTA on the availability of residual Cu is discussed in next chapter.

CHAPTER 8 EFFECT OF LIME, EDTA AND NITROGEN FERTILISER ON THE AVAILABILITY RESIDUAL COPPER

Publication arising from this chapter:

Khan, M. A. R., N. S. Bolan and A. D. Mackay 2000. Effect of liming and nitrogen fertiliser on the plant availability of residual Cu in pasture soils. In Proceedings of the Second International Conference on Geospatial Information in Agriculture and Forestry, Lake Buena Vista, FL, USA. pp. II-266 – 274.

8.1 INTRODUCTION

The results in chapters 3, 4, 5 and 6 indicated that copper (Cu) is very strongly bound to soil organic matter, and hence the plant availability of Cu added through fertiliser is likely to decline with increasing time of contact between the soil and the Cu fertiliser. Farmers require accurate information on the length of time that a Cu application remains fully effective in order to supply the Cu required for the grazing animal. Nitrogen fertiliser is used in pastures for encouraging out of season pasture growth. Lime is used to overcome the problems associated with soil acidification. The application of N fertilisers to pasture soils marginally supplied with Cu may induce a Cu deficiency in plants. Krähmer and Sattelmacher (1997) reported that the increased Cu uptake with increasing N levels might be explained by the release of amines into the root apoplast and the rhizosphere, particularly under Cu sufficient conditions. These may mobilise Cu by the formation of soluble Cu amine complexes.

Among the various soil properties, pH has the largest effect on the adsorption of cations by variable-charge surfaces. The net negative charge on variable-charge surface materials increases with increasing pH; as a consequence, the adsorption of cations increases. In soils, where lime is applied, both the pH and the Ca concentration in the soil solution increase. Whereas an increase in pH can decrease the adsorption, an increase in Ca concentration has the opposite effect on the adsorption Cu, the resultant effect of liming on Cu adsorption is likely to depend on the concentration of Ca in the soil solution. Bivalent transition metal cations exhibit a similar pH dependent sorption behaviour; and many authors have reported that the amount of sorbed metals increases with the raising of solution pH within a certain range. The effect of pH on adsorption is

confounded by the effect of Ca added through liming (Friesen *et al.*, 1980). The amount of Cu in the soil solution decreases with increasing pH because of stronger Cu adsorption (Lindsay, 1974; Section 3.3.3.5).

Chelating compounds, such as EDTA are generally used to enhance the mobilisation of metal ions in soils. Like the natural complexing agents (humic and fulvic acids) that enhance the migration of metals, synthetic chelating agents (eg. EDTA, DTPA etc.) combine with trace metals to increase the total levels of these metals in the soil solution, thereby increasing the nutrient availability by increasing both diffusion and the mass flow of these metals to plant roots (Lindsay, 1974). The residual value of Cu fertilisers as affected by N fertiliser, lime and EDTA was investigated in a glasshouse trial with the following objectives.

- To determine the residual effectiveness of fast and slow release Cu fertilisers as influenced by N fertiliser addition.
- To examine the availability of residual Cu to plants in response to changes in soil pH through liming.
- To evaluate the availability of residual Cu to plants through chelating agents.

8.2 MATERIALS AND METHODS

8.2.1 *Soil collection and preparation*

Soil samples were obtained at the termination of a glasshouse plant growth experiment (Chapter 4). From that trial, two contrasting soils (Manawatu and Ngamoka) treated with both slow (CuO) and fast release (CuSO₄) Cu sources, at four Cu levels (0, 5, 10 and 20 kg Cu ha⁻¹) were chosen for this experiment. Two hundred and fifty-gram soil samples were placed in pots and the soil in the pots was maintained at 80% field capacity for equilibration.

8.2.2 *Plant growth experiment and treatments*

A factorial design was used in this glasshouse trial. The treatments included two soils (Manawatu and Ngamoka), two Cu sources (CuSO₄ and CuO), four residual Cu levels (0, 5, 10 and 20 kg Cu ha⁻¹), three levels of N fertiliser (0, 50 and 100 kg N ha⁻¹; Plate 8.1), three levels of lime (0, 1000, 2000 kg ha⁻¹; Plate 8.2) and three levels of EDTA (0,

10 and 20 kg ha⁻¹; Plate 8.3). The treatments were replicated three times. N fertiliser was applied as a solution 45 days after sowing. Lime and EDTA were incorporated 100 days prior to sowing. After equilibration with water, twenty-five seeds of ryegrass (*Lolium perenne* cv. Super Nui) were sown in each pot. The first Cu-free nutrient solution was given 20 days after sowing. The nutrient solution was applied twice a week.



Plate 8.1 Glasshouse trial of N effects on residual Cu availability in ryegrass.



Plate 8.2 Glasshouse trial of lime effects on residual Cu availability in ryegrass (Ballantrae high fertility known as Ngamoka soil).

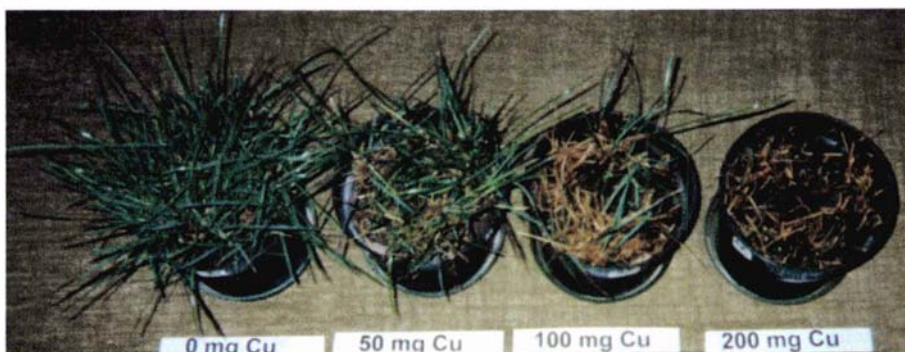


Plate 8.3 Glasshouse trial of EDTA effects on residual Cu availability in ryegrass.

8.2.3 *Harvesting and soil sampling*

The ryegrass was harvested 60 and 90 days after sowing. The plants were cut to a height of approximately 2.5-3.0 cm above the surface of the soil. The samples were dried at 70 °C in a forced air oven. The dry weights of the pasture samples were recorded, the samples were then ground using a coffee grinder and kept in airtight polyethylene bags for chemical analysis. The soil samples were collected at the end of experiment (after 106 days). The soil samples were air dried, ground and passed through 2.00 mm sieve. The soil samples were analysed for total, exchangeable and free ionic Cu.

8.2.4 *Soil analysis*

Soil pH (1:2.5 H₂O) was measured using a glass electrode. Total Cu was determined by wet digestion (Section 5.2.3). Soil samples were extracted either by Mehlich-1 (Section 3.2.10.2), Mehlich-3 (Section 3.2.10.3) or TEA-DTPA (Section 3.2.10.5) For the measurement of exchangeable Cu and ionic Cu, the soil samples were extracted with 0.01M Ca(NO₃)₂. The filtrates were analysed for total Cu and free Cu²⁺ using F-AAS and a cupric electrode, respectively, with the appropriate standards.

8.2.5 *Ryegrass samples for Cu analysis*

The method of the preparation of the plant samples for Cu analysis is described in Chapter 4 (Section 4.2.5).

8.2.6 *Chemical Analysis*

Copper in the plant digests and soil extractants was analysed using F-AAS. Details of other procedures are discussed in Section 4.2.6.

8.2.7 *Statistical analysis*

Statistical analysis of the data was carried out by the Statistical Analysis System (SAS). Details are discussed in section 4.2.7.

8.3 RESULTS AND DISCUSSION

8.3.1 *Soil characteristics*

Both the soils are slightly acidic with the Ngamoka soil (5.9% organic carbon) containing twice the amount of organic carbon compared to the Manawatu soil (2.9% organic carbon). Soil characteristics are discussed in Chapter 3 (Section 3.3.2).

8.3.2 *Dry matter yield and Cu concentration*

8.3.2.1 *Effect of N fertiliser*

Figure 8.1 shows that the nitrogen treatment at 100 kg N ha⁻¹ produced the highest DM yield and Cu concentration at both harvests and it was significantly ($p < 0.05$) different from the control treatment. The 100 kg N ha⁻¹ level contained the highest amount of Cu at each harvest, but it was not significantly different from the 50 kg N ha⁻¹ treatment. Similar results were obtained by Krähmer and Sattelmacher (1997) who reported that increasing rates of N fertiliser increased the Cu concentration in plants under Cu sufficient conditions.

8.3.2.2 *Effect of lime*

The lime treatment at 2000 kg ha⁻¹ produced the highest DM yield in both harvests and it was significantly ($p < 0.05$) different from control. The effect of lime levels on increasing yield was more pronounced at the higher application rate in the Manawatu

soil. Thomson (1982) and Edmeades *et al.* (1984) found a correlation ($r=0.6$) between lime and pasture production. Increases in DM yield with increasing rates of lime were also obtained by others (Hogg *et al.*, 1988; Redente and Richards, 1997; Stout *et al.*, 1997). The lime level of 1000 kg ha^{-1} soil resulted in the highest amount of Cu concentration at harvest 1 and that was significantly different from the other treatments (Figure 8.1). This was attributed to the alleviation of a calcium deficiency. The application of lime to soils significantly increased soil pH values. The lime level of 2000 kg ha^{-1} decreased Cu concentration at all harvests. Cu concentration in the ryegrass was found to be higher at the lower level than at the higher levels of lime (Lévesque and Mathur, 1983). The reduction in plant Cu concentration in response to liming tended to be greater where the soil pH was relatively high. The decrease in the concentration of Cu in the plant resulting from an increase (0.6 unit) in soil pH due to the addition of lime is in general agreement with the findings of others. Hooda *et al.* (1997) observed that the Cu content of carrots and spinach produced on limed soils was decreased by 28% and 27%, respectively, compared to those grown on unlimed soils. Alva *et al.* (1993) reported that the addition of Ca as both lime and gypsum lowered Cu uptake in citrus plant thus ameliorating Cu toxicity.

8.3.2.3 *Effect of EDTA*

Dry matter yield decreased with increasing levels of EDTA. Addition of EDTA resulted in an increase in Cu concentration in ryegrass (nearly 3 fold) resulting in phytotoxicity (Figure 8.1). Albasel and Cottenie (1985) reported that chelating agents increased the uptake of Cu in perennial ryegrass (*Lolium perenne* L) and Italian ryegrass (*Lolium multiflorum*), when humic podzols soils are contaminated with 40 mg Cu kg^{-1} . A high concentration of Cu is toxic to plants, because it directly or indirectly affects the metabolic processes such as respiration, photosynthesis, CO_2 fixation, and gas exchange (Van Asche and Clijsters, 1990; Ouzounidou *et al.*, 1997). Increasing levels of residual Cu increased the Cu concentration in shoots. Cu concentrations in plants from the Manawatu soil were significantly higher, than those from the Ngamoka soil with the addition of EDTA. Hunter (1981) reported that when seedling maize roots were immersed for one hour in a Cu solution (0.13 mM CuSO_4) growth almost completely stopped due to the Cu treatment.

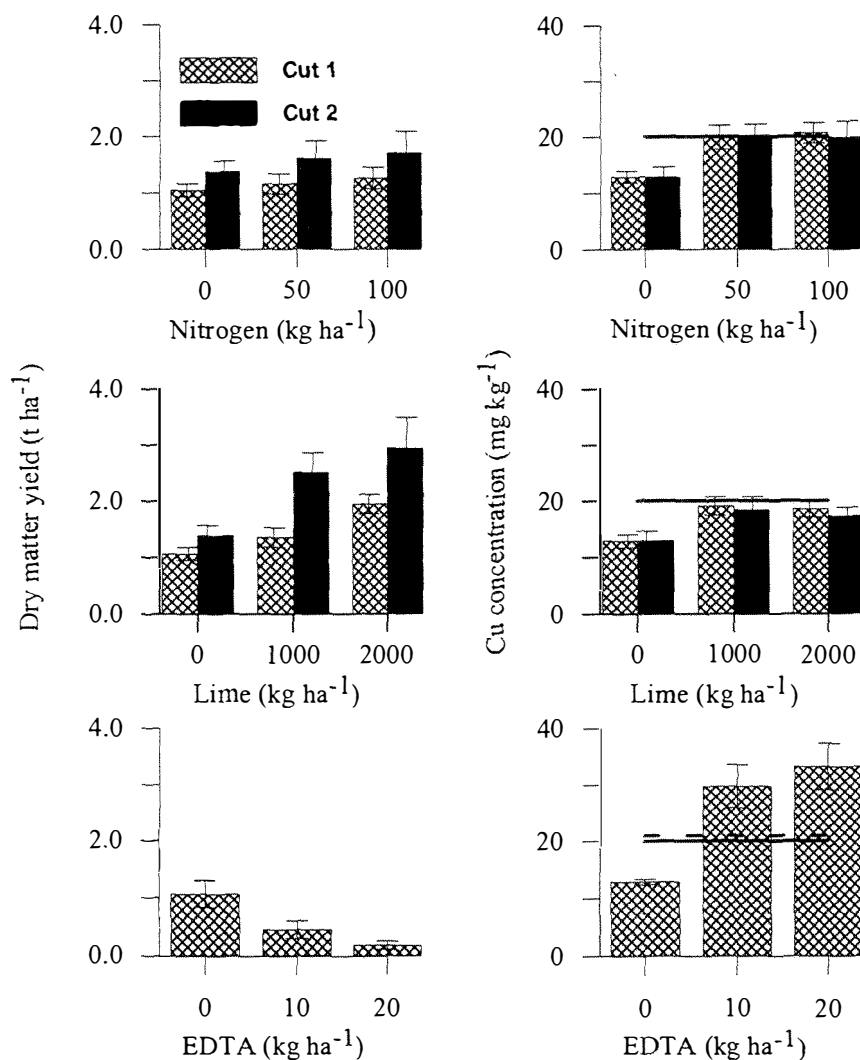


Figure 8.1 Effect of N fertilisers, lime and EDTA on DM yield and Cu concentration at different harvests for both soils. The optimum (—) and the toxic (- - -) levels of Cu in the plant are indicated in the figure. Data are means \pm SE, $n=48$.

8.3.3 Effect of soil types on DM yield and Cu concentration

Both the DM yield and Cu concentration for the Manawatu soil were significantly higher than for the Ngamoka soil at each harvest (Figure 8.2) with the addition of N fertiliser. The effectiveness of liming in reducing the Cu content in ryegrass varied between the soils. The Ngamoka soil contains more organic matter and lime solubilises the organically bound Cu. Addition of lime caused an increase (0.6 unit) in soil pH. The Cu concentrations measured for ryegrass grown in both soils were similar at harvest 2.

Brennan *et al.* (1980) found no specific relationship between the decline in the availability of Cu and soil pH, clay content, or sesquioxide content. On the other hand, King (1988) found both Fe oxide and clay content are important in determining the loading rate of heavy metals in soils.

Soil pH affects the distribution of Cu in soils. McLaren *et al.* (1983a) reported an increase in Cu sorption as pH increased, primarily due to a greater association with soil oxides. In general, lime applications reduce plant uptake of most metals (except Mo and Se), at least to some extent. This is due to the precipitation of metals in soils as insoluble hydroxides and carbonates, and to increase sorption resulting from increased charge density on pH dependent sites.

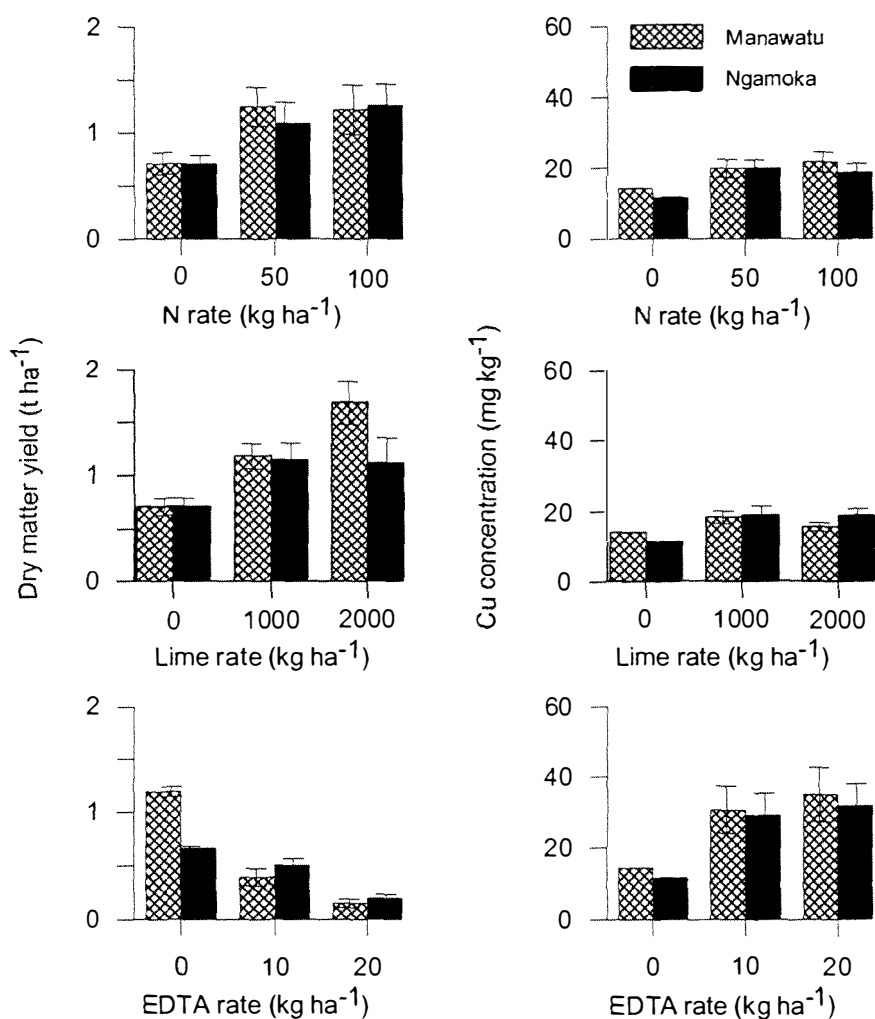


Figure 8.2 Effect of soil types on DM yield and Cu concentration as affected by N fertilisers, lime and EDTA addition at various level of Cu from different sources. Data are means \pm SE, $n=24$.

8.3.4 *Effect on exchangeable Cu*

Both N and lime applications decreased the soil exchangeable Cu of the Ngamoka soil but the Manawatu soil showed an opposite effect (Figure 8.3a and 8.3b). EDTA increased the amount of exchangeable Cu in both soils (Figure 8.3c). The increase in pH due to liming resulted in a decrease in exchangeable Cu. The Ngamoka soil binds Cu strongly due to a high amount of organic matter. Again the Ngamoka soil showed less exchangeable Cu with increasing rates of EDTA addition compared to the Manawatu soil. These differences were attributed to the differences in the characteristics of the two soils. Marschner *et al.* (1995) reported that as a result of a pH increase the water soluble fraction of Cu was reduced by 30% by lime amendment. Guster *et al.* (1983) indicated that liming increased the soil pH and reduced the exchangeable Cu. Komisarek *et al.* (1990) reported that increasing lime rates reduced the water soluble and exchangeable Cu contents. Ma and Uren (1998) observed that additions of CaCO₃ increased the soil pH from 7.08 to 7.68 and decreased the exchangeable Cu concentration.

Soil properties, primarily pH and organic matter content, influence the severity of Cu toxicity (Alva *et al.*, 1995; Mozaffari *et al.*, 1996). Rhoads *et al.* (1989) conducted two experiments with increasing Cu rates from 44 to 2800 mg kg⁻¹ in the pH range of 5.3 to 6.6. In both experiments, the growth of tomato plants decreased significantly with an increase in the Cu rate above 150 mg kg⁻¹ at soil pH levels below 6.5. At soil pH >6.5 significant growth reduction occurred only when the Cu rate was increased to 330 mg kg⁻¹. Other studies also showed that Cu concentration in the plant and Cu uptake were inversely related to the soil pH. This is due to a reduction in the solubility of Cu, and its availability to plants with an increase in soil pH (Jahiruddin *et al.*, 1986; Lindsay, 1991). Alva *et al.* (2000) demonstrated that soil pH influences the phytotoxicity of Cu primarily due to differential distribution of the Cu chemical forms. Exchangeable and sorbed Cu are the most phytotoxic forms, and their concentration increases with decreasing soil pH.

Since the phytotoxic effects of Cu are dependent on soil pH, the critical toxic levels of Cu in soils vary depending on the soil pH. This information is important for an adequate management of topsoils to mitigate the Cu toxicity effects. The effects of lime on the extraction by different chemical test extractants at various rates of residual Cu are

discussed in this chapter. Increasing levels of EDTA increased the exchangeable Cu in soil solution.

Laboratory studies conducted by Dragun *et al.* (1976) revealed that corn plants grown in 10 mM CuSO₄ solution, in equilibrium with the soil solids, resulted in a substantial decrease in height compared to corn grown in solutions with Cu concentrations less than 10 mM. A far less dramatic decrease in plant height was observed for corn grown in 2.5 mM Cu solution. Minnich *et al.* (1987) conducted greenhouse pot experiments and found an increase in Cu accumulation by snapbeans (*Phaseolus vulgaris* L.) in response to increased amounts of Cu as a Cu salt and Cu enriched sludge amended soils. They reported that phytotoxicity was not apparent for any of plants during growth, however they noted that plants grown in 300 mg Cu kg⁻¹ salt treatments had thick stunted root systems at harvest.

While the Cu concentration in soil solution is in the normal range of 0.01-0.6 μM when the total soil content of Cu is 25-40 mg kg⁻¹. The minimum concentration of Cu for specific adsorption has been defined as that concentration of an ion in solution, which causes a reversal of charge of colloids at infinite dilution. The concentration of Cu has been reported as 2.5×10^{-4} M for colloidal SiO₂ at pH 6.5 and 5×10^{-5} M for kaolinite at pH 5, this concentration may be toxic to most plant species (James and Barrow, 1981). Since the availability of Cu is affected by a number of factors including soil pH, clay content, and organic matter, it is not possible to use total or some measure of 'extractable' Cu²⁺ to predict the solution activity of Cu²⁺ affecting plants (Baker and Senft, 1995).

Gimenez *et al.* (1992) reported that the critical toxic levels of soil Cu was 1.0 and 3.0 mg kg⁻¹, respectively, for the sandy and clayey soils used for growing coffee seedlings. In the present study, the Cu concentrations in soil solution ranging from 2.8 to 20.2 mg kg⁻¹ with the addition of EDTA causes toxicity to ryegrass, when the soil pH is below 5.6. Walsh *et al.* (1972) recorded > 20 mg kg⁻¹ Cu was extracted from soil with 0.1N HCl or DTPA and > 15 mg kg⁻¹ Cu was extracted with EDTA; these levels resulted in significant yield reductions of snapbeans.

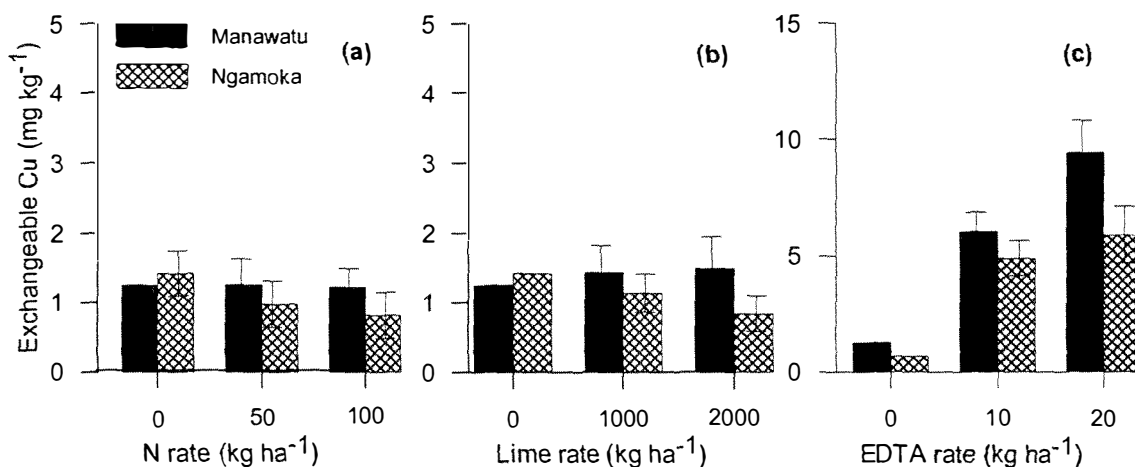


Figure 8.3 Effect of soil types on exchangeable Cu as affected by (a) Nitrogen, (b) Lime, and (c) EDTA. Data are means \pm SE, $n=24$.

8.3.5 Effect of fertiliser Cu sources and levels of residual Cu on DM yield and Cu concentration

Fertiliser Cu sources have a significant effect on DM yield and Cu concentration in ryegrass (Figure 8.4). The residual Cu level of 20 kg Cu ha⁻¹ for CuO fertiliser resulted in the highest concentration of Cu at all harvests and was significantly different from other levels for both N and lime application. This increase in dry matter yield with residual Cu is increased further by the influence of N treatment. However, even in the absence of residual Cu, dry matter yield was increased by the addition of N fertiliser and liming, presumably as a result of alleviating the adverse effect of low soil pH such as aluminium and manganese toxicity on root growth and metabolism (Marschner, 1986). The alleviation of such conditions also resulted in an increased concentration of Cu in spite of the reduced solution concentration of Cu brought about by N and lime addition. Bélanger *et al.* (1986) observed that residual Cu had positive effects on the Cu content of an oat crop grown in soils after four years of fertiliser application. Cox (1992) reported that the residual effect of Cu fertiliser use with time varied between soils. An application of 4.48 kg Cu ha⁻¹ increased the soil extractable Cu above the initial Cu concentration for from 9 to 27 years, the average time across the five soils was about 16 years. Sherrell (1989) reported that the effect of CuSO₄ applied to an established stand of lucerne (*Medicago sativa* L.) on a yellow brown pumice soil was measured over a period of four years. Extractable soil Cu indicated that the residual effect of Cu application might last for a considerable time.

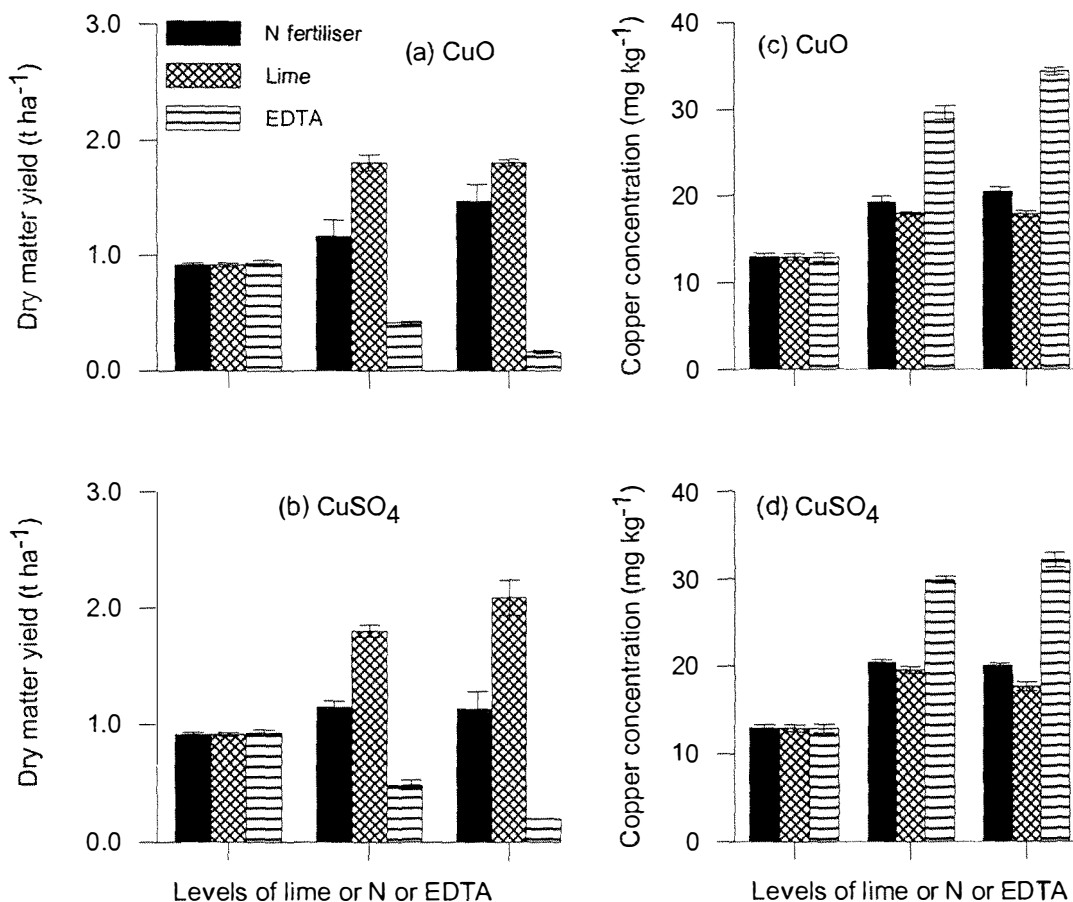


Figure 8.4 Effect of Cu fertiliser sources on DM yield [(a) CuO, (b) CuSO₄] and Cu concentration [(c) CuO, (d) CuSO₄] as affected by lime, Nitrogen or EDTA. Data are means \pm SE, n=24.

The residual Cu rate significantly influenced Cu concentration in plants with the addition of EDTA (Figure 8.5c). The concentration of Cu in the shoots also increased with increasing rates of residual Cu by the addition of lime and N fertiliser. The regression slope between the shoot Cu concentration and residual Cu rate was greater for the Manawatu soil than for the Ngamoka soil. The concentration of Cu was much greater at high rates of residual Cu. This is in agreement with typical plant responses to heavy metal toxicity conditions.

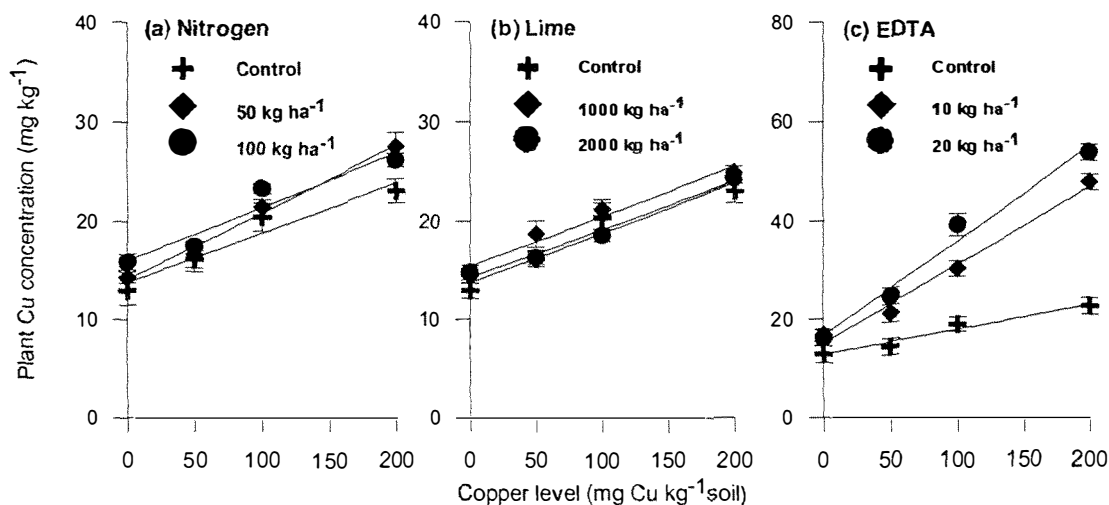


Figure 8.5 Effect of residual Cu levels on Cu concentration either treated with N (a), lime (b) or EDTA (c). Data are means \pm SE, $n=12$.

8.3.6 Effect of soil test extractants on plant Cu concentration

The amount of plant available Cu, as measured by the three soil test extractants, is affected by N, lime and EDTA. The r values between Cu concentration in the plants and Cu in the soil test extractants are 0.84, 0.91 and 0.67 for M1, M3, and TEA-DTPA, respectively, as affected EDTA (Figure 8.6). The application of EDTA resulted in the highest amount of plant available Cu, as measured by the three soil test extractants. The tendency to form complexes between Cu and soluble organic matter increases with an increase in pH. Applications of high levels of Cu as either CuSO₄ or CuO increased the DTPA extractable Cu in both soils.

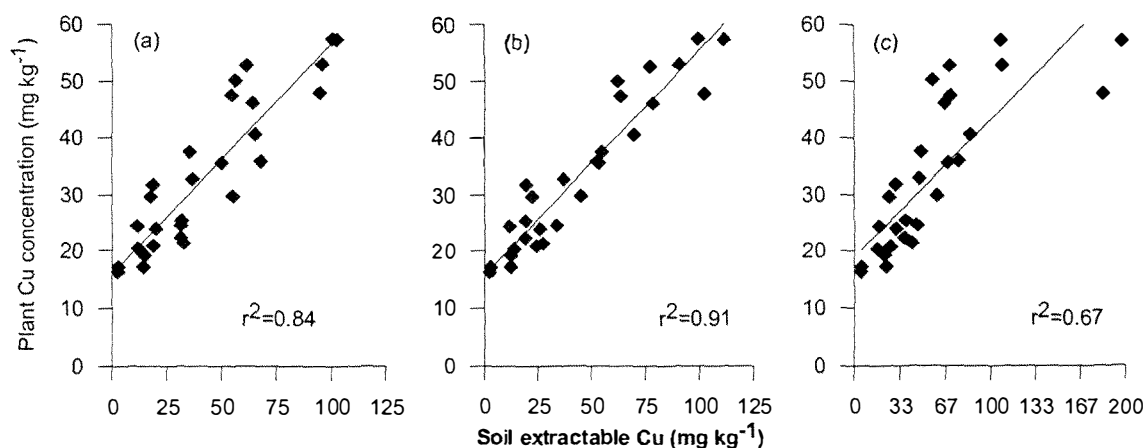


Figure 8.6 Relationship between Cu concentration in plants and Cu extracted by (a) M1, (b) M3 and (c) TEA-DTPA extractants as affected by EDTA.

Lime treatments of 2000 kg ha⁻¹ reduced M1, M3 and TEA-DTPA extractable Cu by 33%, 50% and 23% respectively. In this experiment lime additions decreased the exchangeable Cu by 32%, 38%, 23% and 15% for the 0, 5, 10 and 20 kg Cu ha⁻¹ levels (Figure 8.7). Thus Cu lost from the exchangeable fractions changed into less soluble, less bioavailable forms as the pH was increased. This finding agreed with Sims (1986), who reported that lime additions reduced exchangeable and sorbed Cu, and increased Cu in more tightly bound forms. The two major factors influencing soil solution Cu and plant Cu concentration in this trial were soil pH and residual Cu. Alva (1992) reported a good correlation between the concentrations of Zn, Fe, Mn and Cu extractable by neutral NH₄OAc, M1, M3 and AB-DTPA in soils from citrus groves in Florida. Reed *et al.* (1993) evaluating the Cu availability for corn using the M3 extracting reagent, found this procedure very promising to detect the deficiency and toxicity levels of Cu in soils.

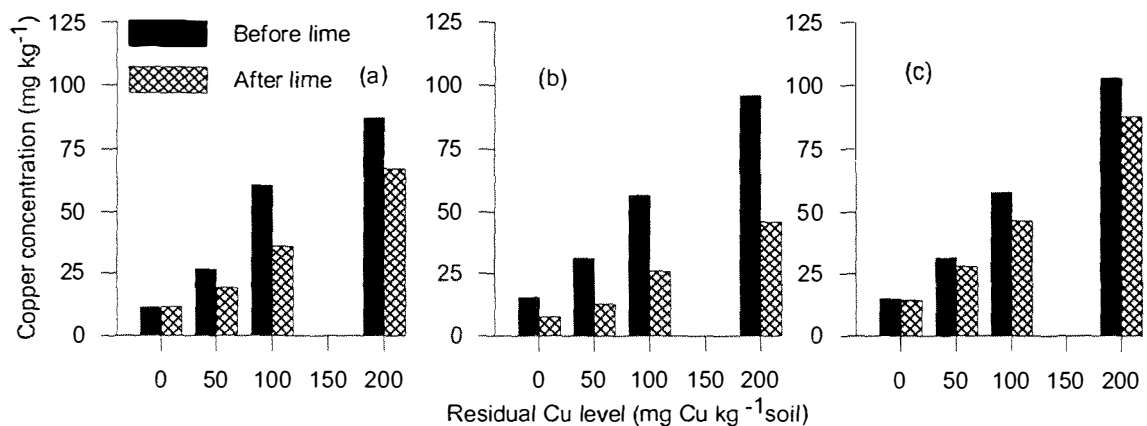


Figure 8.7 Concentration of Cu extracted by (a) Mehlich 1 (M1), (b) Mehlich 3 (M3) and (c) TEA-DTPA extractions prior to and after the addition of lime.

Correlation studies showed a positive relationship between the exchangeable Cu as affected by N, lime and EDTA additions and Cu extracted by the various extractants (M1, M3 and TEA-DTPA) before and after the application of lime (Figure 8.8). Immobilisation of Cu increases with increasing organic matter (Stevenson and Fitch, 1981). Lime reduced the extractable Cu in all the extractants, but it was more pronounced in the M3 extractant.

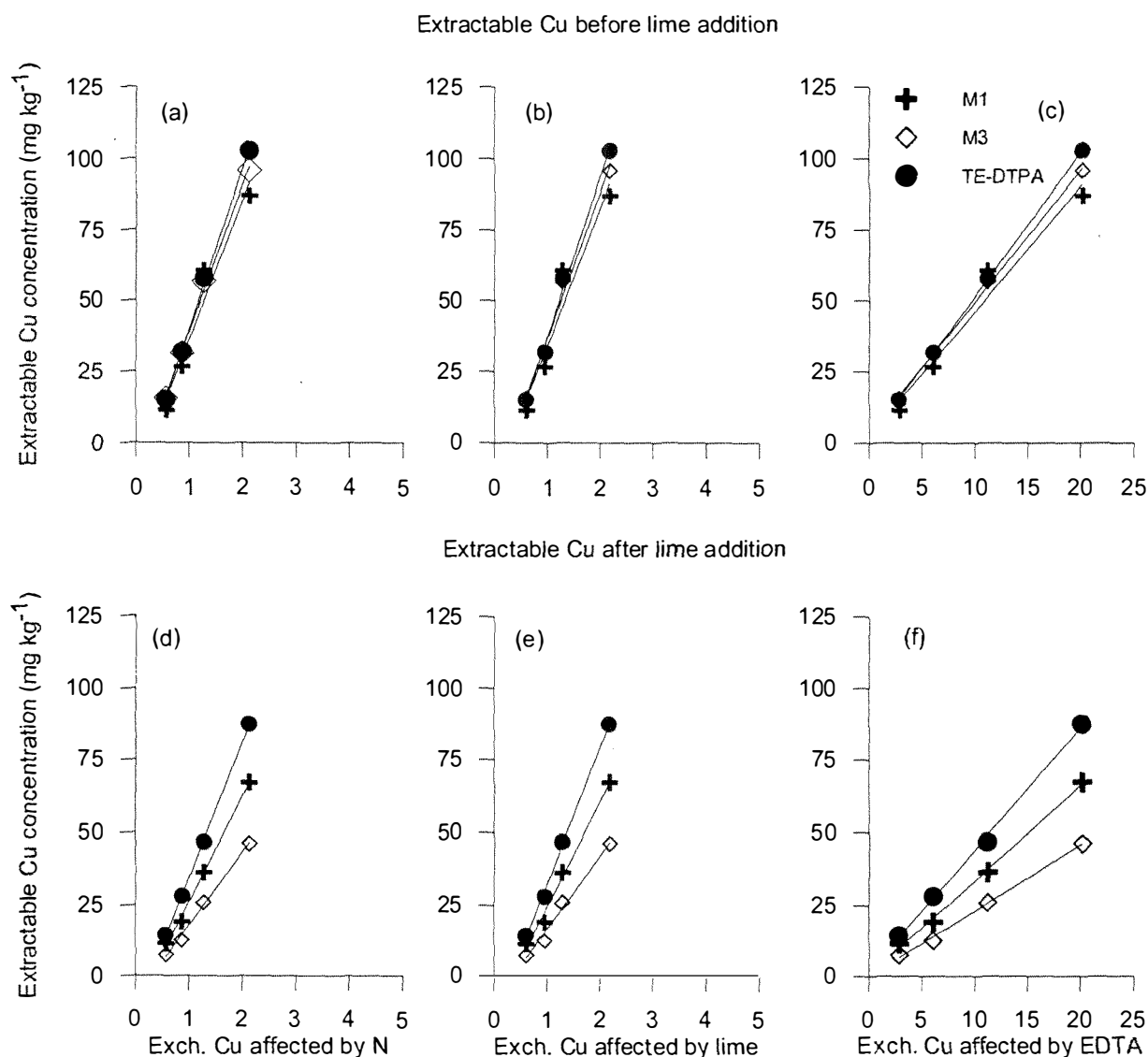


Figure 8.8 Relationship between soils exchangeable Cu as affected by N (a, d), lime (b, e), and EDTA (c, f) additions and Cu extracted by the M1, M3 and TEA-DTPA extracting procedures prior to and after lime application.

There were significant linear correlations between Cu concentration in plants, and exchangeable ($r=0.91$) and free Cu ($r=0.87$) in the soil solution for the lime treatment. And between Cu concentration in plants, and exchangeable ($r=0.89$) and free Cu ($r=0.79$) in the soil solution for the nitrogen treated soil (Figure 8.9). Increasing levels of residual Cu tended to increase the total, ionic and exchangeable Cu concentrations in the soil solution. With the addition of EDTA the plant Cu concentration showed a positive relationship with exchangeable Cu ($r=0.90$) and free Cu ($r=0.94$) (Figure 8.10). A rise in soil pH due to lime application resulted in a decline in both ionic and exchangeable Cu concentrations and an increase in the proportion of Cu present as

soluble organic complexes. It might therefore have been expected that plant Cu concentrations would decrease with an increase in soil pH. However plant Cu concentration was the highest at 1000 kg ha⁻¹ level. A possible reason for the increase of Cu is the displacement of Cu from cation exchange sites by large amounts of base cations (Cu²⁺) released into the soil solution during the dissolution of lime.

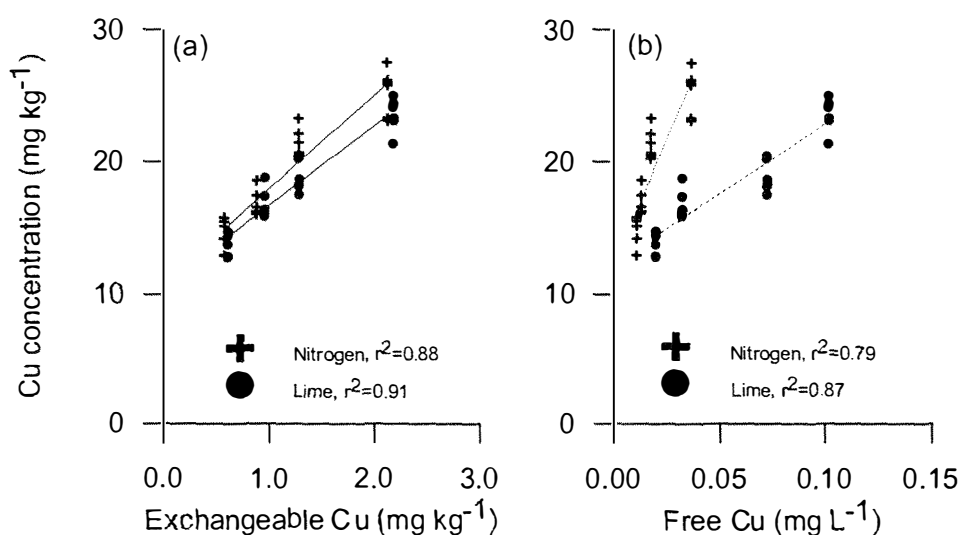


Figure 8.9 Relationship between Cu concentration in plants and (a) exchangeable Cu and (b) free Cu in soil solution with addition of N (+) and lime (●).

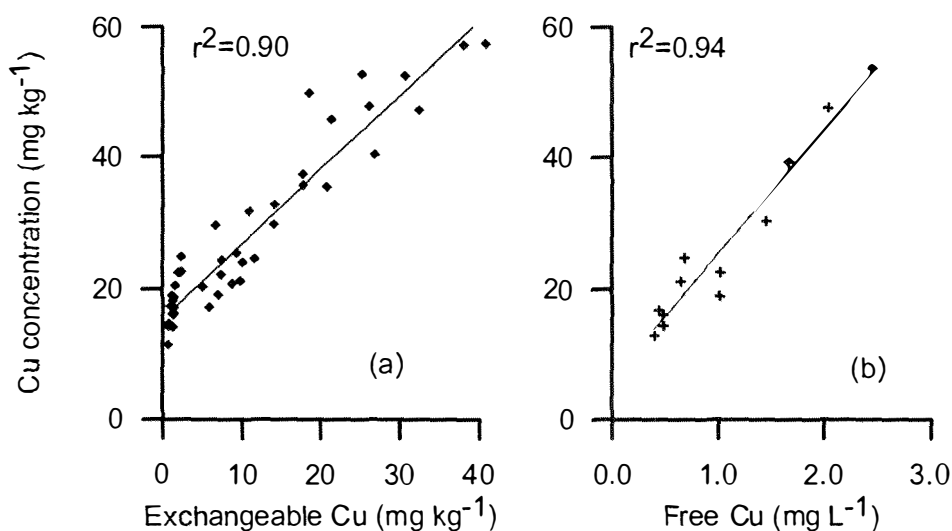


Figure 8.10 Relationship between Cu concentration in plants and (a) exchangeable Cu and (b) free Cu in soil solution with addition of EDTA.

8.4 CONCLUSION AND FURTHER STUDY

Both lime and N fertiliser increased the DM yield, but EDTA decreased the DM yield of ryegrass with increasing level of residual Cu. The lowest level of N increased the Cu concentration and the highest level of lime decreased the Cu concentration. The Cu concentration in ryegrass increased with increasing levels of EDTA. Liming improves the soil condition by alleviating the detrimental effects of soil acidity. The effect of N on Cu concentration persisted beyond the first cut only at the highest N addition level. The present study revealed that the application of 1000 kg lime ha⁻¹ and 50 kg N ha⁻¹ was very effective in enhancing the plant availability of residual Cu in soils without any toxicity; but EDTA increased the plant available Cu to toxic levels. Residual Cu had a positive effect on Cu concentration in the herbage. There were positive correlations between Cu concentration in plants and Cu in the soil test extractants. The free and exchangeable Cu in soils were strongly correlated with plant Cu concentration. The highest application rate of lime and N fertiliser decreased the exchangeable and free Cu in soil, but EDTA showed the opposite effect.

The plant availability of Cu to plants is often poorly related to the quantity of the element in the soil. Soil properties such as pH, organic matter, CEC, plant species, and environmental factors, such as soil water content, temperature and light, greatly influence the likelihood that a plant will exhibit Cu deficiency or toxicity symptoms. Changes in the environment often have a greater effect on Cu concentration in plants. The effects of seasonal responses on Cu availability and the transformation of Cu from two different sources of Cu fertiliser are discussed in the next chapter.

CHAPTER 9 SEASONAL RESPONSE OF COPPER AVAILABILITY IN PASTURE

Publication arising from this chapter:

Khan, M. A. R., N. S. Bolan and A. D. Mackay 2000. Seasonal variation of Cu availability in pasture. pp 171-186. *In* Soil research—a knowledge industry for land-based exporter. L.D. Currie, M.J. Hedley, D.J. Home and P. Loganathan (Eds). Occasional Report No. 13. Fertiliser and Lime Research Centre, Massey University, Palmerston North.

9.1 INTRODUCTION

Pasture provides the main source of Cu for grazing animals. There is a need to define the rates of change in the effectiveness of Cu fertilisers over the range of soil and climatic conditions encountered in New Zealand. Soil moisture, soil temperature, air temperatures and solar radiation are important parameters that affect the growth of plants. The metabolic disorders caused by Cu in grazing animals may be due to excesses or deficiencies of Cu in the diet. These disorders are sometimes associated with ingestion of normal amounts of Cu. Retention of Cu by the animal is influenced by Mo and S and these elements are closely implicated in the development of Cu disorders. The timing of Cu application may also influence pasture Cu concentrations and therefore, the availability of Cu and the uptake of Cu by pasture and the grazing animal varies with the seasons. Little information has been published on the seasonal effect of Cu applications on pasture and its impact on the overall Cu concentration, and status in pasture and the grazing animal. There is no published work on seasonal responses to added Cu on pasture Cu concentration.

The concentration of nutrient ions extracted by various chemical extractants varied considerably over the course of a growing season under field conditions (Haines and Cleveland, 1981; Schnitzer, 1991; Barrow, 1992). Questions arise as to whether climatic and soil moisture conditions affect the extractability of Cu. Soil samples for microelement extraction are often taken with little regard to field moisture content and stored under a variety of conditions (Shuman, 1980). In order to study the effects of these practices on extractable Cu, four different soils were incubated under different soil moisture and temperature conditions.

It has often been observed that a lift in Cu concentration in pasture occurs under field conditions, even after a long period of fertiliser application (A.D. Mackay, Personal communication). This is attributed mainly to the seasonal influences on pasture growth, and Cu transformation in soils. Variation of pasture Cu concentration due to seasonal responses to added Cu is not fully understood. This information is required so that the needs of crops, pastures and the grazing animal can be met, and agricultural production maintained. In this study, the effectiveness of two different Cu fertilisers (CuSO_4 and CuO) in raising the Cu concentration in pasture was examined at different times of the year in the Tokomaru soil. The variation of Cu transformation within the trial periods was also examined.

9.2 MATERIALS AND METHODS

9.2.1 *Field trial*

9.2.1.1 *Experimental site*

The study area was at the Massey University Pasture growth unit (Plate 9.1). The average annual rainfall for this area is 1200 mm. The soil is a Yellow Brown/Yellow Grey Earth intergrade and related (Typic Fragiaqualf). There were two Cu fertiliser sources (CuSO_4 and CuO), each applied at 3 rates (0, 2, and 5 kg Cu ha⁻¹) laid out in a randomised design, with three replicates. Seven trials were conducted and the dates of each trial are given in Table 9.1. Plots were of uniform size (1 m²).

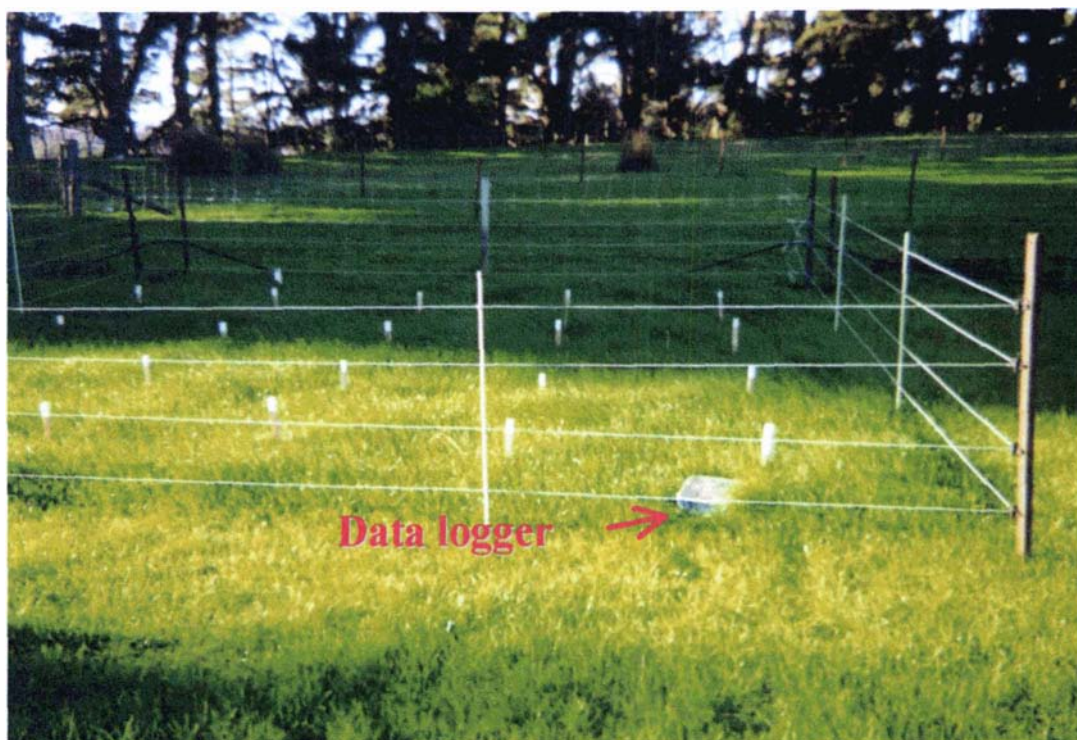


Plate 9.1 Location of the field trial.

Table 9.1 Time frame of the different trial periods.

Trial numbers	Date of fertiliser application	Date of harvesting	Seasons
Trial 1 (T ₁)	19 th March	29 th April	Autumn
Trial 2 (T ₂)	29 th April	10 th June	Autumn
Trial 3 (T ₃)	10 th June	22 nd July	Winter
Trial 4 (T ₄)	22 nd July	2 nd September	Winter
Trial 5 (T ₅)	2 nd September	14 th October	Spring
Trial 6 (T ₆)	14 th October	24 th November	Spring
Trial 7 (T ₇)	26 th November	6 th January	Summer

9.2.1.2 *Soil and pasture sampling*

A set of 5 soil cores (25 mm diameter) to a depth of 75 mm was collected from each plot prior to and 6 weeks after fertiliser application. The soil samples were air-dried and

the soil aggregates were crushed using a porcelain mortar and pestle. These samples were used for the extraction of Cu.

Pasture samples were collected 6 weeks after fertiliser application. The pasture samples were cut using a standard 0.09 m² quadrat to the height of the quadrat edges using hand shears. This was approximately within 2.5 cm above the ground level. The samples were weighed and washed with reverse osmosis (RO) water and dried at 70°C in a forced air oven. The dry weights of the pasture samples were recorded and the samples were then ground using a coffee grinder and kept in airtight polyethylene bags for chemical analysis.

9.2.1.3 *Soil moisture, soil temperature and climate data*

Soil samples were placed in an oven at 105°C overnight and the gravimetric moisture content was recorded before and after each set of trials. A data logger was used to record soil temperature. Daily hourly rainfall, radiation and air temperature for the site were obtained from New Zealand Meteorological Services Ltd.

9.2.1.4 *Fractionation of Cu and soil analysis*

Fractionation of soil Cu was carried out in T₂, T₄, and T₇ soils according to the sequential extraction method described (McLaren and Ritchie, 1993) in chapter 3 (Section 3.2.6). Hydrochloric acid was used instead of hydrofluoric acid in the residual fraction. Soil pH (1:2.5 H₂O) and the exchangeable soil Cu [0.01M Ca(NO₃)₂] were measured.

9.2.1.5 *Pasture analysis*

Methods for the preparation of ryegrass samples for chemical analysis were described in section 4.2.5.

9.2.1.6 *Chemical analysis*

Copper in the plant digests and soil extractants was analysed using F-AAS. Details of these procedures are discussed in section 4.2.6.

9.2.2 *Incubation study*

The effect of soil moisture content and temperature on the transformation of Cu was examined under controlled conditions. Four pasture soils (Manawatu, Tokomaru, Ramiha and Ngamoka) taken from the A horizons were air dried and passed through a 2 mm sieve. Soil samples were equilibrated with a solution containing known amounts of Cu as $\text{Cu}(\text{NO}_3)_2$. Soil samples were incubated at four temperatures (5, 10, 15 and 20°C) and at four moisture levels (10, 20, 30 and 40%) for four weeks in polythene bags. After incubation, the soil samples were air dried, passed through a 2 mm sieve and used for Cu analysis.

9.2.3 *Statistical analysis*

Statistical analysis of the data was carried out by the Statistical Analysis System (SAS). Details are discussed in section 4.2.7.

9.3 RESULTS AND DISCUSSION

9.3.1 *Climatic data*

Mean average hourly rainfall for the experimental period (0.10 mm) was above the average rainfall for the previous 10 years (0.07 mm) (Figure 9.1). Gravimetric soil moisture content at (0-150mm depth) increased from 130 g kg⁻¹ soil for T₁ to 290 g kg⁻¹ soil for T₃ and then decreased to 190 g kg⁻¹ soil for T₅ and again increased for T₇ (Figure 9.1). Mean daily soil temperature (75-mm depth) was highest (>15°C) at the beginning (T₁) and the end (T₇) of the experiment and decreased to 6.4°C for T₄. Mean average hourly air temperature was highest for T₁ (15.4°C) and it decreased to 7.9°C for T₄ (Figure 9.1). Average daily solar radiation was lowest during T₃ (5.65 MJ m⁻² day⁻¹) and highest during T₇ (19.87 MJ m⁻² day⁻¹).

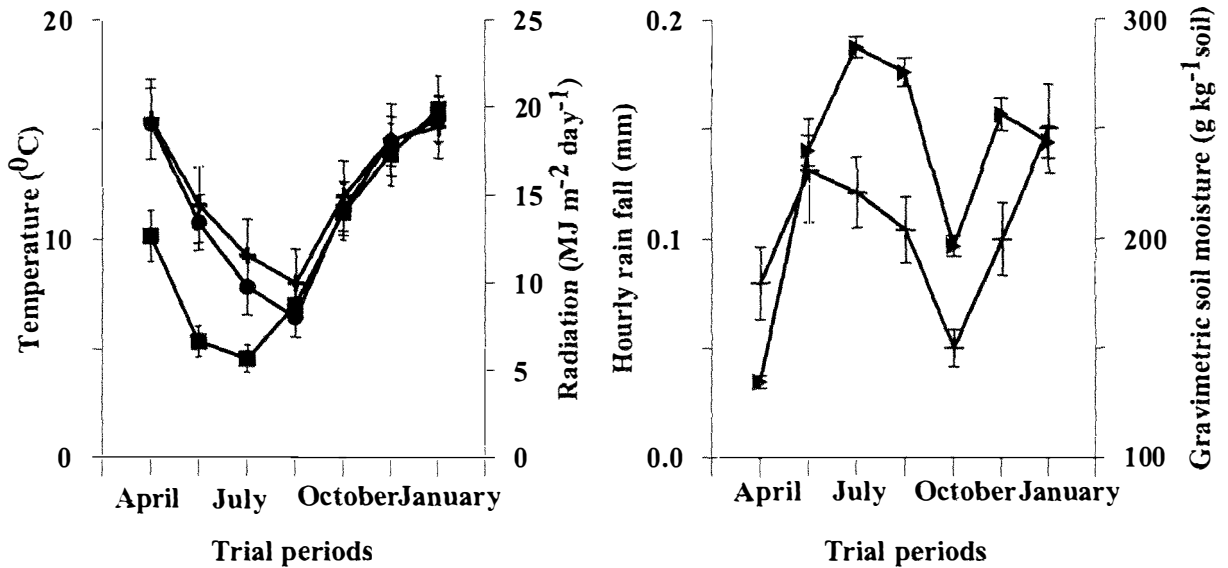


Figure 9.1 Weather data for soil temperature (●), air temperature (+), radiation (■), gravimetric soil moisture (▼) and rain fall (+) during different trial periods. (Data are mean \pm SE for the different parameters at each trial period).

9.3.2 Pasture growth rate

Pasture growth was not affected by either Cu source or rates of Cu fertiliser application throughout the trial period. This is consistent with reports that pasture responses to Cu only occur when the Cu concentration in the pasture is $<4 \text{ mg kg}^{-1}$ (Sherrell and Rawnsley, 1982). There are very few reported incidences of Cu concentration $<4 \text{ mg kg}^{-1}$ in New Zealand pastures. For example, for New Zealand pasture, the exceptions are peats ($<3 \text{ mg kg}^{-1}$;) and pumice soils ($<4 \text{ mg kg}^{-1}$);. Forbes (1978), in a survey of lucerne on Yellow Brown Pumice soils observed very few stands with $<4 \text{ mg kg}^{-1}$ Cu concentration in the herbage. Khan *et al.* (1996) observed that Cu applied, as CuSO_4 fertiliser has no significant effect on pasture growth rates under field conditions.

Pasture growth rate was the highest for T_7 and the lowest for T_3 (Figure 9.2). Pasture growth rate closely followed temperature and solar radiation, with the exception of T_2 , where the lift in pasture growth appears to be in response to an increase in soil moisture with a decline in soil temperature and solar radiation. Hunt and Halligan (1981) grew perennial ryegrass plants in nutrient solution under 6 levels of irradiance at 6 constant temperatures (7 to 33°C) to provide growth response curves to light and temperature over a range of growth conditions. Growth analysis revealed that at 7, 10, 17 and 20°C ,

changes in leaf area ratio compensated for changes in the net assimilation rate over a range in irradiance for which the growth rate was maintained near maximum.

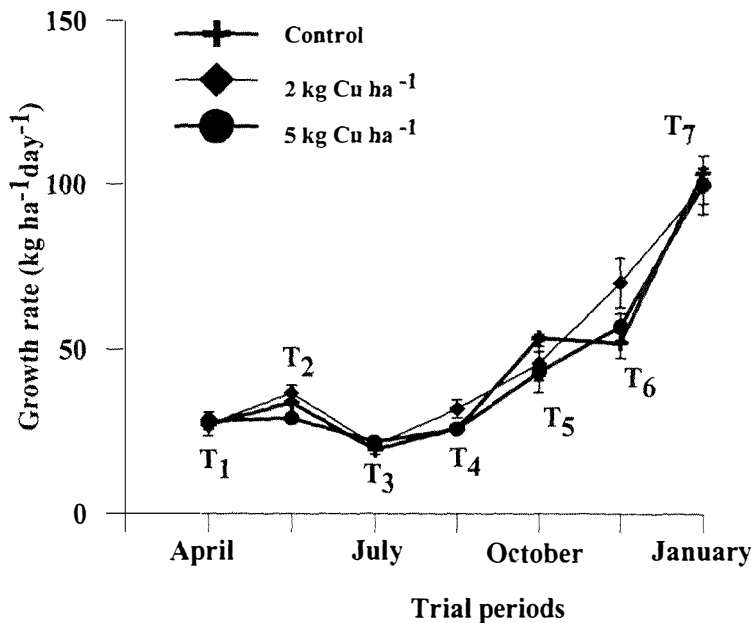


Figure 9.2 Pasture growth rates as influenced by different levels of Cu fertiliser at different periods after fertiliser application. Data are means \pm SE, $n=3$.

Growth rate was highest at 15.1⁰C (except T₁) and lowest at 9⁰C air temperature (Figure 9.3a). During T₁ (March-April), the growth rate was very low, which may be attributed to the low soil moisture content (120 g kg⁻¹ soil). Pollock (1990) has found that air temperature exerts a much larger effect on increasing plant growth than it does on the chemical reactions of metabolism. Low temperatures appear to increase cell wall rigidity, which impairs turgor-driven cell extension processes. Naturally expansion growth is a significant component of crop yield. However, in the absence of other constraining factors, and low temperature induced injury, yield may not be diminished by a short duration of low temperature exposure, only delayed. Sarathchandra *et al.* (1989) observed greater immobilisation of nitrogen and build-up of microbial biomass at low soil temperatures (6-10⁰C) than at higher soil temperatures (11-18⁰C), resulting in low pasture growth. Cossens and Brash (1981) reported that low temperature was the main factor restricting the pasture growth rate in a 7 years study in Central Otago, New Zealand. Campbell *et al.* (1999) reported that the growth was increased by increasing temperature from 5 to 25⁰C of seven white clover cultivars in controlled environmental chambers.

Growth rate was the lowest at 7.8⁰C and the highest at 15.1⁰C (except T₁) soil temperature (Figure 9.3b). Growth rate was lowest at the highest moisture level for T₃, which may be attributed to this period having the lowest air temperature and solar radiation. Baker and Younger (1986) demonstrated the effect of soil temperature on the spring growth of perennial ryegrass swards at three contrasting sites. They observed a highly significant relationship existed between leaf extension rates and soil temperature at each site.

Soil moisture decreased the growth rate at the highest level of moisture content (Figure 9.3c). Roberts and Thomson (1984) reported that pasture growth rates (mainly for *Lolium perenne* L and *Trifolium repens* L.) for 8 years for a site on the Waimate plains of South Taranaki, New Zealand under 28 day cutting intervals were highest in late spring and rapidly declined over summer. Autumn growth rates were reasonably constant, then declined through winter to the lowest growth rates in mid July. The greatest variation in growth rates between years occurred over the summer and early autumn. Soil moisture and rainfall data indicated that variable summer rainfall caused soil moisture levels to become the critical factor affecting growth rates. Moir *et al.* (2000) developed a climatic function model, which describes the interacting effects of soil fertility, soil moisture, and evapotranspiration on pasture production.

Growth rates increased with increasing radiation (Figure 9.3d). During T₁ (March-April) although the radiation was at the optimum level, the growth rate was very low, which may be attributed to the low soil moisture content (120 g kg⁻¹ soil) during this period. It is well known that light intensity and temperature influence the quality of pasture plants. The effects of temperature on crop development, and solar radiation on bio-mass accumulation combine to impose well defined limits to potential crop yields under various environmental conditions. The amount of solar radiation defines the maximal limit to crop yield because intercepted solar radiation provides the energy for photosynthetic fixation of CO₂. By assuming an upper limit to the efficiency of CO₂ fixation (or crop biomass accumulation) per unit of intercepted solar radiation, an estimate can be calculated for the limit to crop yield based on the available solar radiation during the growing season. Woledge and Dennis (1982) observed that the photosynthetic rate of leaves was twice as high at 15⁰C than at 5⁰C for perennial

ryegrass (*Lolium perenne* L.) and white clover (*Trifolium pratense* L.). Ryegrass and clover have similar photosynthetic rates, which responded similarly to temperature.

Spaeth *et al.* (1987) were able to account for much of the yield variability between seasons as a consequence of differences in temperature and incident solar radiation. The radiation use efficiency (RUE) and the harvest index were held constant under all conditions in their calculation. Both the pattern of leaf area development (as determined by temperature) and incident solar radiation in these environments influenced the amount of intercepted radiation, which in turn accounted for differences in yield between seasons in nonstressed soybeans grown in Japan. Yields of nonstressed spring wheat in Israel were examined in terms of environmental variation in temperature and solar radiation (Amir and Sinclair, 1991). Calculated yields across nine seasons, assuming a constant RUE were found to agree well with the observed yields ranging from 410 to 660 g m² for nonstressed wheat crops. As with maize, the duration of various phases of wheat development were very sensitive to variations in temperature. With warming temperatures, crop development is rapid and the length of time needed for radiation interception is shortened. The greatest yields in spring wheat were achieved under environments with cool temperatures and high radiation (Amir and Sinclair, 1991).

Maeda and Yonetani (1981) investigated the light interception by an Italian ryegrass population in each season by measuring the light diminution coefficient (LDC). LDC was determined with a negative regression coefficient between the increase in leaf area index and the decrease in relative light intensity. A close positive correlation was recognised in the autumn and spring. Noble (1972) reported that increasing solar radiation increased the pasture growth rate. Differences in solar radiation patterns between the seasons provide an explanation for the differences in growth rate.

In this experiment pasture growth rates followed a strong seasonal pattern with the lowest growth rates in winter (T₃), and the highest in early summer (T₇), which was similar to an earlier field trial (Khan, 1996). Lambert *et al.* (1986) found that poa and ryegrass have maximum growth rates in spring. But most of the low fertility grasses produce maximum growth in late spring and early summer. Similar results were observed by Del Pino Machado (1994), who found that the pasture growth rates ranged

from 108 kg DM ha⁻¹ day⁻¹ in early summer to less than 2 kg DM ha⁻¹ day⁻¹ in late winter.

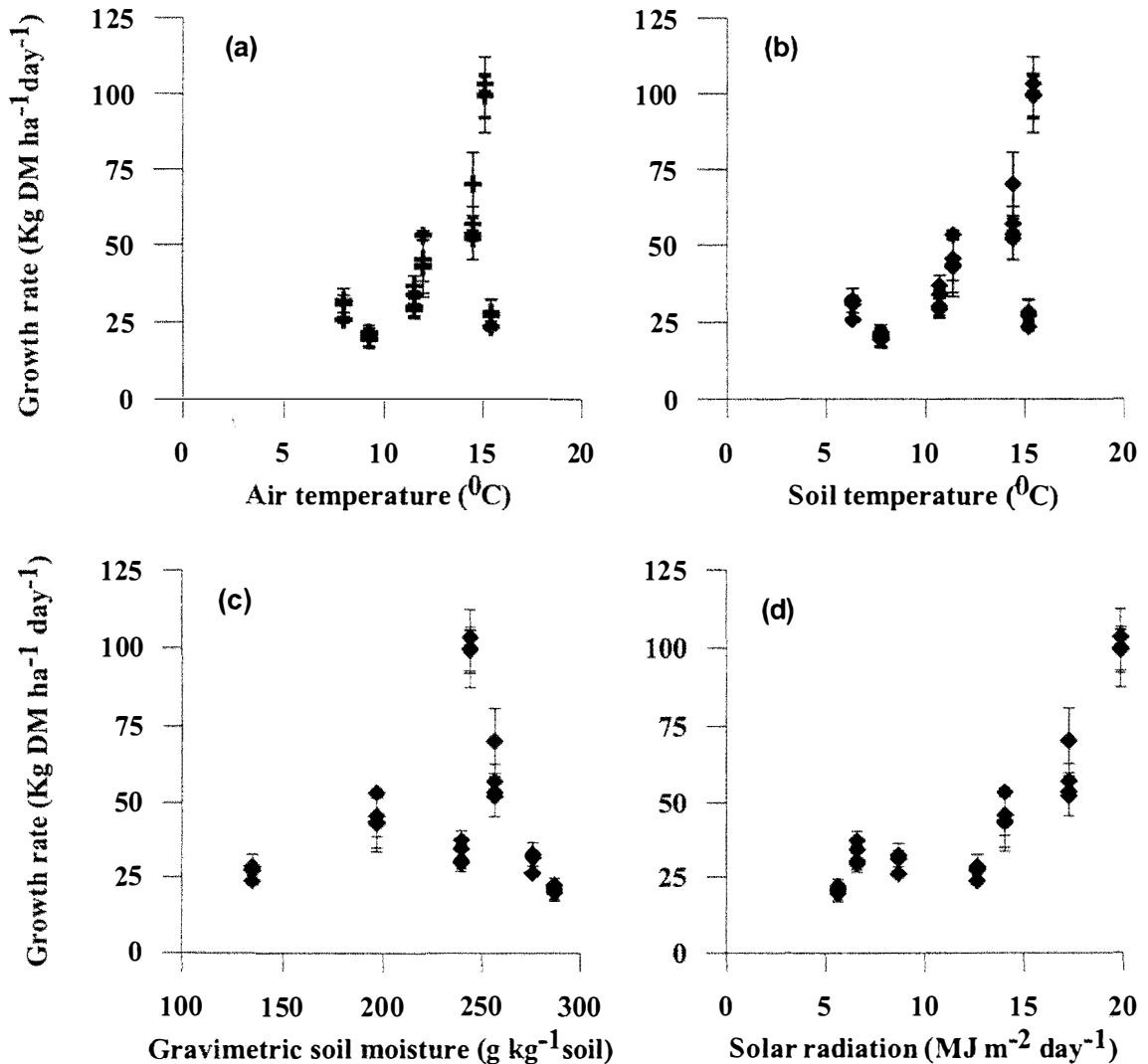


Figure 9.3 Effect of air temperature (a), soil temperature (b), gravimetric soil moisture (c) and solar radiation (d) on pasture growth rates. Data are means \pm SE, n=3.

9.3.3 Pasture Cu concentration

The field study shows differences in seasonal response to added copper. Pasture Cu concentration was highest for T₄ for the CuSO₄ fertiliser and T₅ for the CuO fertiliser (Figure 9.4). Cu fertiliser sources have a significant effect on the Cu concentration in pasture. CuSO₄ resulted in the highest concentration in all trials compared with the CuO fertiliser (Figure 9.4). This might be attributed to the quick release characteristic of the

CuSO₄ fertiliser (Gilkes, 1981). The general use of CuSO₄ reflects its high water solubility and wide availability (Alloway and Tills, 1984; Karamanos *et al.*, 1986). The suitability of CuO for broadcasting has been found to depend on particle size. CuO broadcast onto the soil surface at 5 kg Cu ha⁻¹, and worked into the soil did not correct Cu deficiencies in canola, barley, or wheat during the year of application, but corrected the deficiencies in the following years (Karamanos *et al.*, 1986). The lack of Cu response to CuO treatment was mainly due to the low water solubility of the coarse, granular CuO, which ranged in particle diameter from <0.2 to 3.0 mm. Coarse Cu carriers are also ineffective where inadequate contact occurs between the roots and applied Cu (Gartrell, 1981). A similar result was also observed in a previous glass house trial, where three different sources of Cu fertilisers [CuSO₄, Cu(OH)₂ and CuO] were used in five different soils (Manawatu, Tokomaru, Ramiha, Ngamoka and Mangamahu) (Khan *et al.*, 1998; Section 4.3.3.2).

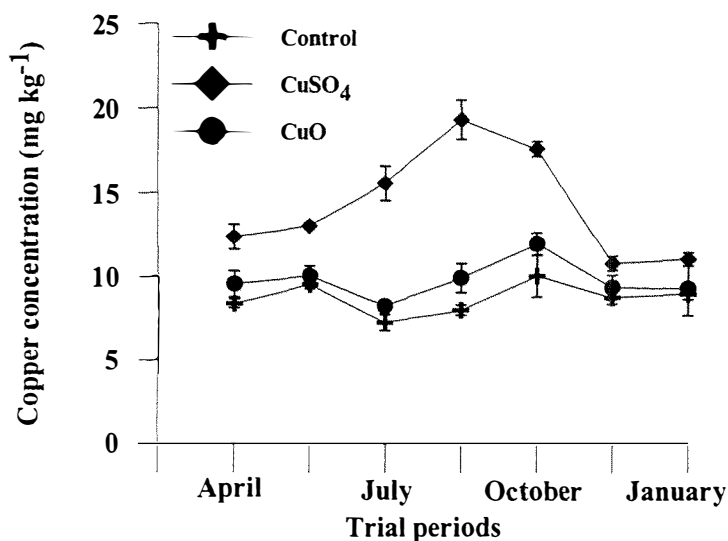


Figure 9.4 Effect of fertiliser Cu sources on Cu concentration at 5 kg Cu ha⁻¹ level. Data are means \pm SE, n=3.

Increasing Cu fertiliser rates increased the Cu concentration in pasture (Table 9.2). Significant seasonal responses to Cu concentration in pasture with added fertiliser was observed (Figure 9.5). The highest Cu concentration was observed in T₄ and the lowest concentration in T₆. The major effect on Cu concentration was due to pasture growth.

Table 9.2 Effect of fertiliser levels on Cu concentration (CuSO₄ and CuO).

Treatments	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 6	Trial 7
Control	8.40c	9.52b	7.27c	8.00c	10.02c	8.75b	8.94b
2 kg Cu ha ⁻¹	10.54b	12.18ab	11.23b	15.62b	13.37b	8.89b	9.82b
5 kg Cu ha ⁻¹	12.36a	13.03a	15.58a	19.29a	17.58a	10.76a	10.99a
5 kg Cu ha ⁻¹ as CuO	9.85bc	10.02b	8.27c	10.17c	11.93bc	9.35b	9.26b

* Treatment means followed by the same letter within a column are not significantly different at the 5% level.

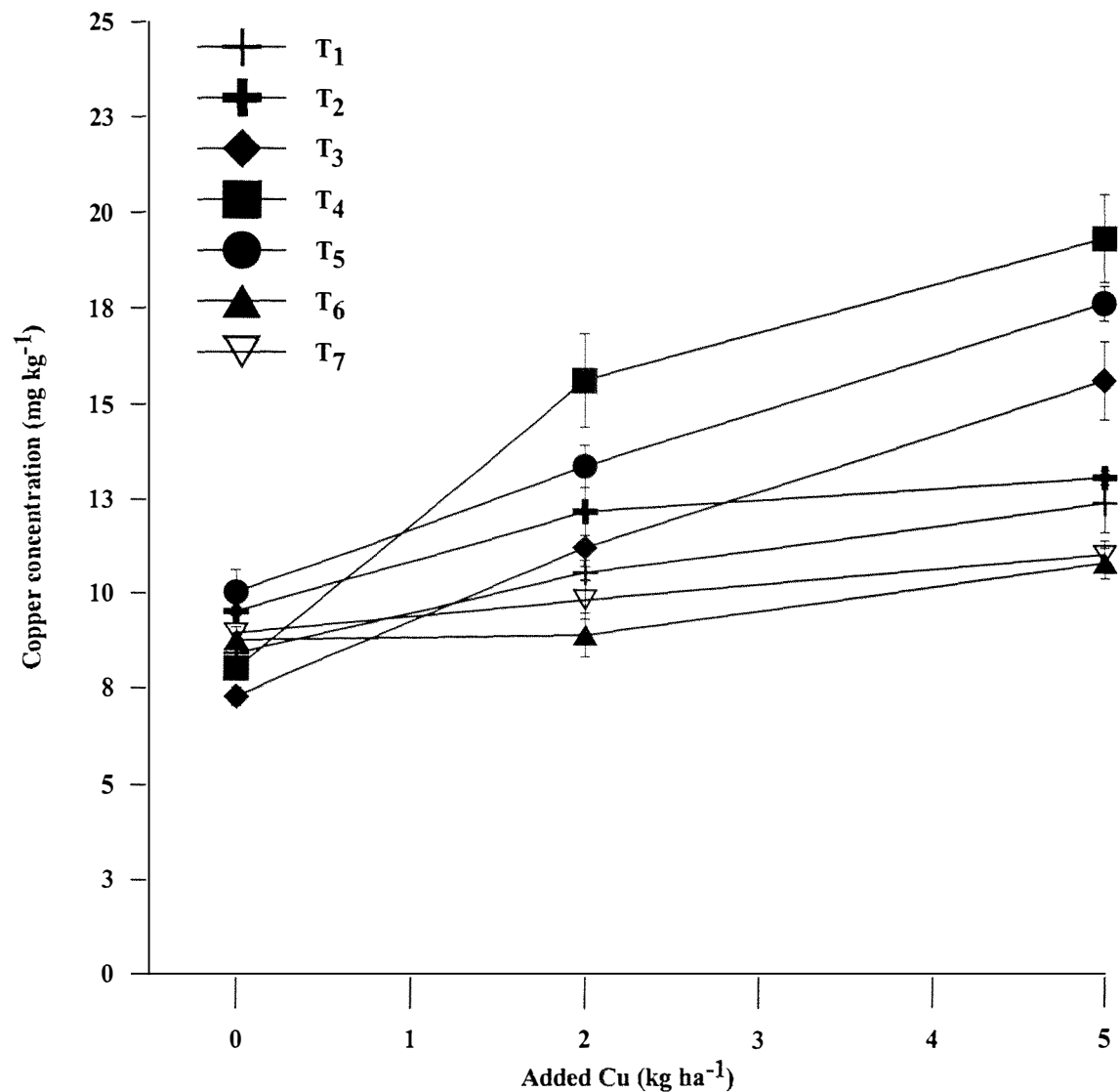


Figure 9.5 Effect of seasonal responses to added Cu on Cu concentration in pasture. Data are means \pm SE, n=3.

The Cu concentration was highest at 7^oC and lowest at 14.5^oC air temperature. The Cu concentration decreased with increasing air temperature (Figure 9.6a). It is apparent that

substantial mobilisation of Cu occurs in the root zone from T₄ to T₇ due to the higher growth rate of the pasture. Merry *et al.* (1986) observed that pasture plants grown at 22°C produced a higher DM yield and Cu concentration than plants grown at 12°C. But in this study, the highest Cu concentration at low air temperatures was obtained due to a low pasture growth rate. The Cu concentration was highest at 6.4°C and lowest at 14.5°C soil temperature at 5 kg Cu ha⁻¹ level (Figure 9.6b). The Cu concentration was highest during winter and lowest during late spring and early summer. Reddy *et al.* (1981) reported strong seasonal variations in Cu concentrations in pasture, the concentrations of Cu were low in spring and early summer when compared to winter. They also observed that increasing the soil temperature from 12 to 22°C increased both dry matter production and the concentrations of Cu in subterranean clover on lateritic podzolic soils. The variation in soil temperature during the trial periods, observed in the present study, is likely to affect the availability of organically bound Cu. Organically bound Cu in soil is present in both soluble and insoluble forms (Stevenson and Fitch, 1981). Mobilisation-immobilisation reactions are undoubtedly temperature dependent, and affect the solubility of soil solution Cu. Differences in soil temperature patterns between the seasons provide an explanation for the differences in Cu concentrations in plants. Reay and Waugh (1983) observed Cu concentrations fluctuated monthly in seasonal variation trials. They also observed that Cu concentration, which fluctuated without seasonal trends in ryegrass leafblades, correlated with organic-N concentration.

Soil moisture content was highest at T₃ and lowest at T₁. It suggested that excessive moisture content, low air and soil temperature may also lead to low Cu concentrations in the T₃ period (Figure 9.6c). The concentration of Cu increased with advancing time until T₄ and then it gradually declined. Soil moisture increased until T₃, then it decreased and again increased till T₇. Copper concentrations increased progressively to a maximum value in September (T₄) due to adequate soil moisture. The Cu concentration was not directly related to radiation, but there was a tendency for Cu concentrations to decrease at the highest level of solar radiation (Figure 9.6d). Copper concentration decreased with increasing growth rate of pasture (Figure 9.7).

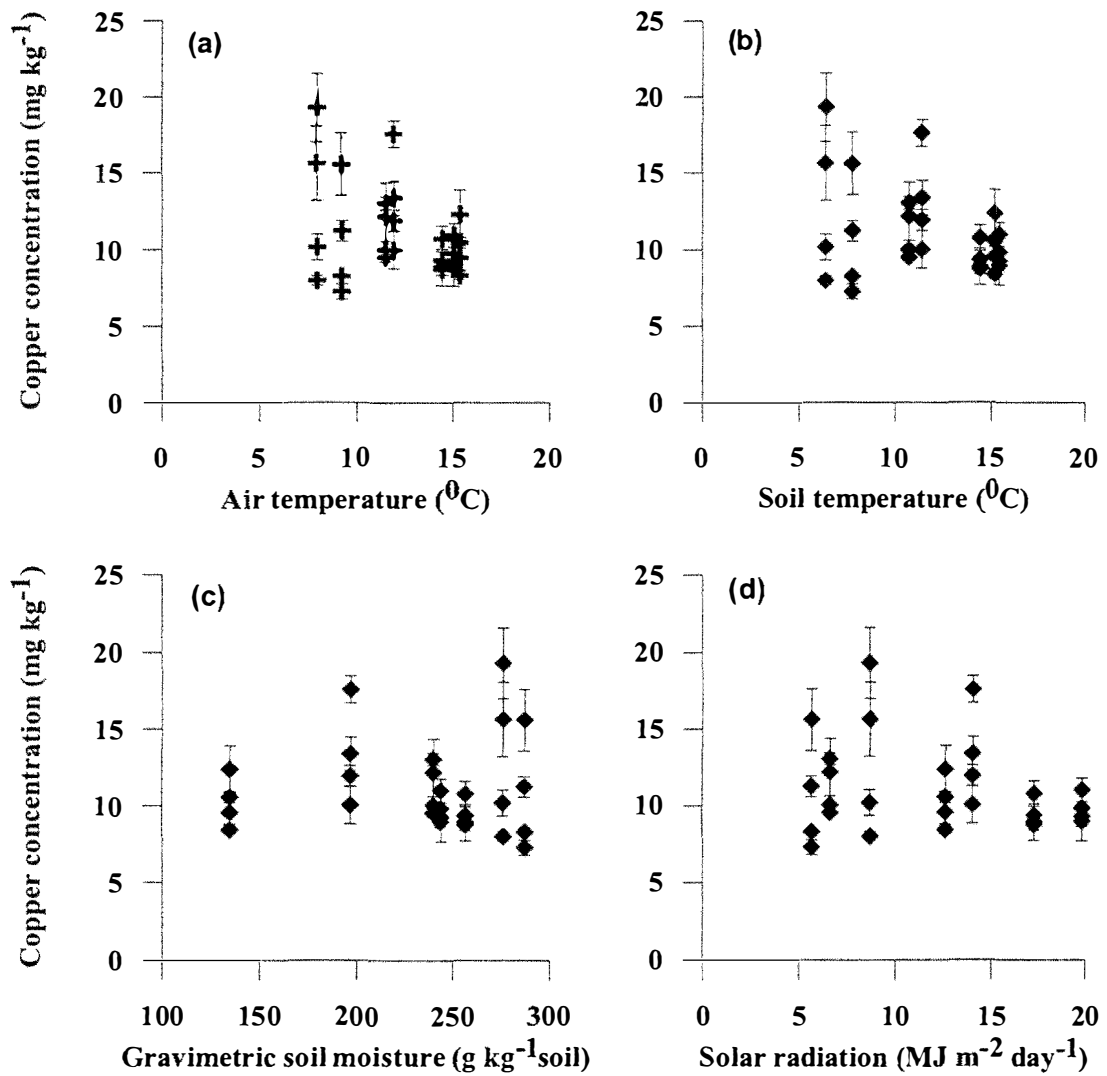


Figure 9.6 Effect of air temperature (a), soil temperature (b), gravimetric soil moisture (c), and solar radiation (d) on Cu concentration. Data are means \pm SE, $n=3$.

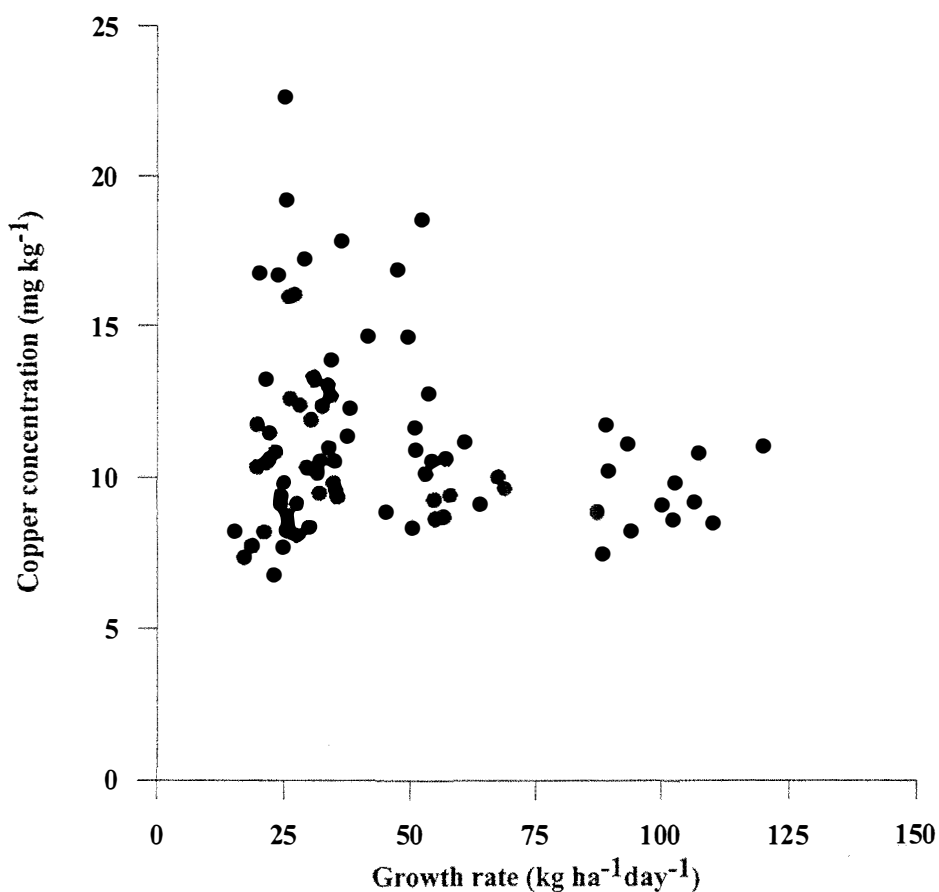


Figure 9.7 Relationship between pasture growth rate and Cu concentration.

9.3.4 Pasture Cu uptake

Copper fertiliser sources have a significant effect on Cu uptake in pasture. Cu uptake in pasture increased with increasing levels of Cu fertiliser application. The highest Cu uptake was observed for T₇ and the lowest uptake for T₃ (Figure 9.8). The total Cu uptake by plants from T₄ to T₇ increased at all levels of Cu fertiliser application. The increase in Cu uptake in these trials could be caused by an increased growth rate. It is apparent that substantial mobilisation of Cu occurs in the root zone from T₄ to T₇ due to the higher growth rate of pasture.

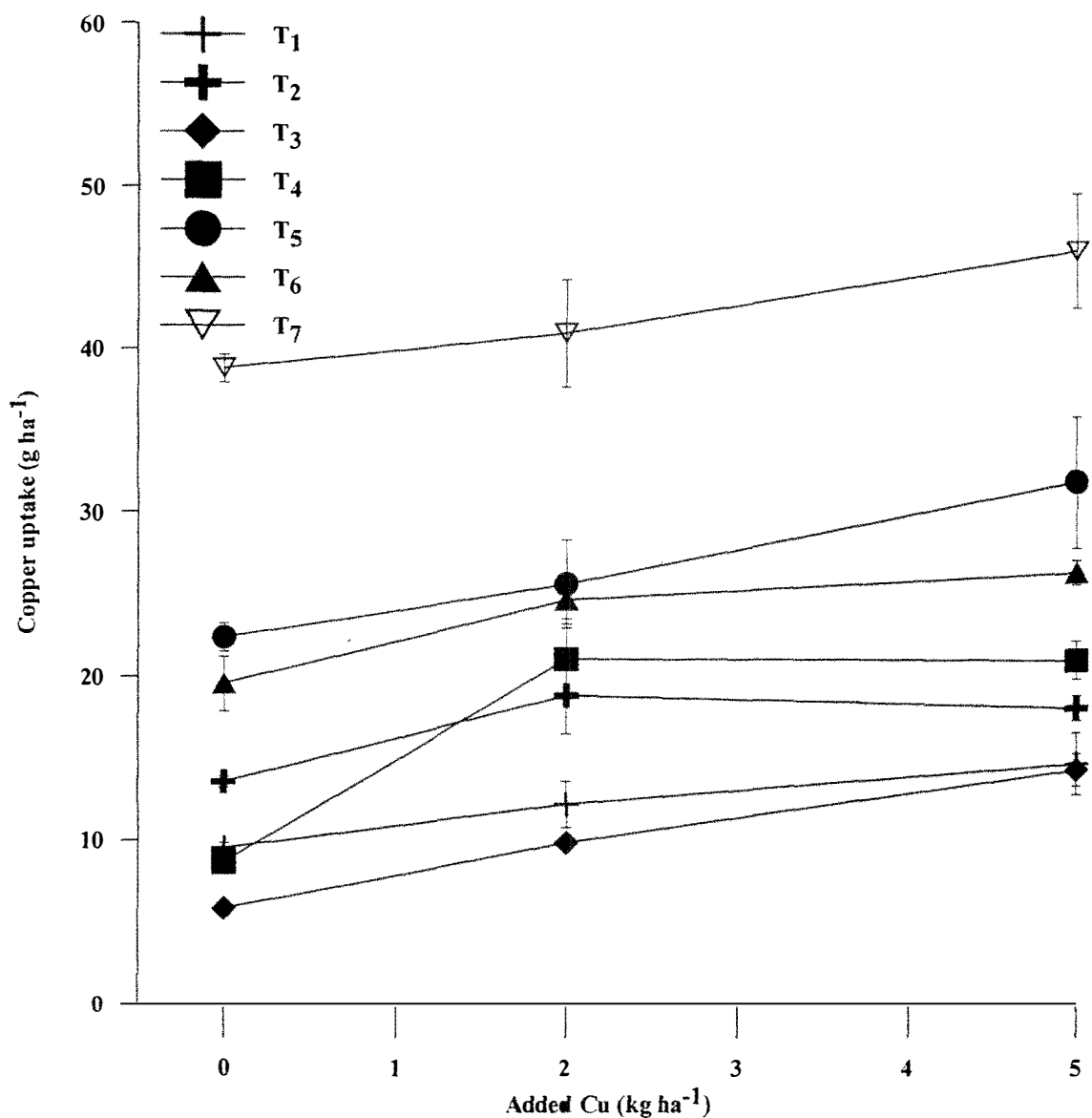


Figure 9.8 Effect of seasonal responses to added Cu on total Cu uptake in pasture. Data are means \pm SE, $n=3$.

Increasing air temperature increased Cu uptake. The Cu uptake was highest at 14.5°C and lowest at 7°C air temperature (Figure 9.9a). Increasing soil temperature also increased Cu uptake (Figure 9.9b). The uptake of both native and added Cu by carrots growing in an acid soil under green house conditions was increased, when the soil temperature was increased from 8 to 20°C (MacMillan and Hamilton, 1971). Brennan *et al.* (1984) conducted a glasshouse experiment in which wheat plants were grown in soil samples incubated with a Cu fertiliser at various temperatures for different periods. They observed that the dry matter yield and Cu uptake decreased when the incubation temperature increased from 10 to 30°C and with a longer period of incubation.

Root extension rates, size and morphology are strongly affected by soil temperature. An increasing temperature lowers the resistance of roots to water uptake, increases rates of ion diffusion and affects the permeability of membranes to cations and anions (Merry *et al.* 1986). Over the range of soil temperatures encountered during the trials, microbial activity and organic matter breakdown could be expected to increase the rate of mineralisation. In field conditions, some of the fluctuations in the concentrations of Cu in the plants may be explained by the dilution caused by increased growth rates. Changes in soil and air temperature, and light intensity affect both the uptake of nutrients by roots and their subsequent translocation to the tops. Temperature affects a number of factors that are important for metal ion absorption. These include the stability of the metal species, the ligands and the ligand-metal complexes as well as the solubility of the metal ions. The low root temperature limits plant growth through reduced rates of nutrient uptake. Engels and Marschner (1996) reported that the net translocation rates of Fe and Cu at all root zone temperatures were markedly enhanced by increasing the temperature of the shoot base. Accordingly, the concentrations of Fe and Cu in the shoot were not greatly affected by root zone temperatures, irrespective of the air temperature. These results indicate that the ability of roots to supply Fe and Cu to the shoot are internally regulated by the growth related shoot demand per unit of roots.

Soil moisture has a profound effect on Cu concentration, which decreased at the highest moisture level. Pasture Cu uptake reached a maximum at about 190-230 g kg⁻¹ soil moisture content (Figure 9.9c). Increases in soil moisture lead to a marked change in the ion pair formation, free hydrated metal concentrations, and the formation of complexes (Fotovat *et al.*, 1997). Soil moisture increased until T₃, then it decreased, and again soil moisture increased till January (T₇). Copper concentrations in plants increased progressively to a maximum value in September (T₄), due to optimum soil moisture content. At optimum soil moisture levels, the Cu concentrations are generally high in the root zone soil solution. It is likely that this mobilisation resulted from the biological production of chelating ligands. Differences in the extent of mobilisation of Cu in the root zone between pasture species growing on the same soil have been observed (Linehan *et al.*, 1989). This may reflect differences in the exudation of ligands and /or differences in the microflora in the root zone. It is clear that mobilisation within the root zone provides a reservoir of soluble micronutrients available for uptake by plants. Sinclair *et al.* (1990) reported, from nutrient inflow calculations and estimates of the

volume of the root zone, that the quantity of Cu present in the root zone solution is rapidly absorbed by the growing crop. They also reported that the micronutrients in soil solution are depleted more rapidly than would be expected from nutrient inflow rates. The rate of depletion is driven by nutrient uptake generated by plant growth, it will be modified and limited by the balance between mobilisation and immobilisation within the root zone, which is dependent on microbial activity controlling the flux of organic ligands. Wardle and Parkinson (1990) found that the microbial biomass during the growing season was significantly positively related to soil temperature, but only when the effects of soil moisture variation had been corrected.

Seasonal changes in nutrient concentrations in plants have often been attributed to differences in growth rate (Loneragan, 1975) or changes in soil moisture content, which may affect transport processes in the soil (Wilkinson, 1972). A decline in soil moisture content would reduce the rate of diffusion, and consequently the uptake of Cu by plants. Porter *et al.* (1960) reported that the rate of diffusion decreases 6 and 25 fold at 1 and 15 atmospheres of soil moisture potential, respectively. This, of course, reduces uptake substantially, especially when roots are not growing rapidly enough to exploit new volumes of soil. Nambiar (1977) showed that ryegrass absorbed significant amounts of Cu from nearly air-dry soil, when the roots had access to subsoil water. A prolonged period of drying tended to cause a small decrease in the Zn and Cu concentrations of the shoot, particularly Cu.

Cu uptake increased with increasing radiation (Figure 9.9d). During T₁ (March-April) although the radiation was at the optimum level, the growth rate was very low, which may be attributed to the low soil moisture content (120 g kg⁻¹ soil) during this period. A significant positive relationship was observed between Cu uptake and pasture growth rate ($r^2 = 0.83$) (Figure 9.10).

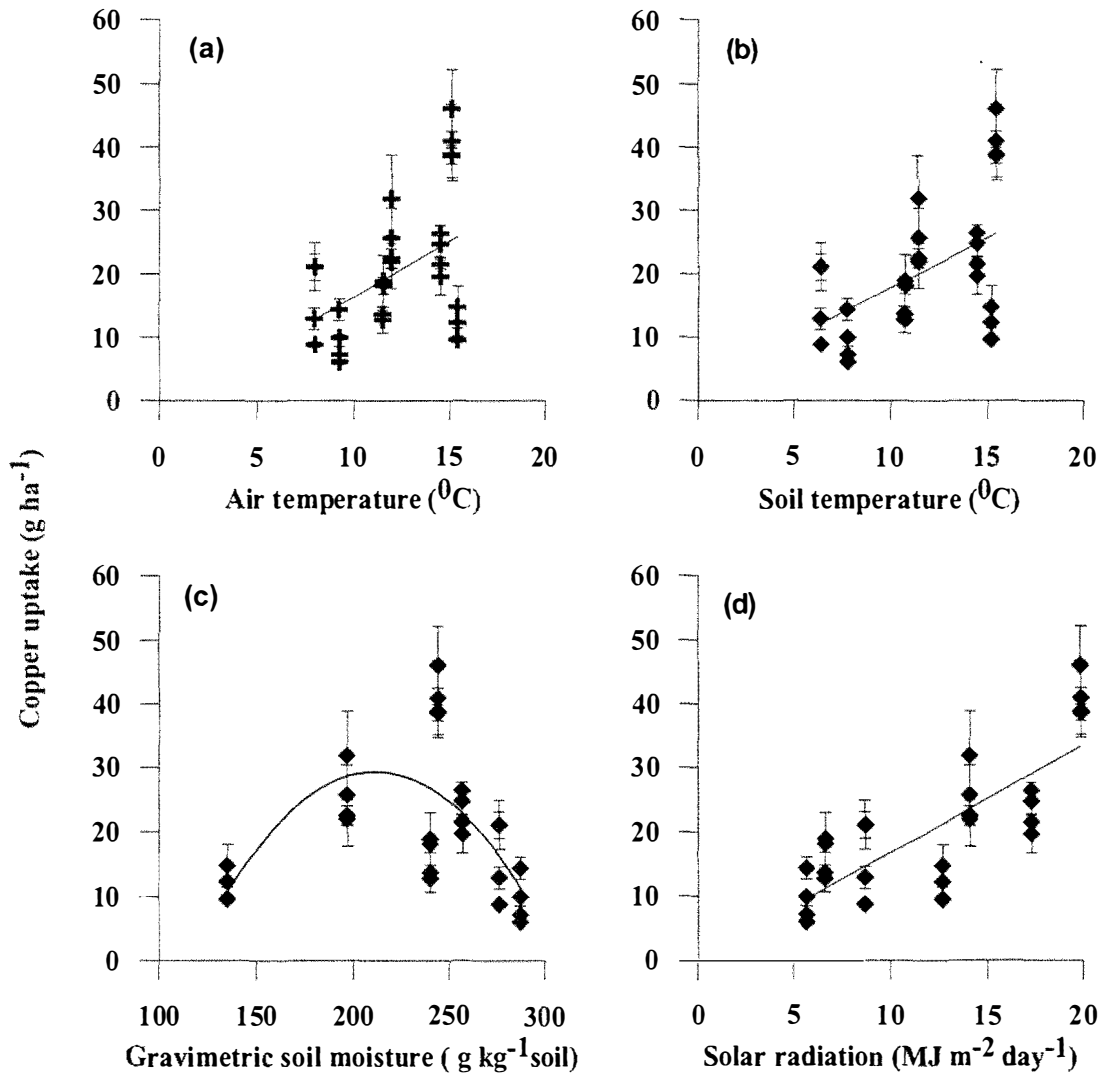


Figure 9.9 Effect of air temperature (a), soil temperature (b), gravimetric soil moisture (c), and solar radiation (d) on Cu uptake. Data are means \pm SE, $n=3$.

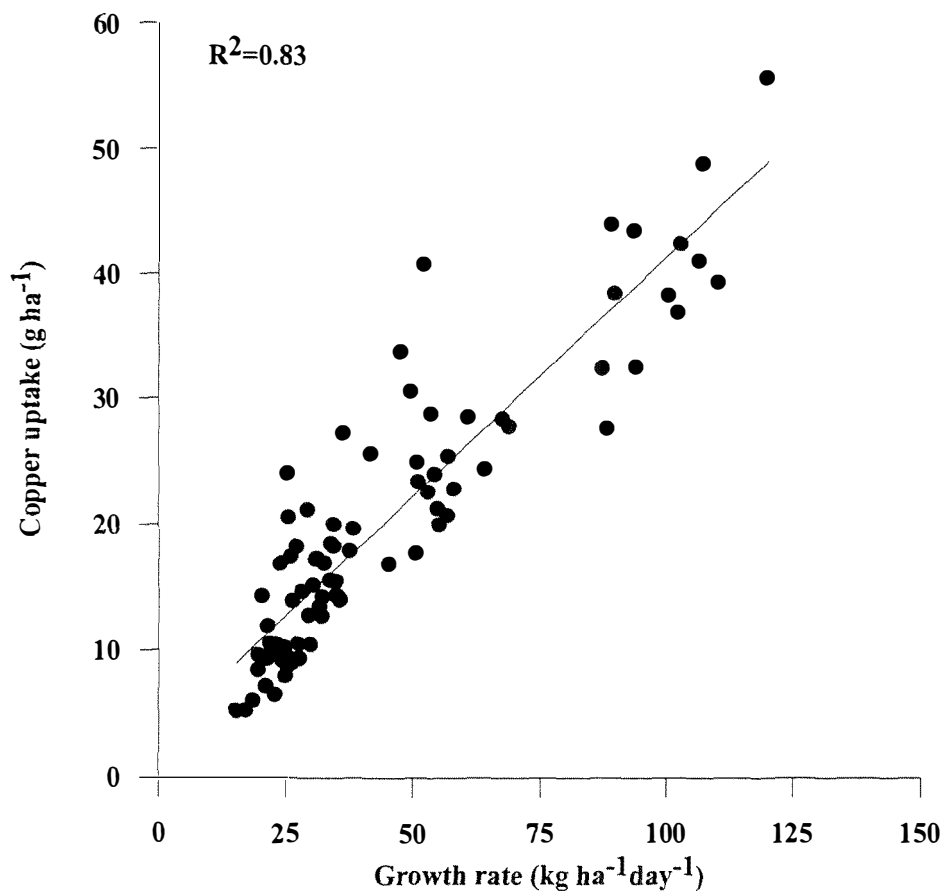


Figure 9.10 Relationship between pasture growth rate and Cu uptake.

9.3.5 *Effect of soil temperature and moisture on Cu concentration in soils*

The effects of different soil temperatures at various levels of soil moisture for the different soils under incubation study are illustrated in Table 9.3. The highest Cu extractability was achieved at 15⁰C soil temperature and 30% soil moisture content. Reddy *et al.* (1981) observed that the concentration of CaCl₂ extractable Cu increased with increasing soil temperature. Lindsay and Norvell (1978) reported that an increase in temperature from 10 to 30⁰C during extraction increased DTPA extractable Mn, Zn, Cu and Fe. Williams and McLaren (1982) reported that there was more extractable Cu and solubilised organic matter when temperature was raised from 10 to 30⁰C. They suggest that this extra Cu originated from the organic matter. Changes in soil temperature thus alter the rates and shift the equilibria of chemical reactions between metal ions and soil constituents (Barrow, 1992). Haynes and Swift (1991) have demonstrated that drying increases the amount of Cu, Fe, Zn, and Mn that can be extracted by EDTA, DTPA, and HCl. Oven drying usually has a more pronounced effect than air drying. They suggest that physical disruption and reformation of organo-

mineral associations is the main cause for increases in micronutrient extractability. Han *et al.* (2001) observed in their incubation study that soil samples kept under saturated conditions had higher Cu concentrations in the less labile fractions than those subjected to wetting-drying cycles.

Table 9.3 Mean Cu concentration in soil solution at various levels of soil temperature and moisture contents for four different soils.

Soil temperature (°C)	Copper concentration (mg kg ⁻¹)	Soil moisture (g kg ⁻¹)	Copper concentration (mg kg ⁻¹)
5	0.37	100	0.32
10	0.37	200	0.33
15	0.39	300	0.41
20	0.35	400	0.34

9.3.6 Seasonal effect on soil pH

The change in Cu concentration in plants varies from trial to trial, it always exhibits a increased or decreased value, which may be sustained for a period, but then decreased towards the low winter values (T₃), and then progressively increased to a maximum value (T₄). Soil pH was monitored during each trial period. Soil pH decreased with decreasing air and soil temperature and then increased slowly when all climatic parameters except soil moisture increased. The lowest (pH 5.0) and the highest (pH 5.75) values were obtained at the beginning of T₃ and T₆, respectively (Figure 9.11). There were many changes in the root zone of the pasture including a substantial degree of acidification. After T₃, the exchangeable Cu in the soil decreased with increasing soil pH (Figure 9.11). However, pH has only a small effect on the mobilisation of Cu into the soil solution (Sanders, 1982; Chapter 3), so it is likely that the mobilisation resulted from the biological production of chelating ligands. Natural complexing substances produced during microbial breakdown of organic matter have the ability to complex Cu into soluble, plant available forms (Stevenson and Fitch, 1981). Nielsen (1976) reported that the plant root might also increase soluble Cu concentrations through an increase in soluble organic matter in the soil resulting from the release of root exudates. Scheffer and Schachtschabel (1989) reported that metal speciation could be affected during a season by changes in soil pH through proton production and consumption by biological

processes, acid deposition and subsequent alteration of pH dependent chemical equilibria, eg. dissolution-precipitation, adsorption-desorption phenomena and metal-organic complex stabilities in the solid and solute phase.

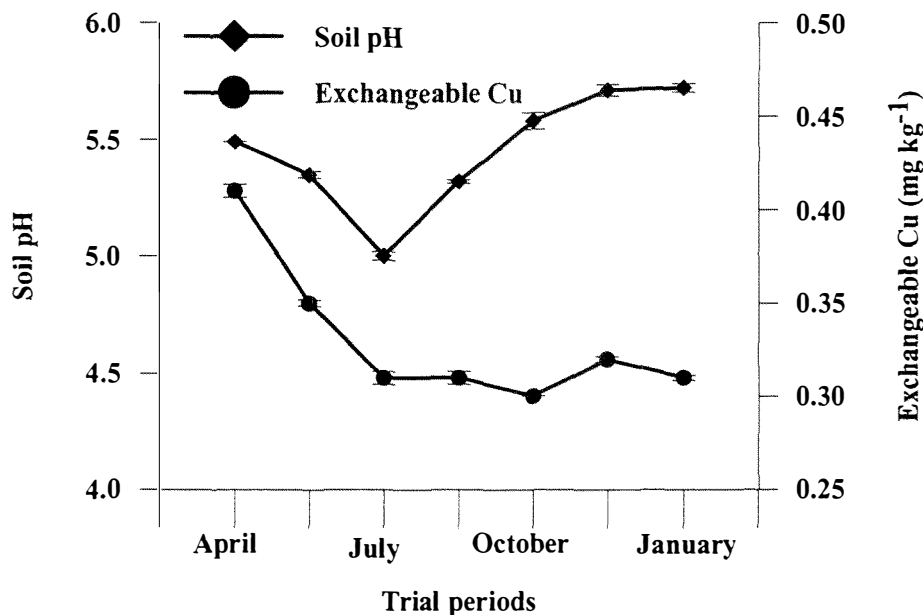


Figure 9.11 Soil pH and exchangeable Cu at different trial periods. Data are means \pm SE, n=3.

Soil pH has little influence on Cu distribution among the different Cu fractions in soils (Sims, 1986; Mathur and Lévesque, 1988; Shuman, 1986). El-Kherbawy and Sanders (1984) found that pH did not influence Cu in soil solution, but increasing the pH decreased Cu in the exchangeable fractions, making it less bio-available. Increasing soil pH redistributed Cu from the exchangeable and organic fractions to the less bio-available Mn-oxide and Fe-oxide fractions (Sims and Patrick, 1978). Thus Cu lost from the exchangeable fractions changed into less soluble, less bio-available fractions as the pH was increased. This finding was corroborated by Sims (1986), who reported that lime additions reduced exchangeable and sorbed Cu, and increased Cu in the more tightly bound fractions.

9.3.7 *Effect on exchangeable Cu*

The exchangeable Cu in the field soil of the control treatment increased with increasing soil temperatures, but decreased with increasing soil moisture (Figure 9.12). The soil

moisture content at 0-75mm depth was lowest (120 g kg^{-1} soil) at T_1 and highest (340 g kg^{-1} soil) at T_3 . Soil moisture plays a greater role than temperature in the extractability of Cu. Shuman (1980) observed that an increase in temperature resulted in an increase in extractable Cu, when soils were incubated at 0-bar moisture. Plant roots release organic substances capable of complexing micronutrient cations, although these organic complexing ligands may have a transient existence because rhizosphere microorganisms utilise them as carbon sources. Despite this such ligands might, if produced continuously, play a significant role in micronutrient mobilisation. The release by microorganisms of relatively small amounts of chelating ligands could thus mobilise significant quantities of micronutrient cations.

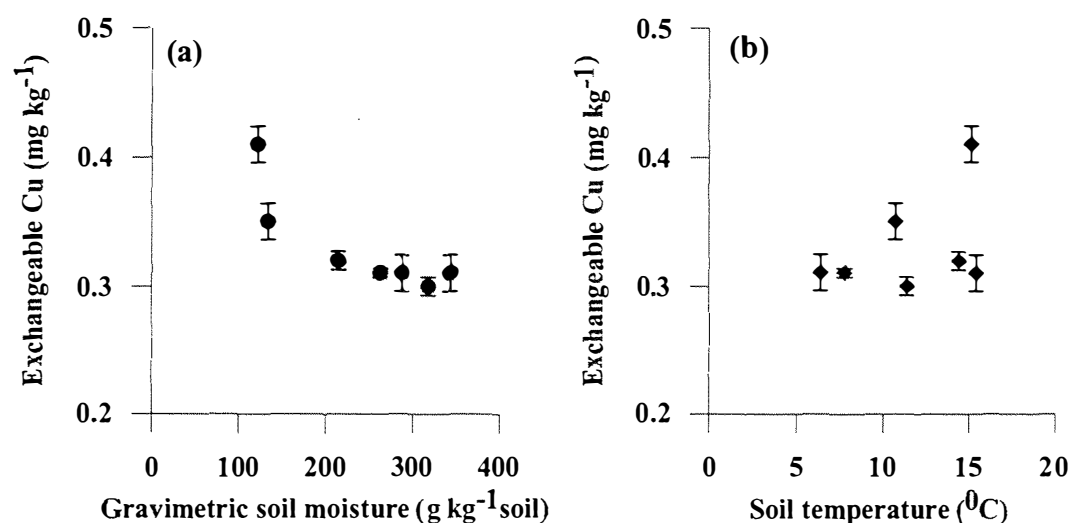


Figure 9.12 Effect of (a) soil moisture and (b) soil temperature on exchangeable Cu at different trial periods. Data are means \pm SE, $n=3$.

9.3.8 Fractionation of soil Cu

Fractionation of soil Cu for the control (0 kg Cu ha^{-1}) and 5 kg Cu ha^{-1} level at time 0 and 42 days after fertiliser application is presented in Figure 9.13. The study indicated that the substantial proportion ($>80\%$) of Cu is present in the oxide and organic fractions and only small amounts in the exchangeable and residual fractions. The mean concentrations of Cu in the different fractions decreased in the order: oxide bound $>$ organic $>$ residual $>$ exchangeable. Addition of Cu fertiliser resulted in an increase in the concentration of Cu in all the fractions, but the effect was more pronounced in the oxide and organically bound fractions.

The concentration of Cu in the organically bound fractions was highest at T₄ and lowest at T₇. This may be attributed to higher microbial activity at T₇ when the soil temperature was high. The reaction of freshly applied Cu with soil constituents plays a major role in the availability of Cu for pasture. This is often cited as a mechanism for reduced availability of Cu fertiliser and the long-term residual effect of Cu addition (Brennan *et al.*, 1983). McLaren and Ritchie (1993) observed that the organically bound and iron oxide bound Cu fractions account for most of the applied Cu in lateritic sandy soils of Western Australia, irrespective of the rate of application. Similarly Mullins *et al.* (1982a) and Liang *et al.* (1991) reported that most of the added Cu remained as organically bound and sesquioxide occluded Cu. Therefore, organic matter and oxide are the major components responsible for the adsorption of added Cu. Schnitzer (1991) reported that metal availability could be affected seasonally by losses and gains of binding sites with the fluctuation of the organic matter content, and changes of functional groups at the surfaces of organic matter due to decomposition and humification processes. The effect of freshly applied Cu in the different trials showed a variation of Cu concentration in the various fractions, particularly in the exchangeable and organic fractions, which indicates that ion exchange and the formation and dissolution of organic complexes resulted in the variation in Cu availability during the different trial seasons.

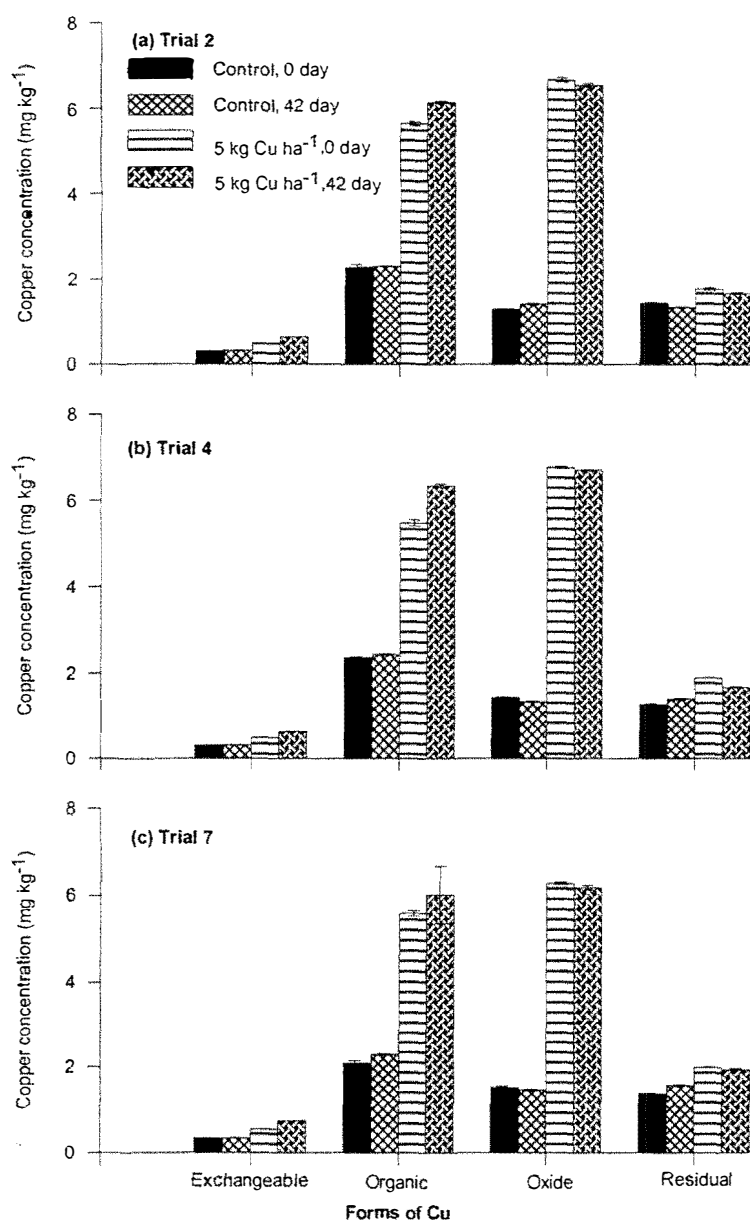


Figure 9.13 Fractionation of soils native Cu and Cu added as (CuSO_4) at different trial periods. Data are means \pm SE, $n=3$.

9.4 CONCLUSIONS

The patterns of Cu concentration in pasture were related to dry matter accumulation, reaching a maximum at T_7 . Differences in air and soil temperature, soil moisture content and solar radiation patterns within the trial periods appear to provide an explanation for the differences in pasture growth and Cu concentrations for a freshly applied Cu fertiliser. From the present study, it can be concluded that the ability of plants to take up Cu was internally regulated by shoot growth and externally affected by the transformation of Cu in the soils.

CHAPTER 10 SUMMARY, CONCLUSION AND FURTHER STUDY

Summary and conclusions

10.1 LITERATURE REVIEW

The review of the literature revealed the current level of understanding of the soil, pasture, and fertiliser management factors that affect Cu concentration in plants, soils and animals (Chapter 2). Cu in plants functions as part of the prosthetic group of enzyme systems, and as a facultative activator of enzyme systems. Cu deficiency is almost entirely confined to grazing animals due to low levels of Cu in the herbage and/or by elevated intakes of Mo, S and Fe. Fractionation and sorption-desorption studies have shown that Cu is strongly adsorbed by organic matter, and Al, Mn, and Fe oxides. Sandy soils low in organic matter and soils derived from volcanic ash are deficient in plant available Cu.

The effectiveness of pasture topdressing with Cu depends on the nature of the Cu fertiliser and soil properties. Fractionation schemes provide an insight into the forms and distribution of Cu and its availability. The proportions of the different Cu forms in soils vary considerably, depending on the soil and the fractionation technique used. Common chemical extractions such as Mehlich 1, Mehlich 3, TEA-DTPA, 0.1 M HCl, and 0.01 M $\text{Ca}(\text{NO}_3)_2$ are commonly used to predict the plant available Cu with varying degrees of success.

The availability of plant Cu declines with increasing time of contact between the soil and Cu fertilisers. The rate of decline in the availability of Cu depends on the constituents of soil and the solubility of the Cu fertilisers. N and P fertiliser compounds tend to exacerbate the Cu deficiency in cereal crops, especially when the Cu levels in the soils are low. In New Zealand, the pastoral system is legume based, P fertilisers are used to enhance N fixation; N fertilisers are also used on pastures to encourage out of season pasture growth; and lime is used to maintain the soil pH. Cu concentrations in forage and pasture crops depend on soil availability of Cu, plant species, stage of

growth, time of year, and fertiliser application. The seasonal pattern of pasture Cu concentration differs with location, soil type, climate, pasture species and management practices.

This study was conducted with the overall objective of quantifying the retention and transformation of Cu in soils and the availability of Cu to pasture. Laboratory studies investigated the adsorption and desorption of Cu in pasture soils. This was followed by a series of glasshouse and field trials with different Cu fertilisers, which examined the availability and transformation of Cu in pasture soils. Various chemical extractions were used to predict the plant available Cu. The effect of different amendments on the availability of residual Cu, and the seasonal response to plant availability of Cu through added fertiliser and transformation of Cu in the soil under field conditions were examined.

10.2 ADSORPTION AND DESORPTION OF COPPER IN PASTURE SOILS

10.2.1 *Adsorption study*

The adsorption of Cu at various pH values was measured using five pasture soils (Chapter 3). In all the soils, sorption of Cu increased with time. The differences in the sorption of Cu between the soils are attributed to the differences in the chemical characteristics of the soils. The Cu sorption followed: Ramiha > Manawatu > Ngamoka > Tokomaru > Mangamahu. The Ramiha, Ngamoka and Mangamahu soils are dominated by both organic matter and oxide components. These three soils also contained relatively high amounts of Al and Fe oxides. Higher pH values, organic matter, and silt and clay content of the Manawatu and Ramiha soils contributed to the higher sorption capacity of these soils. Copper sorption, as measured by the Freundlich equation sorption constants (K and N) was strongly correlated with soil properties, such as silt content, organic carbon and soil pH.

To investigate the relative contribution of different soil components in the sorption of Cu, sorption was measured after the removal of various other soil components; organic matter and Al and Fe oxides are important in Cu adsorption. The relative importance of

organic matter and oxides on Cu adsorption decreased and increased, respectively, with increasing solution Cu concentrations.

In all soils, Cu sorption increased with increasing pH. Freundlich constant (K) values increased with increasing soil pH in all soils. The solution Cu concentration decreased with increasing soil pH. The Tokomaru soil showed the highest solution Cu due to low levels of organic matter and CEC.

10.2.2 *Desorption study*

The cumulative amounts of native and added soil Cu desorbed from two contrasting soils (Manawatu and Ngamoka) during 10 successive 2 hrs and 24 hrs desorption periods showed that the differences in the desorbability of Cu were a result of differences in the physico-chemical properties of the soil matrix. The Ngamoka soil contains a higher amount of organic matter and demonstrated less desorbability of native Cu than the Manawatu soil. Soil samples were incubated for 0, 8 and 28 days with 50 mg Cu kg⁻¹ soil. Subsequent successive desorption procedures from day 0 incubated soil resulted in large increases in the amounts of Cu desorbed, compared to samples where only native Cu was present. The Manawatu soil desorbed more Cu than the Ngamoka soil after both the 2 hrs and 24 hrs desorption periods. This suggests that soil organic matter complexes Cu added through fertiliser, resulting in decreased desorption. The proportions of added Cu desorbed during 10 desorption periods were low, ranging from 2.5% in the 24 hrs to 6% in the 2 hrs desorption periods. In both soils, the desorption of Cu decreased with increasing contact times between the soil and the added Cu. The effect of contact time was more pronounced in the Ngamoka soil, which contains a higher level of organic matter.

The desorption of Cu decreased with increasing soil pH. The desorption decreased from 35%, 29%, 18% and 10% at pH 5 to 13%, 7%, 3% and 5% at pH 8 for the Tokomaru, Ngamoka, Manawatu and Ramiha soils, respectively. The irreversible retention of Cu might be the result of complex formation with Cu at high pH. The Tokomaru soil contains less a amounts of soil organic matter and a lower CEC resulting in a large amount of desorption at both levels (600 and 1000 mg Cu L⁻¹) of added Cu. The Ramiha

soil contains a high amount of organic matter and a high CEC resulting in the lowest desorption of Cu among the soils at pH 5.0.

10.3 PLANT AVAILABILITY OF DIFFERENT COPPER SOURCES IN PASTURE SOILS

The treatments used in the glasshouse experiment (Chapter 4) include: five soils (Manawatu, Tokomaru, Ramiha, Ngamoka and Mangamahu), three Cu sources [CuSO_4 , $\text{Cu}(\text{OH})_2$ and CuO], and four Cu levels (0, 50, 100 and 200 mg kg^{-1} soil) replicated four times. The summary of results is as follows:

- Soils have significant effect on DM yield and also on Cu concentration at all harvests.
- Cu concentration and uptake correlated with soil pH, organic matter and the clay content of the soil.
- Increasing levels of Cu increased the plant Cu concentration at all harvests.
- The high Cu level (200 mg Cu kg^{-1} soil) caused a significant decrease in dry matter yield, but at the levels of 0, 50 and 100 mg Cu kg^{-1} soil resulted in similar yields.
- Cu sources as fertiliser have a significant effect on DM yield, and also on Cu concentration at all harvests.
- CuSO_4 shows a significantly lower DM yield and higher Cu concentration than the other two fertilisers at harvest 1.
- Except for harvests 1 and 2, CuO resulted in significantly higher Cu concentrations at all harvests.
- Cu uptake in the ryegrass decreased with increasing time of contact between the fertiliser and the soils.
- Cu uptake is directly related to the DM yield of ryegrass. The recovery of Cu by plant uptake was highest for the $\text{Cu}(\text{OH})_2$ fertiliser. Recovery of Cu was the highest at the lower levels of fertiliser application.

10.4 TRANSFORMATION AND PLANT UPTAKE OF COPPER IN DIFFERENT SOILS

Soils from the previous glasshouse trial (Chapter 4) were used to examine the transformation of Cu in soils (Chapter 5). The Manawatu and the Tokomaru soils

contained the highest and the lowest concentrations of total native Cu, respectively. The Cu in the soils was fractionated into its different forms. The fractionation procedure achieved nearly complete (>80%) recovery of the total Cu from the soils. The mean concentrations of Cu present in the different fractions decreased in the order: oxide bound > organic > residual > exchangeable. All fractions declined with time after fertiliser application, indicating that the development of a strongly bound fraction that is not extractable with triacid digestion.

The sand fractions contributed the lowest amount of Cu from all soils compared to the silt and clay fractions. In the Manawatu and Ramiha soils the silt fraction contained the highest amount of Cu, but this was not the case with the other three soils, where the clay fraction held the most Cu. The silt and clay fractions contributed the highest amounts of Cu in these five soils.

The organically bound Cu was highest in the organic soils compared to the mineral soils. In most of the soils, organic and oxide bound Cu initially increased and then decreased from time of fertiliser application. Increasing soil organic matter showed a negative relationship with exchangeable Cu but a positive relationship with the other fractions. Increasing CEC and clay content showed a negative relationship with the exchangeable Cu, but a positive relationship with the other fractions. Since a majority of soil Cu is associated with organic matter, it would be expected that organic matter additions would cause a redistribution of Cu, leading to an increase of the organically bound Cu.

The concentration of Cu in the different fractions was affected by the nature of the Cu fertiliser. Soon after fertiliser application, CuSO_4 resulted in the highest concentration of Cu in the organic fraction. However with increasing time after fertiliser application the organic and the oxide bound Cu decreased in both (CuSO_4 and CuO) fertilisers.

The relationship between the amounts of Cu in the individual fractions of different soils, and the plant Cu uptake, showed that organic and oxide bound Cu were correlated with plant Cu uptake. Cu associated with oxides and organic matters are more important to Cu availability than Cu in the other fractions. The Cu concentration and total uptake by ryegrass varied directly with the amounts of Cu in the different fractions (organic,

oxides), total Cu, EDTA extractable Cu, CEC and clay content. The availability of applied Cu for uptake by ryegrass declined with time of contact between the soil and the applied Cu. A significant relationship was obtained between EDTA-extractable Cu and plant Cu concentration and Cu uptake.

10.5 SOIL TESTS TO PREDICT THE AVAILABILITY OF COPPER

The efficiency of various soil test extractants (0.01M $\text{Ca}(\text{NO}_3)_2$, 0.1M NaNO_3 , 0.01M CaCl_2 , 1.0M NH_4NO_3 , 0.1M HCl , 0.02M SrCl_2 , Mehlich-1, Mehlich-3, and TEA-DTPA.) to predict the availability of Cu is discussed for two contrasting soils treated with two sources of Cu fertilisers (Chapter 6). CuO fertiliser resulted in higher concentrations of exchangeable Cu at 191 days after fertiliser application, which was significantly more than the CuSO_4 fertiliser. This may be due to the slow releasing characteristic of CuO fertiliser. It appears that the organic and oxide bound fractions are in equilibrium and they constitute the major potential plant available Cu pool in soils.

The efficiency of various chemical reagents in extracting the Cu from the soil followed this order: TEA-DTPA > Mehlich-3 > Mehlich-1 > 0.02M SrCl_2 > 0.1M HCl > 1.0M NH_4NO_3 > 0.01M CaCl_2 > 0.1M NaNO_3 > 0.01M $\text{Ca}(\text{NO}_3)_2$. Higher amounts of Cu were extracted by the soil test extractants from the Manawatu than the Ngamoka soil. This may be due to the low level of organic matter in the Manawatu soil. In pasture soils, high levels of Cu application have resulted in an increase in extractable soil Cu.

The ratios of exchangeable: organic: oxide bound: residual forms of Cu in M1, M3 and TEA-DTPA for the Manawatu soil are 1:20:25:4, 1:14:8:2 and 1:56:35:8, respectively. The values of the Ngamoka soil are 1:14:6:4, 1:9:5:2 and 1:55:26:17, respectively. The ratios of different forms of Cu suggest that the Cu is residing mainly in the organic form and it decreases in the order: organic > oxide > residual > exchangeable.

In both soils, more than 98% of the Cu extracted by the three soil test reagents was in a complexed form and only a small amount of Cu remains as the ionic form. Manawatu soil had a significantly higher concentration of free Cu^{2+} than the Ngamoka soil. The M1, M3 and DTPA extracted 44%, 56% and 65% of the organically bound Cu from the Manawatu soil as compared to 63%, 77% and 72%, respectively from the Ngamoka

soil. M3 and TEA-DTPA extracted more organically bound Cu than M1. TEA-DTPA and M1 extracted more oxide bound Cu and TEA-DTPA and M3 extracted more residual Cu and exchangeable Cu. A large variation in the extractable Cu was observed for the soil samples. There was a highly significant relationship between the concentrations of Cu extracted by the three soil test extractants. This indicates that in soils that have received recent Cu additions, M3, TEA-DTPA and M1 extractants are equally effective for extracting Cu from the soil.

The determination of the coefficients obtained from the regression relationship between the amounts of Cu extracted by M1, M3 and TEA-DTPA reagents suggests that the behaviour of extractants were similar. This similarity can be explained by the fact that M3 and TEA-DTPA solutions contain the complexing agents that extract the Cu complexed with organic matter, an important soil pool of Cu, whereas M1 may extract the same Cu ions preferentially from mineral surfaces.

10.6 EFFECT OF LIME, EDTA, NITROGEN AND PHOSPHATE FERTILISERS ON THE AVAILABILITY OF COPPER.

10.6.1 Effect of nitrogen and phosphorus fertilisers on the availability of native copper.

Two contrasting soils (Manawatu and Ramiha) were used in a glasshouse experiment to examine the effect of N and P fertilisers on the availability of native Cu (Chapter 7). The Ramiha soil resulted in higher dry matter yields and a lower Cu concentration, but higher Cu uptake when compared to the Manawatu soil. The variation in Cu concentrations in ryegrass between the two soils varied with differences in shoot growth and organic matter content of these soils. Increasing levels of N increased the DM yield, Cu concentration and Cu uptake. An increase in N uptake increases the amino acid concentration in the roots leading to greater translocation of Cu from roots to shoot. Increasing levels of P significantly increased the DM yield and decreased the concentration of Cu in the plants but increased Cu uptake. The decrease in Cu concentration may be due to dilution of Cu in plant tissue through the promotion of plant growth by phosphate fertilisers. It is also possible that reduced exploitation of the soil by mycorrhizae at high P levels may also result in reduced Cu concentration in plants.

10.6.2 Effect of lime, EDTA and nitrogen fertiliser on the availability of residual copper.

Soil samples obtained at the termination of the first glasshouse plant growth experiment (Chapter 4) were used to examine the effect of lime, EDTA and N fertiliser on the uptake of residual Cu from two fertiliser (CuSO_4 and CuO) sources, applied at four Cu levels to two contrasting soils (Manawatu and Ngamoka) (Chapter 8). The DM yields were significantly higher for the Manawatu soil than the Ngamoka soil at each harvest with the addition of N fertiliser and lime. Copper concentration was higher in the Manawatu soil with the addition of N fertiliser and EDTA, but lower with a lime application.

Fertiliser Cu sources have a significant effect on DM yield and Cu concentration in ryegrass. CuSO_4 fertiliser resulted in higher dry matter yields and a lower Cu concentration. Lime and EDTA applications resulted in the highest and the lowest DM yields, respectively, but had the opposite effect on Cu concentration. The residual Cu level at 20 kg Cu ha^{-1} for CuO fertiliser giving in the highest concentration of Cu at all harvests, and this was significantly different to the others at all harvests for N, lime and EDTA applications. The residual Cu rates significantly influenced the Cu concentration in plants with the addition of EDTA. Additions of N fertiliser and lime also resulted in an increase in the Cu concentration in shoots with increasing rates of residual Cu.

The treatment level of 100 kg N ha^{-1} produced the highest DM yield and Cu concentration at both harvests, and it was significantly ($p < 0.05$) different from the control treatment. The lime treatment at 2000 kg ha^{-1} produced the highest DM yield at all harvests and it was significantly ($p < 0.05$) different from the control. The lime level at 1000 kg ha^{-1} resulted in the highest Cu concentration at harvest 1, and that was significantly different from the other treatments. The lime level at 2000 kg ha^{-1} decreased Cu concentration at all harvests. Dry matter yield decreased with increasing levels of EDTA application. Additions of EDTA resulted in an increase in Cu concentration in ryegrass.

The amount of plant available Cu, as measured by the three soil test extractants, is affected by N, lime and EDTA. Significant relationships between Cu concentration in plants and Cu in the soil test extractants were observed.

Both N and lime applications decreased the exchangeable Cu and the free ionic Cu at the highest application rate in the Ngamoka soil, but EDTA increased the amount of exchangeable Cu resulting in phytotoxicity of ryegrass. The Ngamoka soil showed less of an increase in exchangeable Cu with increasing rates of EDTA addition compared to the Manawatu soil.

Higher amounts of Cu was extracted from the Manawatu soil by the M3 and TEA-DTPA extractants before liming. Lime reduced the extractable Cu by the M1, M3 and TEA-DTPA extractants, and it was more pronounced in M3. EDTA increased the amount of plant available Cu, as measured by the M1, M3 and TEA-DTPA extractants.

10.7 SEASONAL RESPONSE OF COPPER AVAILABILITY IN PASTURE

Two Cu fertiliser sources (CuSO_4 and CuO), each applied at 3 rates with three replications were used in the field experiment in the Tokomaru soil (Chapter 9). Seven trials were conducted at different times within the same paddock. Both sources and rates of Cu fertiliser application throughout the trial period did not affect pasture growth. Pasture growth rate was highest in summer and lowest in winter. Pasture growth rates closely followed temperature and solar radiation, with the exception of the autumn period where the lift in pasture growth appears to be in response to an increase in soil moisture. Cu fertiliser sources have a significant effect on Cu concentration in pasture. CuSO_4 resulted in higher Cu concentrations in all trials compared to the CuO fertiliser. This might be attributed to the quick releasing characteristic of CuSO_4 fertiliser.

Application of Cu fertilisers increased the Cu concentration in pasture. The Cu concentration was highest at 6.4°C and lowest at 14.5°C soil temperatures at the 5 kg Cu ha^{-1} level. Soil moisture has a profound effect on Cu concentration and it decreased at the highest moisture level. There was a strong seasonal variation in Cu concentration in

the pasture; the concentrations of Cu were low in spring and early summer as compared to late winter.

Copper fertiliser sources have significant effect on Cu uptake in pasture. Cu uptake in pasture increased with increasing levels of Cu fertiliser applied. The highest Cu uptake was noticed in summer and the lowest in winter. It is apparent that substantial mobilisation of Cu occurs in the root zone in late winter / early summer due to the higher growth rate of pasture during this periods. Increasing air and soil temperature, and solar radiation increase the Cu uptake.

The concentration of Cu in the organically bound fractions was the highest in late winter and the lowest in summer. This may be attributed to increased microbial activity in summer due to high soil temperatures leading to increased mineralisation of organically bound Cu. The reaction of freshly applied Cu with other soil constituents plays a major role in the availability of Cu for pasture. This is often cited as a mechanism for reduced availability of Cu from fertiliser and the long-term residual effect of Cu application. The variation of soil temperature during the trial periods, observed in the present study, is likely to affect the availability of organically bound Cu. Organically bound Cu in soils is present in soluble and insoluble forms. Mobilisation-immobilisation reactions are temperature dependent and affect the solubility of soil solution Cu. The effect of applied Cu in each trial showed variations in the Cu fractions, particularly in the exchangeable and organic forms, which suggests that reactions of Cu with soil organic matter causes differences in Cu availability in the different trial seasons.

Differences in air and soil temperatures, soil moisture and radiation pattern within the trial periods provide an explanation for the differences in pasture growth and Cu concentrations for freshly applied Cu fertiliser. It can be concluded that the ability to take up Cu is internally regulated by the growth of plant shoots and externally affected by the transformation of Cu in soils.

10.8 SUGGESTION FOR FUTURE STUDY

The total Cu content in soils is not available for plant uptake due to its strong complex formation with organic matter as observed in the different experiments. The chemical forms of Cu extracted by soil test reagents from pasture soils varied considerably with the organic matter content. Further studies should be carried out to determine the influence of other soil properties (eg. nature and amount of clay minerals and forms of organic matter) on the estimation of Cu availability so that a better standardisation of the methods could be achieved. The concentration and Cu uptakes are strongly related to soil properties such as pH, organic matter, CEC, clay and silt content. Positive relationships generally existed between organic and oxide bound Cu and plant uptake, indicating that these two forms are the potential sources of plant available Cu. The uptake of native Cu and residual Cu from two contrasting soils showed that N and lime at (50 kg N ha⁻¹ and 1000 kg lime ha⁻¹) levels increased the Cu concentration, and EDTA also increased the plant available Cu to toxic levels. The effect on Cu concentration persisted beyond the first cut only at the highest N addition level. Low levels of lime induce the mineralisation of organic matter and so release the organically complexed Cu into the soil solution. Low levels of EDTA should enhance the optimum level of available Cu. The effect of N, lime and EDTA on the availability of residual Cu in ryegrass needs further investigation.

The transformation of Cu under both glasshouse and field conditions resulted in a decrease in the organic forms of Cu with time after fertiliser application. Further study of seasonal responses to pasture Cu concentration and the transformation of Cu under various soil temperature and moisture regimes, under both field and glasshouse conditions should be undertaken.

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