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AN EVALUATION OF A SLOW RELEASE TRACE ELEMENT FERTILISER FOR THE PREVENTION OF COPPER DEFICIENCY IN SHEEP

by

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ERRATA

All herbage trace element concentrations quoted are in mg/kg DM and not mg/kg as shown.

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SUMMARY

Copper (Cu) deficiency in ruminants continues to be a common trace element disorder in Great Britain despite the fact that its existence was first recognised over 50 years ago. As the direct treatment of animals is demanding, the convenience and simplicity of a soil treatment to raise herbage Cu status is an attractive proposition. However previous studies have indicated that soil application of Cu compounds although increasing plant uptake of Cu were seldom dependable in overcoming long term livestock deficiencies. The objective of this work was to evaluate a novel slow release trace element fertiliser (Cu fertiliser) for the prevention of Cu deficiency in sheep. The Cu fertiliser is an unrefined and unprocessed by-product of the brass manufacturing industry and is not a specially formulated fertiliser.

Chemical and physical characterisation of the Cu fertiliser have shown that it contains approximately 2% Cu which is distributed throughout a wide particle size range. In addition the Cu fertiliser is only sparingly soluble in water. The characterisation studies suggested that the Cu fertiliser when applied to soil should act as a slow release source of Cu to plants. The fertiliser was also found to contain zinc, lead, iron, cadmium, nickel and manganese.

Application of the Cu fertiliser, both incorporated into the soil and broadcast onto an established sward increased herbage Cu concentrations. Both pot and field trials showed that increasing the Cu fertiliser application rate produced significant increases in herbage Cu concentraton. A similar effect was shown for zinc. These

increases were not accompanied by detectable increases in herbage Pb, Fe, Cd, Cr, Ni or Mn concentrations. In the case of the field trial these results were maintained in three successive years after a single application in December 1985.

Glasshouse pot trials showed that lowering the soil pH caused significant increases in herbage Cu concentrations of supplemented soils. These results were not repeated in the field. The increased herbage Cu concentrations in the pot trials occurred on four different soil types. Waterlogging had no effect on the rate of release of Cu from the fertiliser.

In a field trial with ewes and lambs grazing a sward with known low Cu availability a single application of the Cu fertiliser (370 kg/ha) was successful in raising and maintaining plasma Cu concentrations above the deficiency threshold in ewes throughout pregnancy, lambing and weaning, and in their lambs. The single application elevated plasma Cu concentrations within six weeks and was effective for two years, during which time its effect was comparable to two annual oral administrations of Cu needles. In the third year the mean plasma Cu concentration of ewes on treated pasture, although still higher than that of the control ewes, dropped below the deficiency threshold. Tissue analysis from dead ewes in the second year of the trial showed that ewes on the Cu fertiliser treated pasture had a much higher Cu concentration in their liver than those on untreated pasture.

Herbage analysis from the animal response trial showed Cu concentrations above the phytotoxic threshold and up to ten times those found in pot and herbage trials in the first 6 months after

application of the Cu fertiliser. These data suggested that the Cu fertiliser was contaminating the herbage and was thus available for ingestion.

Lead adherence and ingestion trials demonstrated that the Cu fertiliser can adhere to herbage and when ingested by sheep at 10 g/day does increase plasma Cu concentrations without any known deleterious or toxic effects on the animal.

A second field trial using a lower Cu fertiliser application rate (250 kg/ha), and hence reducing the potential for surface adherence, resulted in a comparable increase and maintenance of the plasma Cu concentrations of sheep grazing the treated pasture to that found in the first trial. The replacement of the 20 sheep originally on the treated pasture by 20 new sheep, one year after the Cu fertiliser application also resulted in an increase in their plasma Cu concentrations. This occurred within 10 weeks of their introduction to the treated pasture and was maintained above the deficiency threshold over the subsequent winter.

After the initial surface coating effects resulting from the application of the Cu fertiliser disappeared after 6 months in the first field trial, a 2 to 3 mg/kg advantage in herbage Cu concentrations was maintained throughout the monitoring period, from November 1985 to June 1988, over the untreated pasture.

This trace element fertiliser did give long term increases in the Cu concentration of perennial ryegrass and mixed herbage. However in the animal response trials it is proposed that the initial increase in sheep plasma Cu concentrations were due to direct ingestion of the

fertiliser from coated herbage and from Cu fertiliser mixed with surface soil whilst the sward growth was poor. Subsequent maintenance of sheep plasma Cu concentrations appears to be due to reserves of liver Cu accumulated during times of higher Cu intake together with the increased level of Cu available from the herbage and the possibility of further Cu fertiliser ingestion as soil ingestion in subsequent years. The increased herbage Cu concentrations alone do not appear to be sufficient to provide adequate long term protection from Cu deficiency. Despite this the Cu fertiliser did give up to 2 years protection which is much longer than that provided by oral administration of Cu needles.

CHAPTER I. INTRODUCTION

Copper (Cu) is an essential nutrient for the growth of both plants and animals. Although Cu deficiency has been recognised as a nutritional disorder in animals, especially ruminants, for over 50 years it still continues to cause problems throughout Great Britain (Purves and Ragg, 1962; Reith, 1975; S.A.C., 1982) and many other parts of the world (see review by Gartrell, 1981). Losses attributable to Cu deficiency in ruminants are now recognised in virtually all countries in which the possibility has been investigated. Despite the fact that no reliable estimates of the extent of its incidence or economic significance are available for any of these countries it is evident that its impact ranges from situations in which livestock production over extensive geographical areas is impossible unless deficiency is rectified to strictly local problems which may be accompanied by clinical signs of deficiency or merely by sub-optimal performance.

Copper has a number of important biochemical functions in the ruminant. These are reflected by the various manifestations of Cu deficiency. The classic symptoms of Cu deficiency in sheep are well documented and include; anaemia, bone disorders, neonatal ataxia (swayback), loss of wool pigmentation and defective keratinisation of wool (Underwood, 1981). Copper deficiency occurs when the grazing animal does not absorb enough dietary Cu to meet its metabolic requirements. A number of factors may limit the natural availability of Cu to the animal and thus lead to the establishment of the deficient state. In many cases, because of the particular geological features of the land, the soil and thus the herbage on it are

inherently low in Cu and this leads to Cu deficiency in grazing livestock. In Britain Cu deficiency is particularly associated with soils derived from sandstone, limestone and granite or from drifts derived from these rock types (Tills and Alloway, 1981; Reaves and Berrow, 1984; Archer and Hodgson, 1987).

In addition Cu deficiency can occur on soils of moderate or high Cu content but where most of the Cu is in forms unavailable to the plant, usually associated with organic macromolecules (Caldwell, 1971; Gartrell, 1981).

In grazing sheep absorption of Cu from herbage is dependent upon the herbage molybdenum (Mo) and sulphur (S) concentration (Grace, 1983). High Mo and S concentrations in herbage reduce the availability of Cu to sheep and cause deficiency. Thus Cu deficiency in sheep can also occur in areas with no inherent Cu deficiency in the soil or herbage through the antagonistic effects of Mo and S. Copper absorption can also be inhibited by the antagonistic effects of cadmium, iron and soil ingestion (Mills **et al**, 1972; Campbell **et al**, 1974; Suttle **et al**, 1975).

Copper deficiency may also arise secondarily, often in areas with no naturally occurring Cu deficiency, through the effects of improving hill pasture (Evans, 1983). Pasture improvements lead to increased herbage production which can depress herbage Cu concentrations through a dilution effect. In addition it can also increase the Mo and S concentrations of herbage, which when present in significant amounts cause Cu deficiency. It is generally thought in Scotland that losses from swayback have increased mainly due to the introduction of

measures to increase the fertility and productivity of land (S.A.C., 1982).

As with all trace element deficiencies Cu deficiency more often manifests itself in covert or subclinical forms such as illthrift and low production. Therefore unless a previous history of Cu deficiency such as swayback in lambs leads to the introduction of some form of Cu supplementation and improves the general condition of the animal the situation will go unnoticed. Thus the total incidence of Cu deficiency is unknown and it is difficult to determine actual numbers of ruminants affected.

A number of methods have been made available to the farming community for the treatment or prevention of Cu deficiency in ruminants. The main methods of treatment include application of Cu compounds to pasture, provision of salt licks, Cu injections, addition of Cu to drinking water and oral administration of $CuSO_4$ of Cu needles. Each method has its limitations and has met with varying degrees of success.

A wide variety of Cu sources have been evaluated for their effectiveness as fertilisers for the correction of Cu deficiency. They work either by increasing the concentration of plant available Cu in the soil or by supplying Cu directly to the plant foliage. The application of Cu containing fertilisers has been shown to be an effective method of correcting Cu deficiency in plants, especially cereal crops (Graham and Nambier, 1981; Alloway and Tills, 1984). However, pasture treatment to prevent Cu deficiency in grazing livestock has had only limited success to date. Broadcasting of $CuSO_4$

has been the most frequently tried method, due to its water solubility, relatively low cost and wide availability; however, Cu applied in this form is usually rapidly immobilised in the soil and made plant unavailable. As a result the small increases in herbage Cu concentrations obtained are usually of insignificant value to the grazing animal (Reith, 1975) especially where Cu intake is impaired or metabolism reduced by Mo or S or soil ingeston. Other pasture treatments have been shown either to produce similar results to those of $CuSO_4$ or are too expensive for the treatment of large areas. Thus soil or pasture Cu treatment has not been considered to be a particularly effective way of overcoming a livestock deficiency.

In addition to pasture treatment a variety of direct animal treatments have been used which are administered either orally or by injection. However each of these methods has its limitations. The provision of Cu licks or the addition of Cu to drinking water do not guarantee an adequate, or even any consumption, of Cu by each individual animal, whilst oral methods such as dosing with $CuSO_4$ still have to overcome any effects of dietary factors which modify Cu absorption. In theory subcutaneous or intramuscular injections of Cu compounds should have an advantage when Cu deficiency is caused by antagonists such as Mo and S. However they only provide short term supplementation as the amount which can be administered in a single dose is limited due to problems of possible acute systemic toxicity and localised carcass damage (S.A.C., 1982). Thus their drawback is that repeated injections are necessary to maintain normal plasma Cu concentrations. This creates a lot of extra work for the farmer involved. A relatively new method for the prevention of Cu deficiency is the oral

administration of copper oxide needles. The needles lodge in the ruminant abomasum and the Cu is slowly released over 2 to 3 months. The method has been successfully tested in sheep (Whitelaw **et al**, 1980; Suttle, 1981a). This requires only a minimal amount of collection and handling whilst supplying Cu over a substantial period of time. However it still does not overcome the problem of dietary factors which may modify Cu absorption.

Thus every method used to prevent Cu deficiency to date has its limitations. Direct treatment of the animal is demanding in terms of time and cost whereas pasture treatment is either expensive or the herbage Cu concentration increases obtained are insufficient and too short lived to be of significant value to the grazing animal. Thus the choice of method used will depend on the system of animal husbandry practised, the severity of the Cu deficiency experienced and on the individual preferences of the farmer. If herbage Cu status could be significantly increased by a soil applied product which is inexpensive and has long term residual effects then several of todays problems would be overcome.

The objective of this project was to evaluate the potential of a new Cu containing material, as a slow release trace element fertiliser, for the prevention of Cu deficiency in sheep. The material, hereafter known as the Cu fertiliser, is an unrefined and unprocessed by-product of the brass manufacturing industry which may have properties that make it suitable for use as a Cu source and is not a specially formulated fertiliser. A wide ranging investigation was carried out to follow the effects of the Cu fertiliser from the soil through the plant to the animal. The work included study of:

(a) The fertiliser chemical composition, solubility and rate of release of Cu.

(b) The effects of different application methods and rates on the supply of Cu from the fertiliser to herbage.

(c) The soil factors which affect the availability of Cu in soils treated with the Cu fertiliser.

(d) The residual value of the Cu fertiliser for supplying Cu to the plant and animal.

(e) The comparative response of sheep grazing pasture naturally low in Cu with that of sheep grazing comparable pasture treated with the Cu fertiliser.

As no previous studies had been carried out on the material the project should ideally have started with the laboratory and glasshouse studies. However if the Cu fertiliser was to be of any value it had to show a residual value, i.e. it must continue to prevent Cu deficiency in sheep, for over two years. Therefore it was important to begin long term field trials as soon as possible so as to fit within the time constraints imposed by a post-graduate scholarship.

CHAPTER 2. COPPER IN SOILS, PLANTS AND ANIMALS WITH PARTICULAR REFERENCE TO PROBLEMS OF COPPER DEFICIENCY

2.1 SOIL COPPER

2.1.1 Copper Content of Soils

The Cu content of a soil is determined principally by the lithology of its parent material. The average Cu content of rocks is 70 mg/kg but Cu is one of the few metals to have generally a lower concentration in the soil than in the parent material from which it is formed, soils having an average Cu content of 20 mg/kg (Hodgson, 1963). However soil Cu contents can range from 2-100 mg/kg (Swaine, 1955).

In igneous rocks Cu can occur as the native metal (Cu) but more frequently exists as sulphides such as chalcolite $(CuS)_2$ and chalcopyrite $(CuFeS)_2$, it also concentrates in ferromagnesian silicates (Kraustopf, 1972). Copper is, therefore, more abundant in the sulphide rich basaltic rocks than in granitic rocks (Table 2.1). Sedimentary rocks tend to have a lower Cu content than basaltic rocks as they have been strongly weathered in recent geological times. Soils with low total Cu contents tend, therefore, to be formed from sandstone, limestone and granite or from drifts derived principally from these rock types.

Anthropogenic contributions such as environmental pollution and direct application of organic waste products, e.g. sewage sludge and pig slurry tend to raise Cu levels. However, these are very localised effects.

Table 2.1

Average copper content in soils and rock (from Hodgson, 1963 and Knezek and Ellis, 1980)

	Average Cu (mg/kg)
Earths Crust	70
Basaltic Rocks	100
Granitic Rocks	10
Sedimentary Rocks	23
(i) Limestone	4
(ii) Sandstone	30
(iii) Shale	45
Soil	20

Another factor which affects the Cu content of soils is their age. Older soils are more weathered and as such tend to have lower Cu levels due to leaching.

The soils of Great Britain are relatively young, having largely developed only in the last 12,000 years; therefore soil Cu levels are very closely related to those of the parent materials. The complexity of the geology has resulted in soil parent materials with very varied trace element contents and there is a wide range of Cu levels in British soils (Mitchell, 1974). In Scotland the total Cu concentration of soils has been measured by Reaves and Barrow (1984), who give an average value of 10 mg/kg and a range of 0.93 to 100 mg/kg. Areas of granite and sandstone had the lowest levels of Cu in the soil. Similarly in England, Archer and Hodgson (1987) found the lowest concentration of Cu in soils derived from sandstone, but low

levels were also found in those on chalk, limestone and glacial sand parent materials. They give a range of 1.8 to 215 mg/kg for total soil Cu content with an average of 18.4 mg/kg. The highest values occurred in mining areas and in alluvial soils.

2.1.2 Forms of Copper in the Soil

Copper exists in many forms in the soil, most of which are not available to the plant. Therefore, the total Cu content is a poor indicator of Cu availability to plants and animals. In a comprehensive study McLaren and Crawford (1973a) distinguished five fractions or pools of soil Cu:

(a) Soil solution and exchangeable copper.
(b) Copper bound to specific sites on soil minerals.
(c) Organically bound copper.
(d) Copper occluded by metal oxides.
(e) Residual copper (mainly in clay lattice structures).

Only soil solution Cu, comprising free ions and soluble complexes is immediately available for plant uptake. The remaining pools of Cu can, therefore, be classified as either labile (potentially available) or non-labile (not readily available).

(a) Soil solution and exchangeable copper

The Cu concentration of the soil solution is usually very low (1×10^{-6} to 1×10^{-8} mg/l) representing less than 0.1% of the total soil Cu (Hodgson **et al**, 1965; McBride and Blasiak, 1979). In the soil solution more Cu is complexed to soluble organic matter than occurs as free Cu^{2+} ions. The majority of these Cu complexes are with simple

aliphatic acids, amino acids and aromatic acids (Stevenson and Ardakani, 1972). Hodgson **et al** (1966) suggested that more than 98% of soil solution Cu can exist in these complexed forms. However plants take up free Cu^{2+} ions and while it seems likely that Cu^{2+} dissociates itself from these complexes prior to uptake this is not certain.

The amount of free Cu^{2+} ions present in the soil solution is also affected by the soil pH and Eh. The solubility of Cu^{2+} is at a minimum near pH 7 and increases either side of this (McBride, 1981). A combination of low pH and Eh (<0.2) will keep the majority of uncomplexed Cu as Cu^{2+} (Jenkins and Jones, 1980). In addition Cu^{2+} may react with carbonates, phosphates and chlorides in the soil solution (Mann and Deutscher, 1977); however the importance or influence of these to plant availability are unknown. Thus even the small amount of Cu^{2+}, not organically complexed in the soil solution can be further reduced. The effects of this on plant availability of soil solution Cu are unknown but it is most probable that these reactions will reduce the amount of available Cu in a soil.

The concentration of Cu in the solution is too low for it to be controlled by the solubility of the Cu minerals found in soil. It is therefore probably controlled by surface reactions with soil minerals and organic matter.

Exchangeable Cu is held purely by electrostatic forces on the soil cation exchange sites (McBride, 1981). These adsorption sites are largely non specific and the Cu here is in direct equilibrium with the soil solution. As such it is a source of directly available plant Cu. The exchangeable Cu represents less than 10% of the total Cu in most

soils (McLaren and Crawford, 1974). The bulk of soil Cu is held by other means, such as specific adsorption to mineral surfaces and complexed to organic matter.

(b) Copper bound to specific sites on soil minerals

Copper is specifically adsorbed by layer silicate clays and iron, aluminium and manganese oxides (McBride, 1981). It has been shown that despite the presence of excess electrostatically bonded cations that were capable of preventing Cu^{2+} adsorption by simple ion exchange, Cu^{2+} could be specifically adsorbed by amorphous iron and aluminium hydroxides (Kinniburgh **et al**, 1976; Forbes **et al**, 1976). Once specifically adsorbed, Cu is only very slowly released into the soil solution (McLaren **et al**, 1983). However, the Cu is potentially available for plant uptake and as such contributes to the labile pool of soil Cu. The mechanism of adsorption called chemisorption, unlike the loose electrostatic association observed with the exchangeable Cu, is thought to involve the formation of a direct surface Al-O-Cu or Fe-O-Cu bond (McBride, 1978a). As the reaction is mainly with the hydroxyl groups, chemisorption is likely to occur at surface edges, where such groups exist. Hence the maximum potential level of chemisorption may be determined by the quantity of free surface hydroxyl groups. In general, micro-crystalline and amorphous oxides should bind more Cu^{2+} per unit weight than crystalline oxides as they have a larger surface area. Iron oxides in some soils have particle sizes of 100A or less (Bigham **et al**, 1978). Therefore it is probable that relatively small amounts of Fe or Al oxides will have a disproportionate effect on Cu^{2+} adsorption due to their large surface area when compared with layer silicate clay such as mortmorilonite

which have particle sizes of over 200A.

Manganese oxides like those of Fe and Al specifically adsorb Cu^{2+} in the soil, the level of adsorption increasing as soil pH increases (Murray, 1975). The affinity of synthetic Mn oxides for Cu^{2+} is even stronger than that of Fe or Al oxides (McKenzie, 1980). Although synthetic Mn oxides may be quite different from Mn oxides in soils, a correlation has been demonstrated between the amount of specifically adsorbed Cu^{2+} in soils and their free manganese oxide content (McLaren and Crawford, 1973b). Therefore chemisorption of Cu to Mn oxides is an important process despite the presence of greater quantities of Fe and Al oxides in soil (McBride, 1981).

Most of the above studies have been carried out using Cu^{2+} rather than the more abundant anionic complexes of Cu with organic ligands which are found in the soil solution. However, little is known of the role of such complexes in the adsorption of Cu in soils. Some organic compounds may be strongly adsorbed on mineral surfaces, others not at all. Similarly little attention has been paid to studying the rates of adsorption and desorption of Cu in this fraction of the soil. The rate at which Cu reacts could be important in determining the long term effectiveness of Cu fertilisers, while the rate of desorption of Cu back into the soil could be important in determining the supply of Cu to plants. Thus the full picture of Cu adsorption is not yet clear.

(c) Organically bound copper

Complexation of Cu by organic matter has long been recognised as the most important method of Cu retention in the majority of soils.

Organic constituents in soil may form both soluble and insoluble complexes with Cu (McBride, 1981). The proportion of Cu in such complexes is estimated to be from a fifth to one half of the total soil Cu although levels can be much higher in peat soils (Stevenson and Fitch, 1981).

The Cu^{2+} is directly bonded to two or more organic functional groups (carboxylic, carbonyl or phenolic groups) so that it is immobilised in a rigid inner sphere complex (McBride, 1978). In peaty soils there are large numbers of porphyrin rings which are capable of forming Cu complexes of exceptionally high stability (Goodman and Cheshire, 1976).

A wide variety of compounds are involved in Cu^{2+} complexation. These include simple aliphatic acids, amino acids, phenolic acids, humic acids and fulvic acid (Stevenson and Ardakani, 1972). However, little data is available on the quantitive relevance of these complexes or on the factors affecting the availability of their Cu to plants. Abundant evidence does exist for the key role played by humic and fulvic acids in Cu^{2+} complexation.

In a review, Stevenson and Fitch (1981), suggest that fulvic acid compounds are important as the soluble forms of organic Cu in the soil, although increasing saturation with Cu will result in the formation of an insoluble complex. Each addition of Cu reduces the charge on the acid causing the molecule to collapse, reducing its solubility. In contrast humic acids are important for the binding of Cu to organic matter in largely insoluble forms. The stability of the Cu-humate complex increases with an increase in the degree of

humification. Stevenson and Fitch (1981) then envisioned a sequence of events, in which Cu present in low concentration is immobilised by humic acid complexation. When these sites are saturated an increasing amount of the Cu will be solubilised through the increasing action of fulvic acids. The implications of this are that soil solution Cu concentrations are reduced by the formation of insoluble humic acids complexes. If excess Cu^{2+} is present complexation will serve to maintain Cu^{2+} concentration at less than phytotoxic levels. Finally under conditions where Cu^{2+} may precipitate out of the soil solution (e.g. calcareous soils) complexation will serve to maintain Cu in a soluble form.

Organic matter complexation provides specific sites for retaining Cu^{2+} in a largely non exchangeable form (McBride, 1981). The Cu held on humus macro molecules is very stable and is retained in a non-labile form.

However, organically bound or chelated Cu^{2+} is probably the largest contributor to the exchangeable and soil solution Cu fractions (McLaren and Crawford, 1974; Kline and Ruse, 1966). Thus, except for peat or peaty soils, organic solids serve the function of holding the majority of complexed Cu in a kinetically available but thermodynamically stable (insoluble) form.

(d) Copper occluded by metal oxides

Chemical fractionation schemes show that after removal of exchangeable and organically complexed Cu, a significant amount of the total Cu content often remains (Shuman, 1979). As coprecipitation of Cu^{2+} in Al and Fe hydroxides occurs readily during pedogenesis (McBride,

1978a) it is likely that a fraction of soil Cu may be "buried" or occluded in various mineral structures. Substitution for Fe^{3+} or Al^{3+} by Cu^{2+} results in a positive charge deficit which must be balanced by a structural defect or an adsorbed cation thus further burying Cu.

Occluded Cu exists in a largely non-diffusable form (McLaren and Crawford, 1973b). Therefore it will only be released through weathering of the soil minerals; as such Cu held here will be in a non labile, plant unavailable form.

(e) Residual copper (mainly in clay lattice structures)

Dissolution of soil clay lattice structures by strong acids often releases a significant amount of Cu (McLaren and Crawford, 1973a; Shuman, 1979). This can account for over 50% of the total soil Cu content, especially in soils low in organic matter or from horizons lower down in the profile. This Cu originated from isomorphous substitution of Al^{3+} or Si^{4+} by Cu^{2+} in the octahedral positions in layer silicate clays. These are secondary mineral crystals and once formed are very stable. The Cu here is non labile and will only be released through weathering.

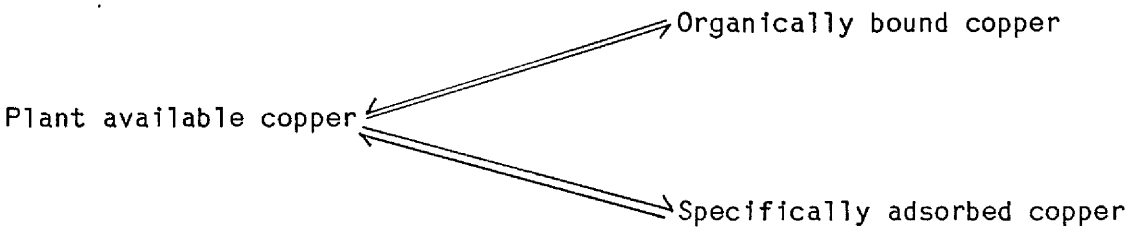
(f) Implications for plant availability

Only soil solution Cu is immediately available for plant uptake, but even here only a small amount is in the plant available free ionic Cu^{2+} form. Most soil solution Cu exists as soluble organic complexes which are thought to release Cu^{2+} by dissociation prior to uptake. The concentration of Cu in the soil solution is primarily controlled by the organically complexed and specifically adsorbed copper as the amount of exchangeable Cu is very low and the soil solution Cu content

is too low to be controlled by the solubility of Cu minerals. The plant available Cu is in equilibrium with the labile pool which comprises the specifically adsorbed Cu and organically bound Cu (Fig. 2.1), the latter forming the greater proportion. However, no distinction can be demonstrated between the contribution made by specifically adsorbed Cu and organically bound Cu to the maintenance of the plant available copper pool (McLaren and Crawford, 1973a and b).

Figure 2.1

Equilibrium between plant available Cu and the labile pool of Cu



In summary the labile pools of Cu comprise the major sites of adsorption, especially the organic and mineral cation exchange complexes and hydrous oxides or Mn, Fe and Al. The adsorption and desorption of Cu from the sites controls the concentration of Cu in the soil solution and hence plant availability.

The non-labile (plant unavailable) forms of Cu are ions in primary and secondary mineral crystals, i.e. occluded and residual Cu, and stable organic complexes of Cu on humus macro-molecules. These are the principle slow release reservoirs of Cu in the soil; the Cu held in them is only released through weathering and slow microbial decomposition of macro organic complexes. The rate of release is

influenced by other factors such as pH; under acidic soil conditions weathering is intensified and chelates start to decompose, thus more Cu is released.

2.1.3 Estimation of Plant Available Copper in Soils

Soil analysis has been widely used to estimate Cu availability to plants. Much research has concentrated on devising soil tests for detecting and confirming the existence of Cu deficiency. A large and diverse number of soil Cu tests have been advocated over the years; however, due to the diversity of soils worldwide no particular test has achieved uniform recognition as an index of soil Cu status (Robson and Reuter, 1981). In general, the soil Cu extractants used in different regions and countries around the world have been selected because they are most appropriate for the local soils, crops and environmental conditions. In Great Britain the "labile" or "available" Cu pool is predominantly organically bound, therefore extractants containing a chelating or complexing agent have been widely used to estimate plant available Cu. Extraction of soils with ethylenediaminetetra-acetic acid (E.D.T.A.) has been sucessfully used for this purpose (Borggard, 1976; Robson and Reuter, 1981; Berrow and Reaves, 1985). Good correlations between E.D.T.A. extractable Cu in soil and plant uptake in pot experiments have been widely reported (Oien, 1966; Beyers and Hammond, 1971; Dolar **et al**, 1971; Tills and Alloway, 1983b). In a comparison of eight extractants, Tills and Alloway (1983b) found that E.D.T.A. gave the best prediction of plant Cu uptake. A number of other workers have reported that E.D.T.A. provides more reproducible results than other extractants for the determination of plant available Cu in British soils (Henrikson and

Jensen, 1958; Davies and Carlton-Smith, 1983). In addition, King and Alston (1975), concluded that although other extractants such as calcium nitrate and diethylenetriaminepenta-acetic acid (D.T.P.A.) were equally as effective as E.D.T.A. for assessing available soil Cu, they found that the extraction and measurement of Cu with E.D.T.A. was performed most simply and accurately and recommended it for routine soil testing.

The E.D.T.A. test has now superceded the use of dilute salt and acid extractants which were used previously, and which are now thought to underestimate (e.g. Ca $(NO_3)_2$) or overestimate (e.g. HCl) the available Cu status. Moreover, acidic solvents remove Cu from pools which are not plant available (Martens, 1968), give less reproducible results (Henrikson and Jensen, 1958) and are clearly unsuitable for extracting calcareous soils.

Other soil Cu extractants currently in use throughout the world are IM HCl in France (Juste and Solda, 1977), dilute HNO_3 in the Netherlands (Westerhoff, 1955) and D.T.P.A. in North America (Lindsay and Norvell, 1978). All are specific for the soil types found in their respective countries.

2.1.4 Diagnosis of Copper Deficient Soils

Using the E.D.T.A. extraction technique (Reith, 1968; Berrow and Reaves, 1985) the Scottish Agricultural Colleges Advisory Service has compiled a table for the classification of soil copper status (Table 2.1.2). A similar E.D.T.A. extraction is used by A.D.A.S. in England and Wales.

Table 2.1.2

Classification of soil copper for soils of all drainage classes containing up to 12% organic matter

Soil status	Extractable Cu (mg/kg)	Probability deficiency or toxicity in crops
Very low	<1.0	Deficiency probable
Low	1.0-1.6	Deficiency possible
Moderate	1.7-8.5	No deficiency expected
High	8.6-8.0	No deficiency
Excessive	>8.0	Toxicity may occur

(M.I.S.R., 1985)

On soils containing over 12% organic matter the threshold concentration for probable Cu deficiency is 2.5 mg/kg. However, herbage analysis will be necessary to confirm Cu deficiency in most cases.

2.1.5 Distribution of Copper in the Soil Profile

Due to its strong association with organic matter and metal oxides, Cu is one of the least mobile of the trace elements. Soil profiles, therefore, show little variation in total Cu levels with depth. However, movement of Cu from highly leached surface horizons in association with organic Fe and Al complexes have been observed in podzols (McBride, 1981). In addition highly organic surface horizons often contain higher Cu levels due to the accumulation of Cu in the residues of plant tissues.

However, the distribution of plant available Cu as a function of depth is of more interest than that of total Cu, and usually varies to a much greater extent (Swaine and Mitchell, 1960). It tends to be higher in the top soil which has a higher organic matter content than the rest of the profile. Available Cu tends to decrease with depth (Krahmer and Bergmann, 1978; Karim **et al**, 1976). The association of Cu with organic matter in soil maintains Cu, released from minerals initially, in a complexed form near the soil surface which is then available for plant uptake.

2.1.6 Copper Deficient Soils

Copper deficiency in crops occurs when there is an inadequate amount of Cu in the soil solution for plant uptake. Cu-deficient soils can be divided into two groups; those inherently low in total Cu and those with a normal or even high total Cu content where most is in unavailable forms.

(a) Soils inherently low in total Cu content

Inherently low total Cu concentrations in soils are principally due to the soil mineralogy and/or degree of weathering and leaching. The main groups of soils with low total Cu levels are:

(i) Coarse textured soils

Sandy soils contain a high percentage of quartz grains and very little silt or clay. As quartz contains no Cu and these soils are usually strongly leached, and have a low capacity for adsorbing added cations due to the low clay content, they tend to be Cu deficient. Such soils may be derived from sandstone, weathered igneous rocks especially granites or from glacial

tills principally derived from these rock types, i.e. gravels and coarse sand. Most of these soils are naturally acid podzols or acid brown earths. Examples of this type of Cu-deficient soil are found in the Breckland of England (Tills and Alloway, 1981) and on sandstone and granite in Scotland (Reaves and Berrow, 1984).

(ii) Calcareous soils

Soils derived from chalk and limestone also tend to have low Cu contents. This inherently low soil Cu content can be exacerbated by high pH which reduces the amount of Cu^{2+} in the soil solution, and also by the high Ca^{2+} content which dominates the cation exchange sites making the Cu less available to plants (Davies **et al**, 1971). An example of such Cu deficient soils are rendzinas found over the soft chalk of Salisbury Plain in England (Caldwell, 1971).

(b) Soils with a normal or even high total Cu content where most is in unavailable forms

The most common cause of Cu unavailability in soils is the formation of stable complexes with organic macro-molecules (Gartrell, 1981). Obviously this complexation is at its most extreme in peats; acid, neutral and alkaline peats have all produced equally severe Cu deficiency (Pizer **et al**, 1966). However, mineral soils with more than 10% organic matter may also be affected. The large areas of peat in Scotland (Reaves and Berrow, 1984) and the loamy peats of the English Fenland (Caldwell, 1971) are examples of this type of Cu-deficient soil.

In the humic rendzinas of the chalk escarpment in Southern England a combination of high organic matter content, high pH and relatively low inherent Cu give rise to very low levels of plant available Cu (Davies **et al**, 1971).

2.1.7 Geographical Distribution of Copper-Deficient Soils in the U.K.

In Scotland areas of potential Cu deficiency have been mapped on the basis of total soil Cu in the B horizon by the Macaulay Institute for Soil Research. As yet this map has not been officially published but it shows up the large areas of peat covered land in the North of Scotland to be Cu deficient. Areas of Cu deficiency are also found over the granitic rocks of North East Scotland and over the areas of sandstone in the South East and South West of the country. Using this information S.A.C. (1982) has produced a table of soil associations and soil series whose properties are likely to have a significant influence upon the Cu content of summer crops. Soils were then grouped into "risk" categories according to total Cu concentration of their B horizon and their pedological drainage type. The risk of Cu deficiency in crops has been assessed to be high in 26 soil associations and moderate in a further 10 (Reaves and Berrow, 1984).

In England and Wales there is little systematic data on soil Cu-concentrations. However, estimates of areas of copper deficient soil have been made, in part, by regional geochemical surveys (Thornton, 1983). These surveys are based on the systematic collection and analysis of stream sediment samples. The Cu concentration of the sediment reflects that of the soils and rocks of its catchment area. Using this technique a map showing the distribution of Cu

concentration in stream sediments in England and Wales has been produced and is available in published atlases (Webb **et al**, 1978). This data has been applied as a rapid and low cost means of focussing attention on possible areas of Cu deficiency in livestock in which soil, plant and mineral investigations can be concentrated. Cu deficiency in crops and animals is frequently found on land within geochemically defined low Cu areas (Thornton and Webb, 1980). These maps have been successful in predicting relationships between geochemical parameters and bovine Cu deficiency and for assessing whether farms lie in high, medium or low risk Cu deficiency areas (Leech **et al**, 1982; Leech **et al**, 1983; Leech and Thornton, 1987).

2.2 PLANT COPPER

2.2.1 Introduction

It is over 50 years since both Sommer (1931) and Lipman and MacKinney (1931) first showed that Cu was essential for the growth of higher plants. Copper is required in small but critical amounts (pg) by all higher plants for healthy growth and reproduction. It is classed as a micronutrient or essential trace element in contrast to macronutrients such as phosphorus which is required in much larger amounts (mg). Its role in a large number of enzymes (Walker and Webb, 1981) means that it influences nearly all the metabolic pathways.

2.2.2 Uptake and Translocation

The Cu content of most plants is generally between 2 and 20 mg/kg in the dry matter. Thus the amount of Cu required by plants is very small. Rates of uptake from dilute solution are of the order of μmol

h^{-1} g^{-1} fresh weight of root (Graham, 1981).

Cu^{2+} is the species commonly absorbed by plants (Dragin **et al**, 1976), despite the fact that Cu^{2+} is almost entirely complexed in the soil solution. The way in which Cu^{2+} dissociates from the chelate prior to absorption is unknown.

The mechanism of absorption of Cu^{2+} by plant roots is also unknown. Loneragan (1975) has suggested that absorption is under some sort of metabolic control while Graham (1979) has suggested that if it is Cu^{2+} that is absorbed, then energy may only be required to maintain the membrane potential, for the electro chemical gradient favours absorption of Cu^{2+} from very low soil solution concentrations. However, evidence to support either theory is inconclusive and the mechanism remains unclear.

Absorption of Cu from solution is strongly inhibited by Zn (Bowen, 1981) and vice versa (Loneragan, 1975); Bowen (1981) considers Cu and Zn to be absorbed by the same unknown mechanism.

In a review of the distribution and movement of Cu in plants, Loneragan (1981) suggests that in xylem and phloem saps Cu probably occurs complexed with soluble N compounds such as amino acids. This affinity was first demonstrated by Tiffen (1972). The distribution and redistribution of Cu within the plant is controlled by processes in the roots and leaves which release Cu for excretion into either the phloem or xylem saps. Once present in the sap Cu moves freely. However, Cu is not freely mobile within the plant as the movement of Cu is strongly dependent on the Cu status of the plant (Loneragan, 1975).

2.2.3 Distribution of Copper in Plants

The distribution of Cu between roots and shoots varies widely with plant species and environmental conditions. The concentration of Cu in roots is generally higher than that in shoots but the degree of difference varies markedly with the level of Cu supply. Jarvis (1978) has shown that increasing concentrations of Cu in solution, result in more rapid increases in the Cu content of the root than of the shoot for perennial ryegrass grown in flowing solution culture, whilst the roots of plants grown for periods at low Cu concentrations in solution have similar Cu concentrations to the tops. Woolhouse and Walker (1981) suggest that many plants which are able to tolerate high levels of Cu in their environment do so by accumulating Cu in their roots and excluding it from the shoots, thus partially protecting the shoot against changes in the root medium.

Within roots Cu is associated with cell walls; Cu deficient ryegrass has almost all of the Cu in its roots associated with the cell walls (Loneragan, 1981). In roots, high concentrations of Cu may be held against transport to shoots even under conditions of severe deficiency. The processes governing this retention are not understood.

In shoots Cu distribution varies with maturity and this will be discussed later. However, the behaviour of Cu in shoots appears to be coupled with N metabolism. The present evidence suggests that Cu entering leaves is bound by N-compounds which release little or no Cu for phloem transport until they are hydrolysed. As a result green leaves can accumulate high Cu concentrations and retain them against

translocation to areas of new growth even during Cu deficiency (Loneragan, 1981).

Nitrogen fertilisers frequently change the Cu content of plants (see 2.2.6). However, these changes are accompanied by profound effects upon plant growth which obscure any changes in distribution of Cu/N relationships.

2.2.4 The Role of Copper in Plant Physiology

Copper has a role in many plant processes. It is either a constituent of or associated with compounds which are of functional importance in the metabolism of higher plants. In general Cu containing enzymes are concerned with the catalysis of oxidation-reduction type reactions in which oxygen is reduced to water or peroxide. However, Cu redox proteins without oxidase function are found, e.g. plastocyanin.

Copper containing enzymes have key roles in respiration and photosynthesis and Cu proteins are thought to be involved in lignification, anabolic metabolism, cellular defence mechanism and hormone metabolism. Some of these Cu compounds and their functions are listed in Table 2.2.

The effects of Cu deficiency on the various plant metabolic processes will be discussed later.

Table 2.2

The range and function of copper containing proteins in plants (Walker and Webb, 1981)

Protein/enzymes	Function
Cytochrome oxidase	Terminal oxidase of the mitochondrial electron transport chain.
Ascorbate oxidase	Catalyses oxidation of ascorbate.
Phenolase Laccase	Catalyses oxygenation of amino aldehydes.
Diamine oxidase	Catalyses oxidation of polyamines.
Superoxide dismutase	Catalyses the dismutation of superoxide.
Quinol oxidase	Function unknown.
Umecyanin	" "
Mavicyanin	" "
Plantacyanin	" "
Blue proteins	" "
Stellacyanin	An electron carrier.
Plastocyanin	Intermediate electron transporter in photosynthesis.
Azurin	Electron carried in respiratory chain between cytochrome and cythochrome oxidase.

2.2.5 Variation of copper concentration between plant species

Plant species vary considerably in their typical Cu contents. Although these differences may be small they are important in animal nutrition studies. Two species often grown together in pasture, clover and grass, can exhibit marked differences in their trace element contents. In permanent pasture as opposed to temporary leys indiginous uncultivated species such as buttercups and thistles may

also become established and affect animal nutrition. Variations in Cu contents between plant species sampled in one field are given in Table 2.3.

Table 2.3

Variation in Cu content between plant species sampled in one field in June (Burridge et al, 1983)

Plant species	Cu
Mixed herbage	9.8
Ryegrass (**Lolium perenne**)	5.7
Cocksfoot (**Dactylis glomerata**)	7.2
Clover (**Trifolium** sp.)	10.8
Buttercup (**Ranunculus** sp.)	17.8

It is apparent from the above table that a change in the botanical composition of a sward could affect the Cu content of the mixed herbage. The relative species contents depend to some extent on soil conditions. In low Cu soils clover generally contains less Cu than ryegrass whereras on soils high in Cu the reverse is more likely to be the case (Mitchell **et al**, 1957).

2.2.6 Factors Affecting Copper Concentrations in Plants

(a) Age

The concentrations of Cu in plants is generally highest in young seedlings and decreases steadily towards maturity (Gladstone **et al**, 1975; Loneragan **et al**, 1980; Reuter **et al**, 1981). Frequency of cutting also affects the Cu content. Reith and Mitchell (1964)

observed an increase in Cu concentration with successive cutting over one growing season and this has been reported in other work (Reith **et al**, 1984). The Cu content was higher in October than June cuts, midsummer cuts had intermediate values.

(b) Interactions with other ions

Many interactions of Cu with other ions have been reported; usually they result in a suppression of Cu uptake by the plant. Zinc fertiliser application suppresses Cu absorption by cereals (Chaudhry **et al**, 1970) which is consistent with effects in solution studies (see 2.2.2).

Hiatt **et al** (1963) showed that aluminium markedly reduced Cu uptake, but this was overcome if Cu levels were higher. They concluded that Al and Cu were not competing for the same absorption site in the plant but were competing for common soil binding sites at or near the root surface.

Calcium may competitively inhibit Cu uptake under certain conditions (Cathala and Salsac, 1975) while synergistic effects between Cu and Mn have been described (Dekock and Cheshire, 1968). McKay **et al** (1966) have reported that Cu and Mo are antagonists for crops grown in acid soils. However this last point is unclear as other work has shown no Cu and Mo antagonism in the field (Reith, 1984).

(c) Fertilisers

There are several ways in which plant trace element concentrations may be affected by N.P.K. fertilisers:

(i) The amounts of N.P.K. used are relatively high and these may affect soil conditions.

If ammonium sulphate is used this acidifies soils and may increase Cu solubility in the soil (Lucas and Knezek, 1972). However, application of most fertilisers only results in transient changes in soil pH which tend to be localised around individual fertiliser prills. Impurities of Cu in fertilisers can also lead to changes in the Cu content of soils and thus affect plant uptake. This effect depends largely on the fertiliser used but phosphate fertilisers are usually associated with Cu impurities (Swaine, 1962).

(ii) Increased growth in response to fertiliser applications, especially N, can also affect Cu contents. The reasons for this may be firstly that increased root growth means the plant has access to a larger soil volume and secondly, that the stage of growth, i.e. leaf, stem ratio may be altered (Burridge **et al** 1983).

(iii) Plant uptake may be affected by changes in the relative amounts of the major and minor nutrients (Burridge **et al**, 1983).

As one or more of the above mechanisms may be operating it is difficult to explain observed effects of N.P.K. fertilisers on plant trace element concentrations. However, several general affects have been observed. When N is supplied without Cu, the Cu concentration of herbage growing in Cu-deficient soil is decreased (Reith, 1975).

The suppression of clover growth is not responsible for this, as under such conditions clovers usually have lower Cu concentration than do grasses (Mitchell **et al**, 1957). This fact has been substantiated by further work (Reith **et al**, 1984) which found that applying N to Cu-sufficient soil increased Cu content in mixed herbage. Applying N, and Cu as $CuSO_4$ to copper-deficient soil resulted in increased yields and Cu contents (Reith **et al**, 1984). These workers also found that applying P, especially at high rates, resulted in slightly lower Cu contents in herbage. These results are summarised in Table 2.4.

Table 2.4

Effects of applying nitrogen and phosphorous fertilisers on Cu concentrations in plants

Nutrient	Effect on Cu	Comment
Nitrogen	(i) Decreased Cu concentration	Deficiency due to increased growth.
	(ii) Increased Cu concentration	Increased uptake when luxury supplies of Cu in soil and increased growth.
Phosphorus	Decreased Cu concentration	Due to: (i) reduced absorption (Olsen, 1972). (ii) Decrease in mycorrhizal absorption of Cu at high P (Timmer and Leyden, 1980).

(d) Soil conditions

(i) Drainage condition

The concentration of plant available copper in a soil is generally higher in naturally poorly drained soils than adjacent freely drained soils (Reith, 1983). Berrow and Mitchell (1980) compared the Cu content of freely drained and very poorly drained soils of the same parent material. They observed that Cu in the surface horizons of both soil types is in a chelated form and therefore extractable. However, lower in the profile more Cu is in an extractable form and it was in these horizons that the degree of mobilisation increased under poor drainage conditions, i.e. Cu extractibility decreases with depth in freely drained soils but in very poorly drained soils there is little change in extractable Cu levels with depth. Further investigations (Barrow **et al**, 1983) have shown that despite poor drainage causing an increase in extractable soil Cu, the plant levels only increased marginally.

Copper is not subject to oxidation/reduction reaction under changing drainage conditions (Jarvis, 1981). Thus the mechanism of the above effects is unclear. It could be due to indirect effects of drainage on Fe and Mn oxides and the breakdown of organic matter, or simply that the exchangeable Cu of the freely drained soil has been displaced and leached away whereas in the poorly drained soil any displaced Cu could be taken up by other binding sites in the soil as there is less chance of Cu being leached away.

(ii) Soil pH

There is little evidence to suggest that plant uptake of Cu is affected by changes in soil pH. However Cu deficiency following land improvement through liming and reseeding is common (Pizer **et al**, 1966; Tills and Alloway, 1981; Evans, 1983; Whitelaw **et al**, 1979). Laboratory studies have suggested that Cu mobility in the soil and hence availability are greater at low pHs (Jarvis, 1981). There is conflicting evidence as to whether this is carried over into the plant. Lucas and Knezek (1972) state that Cu availability is dependent upon soil pH but does not normally increase appreciably until the pH falls below 5.0. However, Piper and Beckwith (1949) found that the Cu concentrations in several plant species were entirely unaffected by a range of soil pHs from 4.5 to 7.5. This conclusion has been substantiated by Archer (1971) and Gupta (1979) who raised pHs from 4.7 to 6.0 and 5.6 to 7.7 respectively, without any effect on Cu concentration of plants. Thus the majority of research tends to suggest pH has no effect although some will debate this point.

Even when some evidence suggesting a pH effect has been found, the results can be conflicting. This was demonstrated by Mitchell **et al** (1957) who showed that liming a Scottish soil increased the Cu levels in red clover but not in ryegrass.

(iii) Organic matter content

The reactions of copper with soil organic matter have been described (Section 2.1.2). On the basis of fractionation studies (Martin, 1968; McLaren and Crawford, 1973a) it has been

concluded that the bulk of the available soil Cu reserve is associated with organic matter. The supply of Cu, as assessed by soil extraction, often initially increases when organic matter is added to a soil, as it often contains a high Cu concentration (Gupta, 1971), but this may be followed by a gradual decrease in available Cu (Elgala **et al**, 1976) as Cu-organic matter complexes become more stable. Thus although the effects of organic matter may be variable and generally increase the Cu reserve in the soil, increasing organic matter content of a soil tends to decrease plant available copper levels.

2.2.7 Effects of Copper Deficiency on Plant Physiological Processes

The manifestation of copper deficiency on physiological processes can be divided into its effects on various metabolic pathways. Copper influences nearly all the plant metabolic pathways due to its role in a large number of key enzymes (Walker and Webb, 1981).

(a) Carbohydrate metabolism

(i) Photosynthesis

Copper deficiency may induce chlorosis and structural malformations in leaves. Since chlorosis involves the breakdown of chloroplasts which are the organelles for photosynthesis, symptoms of chlorosis can be expected to lead to decreased photosynthetic activity.

Plant chloroplasts contain appreciable amounts of copper, e.g. in clover 75% of total Cu was found in the chloroplasts (Neish, 1939). However evidence suggests that damage of chloroplasts

is a late step in developing Cu deficiency, i.e. it is a secondary effect (Bussler, 1981). If Cu deficiency directly depresses photosynthetic rates, it must do so through some other mechanism. It is not due to a plastocyanin limitation. However in addition to depressing content of chlorophyll a and b in leaves Cu deficiency also depresses B-carotene, lutein, neoxanthine, plastoquinone and Vitamin K contents (Bussler, 1981). Any one of which may have a direct effect on photosynthesis. To date research has been inconclusive.

(ii) Respiration

Since mitochondrial cytochrome-C-oxidase contains Cu, Cu must be involved in respiration. However this enzyme appears to be very stable and the Cu cannot be removed even under Cu-deficient conditions, thus respiration appears to be unaffected by Cu deficiency (Bussler, 1981).

(iii) Carbohydrate distribution

Carbohydrate distribution in the plant does not appear to be affected by Cu deficiency.

(b) Nitrogen metabolism

(i) Protein metabolism

Many Cu enzymes show decreasing activity with Cu deficiency and this is restored if Cu is added to the system (Walker and Webb, 1981). Protein synthesis is also disrupted by Cu deficiency, which results in an increase in soluble N compounds in the plant (Bussler, 1981). However the present evidence suggests that the influence of Cu on protein metabolism is also a

secondary effect of long lasting Cu deficiency (Price **et al**, 1972).

(ii) Nitrogen reduction and fixation

Copper appears to be required for symbiotic N fixation in legumes. Cu deficiency decreases N fixation in clover (Snowball **et al**, 1980).

(iii) Lignin synthesis

Cell wall composition changes with age. Copper is important in cell wall metabolism, having the greatest effect during lignification. Many researchers have described a decrease in lignification under Cu-deficient conditions (Busler, 1981) and this agrees with observed Cu deficiency symptoms (2.2.8). This is due to the phenoloxidase enzymes, which biosynthesise lignin, losing activity under Cu deficiency. Lignification can be inhibited or completely absent in deficient plants leading to bending and distortion of the leaves and stems. Xylem vessels may collpase due to lack of structural thickening leading to restricted water movement and hence wilting (Alloway and Tills, 1984). The Cu enzymes involved appear to be very sensitive to Cu levels in the cell and this may be a direct effect of Cu deficiency.

(e) Reproduction

Pollen sterility may be found in a number of plant species grown under Cu stress (Graham and Nambier, 1981). All the evidence appears to suggest that Cu deficiency affects pollen development rather than that of the ovule. Alloway **et al** (1983) suggest that pollen sterility may

be linked to a tapetal abnormality in the anther due to Cu deficiency, during pollen grain development. In self-pollinating plants such as wheat and barley a reduction in pollen viability will lead to a decreased yield.

Symptoms of Cu deficiency primarily appear on the younger parts of plant as its mobility within plants is low. Recent results indicate that Cu deficiency always starts to develop in younger tissues (Bussler, 1981). The anthers are the younger parts of a plant at the time of pollen formation, therefore it would be expected that the reproductive process may be sensitive to Cu deficiency. If pollen sterility as an effect of Cu deficiency is generalised for all flowering plants then seed production must decrease. Reuter **et al** (1981) found decreased seed production in clover under Cu stress, however a primary role of Cu in seed formation cannot be deduced.

(d) Disease resistance

Graham (1981a) has reported that plants under Cu stress are more susceptible to powdery mildew and ergot. The role of Cu in disease resistance is unknown; the structure of the plant as well as the physiological composition may be involved and it also appears to change with age and degree of Cu deficiency. Bacterial speck in tomatoes (Bonn and Lesage, 1984) and stem melanosis of wheat (Piening **et al**, 1987) have also been associated with Cu deficiency.

2.2.8 Symptoms of Copper Deficiency

Visible symptoms of Cu deficiency are widespread and occur in many different plant species (see review by Caldwell, 1971 and Gartrell, 1981). However Cu deficiencies occur much more frequently in some

species than others. Table 2.5 lists cultivated species and their sensitivity to Cu deficiency.

There also exists some variation between cultivars within a species (Graham and Namber, 1981).

Table 2.5

The relative sensitivities of selected crops to copper deficiency (from Alloway and Tills (1983))

Sensitivity to copper deficiency		
Low	**Medium**	**High**
Beans	Barley	Wheat
Peas	Clovers	Rice
Potatoes	Broccoli	Oats
Rye	Cabbage	Lucerne
Pasture grasses	Cauliflower	Lettuce
Asparagus	Sorghum	Onion
Rape	Tomato	Carrot
Lupins	Turnip	Citrus fruits
Soyabeans	Maize	Spinach

A list of the main symptoms found in several crops is given in Table 2.6. In many species symptoms, including chlorosis, necrosis, distortion of leaves and dieback of leaf tips, are first observed in young shoots (Hewitt, 1963). Wilting is also a common symptom (Bussler, 1981) usually due to structural weaknesses (reduced lignification).

In most annual or perennial sensitive species the most spectacular effects of Cu deficiency are observed during reproductive development; seed and fruit yields are reduced and sterile pollen is produced (Robson and Reuter, 1981). In some cases this is the first visible symptom of deficiency, growth having been normal up to this point. Barley is especially susceptible to this latter problem.

Table 2.6

Principle symptoms of copper deficiency (from Alloway and Tills, 1983)

Crop	Visible symptoms
Forage	
Grasses	Plant stunted, necrosis of tips of young leaves.
Clover	Young leaves become green, wither and die.
Lucerne	Stunted growth. New leaves are bluish green. Leaflets wither and die.
Cereals	
Wheat Barley Oats	Young leaves pale and lack turgor. Leaf become chlorotic with tip turning white and rolling into a spiral "wither tip". Upper half of lamina may wither and break on the healthy part - "whiptail".
Vegetable crops	
Beet Sugar beet Cauliflower	Young leaves small and bluish green in colour. Older leaves become chlorotic.
Potatoes	Leaves wilt due to lack of turgor.
Turnips	Yellowing of foliage which also develop white spots.
Onions	Bulb greenish bronze in colour.
Cabbage	Leaves chlorotic, heads fail to form.

Diagnosis of Cu deficiency by symptoms alone has two major drawbacks. Firstly yield may be reduced either without symptoms or before the symptoms are manifested, and secondly the symptom can often be confused with other nutrient deficiencies, drought stress or damage from sprays or frost (Robson and Reuter, 1981; Graham and Nambier, 1981). Therefore where possible visible symptoms should be verified by soil or plant analysis.

2.2.9 Effects of Copper Deficiency on Crops and Yield

Subclinical Cu deficiency can result in grain yield losses of up to 20% or more without any prior symptoms (Graham and Nambier, 1981). This condition is often referred to as "marginal" copper deficiency or "hidden hunger". Affected plants may be slightly smaller than those grown on Cu-sufficient soils but the size difference is not apparent unless areas of deficient and sufficient soil converge. Thus Cu deficiency is often overlooked and poor yield attributed to other factors such as water stress. The soils causing this are likely to remain undetected unless they are analysed for copper.

Several cases have been reported in Britain where yield increases of up to 20% have been obtained in response to Cu treatment of crops such as oats, barley, sugar beet and carrots, with no visible Cu deficiency symptoms (Reith, 1968; Jordan, 1975; Pizer **et al**, 1966). Tills and Alloway (1981) reported subclinical Cu deficiency in crops on the Breckland area of East Anglia where despite very low total and plant available soil Cu concentrations, no visible symptoms of Cu deficiencies were recognised by the farmer. Similar subclinical deficiencies have been reported on the humic rendzinas of the Icknield

series in Southern England (Tills and Alloway, 1983a).

Herbage growing on Cu-deficient soil is unlikely to show Cu deficiency symptoms itself but the low Cu concentrations may lead to reduced dry matter yields, and may cause deficiency problems in grazing livestock (Gartrell, 1981).

2.2.10 Diagnosis of Copper Deficiency

Copper-deficient soils and affected plants can be identified by symptoms alone (2.2.8). However, as previously mentioned (2.2.8 and 2.2.9) Cu deficiency diagnosis by symptoms alone, although by far the quickest and cheapest approach is not always useful. Another problem is that deficiency symptoms often only become apparent at fairly advanced stages of growth by which time it may be too late to correct. Therefore soil or plant analysis is required to delineate the extent of the deficiency problem.

(a) Soil analysis

Diagnosis of Cu-deficient soils has been described in 2.1.5. Once identified the soil can be treated for the benefit of subsequent crops. However soil tests are empirical and do not always discriminate between responsive and non-responsive situations. They have nevertheless proved reasonably useful in separating deficient from adequately supplied soils. In addition, because plant species vary widely in their ability to extract soil Cu (Graham and Pearce, 1979), it is to be expected that "critical" soil test values will also vary with the type of crop being grown.

(b) Plant analysis

Plant analysis is more reliable than the diagnosis of symptoms and is better than soil tests because it indicates the copper status of the plant itself under a given set of soil and environmental conditions. However, unlike soil analysis, plant analysis can only be used to indicate whether the plant was deficient when sampled. Nutrient supply can either increase or decrease after sampling. Therefore sampling at several stages of growth is required so that any suspected Cu deficiency can be detected and dealt with. In addition delays in analysis may prevent measures being taken in time to correct any detected deficiency.

Robson and Reuter (1981) discuss plant analysis in detail and suggest that for cereal crops, the Cu concentrations in young leaves are much more sensitive and accurate indicators of Cu status than concentrations in whole shoots or grain. The critical concentration of Cu in young leaves shows less change with plant age and is usually unaffected by environmental factors such as water stress, N.P.K. supply and soil type.

Graham and Nambier (1981) quote a range of Cu levels for cereals below which deficiency usually occurs. These include 1 mg Cu/kg DM in the young leaves of wheat plants and 5 and 4 mg Cu/kg in the shoot at tillering for barley and oats respectively. Critical Cu concentrations for pasture herbage species are important for animal nutrition as Cu deficiency rarely affects pasture production and will be discussed later.

(c) Biochemical assays

Nutrient deficiencies in plants can be diagnosed by measuring enzyme activity. A field test based on an assay of ascorbate oxidase has been developed for young wheat leaves (Loneragan, 1982). A similar enzyme test has been developed for use with subterranean clover (Delhanze **et al**, 1982). Both agree well with plant analysis results yet are faster and cheaper. However, further research is required to test their usefulness and general validity as diagnostic tools. In addition the test has to be carried out on leaves at specific stages of growth and therefore lacks the flexibility of soil tests.

(d) Summary

Soil tests are less direct than plant analysis and are therefore subject to errors caused by variation in Cu uptake and utilization by different plant species. However, they are the most convenient of all the diagnostic/predictive tests for micronutrients as the sample can be collected at any time of the year. This enables any necessary corrective treatment to be carried out before the deficiency appears. However, they are only suitable for crops. When the grazing animal is present other factors have to be taken into consideration and plant analysis is more useful (see 2.3.6).

Soil tests are empirical and only through plant analysis can Cu deficiency actually be confirmed. Thus although soil tests are better at predicting Cu deficiency, plant analysis gives a much better indication of the extent of a problem.

2.3 COPPER IN RUMINANTS

The first indications that Cu was essential for growth and the prevention of a wide range of clinical and pathological disorders in animals were found in the early 1930s (Hart **et al**, 1928; Cunningham, 1931; Neal **et al**, 1931). Subsequently, Bennets and Chapman (1937) found that an ataxic disease of lambs (enzootic neonatal ataxia) occurring in parts of Western Australia was an expression of Cu deficiency in the ewe during pregnancy. Since then other expressions of Cu deficiency and extensive Cu-deficient areas have been discovered throughout the world.

2.3.1 Distribution of Copper in the Animal Tissue

The distribution of total body Cu among the various body tissues varies with the age and Cu status of the animal (Underwood, 1977). The liver is the main storage organ for Cu in sheep and may contain between 40 and 70% of total body Cu in animals given an adequate Cu diet. However the liver Cu concentration of sheep can vary widely (50 to 400 mg Cu/kg) even in apparently healthy animals (Underwood, 1977). Adamson **et al** (1983) observed that liver Cu values for lambs raised on apparently similar feeding regimes varied widely (500 to 600 mg Cu/kg D.M. in one group and 500 to 1600 mg Cu/kg D.M. in another). Newly born lambs when compared with adults tend to have higher levels of Cu in their livers (Grace, 1983). In ruminants on Cu-deficient diets the hepatic Cu levels can decrease to very low levels, e.g. 6 mg Cu/kg D.M. or less.

The blood Cu levels of sheep given an adequate Cu diet range from 9.4 to 18.9 μmol/l (Grace, 1983). Under Cu-deficient conditions and when

liver stores become depleted, blood Cu levels can fall to below 4.9 μmol/l (Smith and Coup, 1973).

Little reliable information is available on the range and variability of the Cu content of the wool of sheep. The Cu content varies significantly with sheep breed and diet (Wooliams **et al**, 1983). However a kilogram of wool usually contains 6 to 8 mg of Cu (Grace, 1983).

2.3.2 Metabolism of Copper

Copper has a number of important biochemical functions in the ruminant. These are reflected in the various manifestations of Cu deficiency which will be discussed later.

The absorption of Cu occurs in the intestinal region and particularly in the large intestine (Grace, 1975). In most species Cu is poorly absorbed, the amount is influenced by; the concentration and chemical form of the Cu ingested, the dietary levels of other metal ions and organic substances (see later) and by the age of the animal. Mature sheep normally utilise less than 10% of the Cu ingested while young lambs utilise 4 to 7 times this proportion (Suttle, 1973). The mechanisms that regulate Cu absorption are not well understood.

Copper entering the blood plasma becomes loosely bound to serum albumin and amino acids in which form it is distributed to the tissues. In addition around 90% of Cu in the plasma is found firmly bound as the blue Cu protein ceruloplasmin. Ceruloplasmin is a ferroxidase enzyme involved in iron utilisation and in promoting the rate of Fe saturation of transferrin in the plasma. It does not play

a role in Cu transport (Underwood, 1977).

Some 60% of total red cell copper occurs as the protein erythrocuprein. This protein functions as a superoxide dismutase, i.e. it catalyses the dismutation of superoxide radicals into hydrogen peroxide and oxygen.

The Cu reaching the liver, the main storage organ of the body, is incorporated into the mitochondria, microsomes, nuclei and parenchymal cells in proportions which vary with the Cu status of the animal (Milne and Weswig, 1968). The Cu is then either stored here or is released for incorporation into enzymes.

The protein Metallothionein (MT) is thought to play an important role in the binding of Cu in the liver. Increases in liver Cu levels are associated with an increasing amount of MT and it has been proposed that it has a role in cellular detoxification (Bremner, 1974).

It has also been suggested that MT acts as a temporary Cu store prior to its utilisation in other enzymes (Bremner and Davies, 1974). Similarly it has been shown that MT has an important role in hepatic Cu accumulation in man by binding copper as Cu MT (Elmes, 1987). However attempts to investigate its role in Cu metabolism have been frustrated by lack of suitable analytical procedures for its measurement (Bremner **et al**, 1985). Therefore despite the activity of many researchers the actual function of MT is still unclear.

A high proportion of the ingested Cu is excreted in the faeces while very little is lost in the urine. Bile is considered to be the major excretion route for Cu in the body while digestive tract secretions

also act in this way but to a lesser extent (Grace and Gooden, 1980).

2.3.3 Copper Deficiency and Functions

Situations of marginal Cu status amongst ruminants on upland and hill farms in the U.K. are already common and may in fact increase as pastures are improved (Suttle, 1983b). Cu deficiency occurs when the grazing animal does not absorb enough dietary Cu to meet its metabolic requirement. A number of factors may limit the natural availability of trace elements to the animal and thus lead to the establishment of dietary deficient states.

In many areas, because of the particular geological features of the land, the soil and thus the herbage on it are inherently low in Cu and this leads to Cu deficiency in the grazing livestock. Pasture deficiency may also arise secondarily, often in areas with no naturally occurring Cu deficiency, though the effects of improving hill pasture (Evans, 1983). The basic aim of pasture improvement is to replace swards of low dry matter production. This involves removal or suppression of indigneous plant species and the application of lime, fertiliser and new seed. When this is carried out on soils with low or marginal Cu levels, the reseeding and increased production depresses herbage Cu concentrations through a dilution effect (Evans, 1983). However Cu deficiency in ruminants can also occur when reseeding has resulted in herbage with Cu concentrations similar to or even higher than they were before improvement. This occurs as liming increases the availability of Mo and S to the herbage. These two elements are antagonists which if present in sufficient amounts can reduce Cu absorption by ruminants (see 2.3.6). Thus despite

sufficient Cu in the herbage, the antagonistic effect of the increased Mo and S concentrations, resulting from the improvements, leads to Cu deficiency in the grazing livestock.

Another secondary Cu deficiency problem in ruminants is found on teart pastures where the Cu deficiency occurs as a result of inherently high Mo concentrations in the soil and herbage.

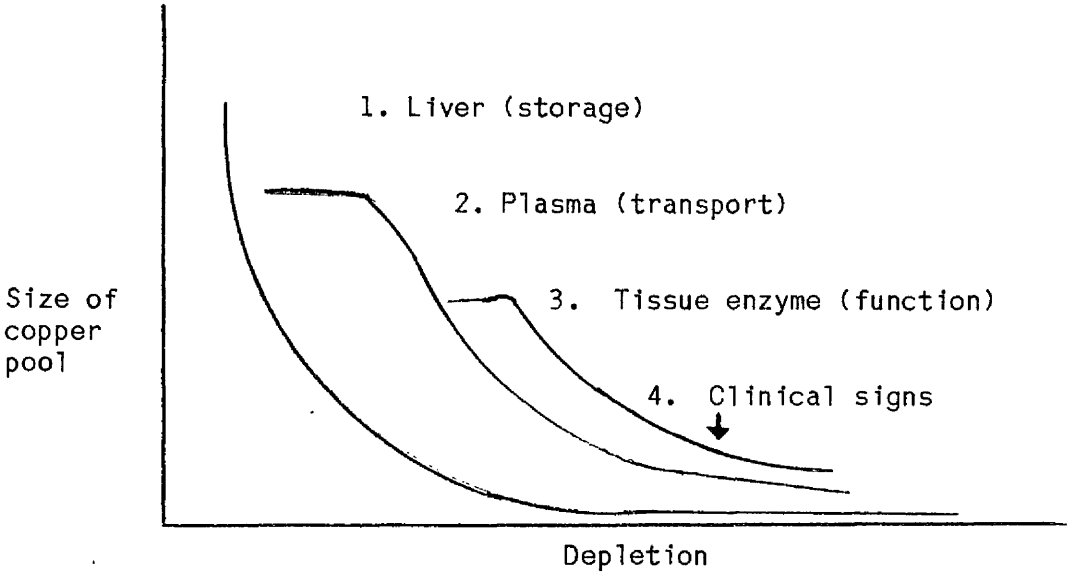
When animals have insufficient Cu in their diet to maintain body stores, they become Cu depleted. The animal may firstly react to conserve those stores which remain by increasing absorption efficiency and decreasing endogenous losses. In this way the animal may be sustained for some months, especially where it has substantial initial reserves, without becoming Cu-deficient.

Eventually however continued depletion will lead to deficiency and this is marked by biological signs that the homeostatic mechanisms are no longer maintaining a constant physiological activity (Suttle, 1983). For example, plasma Cu concentrations in sheep which are usually above 9.4 μmol/l begin to decline when the diet does not meet the animals requirement for Cu. A transition from a deficiency state to one of disorder occurs later and clinical symptoms become apparent.

A schematic representation of the sequence of events which may occur when sheep are fed a Cu-deficient diet is given in Figure 2.2. The absence of body stores or the presence of antagonists may restrict the depletion stage. Advancing maturity may extend the depletion and deficiency stages. The figure also provides an explanation for the poor correlation between the trace element content of the diet and the incidence of clinical disease at any moment in time.

Figure 2.2

Sequence of events in Cu depletion (Suttle, 1983b)



The number of important biological functons that Cu has is reflected in the various manifestations of Cu deficiency listed below:

(a) Anaemia and iron metabolism

Copper is involved in at least two stages in the metabolic events leading to haemoglobin synthesis. Therefore anaemia is a common expression of Cu deficiency where the deficiency is severe or prolonged. Cu deficiency reduces the amount of ceruloplasmin (ferroxidase) in the plasma thereby reducing the amount of Fe^{3+} formed and stopping Fe mobilisation between the plasma Fe and the tissue stores (Frieden, 1978). Thus Cu through ceruloplasmin has a critical role in mobilising absorbed Fe for haemoglobin synthesis and this is reduced under Cu-deficient conditions. The release or iron from liver parenchymal cells is also reduced.

(b) Bone disorders

Bone disorders are not usually a conspicuous feature of Cu deficiency in ruminants, however, fractured bones have been reported and Suttle **et al** (1972) has reported osteoporosis (abnormal porosity of bone) in bones from 10 week old lambs born to ewes which had been maintained on a Cu-deficient diet for 2 years. The authors concluded that osteoblastic activity (the bone cell forming processes in the foetus) is one of the first functions to be impaired in lambs born to Cu-deficient ewes.

These skeletal abnormalities appear to be related to the reduction in the activity of the Cu enzyme lysyl oxidase. This is responsible for the formation of cross linkages in the polypeptide chains of structural proteins such as collagen. Reduced enzyme activity leads to a reduction in the number of cross linkages in the bone collagen (Rucker **et al**, 1969).

(c) Neonatal ataxia (swayback)

Neonatal ataxia is a nervous disorder, characterised by incoordination of movement and high mortality. It has been recognised for many years in different parts of the world and is called swayback in Great Britain, Australia and New Zealand. It was first identified by Bennets and Chapman (1937) and arises from subnormal levels of Cu in the blood and tissues of ewes during pregnancy. The disease may be prevented by Cu supplementation of the ewe.

Swayback has been experimentally produced in lambs born to ewes on a Cu-deficient diet (Lewis **et al**, 1967) and by maintaining ewes of low copper status on a high Mo and S intake (Fell **et al**, 1961; Suttle and

Field, 1968).

Two types of ataxia occur in lambs. The commonest form is "congenital" which appears at birth and the other is "delayed" in which clinical signs do not appear for several weeks. A stiff and staggering gait and swaying of the hind quarter are evident as the disease develops.

The disorder is complex and associated with both lesions in the brain and spinal cord, and the demyelination of the nerve fibres (Cunningham, 1950; Howell and Pass, 1981). The lesions are irreversible and may begin to form as early as 6 weeks before birth and continue until parturition. It has been suggested that Cu deficiency leads to a lowering of the activity of several Cu-containing enzymes, including cythochrome oxidase, which results in a reduction in the amounts of the phospholipids which are important constituents of the myelin sheath around the nerve fibre (Howell and Pass, 1981).

Swayback is characterised by progressive cerebral demyelination, which results in paralysis and all lambs suffering from it die. Death is usually the result of starvation due to an inability to rise and suckle rather than a direct Cu deficiency effect.

(d) Loss of wool pigmentation

Pigmentation of wool is dependent upon the conversion of tyrosine to melanin which is catalysed by Cu-containing polyphenyloxidases (Underwood, 1981). Pigmentation is very sensitive to changes in Cu levels and alternating bands of unpigmented and pigmented wool can be produced in sheep successively deprived of and supplemented with Cu.

Prolonged Cu deficiency can result in the wool of black sheep turning white. These manifestations can occur on dietary Cu intakes sufficient to prevent other deficiency symptoms.

(e) Defective keratinisation of wool

The loss of crimp in wool is a characteristic of Cu-deficient sheep, particularly in the fine wool breeds such as the Merino. As the animal's Cu reserves are depleted the characteristic crimp of the wool is lost and fibres emerge as almost straight hairlike growth. This is termed stringy or steely wool (Lee, 1956). The physical properties of wool including crimping are dependent on the formation of the disulphide bonds which provide the cross linkages in the keratin structure and the orientation of the long chain keratin fibrillae in the fibre. These processes have been shown to be dependent on the presence of Cu (Burley and Dekoch, 1957).

2.3.4 Diagnosis of Copper Deficiency

This section will deal primarily on the diagnosis of Cu deficiency in sheep as the project was carried out with particular reference to these animals.

Although clinical symptoms of Cu deficiency in sheep are highly characteristic, e.g. swayback, they only occur under conditions of severe or prolonged deficiency. In addition, the incidence and severity of Cu deficiency varies from year to year (Scottish Agricultural Colleges, 1982). Higher incidences of swayback are associated with mild wet weather in February and March while long periods of cold weather or snow tend to decrease the incident rate probably due to the provision of supplementary feeding.

Copper deficiency more often manifests itself in covert or subclinical forms, e.g. ill thrift, anaemia or osteoporosis. Therefore unless a previous history of Cu deficiency as swayback exists, supporting diagnostic techniques are required to predict the possibility of Cu deficiency. In addition although swayback is highly characteristic of Cu deficiency it is not always specific and a final diagnosis cannot be made until after a pathological examination.

(a) Identification procedures

(i) Soil and pasture analysis

Unambiguous diagnosis of risk to livestock is only possible under very limited conditions where soil Cu levels are very low. The Scottish Agricultural Colleges (1982) have listed the main soil associations of Scotland and designated the risk factor of low Cu concentrations in summer herbage for each soil series within the association. Low risk soil series would be expected to give 5 to 9 mg/Cu/kg D.M. in summer herbage, moderate risk series 4 to 8 mg Cu/kg D.M. and high risk series 3 to 6 mg Cu/kg D.M. However most cases of Cu deficiency in sheep occur where soil Cu levels are apparently fully adequate.

Herbage Cu analysis, whilst not affected by soil contamination, is subject to fluctuations due to species and seasonal variations and analysis therefore only indicates the immediate situation. In fact herbage Cu levels can be misleading unless other elements with which Cu interacts, particularly molybdenum and sulphur, are also determined. However a herbage Cu content of over 10 mg/kg D.M. is unlikely to result in Cu-deficient sheep (Underwood, 1981).

(ii) Blood and liver analysis

Blood and liver analysis for Cu provides a basis for assessing the Cu status of an animal. The liver is the main storage organ of the body for Cu so that liver Cu concentrations would be expected to provide a useful index of the copper status of the animal. However liver Cu concentrations values vary greatly with the species and age of animal and also with the nature of the diet (Underwood, 1981). In addition liver sampling requires surgery and is therefore not often undertaken in the U.K. and in fact is usually done only after death of the animal. Liver Cu determination on post mortem material is therefore only useful for confirmatory diagnosis.

Whole blood or plasma Cu determinations are probably the most useful methods for establishing hypocupraemia (Cu deficiency) in livestock. They are relatively easily obtained and can be taken repeatedly over a long period of time. However, plasma copper levels do vary between sheep breeds (Wiener and Field, 1974; Wiener **et al**, 1978) even when maintained together as a single flock. Scottish Blackface sheep have been shown to have a lower plasma Cu status than Welsh Mountain sheep when both breeds are kept under the same conditions (Wiener **et al**, 1969).

Wiener and Field (1974) showed that there is a steady decline in plasma Cu status of a grassland flock from November through to June followed by a partial recovery over the summer, when the sheep are free from complications of pregnancy and copper supplementation. The values are further influenced by age, and Mo and S intakes (Underwood, 1981). Despite these limitations

plasma Cu levels are easily followed over a season and are still the single most useful diagnostic tool. However data from blood and liver analysis cannot be used to discriminate animals in which clinical deficiency is imminent from those likely to remain relatively healthy despite a low Cu status. The factors provoking the appearance of clinical signs in low Cu sheep have not been identified. Thus, decisions as to appropriate threshold values for the Cu content of blood or liver are arbitrary but are of value for identifying groups of animals in which the risks of development of Cu deficiency are high. The criteria in Table 2.7 are suggested for the interpretation of tissue Cu analysis results.

(iii) Blood copper enzyme activity

As previously mentioned (section 2.3.2) several Cu containing enzymes are present in the blood. A loss of enzyme activity when Cu supply is inadequate offers an alternative possibility for the measurement of Cu status. Todd (1970) showed that ceruloplasmin estimations could provide an effective Cu determination. However as some 80% of plasma Cu exists as ceruloplasmin and a high correlation exists between values of ceruloplasmin and plasma Cu status this offers little advantage in sensitivity.

Plasma monoamine oxidase and liver cytochrome oxidase activities also provide indices of Cu status but Mills **et al** (1976) concluded from studies with calves that "existing biochemical techniques are still of limited value for predicting the speed or extent to which an individual will

develop overt signs of deficiency".

Table 2.7

Suggested criteria for the interpretation of tissue copper analysis (from S.A.C., 1982 and Grace, 1983)

Tissue	Interpretation
Plasma or whole blood Cu (μmol Cu/l)	
18.9-9.4	Normal.
9.4-4.7	Clinical symptoms possible, covert pathological changes may already be established.
<4.7	Clinical symptoms likely, covert pathological changes almost certainly established.
Liver Cu (mg Cu/kg D.M.)	
>30	Cu deficiency unlikely.
30-10	Inadequate hepatic Cu reserves; clinical and covert pathological changes may become established.
>10	Increased probability of pathological and clinical effects from Cu deficiency.

Suttle and McMurray (1983) have used erythrocyte superoxide dismutase to try and predict hypocuprosis with some success but found it less useful than plasma and liver concentrations if high Mo induced the clinical deficiency.

2.3.5 Requirements for Copper

The Agricultural Research Council (1980) have used a quantitative approach to assess the Cu requirements of sheep. This includes

consideration of all aspects of Cu metabolism including the effects of dietary antagonists on Cu absorption and utilisation. The amount of absorbable Cu required by sheep varies with age, weight, pregnancy and lactation. The absorbable Cu required for 5, 10, 20, 30 and 40 kg growing sheep and an adult animal were 0.2, 0.2, 0.25, 0.50 and 0.22 mg Cu/Day respectively. In pregnancy 0.63 mg Cu/day is required as the minimal intake if deficiency is to be avoided.

They also state that provided the availability of the Cu is not greatly influenced by dietary factors such as Mo and S and the dry matter intakes are adequate then pastures containing 5 to 6 mg/kg should meet the Cu requirements of sheep.

2.3.6 Factors Influencing the Absorption of Copper

Despite apparently adequate pasture Cu levels grazing sheep may still show signs of Cu deficiency or respond to Cu supplements. This type of Cu deficiency is an induced Cu deficiency and reflects an impairment in the absorption or utilisation of Cu as a result of interference by dietary factors (Cunningham **et al**, 1956; Mills **et al**, 1978; Smith and Coup, 1973).

In grazing sheep absorption of Cu from herbage is dependant upon the herbage Mo and S levels (Grace, 1983). Suttle (1974) showed that both organic S (as methionine) and inorganic S directly affect Cu absorption. Increasing the S and/or Mo content will decrease the Cu absorption. Suttle (1983a) concluded that Cu absorption is most inhibited by 4-6 mg Mo/kg D.M. and that inhibition of S^{2-} production at higher levels of Mo may give rise to a recovery in Cu absorption.

The mechanism of the Cu x Mo x S interaction is not fully understood despite over 20 years intensive research. The primary site of their interaction is in the gut (Underwood, 1981). Dick **et al** (1975) proposed that here sulphide and molybdenum form thiomolybdate which then reacts with copper to form highly insoluble copper thiomolybdate $CuMoS_4$. Suttle (1975) and Mills **et al** (1978) also support this hypothesis.

Suttle (1983a) has derived a relationship to estimate the absorptive efficiency of Cu digested by the ruminant in the presence of various Mo and S levels in the herbage. The relationship for summer herbage is:

$$\text{Cu absorption (\%)} = 5.71 - 1.2795 \log_e \text{Mo} + 0.227 \text{ Mo} \times \text{S}$$

where S and Mo are herbage concentrations in g/kg D.M. and mg/kg D.M. respectively. Equations have also been devised describing the effects of Mo and S in semipurified diets (Suttle and McLauchlan, 1976).

Whitelaw **et al** (1979) demonstrated the importance of even small changes in Mo and S concentrations in herbage, in instances where pasture improvements produced increased Mo and S concentrations, which allowed growth responses in lambs supplemented by Cu to be observed. Absorption of Cu from autumn herbage is even lower than from summer herbage (Suttle, 1981c) due to Mo and S increases.

The Cu:Mo ratio is therefore also important in predicting the likelihood of Cu deficiency or toxicity; however there is little agreement on the critical ratio (Underwood, 1981). Alloway (1973) suggests a ratio of more than 4:1 to avoid hypocuprosis in sheep while

Miltimore and Mason (1971) suggest 2:1.

Cadmium and iron have also been shown to reduce significantly the absorption of Cu by ruminants (Mills **et al**, 1972; Campbell **et al**, 1974). Thornton and Abrahams (1981) have shown that soil ingestion is a pathway of metal intake into grazing livestock. Grazing sheep can involuntarily ingest up to 300 g soil per kilogram herbage D.M. especially when herbage coverage is poor over the winter months (Thornton, 1974; Healy **et al**, 1974). These sort of levels were further substantiated by McGrath **et al** (1982b) who found that sheep ingested up to 600 g soil per kg body weight between November and May. Suttle **et al** (1975) have shown that soil ingestion decreases dietary Cu utilisation by sheep. The effects of soil ingestion on Cu absorption are thought to be due to the Fe content of soil (Suttle **et al**, 1982). Suttle **et al** (1984) also suggested that soil ingestion impairs Cu absorption in sheep by trapping S, as heavy metal sulphides such as FeS and releasing sulphide in the acid abomasum. Although the effect of Fe on Cu absorption has been demonstrated, the actual effect of the soil, although thought to be related to Fe in the soil is unclear.

2.4 CORRECTION OF COPPER DEFICIENCY

A wide variety of copper sources have been evaluated for their effectiveness as fertilisers for the correction of Cu deficiency in plants and animals. They work either by increasing the concentration of plant available Cu in the soil or by supplying Cu directly to the plant foliage. In addition to pasture treatment a variety of direct animal treatments have been used either orally or by injection.

2.4.1 Plants

Copper sulphate ($CuSO_4.5H_2O$), cuprous oxide (Cu_2O), copper ores, copper carbonates, copper slags, copper frits, various Cu mixes with superphosphate or N.P.K. fertilisers and a variety of chelated copper compounds have all been found to be equally effective in treating Cu deficiency (Caldwell, 1971; Barnes and Cox, 1973). However wide variations exist in the amount of copper applied to soils for the correction of deficiencies. These variations are explained by several factors such as placement geometry in relation to the root, sorption of copper by soil constituents, reactions of the fertiliser with soil constituents and by the copper requirements of different plants (Gartrell, 1981). Opinions differ about the maximum application rate of Cu, to avoid toxicity, but it is generally recommended to be not more than 45 kg Cu/ha for a mineral soil. Applications to organic soils tend to be larger and more frequent than those to mineral soils (Follet **et al**, 1981).

The most frequently used method of correcting Cu deficiency in plants is to apply copper sulphate to the soil (Follet **et al**, 1981) as it is relatively cheap and convenient to use. It can be applied by; broadcasting over the soil or pasture, incorporation into the soil during cultivation or banded in the vicinity of the seed either before or at the time of sowing. The relative effectiveness of these methods depends on the circumstances because Cu is highly immobile in the soil, so that even in freely draining soils it is rarely leached out of the cultivation layer, therefore where possible it should be well mixed into the top soil if plants are to obtain a sufficient supply of Cu.

The residual effectiveness of Cu fertilisers applied to the soil is high (Caldwell, 1971; Reuter, 1975; Gartrell, 1980) due to the strong adsorption and the complexing of Cu by soil organic matter. This is especially so under arable cropping in which after a poor initial mixing within the rooting zone, cultivation in the following years improves the mixing and allows gradual desorption of Cu^{2+} ions to provide an increased Cu supply capacity. Under these conditions Cu fertilisers may become more effective in the years following application (Reith, 1975; Gartrell, 1980). Thus reapplicaton of Cu fertiliser is only necessary after periods of 5 to 18 years depending on the soil type, crop grown and the initial application rate.

Despite evidence that $CuSO_4$ has given long term control of copper deficiency in cereal crops (Reith, 1968; MacNaeidhe, 1984) a number of problems have been associated with its use, especially on acidic soils. When $CuSO_4.5H_2O$ is applied, a large proportion of the Cu^{2+} ions are rapidly brought into solution and are immobilised by soil adsorption processes. Some of these effects have been alleviated by the use of other less soluble Cu compounds (see below) which dissolve more slowly in acidic soils to release Cu^{2+} ions over a longer time period (Follet **et al**, 1981). The water insoluble Cu compounds include Cu metal, cupric and cuprous oxides and Cu slags (Follet **et al**, 1981). The former three can be expensive especially if used to cover large areas, however cupric oxide is often used as a soil applied fertiliser to correct Cu deficiency in plants (Fink, 1982).

In a review by Alloway and Tills (1984), they state that cupric oxide can correct Cu deficiency in most crops and is particularly effective on acidic soils. It has also been incorporated into superphosphate

fertilisers to produce a range of fertilisers containing 0.2-2% Cu (Gilkes, 1981).

Copper slag is a by-product of Cu smelting and contains Cu in metallic, oxide and silicate forms. However the quality and Cu content of Cu slag can vary widely. Its rate of decomposition and hence effectiveness is surface area dependant and so it is ground very finely but often has to be regranulated to improve ease of application and prevent losses through drift (Fink, 1982). Thus despite its low cost and potential ability to correct Cu deficiency in many crops (Alloway and Tills, 1984) it is not widely used.

Another Cu fertiliser, whose usage is confined mainly to West Germany, is a material called "Excello" which consists of brass particles and cupric oxides mixed with ground Cu slag to produce a Cu fertiliser of consistent quality and Cu content, but at a reasonable cost compared to Cu metal or oxide. All these insoluble Cu fertilisers are broadcast onto the soil and usually give best results if worked into the soil (Fink, 1982). The relatively insoluble nature of these compounds means that they slowly degrade to release a steady supply of Cu ions to plants. In a review by Alloway and Tills (1984) the residual effectiveness of these compounds for crops is given as 3 to 4 years.

Foliar application of Cu is less widely practised and is usually used only when symptoms have appeared or the deficiency is diagnosed by plant analysis (Gartrell, 1981), in which case it is used as an emergency treatment for unpredicted deficiencies. However, foliar Cu applications are preferred in Britain where they suit the system of

crop management and the acidic soils. Copper can be applied in sprays mixed with other agrochemicals thus dispensing with an extra spraying operation solely to apply Cu. Foliar applications ensure that each crop receives an adequate supply of Cu which may be less certain with soil treatment.

A single spray of $CuSO_4$ is usually enough to restore crop vigour (Pizer **et al**, 1966; Reith, 1968; Caldwell, 1971; King and Alston, 1975). Obviously the ability of cereals to recover is dependant on timing. If the treatment is applied too late the crop may have suffered irreversible yield limiting effects. In wheat for example, Cu sprays will not be effective if applied after the end of tillering (Graham and Nambier, 1981).

The forms of Cu most often used in foliar sprays are copper oxychloride and its chelates. Copper sulphate is also used, but owing to its high solubility which can cause phytotoxicity and its corrosive effects on metal spraying equipment its use in foliar sprays is in decline. Foliar sprays only give temporary correction and little residual value may be expected (Gartrell **et al**, 1979). Therefore regular repeat treatments are required.

Seed treatments of dusts or Cu solutions are mostly inefficient and inadequate because the amount of Cu that may be added to the seed without risking injury to the seedling is too small. It is also too localised in the soil to support the crop throughout the growing season (Caldwell, 1971).

In summary the application of Cu-containing fertilisers can be an effective method of correcting Cu deficiency in plants especially

cereal crops. Soil applications are the preferred method for general treatment while the use of foliar sprays, except in Britain, is mainly confined to emergency treatment where the deficiency is not recognised until after planting.

2.4.2 Animals

Although the use of Cu fertilisers can be an effective method of correcting Cu deficiency in plants, the increased Cu concentration especially of pasture is not always of significant benefit to the grazing animal. Long lasting residual effects of fertiliser Cu for pasture and grazing animals have been reported in Western Australia (Gartrell, 1981) but are rare elsewhere in the world. Under the acidic conditions of Great Britain, application of Cu to soil or as a top dressing to herbage have produced only limited increases in herbage Cu concentrations (Mitchell **et al**, 1957; Morgan and Clegg, 1958; Reith and Mitchell, 1964; Archer, 1970; Reith, 1975; Evans, 1983).

The most frequently used herbage treatment is broadcast application of $CuSO_4$ to pasture which has demonstrated increases of up to 4 mg/kg in herbage Cu concentrations (Evans, 1983; Burridge **et al**, 1983). However its effectiveness is influenced by the sward composition. The Cu concentration in clovers can be increased more readily than that of grasses grown in the same sward. The increase in clovers is normally 2-4 mg Cu/kg while the increase in ryegrass Cu concentrations is usually 1-2 mg/kg (Reith, 1983; 1984). Similar results have been obtained in pot experiments by McLaren and Williams (1981); however increases of 3-4 mg Cu/kg in ryegrass have been found in pot trials

(McGrath, 1982a). These increased herbage concentrations are usually short lived, 1-2 years, and are not always sufficient to be of significant value to the animal especially where Cu intake is impaired or metabolism reduced by sulphate or molybdenum or soil ingestion.

Addition of Cu chelates to the soil to alleviate Cu deficiency in pasture have been shown to have an even poorer effect than $CuSO_4$ (McLaren and Williams, 1981). Other methods such as application of Cu metal or oxide are expensive especially if used to cover large areas. Therefore soil or pasture treatment with Cu is not usually considered to be a particularly effective way of overcoming a livestock deficiency.

Partly because of the existence of such problems with soil treatment a number of methods for direct treatment of the animal have been evaluated for their effectiveness in reducing animal deficiency. Each method has its limitations and the choice will depend upon the system of ruminant husbandry practised and the severity of Cu deficiency on the farm. Under range conditions Cu deficiency can be prevented by the provision of salt licks containing 0.5 to 2% $CuSO_4$. These were first introduced by Dunlop **et al** (1939), but the provision of the licks did not guarantee an adequate or even any consumption of Cu by each individual animal.

Consequently oral dosing with $CuSO_4$ was tried as a supplementation method (Hunter **et al** 1945). This has proved successful for the prevention of copper deficiency in sheep. However as for other oral dosing methods its effectiveness may be affected by dietary, e.g. Mo and S, and genetic factors that affect copper absorption rates.

Claims have been made that organically bound Cu is more available than inorganic salts when orally dosed. However, MacPherson and Hemingway (1968) and Regius and Nagy (1972) have shown no difference in the effect of Cu given in the chelate or ionic form to lambs and sheep. Copper chelates suffer from the same limitations as oral $CuSO_4$ dosing.

In theory subcutaneous or intramuscular injections of Cu compounds should have an advantage when Cu deficiency is caused indirectly by antagonists such as Mo and S since the administered Cu bypasses the inhibitors to Cu adsorption in the gut. The Cu complexes that have been evaluated for use with sheep includes copper calcium E.D.T.A., copper methionate, diethylamine copper oxyquinoline sulphonate, copper glycinate (S.A.C., 1982; Suttle, 1981b). All have proved to be effective sources of supplemental Cu. However individual products vary in three respects; in the local lesions they induce, acute toxicity and the extent to which they are retained in the liver. Severe reactions at the site of injection have been associated with many of these products (Suttle, 1981b). In general the copper compounds which cause local lesions at the site of injection (Cu methionates and glycinates) are translocated slowly and therefore of low toxicity but give poor protection in some Cu deficiency cases. The more rapidly translocated compounds (e.g. copper calcium E.D.T.A. and copper oxyquinoline sulphonate) must be given in lower doses to prevent acute toxicity. The frequency of injection will obviously depend on the severity of the copper deficiency but care must be taken not to overdose and induce chronic Cu toxicity.

Recently a new injectible preparation containing cupric oxide powder has been evaluated as a supplement for cattle (Sankoh and Boila,

1987). However it is too early yet to say if it will be successful.

Despite the problems of lesions and toxicity associated with injections they are widely used. Their drawback, however, is that repeated injections are often necessary to maintain normal plasma Cu concentrations thus creating a lot of extra work for the farmer involved.

The use of drinking water as a carrier of Cu for cattle has been found to be effective for supplementing Cu intake (Humphries **et al**, 1983; Farmer, 1983). However it remains to be determined whether it is safe to use for sheep which are much more susceptible to Cu toxicity.

A new method of long acting copper supplementation which eliminates the carcass damage often associated with injection has now been developed in Australia (Dewey, 1977; Deland **et al**, 1979). The method involves the oral administration of copper oxide needles or wire (CuO_N), in lengths of between 5 and 10 mm and with a mean diameter of 0.5 mm, of high specific gravity and low mass in a gelatin capsule into the oesophagus with a tube or balling gun. The needles lodge in the ruminant abomasum and the Cu is slowly released over 2-3 months.

Dewey (1977) found that the oral administration of copper needles successfully increased the liver Cu concentrations of adult sheep as well as raising plasma Cu levels. This treatment has been successfully tested in field trials on hill sheep in Scotland by Whitelaw **et al** (1980). Suttle (1981a) administered 0.5 g CuO_N to hypocupraemic ewes maintained on a Cu-deficient diet; it alleviated hypocupraemia for 111 days when the diet was supplemented with Mo and S and 301 days when the diet was not so supplemented. Whitelaw **et al**

(1982) treated ewes at parturition with 4 g or 8 g Cu needles and found that they remained normocupraemic until beyond weaning when grazing reseeded pastures on which control ewes rapidly became hypocupraemic. In ewes confined to the reseeded pasture during pregnancy, the administration of 4 g CuO_N in midpregnancy prevented the occurrence of swayback in their offspring whereas control ewes produced a high incidence of both the congenital and delayed types of swayback. This work was further substantiated by Whitelaw **et al** (1983) who showed that lambs derived from dosed ewes took longer to become hypocupraemic and also that lambs derived from dosed or undosed ewes and treated at 3 to 5 weeks of age with CuO_N were maintained in normocupraemia and showed no signs of ill-thrift.

Trials in Australia have found that after administration of CuO_N concentrations of Cu in the sheep liver often rise to levels which might be expected to be toxic without the animals appearing to be adversely affected (Ellis, 1980).

These results indicate that both ewes and lambs grazing Cu-deficient pasture can be protected very simply by an annual single dose of Cu needles. The method permits the minimal amount of collection and handling of livestock whilst supplying Cu over a substantial period of time. In addition overdosing is not such a serious problem, as with Cu injections, because of the slow release of Cu.

Soluble glass designed for the slow release of trace elements has been proposed as a method of animal mineral supplementation (Allen **et al**, 1978). Knott **et al** (1985) review the properties of glass which allow this possible function. Alkali-phosphate glass with less than 50%

P_2O_5 is soluble and trace elements such as Zn, Cu, Co and Se can easily be incorporated into the glass and exhibit the property of slow release. Subcutaneous implantation of such boluses have been shown to eliminate Cu deficiency in animals (Allen **et al**, 1979). However the use of an intra ruminal bolus should allow all these trace elements to be made available to animals.

Telfer **et al** (1983) produced a soluble glass bolus containing Cu, Co and Se which, when given by balling gun to sheep, will lodge in the animals reticulum and dissolve at a slow rate releasing Cu, Co and Se over a period of up to 1 year. The bolus prevented Se deficiency in sheep and cattle and produced higher plasma Cu levels (Knott **et al**, 1985). However further studies on the effectiveness of the bolus have produced conflicting results.

Carlos **et al** (1985) and Care **et al** (1985) have shown that administration of the bolus elevated the blood and liver copper status of sheep and lambs and recommended a yearly dosing. Allen **et al** (1985) showed that the boluses remain in the forestomach of sheep for periods of up to 9 months after dosing providing an efficient and practical method of providing long term oral supplementation of Cu.

In contrast Patterson **et al** (1985) found the release rate of the trace elements to vary widely, with complete dissolution occurring in a few days or over 6 months. Judson **et al** (1985) found the dissolution rate to be too slow to provide a boost to liver Cu reserves in sheep. Whilst Allen and Sansom (1985) observed that the sheep glass bolus contained less Cu than the recommended dose of oxide needles and that the rate of release from the boluses was much slower. These factors

have been further substantiated by results which show that the more prolonged release period of Cu from the boluses, compared with other treatments, appears to result in an insufficient Cu supply to the animal (Gallagher and Cottrill, 1985; MacPherson, 1985; Buckley **et al**, 1987). The results suggest that the soluble glass bolus does show promise but that further development is necessary if the full potential of this technique for the control of Cu deficiency is to be realised.

In summary pasture treatment with Cu to prevent Cu deficiency in animals has had only limited success to date. Broadcasting of $CuSO_4$ is the most frequently tried method, however the Cu applied is usually rapidly immobilised in the soil and unavailable to plant (2.4.1). As a result the increases in herbage Cu concentrations obtained are usually too short lived and insufficient to be of significant value to the grazing animal. Other pasture treatments have only demonstrated similar results to that of $CuSO_4$ or are too expensive for the treatment of large areas. Because of this a number of parenteral and oral methods for the prevention of Cu deficiency have been made available. Each method has its limitations, oral methods including Cu needles still have to overcome any effects of dietary factors which modify Cu adsorption, while injections provide only short term supplementation as the amount which may be administered as a single dose is limited due to problems of possible acute systemic toxicity and localised carcass damage. Alternative methods still have to guarantee that each individual animal will obtain its daily Cu requirement. Thus every method used to date has its limitations. Therefore the choice of method will depend on the system of animal

husbandry practised, the severity of the Cu deficiency experienced on the farm and on the individual preferences of the farmer.

CHAPTER 3. MATERIALS AND METHODS

3.1 INTRODUCTION

This chapter deals with the principles of the analytical methods used in this work and gives summaries of each procedure. The reason for, and application of, each particular method used will be discussed in the relevant chapters.

3.1.1 Analytical Quality Control

The value of soil, herbage and blood analysis in the diagnosis of plant or animal Cu deficiency depends largely on the accuracy and precision with which both the sample is taken and the way the determination is carried out.

Accuracy is defined as the proximity of the measured result to the "true" value.

Precision is defined as the reproducibility of a result.

Owing to the extreme heterogeneity of the materials investigated the largest potential hazard in obtaining accurate results in this work would be sampling errors, both in the field and when sub-sampling in the laboratory. Sampling procedures were employed which reduced the risk of these errors especially for soil and herbage and will be discussed in the relevant chapters.

The following quality control procedures were used to assess the accuracy and precision of analytical results:

(i) The use of standard solutions to calibrate and monitor analytical instrument performance and thus maintain optimum sensitivity. These were prepared using commercial standard solutions and were made up in a solution similar to that of the samples. Standards were systematically distributed throughout the batch and made up between 10% and 20% of it.

(ii) Reagent blanks were used throughout to assess background concentrations and possible contamination errors.

(iii) Departmental standard reference materials were included in each batch of soil or herbage analysis to assess the between batch reproducibility and overall precision of results throughout the period of work. A commercial reference material "precinorm" (Boehringer, Manhein, Gmbh, West Germany) was used as above for blood analysis. The results of any one batch were only accepted if the precision was less than or equal to 10%.

(iv) Sample determinations were all replicated at least twice in each batch during analysis to monitor the within batch precision (i.e. reproducibility of the results). The result for any one sample was only accepted if the replicates agreed to within 5% of each other.

3.1.2 Atomic Absorption Spectroscopy (A.A.S.)

A.A.S. has been used almost exclusively as the analytical technique for the determination of trace elements in this work. The major advantage of A.A.S. over most of the comparable techniques used for inorganic analysis is its relative freedom from interferences and the

fact that it often allows the determination of several elements from one sample preparaton. In contrast in electrochemical methods such as polarography most samples require dissolution followed by a preliminary separation of the analyte to obtain sufficient selectivity, i.e. extensive sample pretreatment is required for each element to be determined. The analytical signal in A.A.S. is less sensitive to temperature changes in the atom cell than atomic emission spectrometry. A.A.S. also gives ease of instrument operation and is simple to set up due to the useful wavelength and intensity reference provided by the background light source.

In A.A.S. a sample is converted into an atomic vapour by a chemical flame, and irradicated by a light beam from a hollow cathode lamp source. This source must emit radiation of a wavelength that is specific to the element being analysed. This radiation is isolated by a monochrometer.

The amount of radiation absorbed by the atomic vapour is then measured and converted from an optical signal to an electrical signal by a photo-electric cell and is then related to the concentraton of the specific metal in the sample. The theory and basic principles of A.A.S. are discussed in greater detail by Kirkbright and Sargent (1974) and Page **et al** (1983).

Each element has a set of standard instrumental parameters for use with A.A.S. in order to obtain optimum conditions for accurate and precise results. The parameters for the elements studied in this work are listed in Table 3.1. Detection limits given in Table 3.1 are for flame atomic absorption using an I.L. Video IIAA/AE Spectrophotometer.

Table 3.1

Standard parameters for use with atomic absorption spectrophotometry

Element	Detection limit (g/ml)	Wavelength (nm)	Lamp current	Bandpass (nm)	Flame type	Necessity for background correction
Copper	0.03	324.7	5	1	Air acetylene (oxidising)	No
Zinc	0.008	213.9	3	1	" "	Yes
Iron	0.04	372.0	8	0.3	" "	No
Cadmium	0.01	228.8	3	1	" "	Yes
Nickel	0.06	232.0	10	0.15	" "	Yes
Lead	0.1	217.0	5	1	" "	Yes
Manganese	0.02	403.0	5	0.5	" "	No
Chromium	0.06	357.9	6	0.5	Nitrous oxide	No
Magnesium	0.003	285.2	3	1	Air acetylene (oxidising)	No
Calcium	0.05	422.7	7	1	" "	No

They represent the concentration of the element that produces an absorption reading equivalent to twice the standard deviation of a series of at least ten determinations at or near the blank level.

Atoms only absorb energy at narrow and specific wavelengths. Therefore for each element the light source is adjusted to produce a wavelength specific for that element, e.g. for Cu, when the source emits radiation of exactly 324.7 nm it will be absorbed by copper atoms.

The lamp current is a critical parameter in obtimising A.A.S. as it controls the intensity of the radiation line in the flame. Also because high currents shorten lamp life the optimum current also extends lamp life and is usually one third of the maximum lamp current.

The band pass is simply the width of the observed peak at the detector and is used to isolate the resonance line of interest from nearby non-resonance, weakly absorbing lines. The more non-reasonance lines there are around the line of interest, the narrower the band pass slit width has to be.

The flame is used as a means for producing the atomic vapour and selection of the correct flame type gives accurate and complication free results.

Air-acetylene flames are suitable for most elements and can be divided into three types:

(i) Oxidising flame - this is the hottest flame and contains enough oxidant to obtain a clear blue flame.

(ii) Stoichiometric flame - this flame has tinges of yellow in the flame.

(iii) Reducing flame - the entire flame is yellow, this is the coldest flame.

Some elements form refactory oxides at the temperature (2300°C) produced by an air-acetylene flame. At the highest temperature produced by a nitrous-oxide/acetylene flame (3000°C) free atoms can be produced from the oxides.

Background molecular absorbance occurs in the ultraviolet region of the wavelength spectrum (190-300 mm) which reduces the sensitivity of the determination. Therefore the Smith-Heiftje background correction technique was employed.

3.2 SOIL ANALYSIS

3.2.1 Sampling

Soil samples were taken in such a way as to be representative of the area under consideration using the techniques described in M.A.F.F. (1979, 1983). A tubular corer giving a soil core of 25 mm diameter including the surface mat was used for all soil sampling. Different sampling depths between 0 and 10cm were used and these will be detailed in the relevant later chapters. Samples were collected in greaseproof paper lined paperbags.

3.2.2 Sample Preparation

Soils were riddled through a 5 mm mesh wire sieve and air dried in a fan oven at a temperature less than 30°C. The soil was then ground to pass through a 2 mm sieve using a Rukuhia type soil milling machine. Samples were then stored in paper bags for later chemical analysis.

3.2.3 Moisture Content

The moisture content of field moist soil was assessed as the percentage loss of weight on oven drying at 105°C for 16-18 hours.

3.2.4 pH in Water

Soil pH was determined by the method of Avery and Bascomb (1974) in which the pH of a 1:25 air dry soil:water suspension is measured using a pH electrode and meter. Standard buffer solutions of pH 4 and pH 7 were used to calibrate the meter.

3.2.5 Lime Requirement

The lime requirement of a soil is defined as the weight of calcium carbonate required to raise its pH to a target value.

Two methods were used to determine lime requirement.

(a) For field trials lime requirement was determined using the routine method (unpublished) of the West of Scotland College Soil Science Analytical Laboratory. This is a modified electrometric titration method and gives rapid results. The lime required is interpolated from a comparison of the pH values measured in a 1:25 ratio air dry soil: 0.01 M calcium chloride suspension, and

a 1:25:10 ratio air dry soil: 0.014 M calcium chloride: 0.04 M lime water suspension. Full details of this method are given in Appendix 1.

(b) Lime requirements for fields, as above are crude determinations regardless of methodology. For pot trials a more accurate determination of lime requirement was required as soils were to be limed to a range of specific pH values. As pot work involves known volumes of soil the lime requirement was determined by soil-lime incubations using known smaller volumes of soil. Calcium hydroxide ($Ca(OH)_2$) was used, instead of calcium carbonate as the liming material as it is more soluble and faster acting.

Increments of $Ca(OH)_2$ incubated in aliquots of moist soil were equilibrated at room temperature for 10 days with periodic shaking. Then the pH was measured. The amount of $Ca(OH)_2$ required to bring a soil to a selected pH was then interpolated from a graph of equilibrium pH against $Ca(OH)_2$ addition and scaled up to the larger pot volumes.

3.2.6 Organic Matter Content by Loss of Weight on Ignition

Many methods are available for the estimation of the organic matter content of a soil (Hesse, 1971) but for the purposes of this work estimation by loss of weight on ignition was chosen as it provides a simple and rapid technique.

The method used was that of Avery and Bascomb (1974). The loss of weight of an oven dried soil (105^{o}C) is determined after ignition at

400°C for 16 hours. This temperature was used to reduce the risk of release of absorbed water or ignition of inorganic fractions which occur at higher temperatures, e.g. 680°C (Ball, 1964). As none of the soils were calcareous weight loss due to the destruction of carbonates was considered negligible. Duplicate analyses were always undertaken.

3.2.7 Exctractable Potassium and Phosphorus

Scottish Agricultural Colleges (S.A.C.) fertiliser recommendations (S.A.C., 1985) were employed for all field trials. Therefore extractable Potassium (K) and Phosphorus (P) were determined using standard S.A.C. methods (M.I.S.R. and S.A.C., 1985). These are outlined below.

(a) Extraction

P and K were extracted from the soil using 0.43 M acetic acid in a soil to solution ratio of 1:40 with an extraction time of 2 hours.

(b) Potassium determination

The concentration of K in the extract was determined using a flame photometer (Corning 450); there is no significant interference by other elements (Collins and Polkinhorne, 1952). The photometer was calibrated using potassium sulphate in acetic acid standards. Results are expressed in mg K/litre air dry soil.

(c) Phosphorous determination

The concentration of P was determined colourimetrically using the method of Murphy and Riley (1962). A 1:4 ratio mixture of the acetic acid soil extract and 0.15% acid ammonium molybdate produces a phosphomolydbate complex, which when reduced by ascorbic acid gives a

blue colour. The colour is then measured using a spectrophotometer (Corning 254) which was calibrated using potassium dihydrogen orthophosphate standards. Results are expressed in mg P/litre air dry soil.

3.2.8 E.D.T.A. Extractable Copper

E.D.T.A. extractable copper is generally assumed to represent plant available copper in the soil (see section 2.1.3) and was used in this study for the interpretation and classification of soil Cu levels for crop and animal nutrition.

The method used was that of Reith (1968) in which copper was extracted from the soil using 0.05 M E.D.T.A. at a soil:solution ratio of 1:5 with an extraction time of 1 hour. The Cu concentration in the extract was determined by A.A.S. (section 3.1.2). Results are expressed in mg Cu/kg air dry soil.

3.2.9 Total Trace Metal Concentration

Total copper, zinc, iron, cadmium, lead, nickel, chromium and manganese in soil were determined by aqua regia extraction using method B of Berrow and Stein (1983). At least 80% of the total metal content is extracted (over 90% for lead) by this method; the remaining metals are retained in siliceous residues.

A 1:10 ratio of soil:aqua regia solution (3 parts 6 M HCl:1 part 16 M HNO_3) was stood at room temperature for 16-18 hours before being heated firstly at 80°C for 30 minutes and secondly for 2 hours at 140°C using a Tecator system 40 digestion block. Metal analysis was by A.A.S. (section 3.1.2). Results were expressed in mg metal/kg

soil.

3.3 HERBAGE ANALYSIS

3.3.1 Sampling Methods

Methods of herbage sampling varied and will be described at the appropriate time in subsequent chapters. All samples were collected in sealed polythene bags. All subsequent analysis was carried out on unwashed herbage.

3.3.2 Dry Matter

Herbage was oven dried in paper lined trays at 100°C and the dry matter content calculated in g/kg fresh weight.

3.3.3 Sample Preparation

Dried herbage was milled to pass a 0.5 mm mesh seive using a Tecator Cyclone Sample Mill 792. All samples were stored in greaseproof paper lined bags. Before chemical analysis all samples were dried at 100°C for 16 hours to ensure that all results were based on a 100% dry matter basis.

3.3.4 Trace Element Analysis

(a) Preparation

Preparation for trace element analysis was based on that given in M.A.F.F. (1986) in which the organic matter is destroyed by dry combustion at 470°C for 16 hours and the soluble mineral constituents in the ash dissolved in 6 M hydrochloric acid; any silica present is dehydrated to an insoluble form and filtered out. This dry ashing method is safer and quicker than wet acid digestion.

(b) Trace element analysis

The sample solution prepared as above was used for the determination of copper, zinc, iron, lead, manganese, cadmium, nickel and chromium by A.A.S. (section 3.1.2). Results are expressed in mg element/kg dry matter.

3.3.5 Molybdenum (Mo)

The method used was that of Dixon and Hepher (to be published) in which the organic matter in dried soil herbage is destroyed by ashing at 480^{o} for 16 hours. The soluble mineral constituents in the ash are then dissolved in 6 M HCl and the mixture refluxed for 30 minutes. After filtering the solution is buffered to pH 1 using ammonia. Ammonium pyrrolodine dithiocarbonate (A.P.D.C.) is then used to form an organo-molybdenum complex in the acid. Chloroform is added to bring the Mo into an organic phase which is then separated from the acid using Whatman grade 1 phase separating paper. The chloroform is thereafter allowed to evaporate to dryness. The residue is taken up in HNO_3 and re-evaporated to dryness. This residue is dissolved in 6 M HCl and the molybdenum content determined using an inductively coupled plasma (Thermoelectron Plasma 100). Full details of this method are given in Appendix I.

3.3.6 Sulphur (S)

Herbage sulphur content was determined using the standard S.A.C. method.

The sample is digested in a mixture containing: 2 parts herbage; 1 part digestion catalyst (potassium dichromate in ammonium

metavanadate); 37.5 parts digestion mixture (5 parts nitric acid; 3 parts perchloric acid) for 1 hour at 120°C on a Tecator system 40 digestion block. The nitric acid is then evaporated off at 200°C (complete when colour changes from green to orange). After cooling, 50 ml of water is added and the contents filtered through a Whatman No. 540 filter paper. All sulphur is now in the sulphate form and is precipitated using a barium chloride/polyacrylamide (300:1) mixture and determined turbidimetrically at 660 mm. Results are expressed in g S/kg dry matter.

3.3.7 Chlorophyll

Chlorophyll was determined by the method of Arnon (1949) in which fresh herbage is ground in 80% acetone until all the colour is released from the tissue. The extract is then filtererd and made up to a known volume. The absorbance (A) of the solution at 663 nm and 646 nm is measured using a pye unican SP8-500 U.V./V.I.S. spectrophotometer. Total chlorophyll is then given by the following relationship:

$$\text{Total chlorophyll (mg/l)} = 17.3A_{646} + 7.18A_{663}$$

3.4 BLOOD ANALYSIS

3.4.1 Sampling

Venous blood samples were collected from all animals before field trials began and at regular intervals throughout their course.

When whole blood or plasma was required samples were collected in tubes containing lithium herapin as the anticoagulant. Silicon lined

tubes were used when serum only was required.

3.4.2 Plasma Copper

Copper concentrations in blood plasma, used to assess the copper status of animals involved infield trials, was determined by the following method:

(i) Preparation

Whole blood is centrifuged at 3000 r.p.m. for 20 minutes and the plasma removed as the supernatant.

(ii) Analysis

Copper was determined by analysing a 1:10 plasma:water solution using A.A.S. (section 3.1.2). Results are expressed in μmol Cu/l plasma.

3.4.3 Plasma Magnesium (Mg) and Calcium (Ca)

These two elements were analysed to monitor hypocalcaemia and hypomagnesaemia and are good indicators of the likelihood of associated animal health problems especially around lambing time.

(i) Preparation

As in 3.4.2. (i) above.

(ii) Analysis

Magnesium and calcium were determined by A.A.S. after a 1:75 dilution of plasma with a lanthanum/phosphate (50:1 solution in $HClO_4$) buffer. The use of the buffer is necessary as the sensitivity of A.A.S. to these two elements is depressed by the formation of stable compoudns of Mg and Ca with sulphates,

silicates and aluminates in the flame. These compounds reduce the number of free Ca and Mg atoms which can be read. Lanthanum (La) also forms stable compounds with the above interfering anions; therefore by adding La to the plasma solution in excess, the sought after element, e.g. Ca is released as free atoms which are able to absorb or emit their reasonance energy. Results are expressed in mg/100 ml plasma.

3.4.4 Haemoglobin (Hb)

The method used to assess haemoglobin levels was based on that of Drabkin and Austin (1935) and has been modified into kit form by Sigma Diagnostics, St. Louis, U.S.A. (Procedure No. 525).

Haemoglobin in whole blood is converted by Drabkins reagent (20:4:1 sodium bicarbonate; potassium ferricyanide; potassium cyanide) to cyanmethaemoglobin. The colour intensity of this is proportional to the haemoglobin concentration in blood. Cyanmethaemoglobin concentration is photometrically determined at 540 mm using a Pye Unicam SP8 - 500 U.V./V.I.S. spectrophotometer. Results are given in gHb/100 ml whole blood.

3.4.5 Lead (Pb)

Lead concentrations were measured by a modification of the chelation-extraction technique of Farelly and Pybus (1969) in which lead is extracted directly from whole blood. This method required neither protein precipitation nor any pH adjustments as employed in other more complex methods and is therefore a rapid and simple technique.

A 25:1:10:1:25 suspension of whole blood:saponin:formamide;ammonium pyrolidine dithiocarbonate (A.P.D.C.):methyl isobutyl ketone (M.I.B.K.) is well mixed. The formamide breaks down the blood cells to release the lead which is complexed by A.P.D.C. and taken into the organic phase (M.I.B.K.). The suspension is centrifuged at 3500 rev/min for 20 minutes and the organic layer separated and retained. Due to poor sensitivity obtained by direct aspiration of the organic solent in A.A.S. a resolution in acid was employed.

The organic solvent was allowed to evaporate to dryness at room temperature. The residue was then redissolved in 10 ml of 10% HNO_3 and refluxed for 20 minutes at 50°C. In the acid, sensitivity was improved and the lead concentration was determined using A.A.S. (section 3.1.2). Results are given in μg Pb/ml blood.

3.4.6 Vitamin B_{12} Determination for Cobalt Status

Vitamin B_{12} levels in blood were used to assess the cobalt status of animals involved in field trials.

Vitamin B_{12} in blood serum was determined using a kit developed by Becton and Dickinson (B and D), Orangeburg, New York, U.S.A., which is based on the technique of Lau **et al** (1965). This is a radio assay which measures only physiologically active ("true") vitamin B_{12}. The method uses the principle of competitive protein binding in which unlabelled (test serum) and radioactively labelled (prepared) vitamin B_{12} compete for a limited number of specific binding sites on a vitamin B_{12} binder protein and equilibrium is established. The level of radioactive bound vitamin B_{12} is inversely related to the concentration of non-radioactive vitamin B_{12} in the test sera. After

incubation the bound and free fractions are separated using charcoal and the amount of radioactivity measured by gamma scintillation. Results are expressed in ng vitamin B_{12}/ml blood.

3.4.7 Selenium Status as Determined by Glutathione Peroxidase (GSH-Px) estimation

The only known function of Se in the animal is the destruction of peroxidases through the activity of the selenoenzyme glutathione peroxidase (Suttle and Linklater, 1983). Therefore measurement of GSH-Px in the blood can be used to assess the Se status of animals.

The technique used is a modified enzyme assay based on that of Godwin **et al** (1973). The state of glutathione oxidation by peroxidase in the blood lysate (1:40 whole blood:water) was measured by following the rate of disappearance of nicotinamide adeniene dinudeotide phosphate (N.A.D.P.H.) which is also involved in the reaction and which is present in equal amounts to GSH-Px. Glutathione reductase is included to maintain the presence of glutathione in the reduced form. Activities were measured in 1.5 ml aliquots using a Pye Unicam SP30 spectrophotometer at 340 nm and the calculation is based on the rate of reaction (i.e. absorbance change per minute) over the first 2-20 minutes. Results are expressed as U GSH-Px/ml blood.

3.4.8 Serum Glutamic-Oxalacetic Transaminase (S.G.O.T.)

S.G.O.T. is a liver enzyme which may also be found in the blood of sheep. Elevated S.G.O.T. concentrations in the blood give advance warning of the haemolytic crisis and death due to chronic copper poisoning (MacPherson and Hemingway, 1969). S.G.O.T. concentrations were determined colourmetrically by following the procedure and

employing the reagents as detailed in the Sigma Chemical Company's Technical Bulletin No. 505 (1963) in order to diagnose liver necrosis due to copper poisoning. Results are expressed in Sigma-Frankel units of S.G.O.T. per ml blood plasma.

3.5 MISCELLANEOUS MATERIALS AND METHODS

3.5.1 Chemicals

Distilled water was used in the preparation of all samples, reagents, and standard solutions. "Analar" or "Spectro" grade chemicals were used for all reagents.

3.5.2 Washing

Sample cups, polypropylene bottles and glassware were soaked in 10% nitric acid overnight and rinsed successively in deionised and distilled water. They were then dried in a fan-oven prior to use.

3.5.3 Liquid Feed for Glasshouse Pots

As pots were kept for a considerable length of time there was a requirement for a liquid feed to maintain optimum herbage growth. The feed used was a high nitrogen solution made up as follows:

Stock solution

		g/l
(i)	$Ca(NO_3)_2.4H_2O$	59
(ii)	KNO_3	79
	$NH_4H_2PO_4$	12
	NH_4NO_3	11
	$MgSO_4\ 7.H_2O$	25
	H_3Bo_3	0.17

Equal volumes of stock solutions (i) and (ii) were diluted 1:100 with distilled water and mixed together. This was then fed to pots in 5 ml aliquots when required.

3.6 STATISTICAL ANALYSIS

All statistical analysis was carried out using statistical computer packages on a Comart computer. This involved "Minitab" (Ryan **et al**, 1981) for regressions and correlations, while analysis of variance was obtained using "EDEX" (A.F.R.C., Statistics Unit, Edinburgh. Unpublished).

CHAPTER 4. CHARACTERISATION OF THE CU FERTILISER

4.1 INTRODUCTION

The copper fertiliser is a largely unrefined and unprocessed by-product of the brass manufacturing industry from one foundry in the West Midlands of England. It is a sand based material containing 55% silica as SiO_2 (Birmingham University, Private Communication) which was stored in a clay lined pit. The material is unique in that manufacturing on the site was confined to brass production only and no other metallurgical processes were carried out. The raw material was ground to pass through a 2 mm sieve before use in agriculture.

Little was known of the materials, chemical composition or physical and chemical properties. It was, therefore, necessary to assess some of the basic properties in order to obtain a better understanding of how the Cu fertiliser itself might behave when applied to the soil.

The concentrations of Cu and Zn in the fertiliser were determined. In addition, as the Cu fertiliser is a by-product of a metallurgical process in which there may be contamination by other heavy metals, the concentrations of Fe, Pb, Cd, Mn, Cr and Ni were also determined.

The Cu fertiliser is used as a powder composed of varying particle sizes. The particle size distribution is likely to affect the rate of degradation of the Cu fertiliser in the soil. The relatively larger surface area per unit weight of smaller particles may allow that fraction to degrade quicker. Thus knowledge of the particle size distribution would be valuable in assessing the long term effectiveness of the Cu fertiliser. Analysis of the different size

fractions should give some idea of the way in which the Cu was distributed in the material, for example as a surface coating or free metal particle. In addition, further processing involving the grading of size fractions may allow simple manipulation of the composition and properties of the material.

If this material when used as a Cu fertiliser is to be a viable slow release source of Cu to the plant then it must be sparingly soluble in water. If it is very soluble then the Cu will be rapidly released into the soil solution and immobilised by strong adsorption processes in the soil thus making it unavailable to the plant and will have the same drawbacks as copper sulphate (section 2.4.1). Whereas if it is totally insoluble there will also be no Cu available for plant uptake and so the Cu fertiliser will be of no value. Thus the solubility of the Cu fertiliser will to a large extent determine its effectiveness as a slow release Cu source.

The pH of the soil solution with which the Cu fertiliser will be in contact can vary greatly. Thus the solubility and rate of release of Cu at different pH's was studied using a range of reagents.

4.2 MATERIALS AND METHODS

4.2.1 Analysis of the Cu Fertiliser for Total Trace Element Concentration

Subsamples from six individual 25 kg bags of Cu fertiliser were analysed. The concentration of total Cu, Zn, Fe, Pb, Cd, Mn, Cr and Ni in the Cu fertiliser were determined by the aqua regia technique as used for the total trace element concentration of soils (section

3.2.9). Each analysis was carried out in duplicate.

4.2.2 Particle Size Distribution and Trace Element Analysis

One hundred grams of the Cu fertiliser was brushed onto a 2 mm mesh sieve arranged over a nest of sieves as follows: 1000, 600, 500, 212, 106, 63 μm and receiver. The sieves were covered and shaken on a sieve shaking machine (Endecotts Ltd., London) for 15 minutes. The contents of each sieve were then weighed. The above process was carried out on duplicate samples from two separate bags of the Cu fertiliser and results are given as a percentage of the total.

Each individual fraction obtained was analysed for total trace element concentration.

4.2.3 Solubility and Rate of Release of Cu

The chemical reagents used were:

(i) H_2O (distilled).
(ii) 0.01 M calcium chloride.
(iii) 1 M ammonium acetate.
(iv) 0.05 M E.D.T.A. buffered to pHs 4, 5.5, 7 and 8 using M nitric acid or M ammonium solution.
(v) 0.5 M acetic acid (pH 4).
(vi) 6 M hydrochloric acid (pH 1).

Distilled water was used to look at the water solubility of the Cu fertiliser. However, as the soil solution with which the Cu fertiliser is in contact is a salt solution, 0.01 M calcium chloride and 1 M ammonium nitrate were used to study its solubility in a dilute

salt solution. These two salts were chosen as at these concentrations they are thought to be of the approximate salt concentration found in a normal temperate soil (Schofield and Taylor, 1955). The latter two salt solutions with acetic and hydrochloric acids were used to investigate the effects of decreasing pH on the solubility and rate of release of Cu from the Cu fertiliser. E.D.T.A. buffered to a range of pHs was also used to look at the effects of pH. In addition E.D.T.A. would allow the effects of a chelating agent on the Cu fertiliser to be studied. This may give an indication of the ionic state of the Cu in the Cu fertiliser as the cupric ion is more easily chelated than the cuprous ion (Cotton and Wilkinson, 1976).

The Cu fertiliser was mixed with each of the above reagents in a 1:10 (w/v) ratio and placed on an end over end shaker for shaking times of 1/2, 1, 2, 4, 8, 24 and 48 hours.

After shaking each solution was filtered (Whatman No. 40) and the amount of Cu released into solution from the Cu fertiliser was determined by atomic absorption spectroscopy (3.2). Each extraction was carried out on two separate occasions with 4 replicates in each batch.

4.3 RESULTS AND DISCUSSION

4.3.1 Total Trace Element Concentrations

The total Cu, Zn, Fe, Pb, Cd, Mn, Cr and Ni concentrations in the Cu fertiliser are given in Table 4.1. The most abundant elements are Cu (2%), Zn (2%) and Fe (3%). It also contains 0.3% Pb and smaller amounts of Cd, Mn, Cr and Ni. The concentration of each of these

elements in the Cu fertiliser was relatively uniform among the bags sampled. The Cu concentration in this material is comparable to that found in other "insoluble" Cu fertilisers such as Excello and ground Cu slag which contain around 2.5% Cu (section 2.4.1).

Table 4.1

Trace element composition of the Cu fertiliser

Element	Mean total concentration (mg/kg)	% of total by weight
Cu	22150	2.2
Zn	21460	2.1
Fe	30810	3.1
Pb	3080	0.31
Cd	22	0.002
Mn	218	0.02
Cr	173	0.02
Ni	330	0.03

At present there are no regulations governing either the maximum allowable concentration of any metal in inorganic fertilisers or the amount of a metal that may be applied to the soil in such a form. Guidance could, however, be taken from E.E.C. limits for the maximum allowable concentrations of metals that may be applied to agricultural land in the form of sewage sludge (E.E.C., 1986), which are the nearest comparable recommendations. Table 4.2 shows that at a 370 kg/ha Cu fertiliser application rate, as used in later experimental trials, the concentrations of Cu, Zn, Pb, Cd and Ni applied to the soil are well below the maximum recommended applications of metals in

sewage sludge.

Table 4.2

Comparison of the amounts of each metal applied to land in the Cu fertiliser with E.E.C. limits for sewage sludge

Element	Cu fertiliser⊙ (t/ha/yr)	E.E.C. limit* (t/ha/yr)
Cu	2.7	12
Zn	2.6	30
Pb	0.4	15
Cd	0.002	0.15
Ni	0.04	3

⊙Annual metal loading from application of 370 kg/ha Cu fertiliser assuming a reapplicaton rate of every three years.

*Limit values for the amount of heavy metals which may be added annually to agricultural land in the form of sewage sludge based on a 10 year average (E.E.C., 1986).

4.3.2 Particle Size Distribution and Analysis of Size Fractions

The mean particle size distribution of the Cu fertiliser is given in Table 4.3. The results show that the Cu fertiliser consists of material less than 200 μm in diameter with over 90% of it being less than 500 μm in diameter. Apart from 15.7% which is less than 63 μm in diameter (silt sized) the Cu fertiliser is therefore composed of sand sized particles. The trace element composition of each fraction is given in Table 4.4, it shows that Cu is present in all the fractions and that the relative amounts of the trace elements are similar in each fraction. It will not therefore be possible to remove any of the heavy metals present by simple manipulation of the particle size

distribution.

Table 4.3

Mean possible size distribution

Fraction size (μm)	% of total by weight	Cummulative %
>2000	0	0
1001-2000	1.2	1.2
601-1000	3.2	4.4
501-600	1.8	6.2
213-500	15.3	21.5
107-212	33.8	55.3
64-106	29.0	84.3
<63	15.7	100.0

The presence of Zn at similar concentrations to that of Cu throughout the Cu fertiliser, means that this fertiliser may also be suitable for use as a Zn fertiliser. This possibility may merit further investigation. The presence of Pb, Cd, Mn, Cr and Ni in the fertiliser may give some concern and it was therefore decided to monitor their uptake and concentrations in herbage during this work to ensure that potentially zootoxic concentrations do not result.

4.4.3 Solubility and Rate of Release of Cu

In all reagents the release of Cu showed a similar curve such that a near maximum was reached within 24 hours. Results for each reagent are shown graphically in Figures 4.1 to 4.6. Percentage solubility at 24 hours is used as a comparative index of the solubility and rate of

Table 4.4

Trace element concentration of the particle size fractions

Element	Fraction size (μm)						
	<63	63-106	107-212	213-500	501-600	601-1000	1001-2000
Cu (g/kg)	44.1	28.1	17.3	19.2	27.0	25.8	28.1
Zn (g/kg)	30.1	26.2	17.2	18.2	24.6	24.8	27.3
Fe (g/kg)	48.5	32.5	21.8	23.1	28.3	32.3	38.1
Pb (mg/kg)	4740	3780	2370	2460	3740	3540	4200
Cd (mg/kg)	33	28	18	16	16	22	24
Cr (mg/kg)	210	180	180	150	165	210	255
Ni (mg/kg)	510	360	255	300	300	480	720

Figure 4.1. Concentration of Cu released into water from the Cu fertiliser verus time.

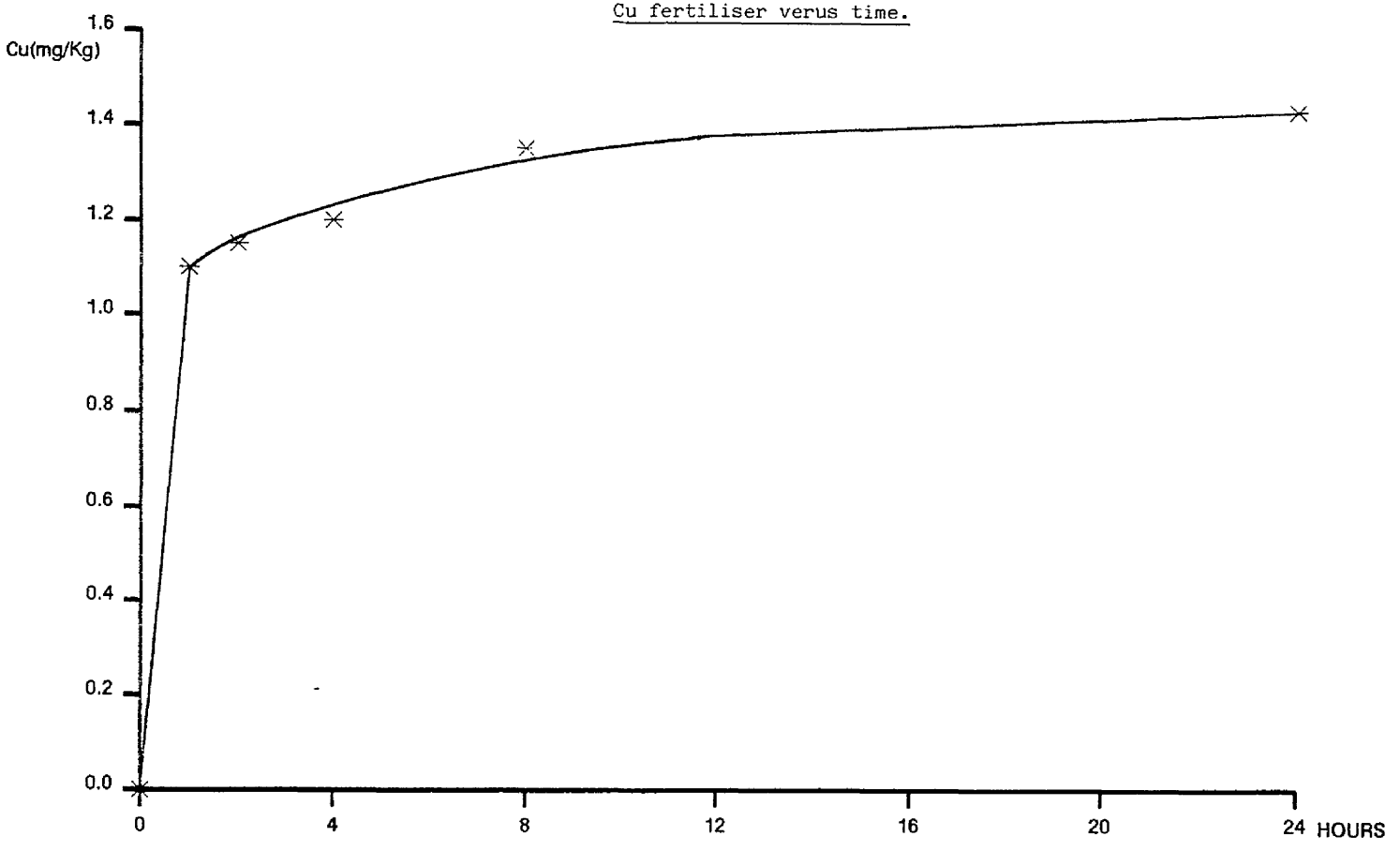


Figure 4.2. Concentration of Cu released into Calcium Chloride from the Cu fertiliser versus time.

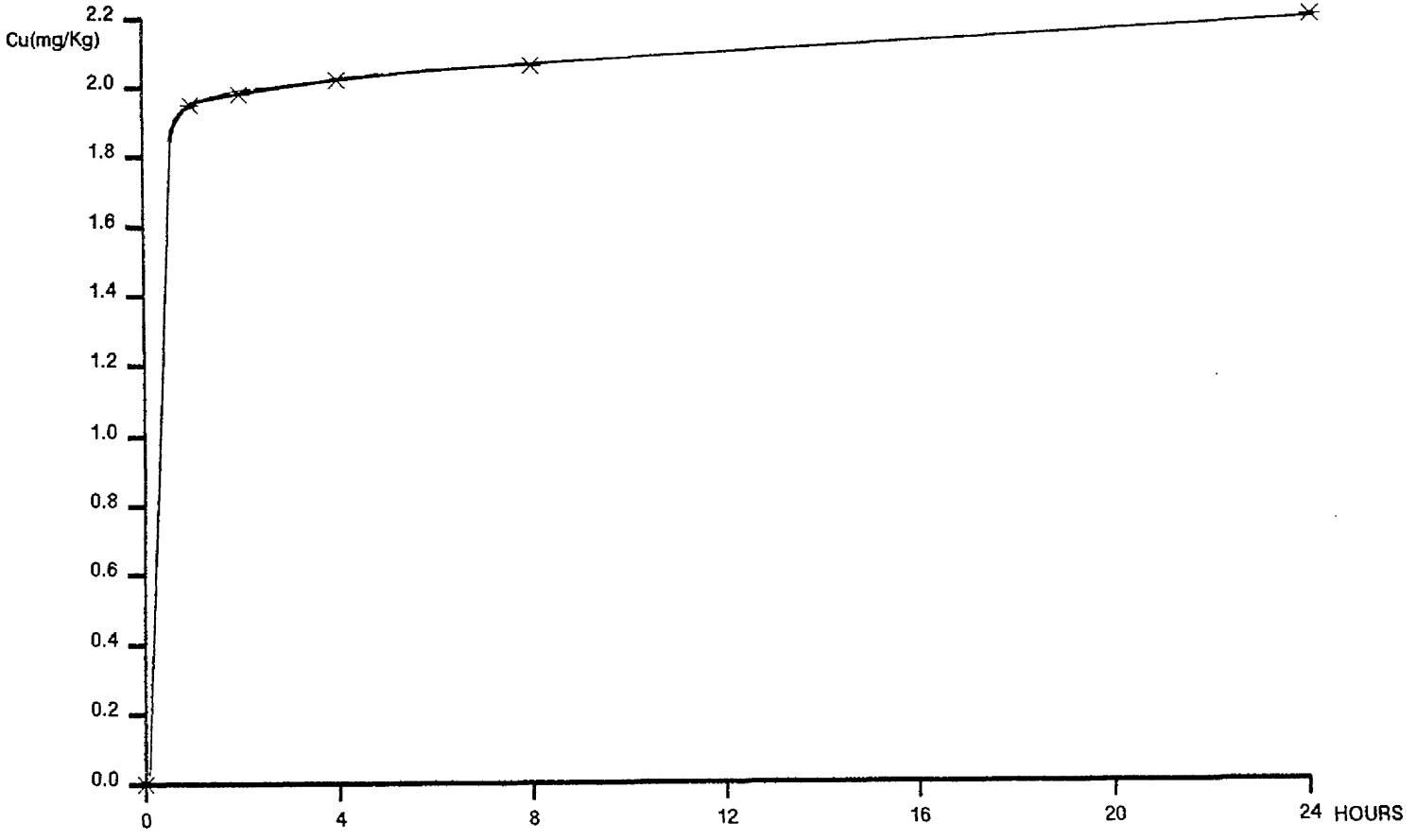


Figure 4.3. Concentration of Cu released into Ammonium Acetate from the Cu fertiliser versus time.

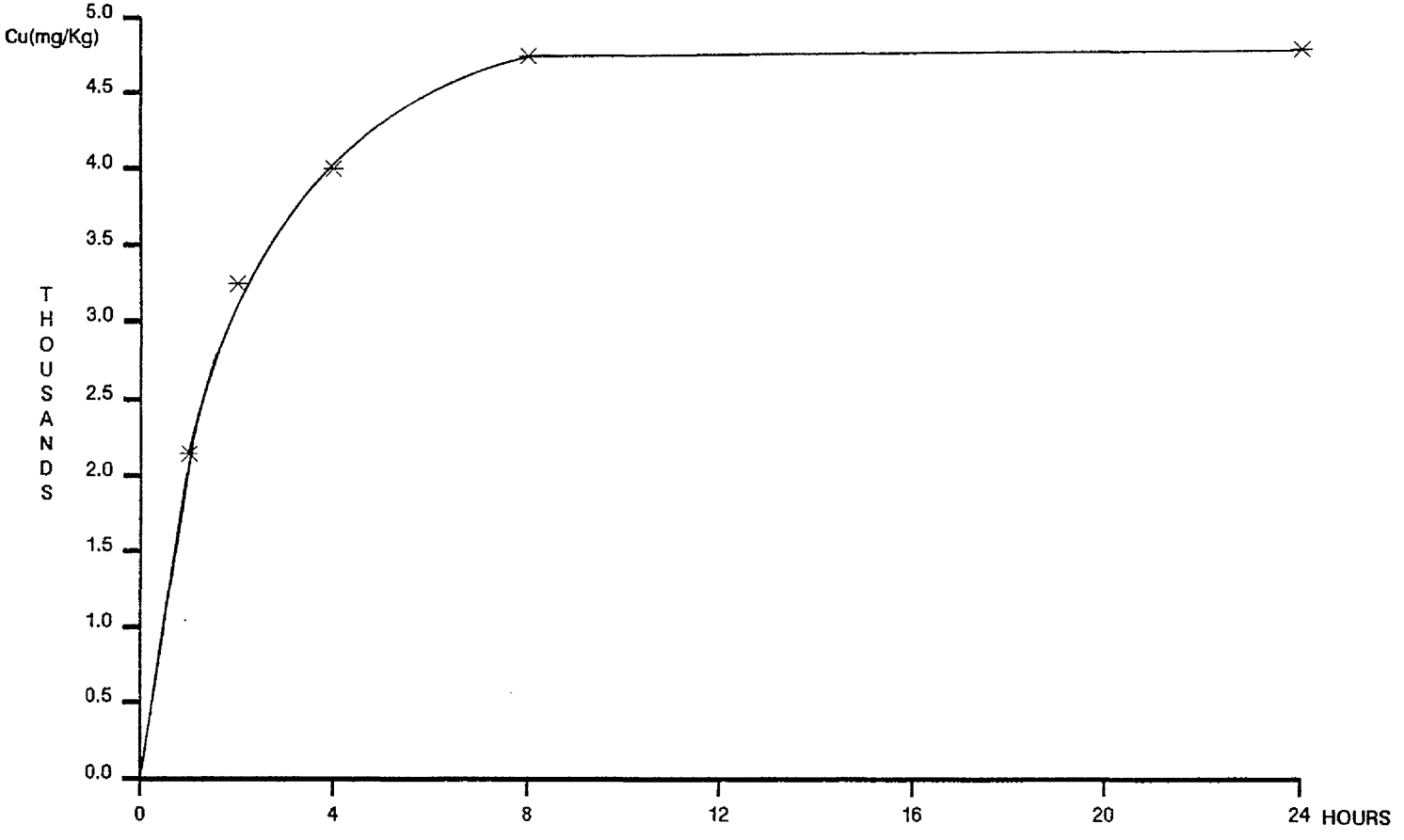


Figure 4.4. Concentration of Cu released into E.D.T.A. from the Cu fertiliser at pHs 4.0,5.5, 7.0 and 8.0 versus time.

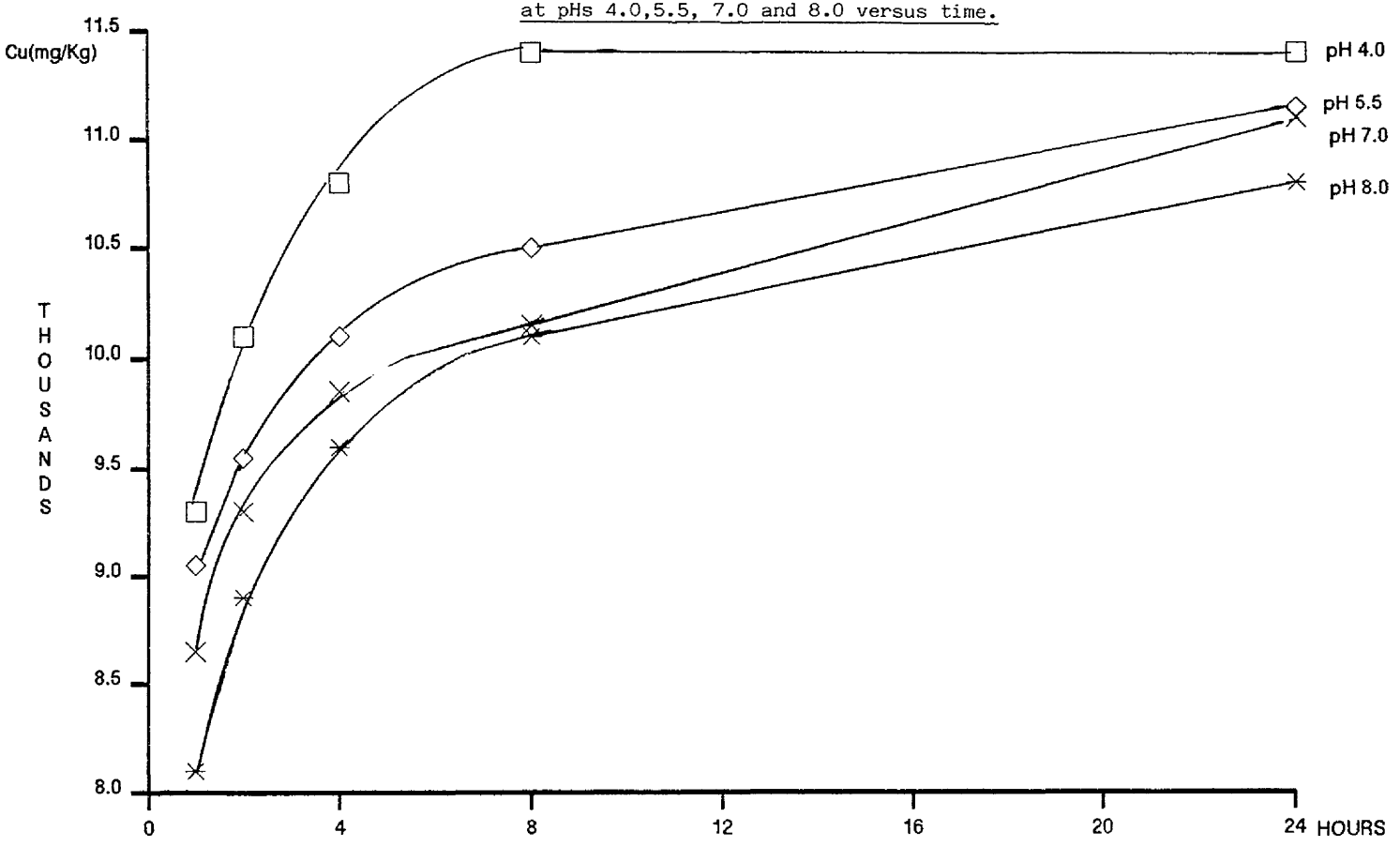


Figure 4.5. Concentration of Cu released into Acetic Acid from the Cu fertiliser versus time.

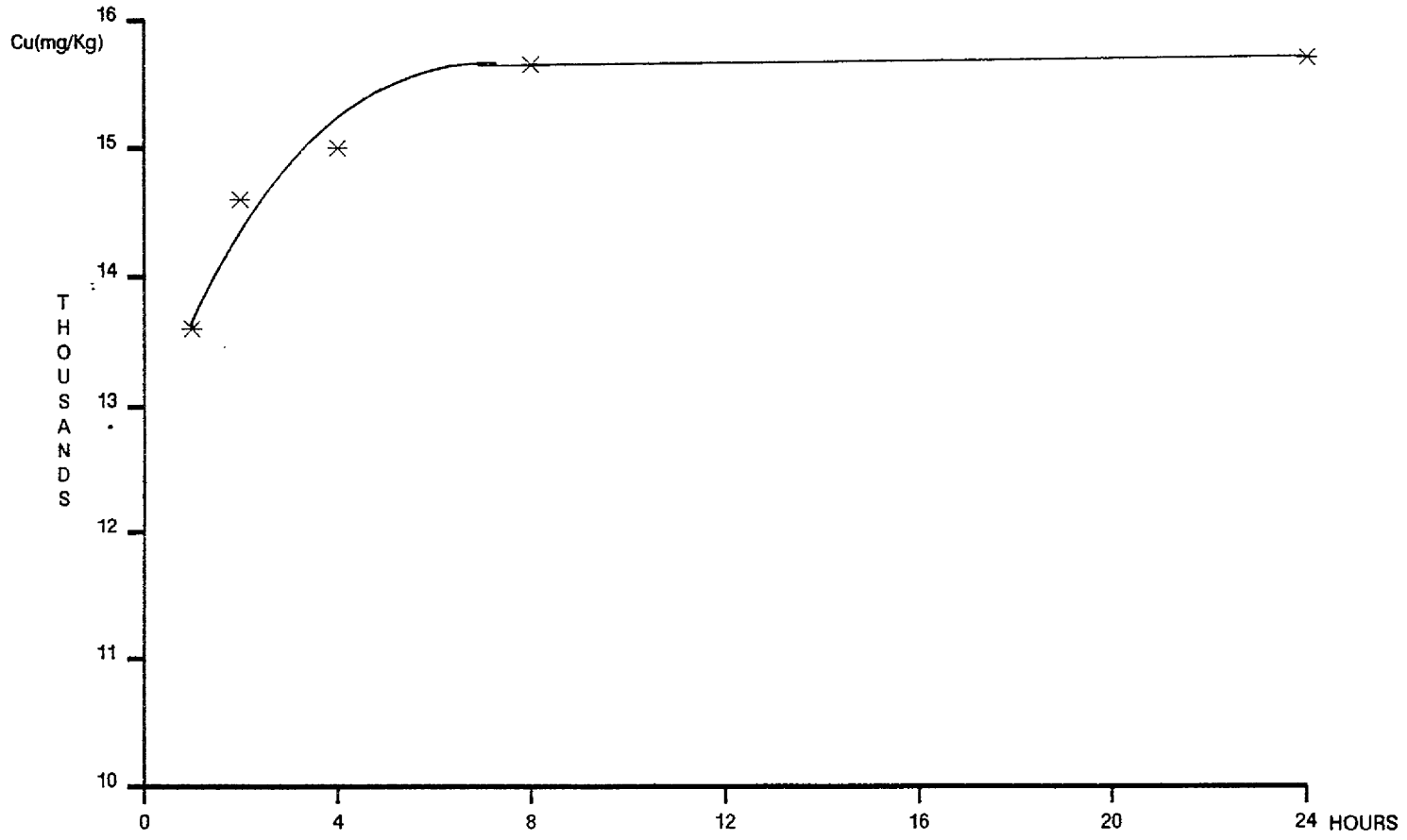
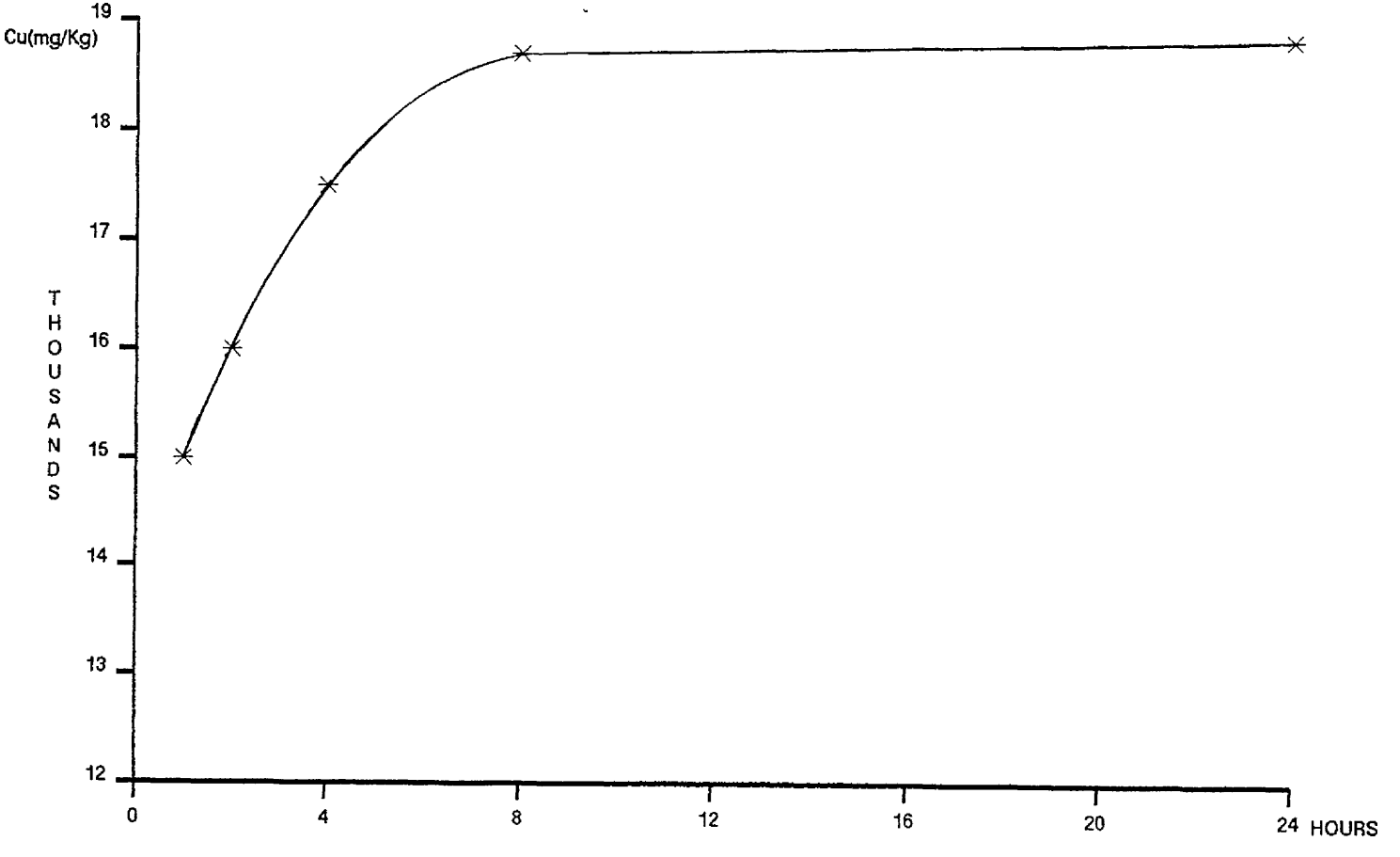


Figure 4.6. Concentration of Cu released into Hydrochloric Acid from the Cu fertiliser verus time.



release of Cu in each reagent (Table 4.5). The solubility and rate of release of Cu were lowest within water and calcium chloride in which the Cu fertiliser was only sparingly soluble. However, as the aqueous solubility of most Cu compounds is very low (Cotton and Wilkinson, 1976) this was not unexpected. The slow release of Cu in water and calcium chloride together with the wide range of particle sizes suggests that in theory the Cu fertiliser should be able to act as a slow release form of Cu to the plant.

Table 4.5

Percentage of Total Cu Released into Solution after 24 hours

Reagent	% Cu released at 24 hours
Water	0.01
Calcium chloride	0.01
Ammonium acetate	22.5
E.D.T.A. pH 4	53.0
E.D.T.A. pH 5.5	51.0
E.D.T.A. pH 7	50.0
E.D.T.A. pH 8	47.0
Acetic acid	70.0
Hydrochloric acid	85.0

Although the rate of release of Cu from the different Cu fertiliser size fractions was not compared it is assumed that the Cu is in the same form in each fraction and as such will release Cu at the same rate. However, the smaller fractions which have the largest surface area should release a greater proportion of their Cu first giving an

immediate source of Cu with the larger particles supplying Cu over a longer time span.

The rate of release of Cu from the Cu fertiliser increased as the solution became more acidic (Figures 4.3 - 4.6). Thus the release of Cu is dependent on pH. This implies that under the acidic soil conditions of Great Britain it is likely that there will be a faster rate of release of Cu from the Cu fertiliser than suggested by its water solubility. Thus Cu may be released more rapidly in acidic soils, however this does not necessarily mean that the availability of that Cu will increase as the extra Cu may be immobilised by the soil. Therefore under acidic conditions more frequent applications may be required if the availability of the Cu to the plant is to be maintained.

The rate of release of Cu was not entirely governed by pH. The chelating agent E.D.T.A. removed 47% of the Cu in the fertiliser at pH 8 and this only increased to 53% at pH 4. Thus Cu can be removed from the Cu fertiliser by chelation. This suggests that the Cu is present in the cupric Cu^{2+} form rather than the cuprous Cu^{+}, as the former is more readily chelated (Cotton and Wilkinson, 1976). The presence of Cu throughout the particle size range of the Cu fertiliser together with the fact that the Cu can be chelated implies that the Cu is present as a surface coat as if it was distributed throughout the particles the Cu would not be available for chelation. This together with the knowledge that the relative amounts of each trace element in the fractions are similar, suggests that the unground material from which the Cu fertiliser is derived is itself a very uniform material. Thus when it is ground to produce the Cu fertiliser, this process

shatters the material to produce the range of particle sizes observed with the total amount of trace element present varying with the amount of sand in a fraction. Due to the high temperatures used in brass manufacturing it is likely that the Cu is present as CuO or CuS or as a mixture of these along with some pure metal, however from the results obtained here it is still impossible to give the actual form in which the Cu is present.

One problem that this work has thrown up is regarding the suitability of using E.D.T.A. to assess plant available Cu concentrations in the soil when Cu fertiliser has been applied. In Great Britain the plant available Cu is predominantly organically bound and so the chelating agent E.D.T.A. has been widely used for its estimation in the soil (sections 2.1.3 and 2.1.4). However, these results show that the Cu fertiliser is very soluble in E.D.T.A. which will extract 50% of the Cu in the Cu fertiliser within 8 hours (Figure 4.4). Thus when the Cu fertiliser is present in the soil E.D.T.A. extraction may not be a suitable method for assessing plant available Cu, as large amounts of Cu will be solubilised from the Cu fertiliser not all of which may actually be plant available. The fact that some of the Cu in the fertiliser can be removed by chelation implies that some of it is plant available.

4.4 SUMMARY

4.4.1 The Cu fertiliser is a sand based material containing approximately 2% Cu, 2% Zn and 3% Fe. It also contains much smaller amounts of Pb, Cd, Mn, Cr and Ni.

4.4.2 The Cu fertiliser contains a wide range of different particle sizes. Copper is present in all the size fractions. The relative amounts of each trace element are consistent throughout each size fraction.

4.4.3 The Cu fertiliser is sparingly soluble in water. Its solubility increases as the solution becomes more acidic.

4.4.4 This fertiliser contains enough Cu to be an effective Cu fertiliser. Its particle size distribution and solubility suggest that when applied to the soil the Cu fertiliser should be able to act as a slow release form of Cu to plants.

4.4.5 The presence of 0.3% Pb and the smaller amounts of the heavy metals Cd, Mn, Cr and Ni in the Cu fertiliser means that in addition to Cu and Zn it will be necessary to monitor these elements in further work to prevent any potentially toxic effects.

4.4.6 E.D.T.A. soil extractions are not suitable for assessing plant available Cu when the Cu fertiliser is present in the soil.

CHAPTER 5. ANIMAL RESPONSE TRIAL, GARMORE FARM

5.1 INTRODUCTION

Copper deficiency in sheep continues to cause problems in Great Britain, despite the fact that its existence was first recognised over 50 years ago. The following trial was conducted to evaluate the effectiveness of the new slow release trace element fertiliser in the prevention of Cu deficiency in sheep. The trial involved the treatment of a 4 ha field, in an area of known Cu deficiency, with the Cu fertiliser. Thirty ewes and their spring-born lambs were then allowed to graze the treated sward. Blood plasma Cu concentrations were monitored to determine if treatment of the sward was effective in preventing Cu deficiency in the livestock. In order to assess the residual value of the Cu fertiliser the animal response trial was continued for a period of 31 months from November 1985 to June 1988.

5.2 OBJECTIVE

The objective of this trial was to evaluate the effectiveness of a single application of the Cu fertiliser to pasture for the prevention of Cu deficiency in sheep and in particular swayback in their lambs. The field trial consisted of two main components:

(a) Ewe response trial

To compare the performance of ewes grazing pasture naturally low in available Cu with that of ewes grazing comparable pasture treated with Cu fertiliser.

(b) Lamb response trial

The spring-born lambs from the ewes in the ewe response trial were used to carry out a similar response trial on the same site over the spring and summer months.

These trials of course cannot be totally separated as the effects in (a) have implications for (b). Thus the trial is one entity which has been split into two for convenience of presentation.

5.3 EWE RESPONSE TRIAL, EXPERIMENTAL METHODS

5.3.1 Site Description

The field site was situated at Garmore Farm, half way up the Campsie hills, approximately 2 miles from Milton of Campsie (O.S. map reference NS643786). The farm has a history of Cu deficiency problems due to a combination of low soil Cu concentrations and relatively high herbage Mo and S concentrations. Swayback in lambs from untreated ewes and growth responses in cattle to Cu supplementaton have been noted in the past (MacPherson, personal communication).

The field was 8 ha (20 acres) in area, at an elevation of 170-200 m and represented the highest area of improved pasture on the farm (Plate 5.1). The soil was a free draining brown forest soil of the Darleith series. The area is subject to an average annual rainfall of 1400 mm. The field was fenced down the middle to create two 4 ha paddocks for the trial (Figure 5.1).

To assess the Cu status and uniformity of fertility across the field site prior to application of the Cu fertiliser, it was divided into 8 sections and a composite soil sample 0-10 cm depth obtained from each section.

Figure 5.1

Plan of Garmore field site

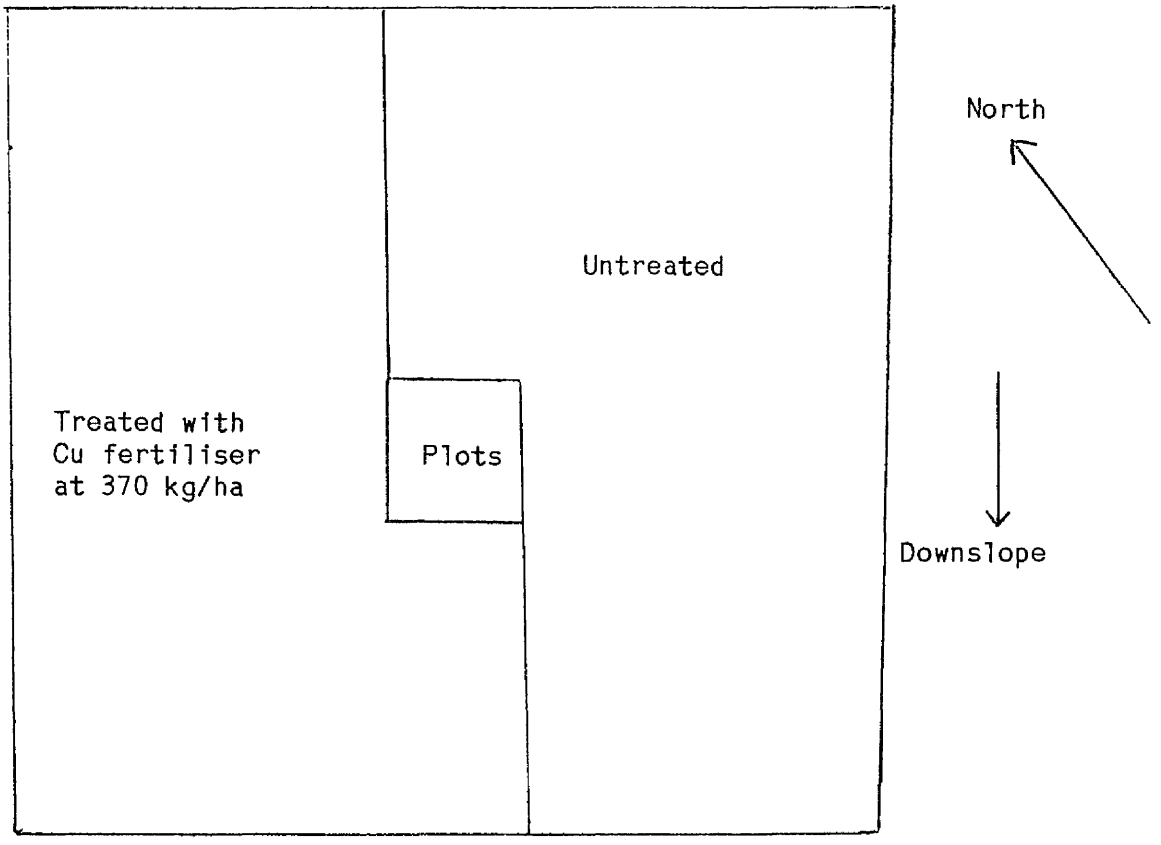


Plate 5.1

Garmore animal response trial field site

These were analysed for E.D.T.A. extractable Cu, pH, % loss on ignition, lime requirement, available potassium and available phosphorus using the procedures given in 3.2. The two halves of the field were found to be of reasonably uniform fertility; the range of results found together with the mean value are given in Table 5.1. These show the soil to have a moderate level of P, moderately high organic matter content and high K. The Cu status of the soil is classified as being low (M.I.S.R., 1985).

Table 5.1

Response trial soil analysis results

Analysis	Range	Mean
Loss on ignition (%)	15.8 - 16.2	16.1
Lime requirement to pH 5.8 (t/ha)	6.9 - 7.1	7.0
E.D.T.A. extractable Cu (mg/kg)	3.4 - 3.9	3.73
Available K (mg/l)	253 - 274	267
Available P (mg/l)	26 - 31	28
pH	5.0 - 5.2	5.13

5.3.2 Fertiliser Application

The paddock furthest from the farm buildings was selected for treatment with the Cu fertiliser. This ensured that no control animal would cross the treated pasture on the way to or from the farm buildings, or handling pens.

The Cu fertiliser was applied as a single broadcast application to the existing sward, at a rate of 370 kg/ha, equivalent to 7.5 kg Cu/ha, on the 19 November 1985. A Vicon Varispreader was used to apply the fertiliser. This is a swinging outlet machine which uses a pendulum action to spread the Cu fertiliser. As such it is virtually unaffected in its spread pattern by slope, unlike the more popular rotating disc fertiliser spreader.

Plate 5.2

Application of the Cu fertiliser to the field site

A light breeze during calibration of the Vicon Varispreader revealed that the Cu fertiliser was prone to drifting. However, the breeze dropped prior to the field application and so caused no drifting problem. Using this machine a good even spread of the fertiliser was obtained, which left an even grey coating on the treated pasture (Plate 5.2).

5.3.3 Ewe Selection

From the farm flock of over 600 Scottish Blackface ewes, 60 were selected to be representative of the age range of the flock. They were then randomly divided into two representative groups of 30 and tagged for identification. A period of rain 10 days after the Cu fertiliser application, which was expected to wash the fertiliser off the herbage, allowed the introduction of one group onto the treated pasture while the other group was concurrently released into the adjacent untreated paddock. This period between Cu fertiliser application and the introduction of the animals was necessary as application of the Cu fertiliser resulted in a grey coating of the herbage, ingestion of which might have led to unnecessary toxicity problems for the livestock.

Any ewes that died or were sold as casts, were replaced in October 1986 and 1987.

5.3.4 Tupping

Two Scottish Blackface Tupps were used for tupping. One was released into each paddock and they were swapped over after 17 days. Tupping took place in November 1985, 1986 and 1987.

5.3.5 Blood Sampling and Analysis

At intervals of approximately six weeks throughout the winter and four weeks over the spring and summer, blood samples were taken from all the animals on the trial. The blood was analysed for the range of parameters given below using the procedures in section 3.4:

(i) Plasma Cu - to assess the Cu status of the ewes.

(ii) Haemoglobin (Hb) - anaemia (low Hb concentration) is a symptom of Cu deficiency in sheep (section 2.2.3) and so was also monitored.

(iii) Plasma Mg - plasma Mg was analysed to monitor potential hypomagnesaemia problems.

(iv) Gluthathione peroxidase (GSH-Px) - concurrent deficiencies of Cu and Se are common in Scotland, therefore GSH-Px as an indicator of Se status was monitored to check that any deficiency symptoms were not due to Se deficiency.

Of the heavy metals present in the Cu fertiliser, Pb is present in the largest concentration. Therefore on each sampling occasion six additional blood samples were taken at random from each group of ewes, and analysed for whole blood Pb concentration. This was to determine whether lead status was elevated due to the Pb content of the Cu fertiliser.

Yearly blood analysis was also carried out for vitamin B_{12} and plasma Ca concentrations. Plasma Ca concentrations were monitored to ensure that there were no hypocalcaemia problems, especially at lambing time

as this is a potentially fatal condition for the ewe and can effect the viability of its lambs. Cobalt and Cu deficiencies often occur together (Voss and MacPherson, 1977) and consequently the Co status of the ewes was determined by vitamin B_{12} assay.

5.3.6 Ewe Treatment

After analysis of the first batch of blood samples taken on 13 November 1985, 45% of all the ewes showed blood plasma Cu concentrations below the deficiency threshold of 9.4 μmol/l (Grace, 1983). Therefore on 8 January 1986, 15 ewes from each side of the response trial site were treated with 5 g Cu needles. This both protected some of the ewes from Cu deficiency and also allowed a comparison of the Cu fertiliser with a method presently used to prevent Cu deficiency (section 2.4.2).

Thus four different treatment groups were created within the response trial:

Group 1 Control, grazing untreated pasture (n=15).

Group 2 Grazing untreated pasture and dosed with 5 g Cu needles (n=15).

Group 3 Grazing Cu treated pasture (n=15).

Group 4 Grazing Cu treated pasture and dosed with 5 g Cu needles (n=15).

For simplicity these will be referred to as the control, Cu needles, Cu fertiliser and Cu needles + Cu fertiliser groups, respectively, in subsequent sections.

As a result of conclusions derived from the first year of the trial (5.5.1), in subsequent years only 15 ewes from the untreated paddock were dosed with 5 g Cu needles on 3 March 1987 and 27 October 1987 respectively.

Thus in years 1986-88 only three treatment groups were used in the trials:

Group 1 Control grazing untreated pasture (n=15).

Group 2 Grazing untreated pasture and dosed with 5 g Cu needles (n=15).

Group 3 Grazing Cu treated pasture (n=30).

For simplicity these will be referred to as the control, Cu needles and Cu fertiliser groups, respectively, in subsequent sections.

5.3.7 Herbage Sampling and Analysis

Over the spring and summer months herbage was sampled at regular intervals from each paddock. Each sample consisted of 20 subsamples taken from randomly distributed points within each paddock. A subsample consisted of all the herbage in an area of approximately 0.5 m square cut at 2 cm from the soil surface. Great care was used to prevent soil contamination and steel sheep shears were used to prevent any trace element contamination from the cutting tool.

Samples were analysed for total Cu (section 3.4) to determine the amount of Cu available to the sheep.

5.3.8 Soil Sampling and Analysis

The behaviour of the Cu fertiliser in the soil was monitored by obtaining soil samples at 0-10 cm depth from each paddock at yearly intervals after application of the fertiliser. These samples were analysed for total Cu concentration.

5.3.9 Post-Mortem

Ewes that died during the trial were sent to the Scottish Veterinary Investigations Unit Laboratory at Auchincruive, where a post-mortem was carried out to establish the cause of death. Liver and kidney samples were taken from each dead animal and analysed for total Cu concentration to determine the Cu status of the animal; samples were also analysed for Zn, Cd and Pb concentratons to determine if there was any possible toxic accumulation of these metals, which may have been caused by their presence in the Cu fertiliser.

5.4 LAMB RESPONSE TRIAL, EXPERIMENTAL METHODS

The trial was conducted on the same site as described in 5.3.1.

5.4.1 Ultrasonic Scanning of Pregnant Ewes

In March of each year ultrasonic scanning of the 60 ewes was carried out to give an indication of the number of lambs expected in the spring.

5.4.2 Lambing

Lambing occurred in the April and May of each year of the trial. After birth each lamb was tagged for identification and correlation with dam treatment. The lambs were monitored by the farmer for any signs of swayback and mortalities were collected for post-mortem.

5.4.3 Treatments

After lambing, in 1986 and 1987, a response trial similar to that using the ewes was conducted. Half of the lambs in the untreated paddock were dosed orally with 1.4 g Cu needles in May, which is the recommended level for this form of treatment. Thus the trial consisted of three treatment groups:

Group A Control, no treatment.
Group B Dosed with 1.4 g Cu needles.
} Born to ewes grazing untreated pasture.
Group C Born to ewes on Cu fertiliser treated pasture.

5.4.4 Blood Sampling and Analysis

Blood samples were taken from each lamb every four weeks from May through to September at which time the lambs were sold. The blood was analysed for plasma Cu and Mg, Hb and GSH-Px; six samples chosen at random from each treatment group were analysed for whole blood Pb (5.3.5).

5.4.5 Liveweights

On each blood sampling date the lambs were weighed and the rate of liveweight gain calculated.

5.4.6 Post-Mortem

Any lambs that died, and which could be recovered, during the trial were sent to the Scottish Veterinary Investigations Unit Laboratory at Auchincruive where a post-mortem was carried out. Histological examinations were undertaken to confirm or otherwise suspected cases of swayback. Liver and kidney samples were analysed as for the ewes (5.3.9).

5.5 RESULTS

5.5.1 Ewe Response Trial, November 1985 to September 1986

The mean ewe plasma Cu concentrations for each of the four treatment groups are given in Table 5.2 and presented graphically in Figure 5.2. All four groups of ewes had similar mean blood plasma Cu concentrations at the start of the trial on 13 November 1985. On this date 45% of the ewes had plasma Cu concentrations below the deficiency threshold of 9.4 μmol/l and these animals were distributed evenly throughout the four treatment groups (Table 5.3).

The control group demonstrated the normal decline in plasma Cu concentrations with time over the winter (section 2.3.4). Over half of the ewes in this group had plasma Cu concentrations below the deficiency threshold throughout late pregnancy, lambing and early lactation (Table 5.3). Their plasma Cu concentrations then rose gradually after lambing until the end of the first year of the trial on 9 September 1986. However, six ewes remained Cu-deficient throughout this latter period.

Table 5.2

Mean ewe plasma Cu concentrations, November 1985 to September 1986

Date	Treatment group:	Mean ewe plasma Cu (μmol/l) Control (n=15)	Cu 0 needles (n=15)	Cu fertiliser (n=15)	Cu fertiliser + Cu needles 0 (n=15)	S.E.D.
13/11/85		10.37^{a}	10.37^{a}	10.66^{a}	12.08^{a}	2.31
08/01/86		8.14^{a}	9.89^{a}	16.10^{b}	16.62^{b}	1.52
19/02/86		7.36^{a}	13.04^{b}	16.24^{b}	15.21^{b}	1.67
25/03/86		*	*	*	*	*
15/05/86		6.67^{a}	9.97^{b}	18.88^{c}	15.76^{c}	1.63
12/06/86		6.24^{a}	7.67^{a}	19.15^{b}	15.92^{c}	1.36
08/07/86		9.64^{a}	10.84^{a}	17.71^{b}	16.55^{b}	1.62
06/08/86		10.94^{a}	12.85^{a}	18.80^{b}	17.47^{b}	1.59
09/09/86		10.85^{a}	12.65^{a}	18.54^{b}	17.84^{b}	1.48

0 Ewes dosed with 5 g Cu needles on 08/01/86.
*Samples were contaminated and could not be analysed.

Means with different superscripts in any row are significantly different at the $p<0.001$ level (analysis of variance, EDEX, A.F.R.C. Statistics Unit, Edinburgh).

Figure 5.2. Mean ewe plasma Cu concentration, November 1985 to September 1986 after a single Cu fertiliser application on 19/11/85.

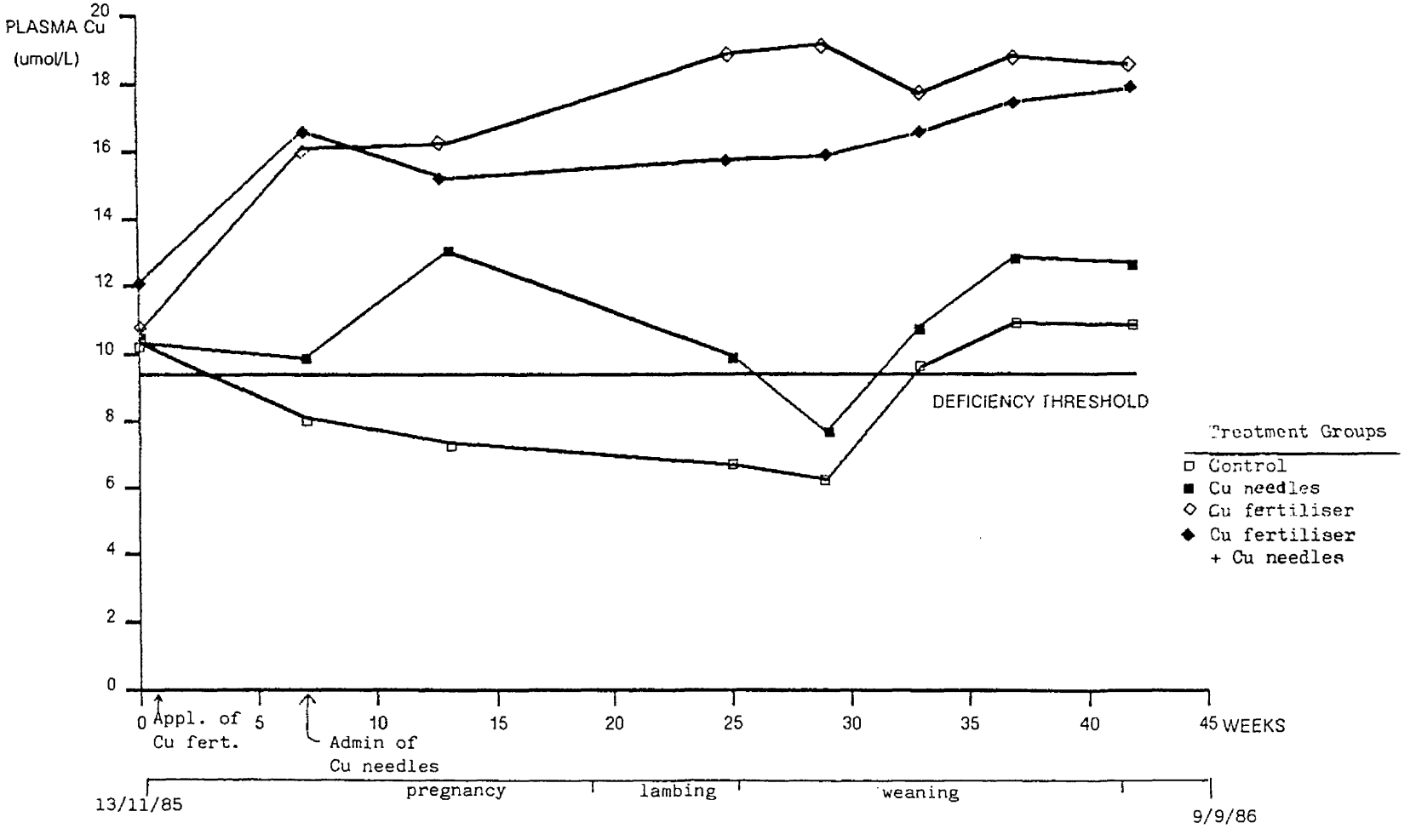


Table 5.3

Number of Cu-deficient ewes in each treatment group,
November 1985 to September 1986

Date	Treatment group:	Number of Cu-deficient ewes (plasma Cu <9.4 μmol/l)			
		Control	Cu needles	Cu fertiliser	Cu fertiliser + Cu needles
13/11/85		7	6	6	8
08/01/86		8	7	0	0
19/02/86		9	1	0	0
15/05/86		8	4	0	0
12/06/86		10	6	0	0
08/07/86		6	4	0	0
06/08/86		6	3	0	0
09/09/86		6	3	0	0

The treatment of ewes grazing untreated pasture with 5 g Cu needles on 8 January 1986 gave an increase in their plasma Cu concentrations within 4 weeks. This treatment gave 10 to 12 weeks protection from the possibility of Cu deficiency over the critical late pregnancy and lambing period when Cu is in most demand by the ewe and its foetus. However, by the end of the trial period on 9 September 1986 the mean plasma Cu concentration was not significantly different from that of the control group.

Within six weeks of the ewes first being allowed to graze the treated sward their plasma Cu concentrations were significantly ($p<0.001$) increased (Table 5.2) and by 8 January 1986 all ewes in this paddock had concentrations above the deficiency threshold (Table 5.3). Mean plasma Cu concentrations of the Cu fertiliser group were maintained above 15 µmol/l throughout the 43 weeks of the trial and were significantly higher ($p<0.001$) than those of the control ewes and the Cu needles group. The maximum mean concentration attained was 19.15 µmol/l which was well below the accepted toxicity threshold of 30 µmol/l (Grace, 1983). Treatment of 15 of the ewes on the Cu treated pasture with 5 g Cu needles had no further effects on plasma Cu concentrations (Figure 5.2) when compared to the Cu fertiliser group.

There was no significant differences in plasma Mg, Hb, whole blood GSH-Px or Pb concentrations among the four treatment groups. Mean results for all 60 ewes on each sampling date are therefore given in Table 5.4. These parameters were all well within the normal ranges (Appendix 2). Similarly the periodic analysis of blood for both Co as vitamin B_{12} and plasma Ca showed no significant differences between the groups and all results were within the normal ranges (Appendix 2).

Therefore trial means only are given in Table 5.5.

Table 5.4

Mean ewe plasma Ma, Hb, whole blood, GSH-Px and Pb concentrations, November 1985 to September 1986

Date	Plasma Mg (mg/100 ml)	Hb (g/100 ml)	GSH-Px (U/ml)	Pb (μg/ml)
13/11/85	2.13	12.44	93.83	-
08/01/86	2.02	13.46	104.11	-
19/02/86	1.99	12.21	85.00	0.07
25/03/86	-	10.92	77.06	0.07
15/05/86	1.90	8.23	142.06	0.07
12/06/86	1.78	10.53	95.60	0.08
08/07/86	2.01	11.44	61.65	0.07
06/08/86	2.00	12.39	70.88	0.08
09/09/86	1.95	12.40	45.66	-
S.E.D.	0.16	0.43	12.10	-

Table 5.5

Mean ewe, vitamin $_{12}$ and plasma Ca concentrations

Date	Vitamin B_{12} (ng/l)	Plasma Ca (mg/100 ml)
8/1/86	1894	-
15/5/86	-	9.74

5.5.2 Lamb Response Trial May to August 1986

Ultrasonic scanning of the pregnant ewes indicated that 45 lambs were expected from the ewes in the untreated paddock and 47 from those in

the Cu-treated paddock. However, bad weather, including drifting snow and very low temperatures, resulted in the loss of many lambs due to hypothermia and starvation. Of the 49 lambs which survived the bad weather, 23 came from the untreated paddock and 26 from the Cu-treated paddock. Such losses were a common feature on farms in Scotland in 1986.

The bad weather also hindered the finding and collection of dead lambs for post-mortem. However, two dead lambs were obtained from the Cu-treated paddock. Results of post-mortem showed one to be stillborn and that the other died of starvation. Analysis of liver revealed Cu, Zn and Pb concentrations of 118, 86 and 0.9 mg/kg respectively in one lamb and 136, 78 and 1.1 mg/kg in the other, all of which were within the normal range of concentrations found in lambs. No cases of swayback were found in 1986.

Half the lambs born to ewes from the untreated paddock were dosed with 1.4 g Cu needles on 15 May 1986. Blood samples were obtained on 15 May, 11 June, 8 July and 6 August and the mean lamb plasma Cu concentrations for each of the three groups on these dates are given in Table 5.6 and shown graphically in Figure 5.3. Soon after birth lambs born to Cu fertiliser ewes had significantly higher ($p<0.001$) mean plasma Cu concentrations (14.4 μmol/l) than those born to ewes in the untreated paddock (10.1 μmol/l) whether or not the ewe was treated with Cu needles. Table 5.6 shows that there were 4, 5 and 1 Cu-deficient lambs in the control, Cu needle and Cu fertiliser groups, respectively, on 15 May. By 11 June only the control group had Cu-deficient lambs, treatment of half the lambs in the untreated paddock with Cu needles having effectively increased plasma Cu concentrations

Table 5.6

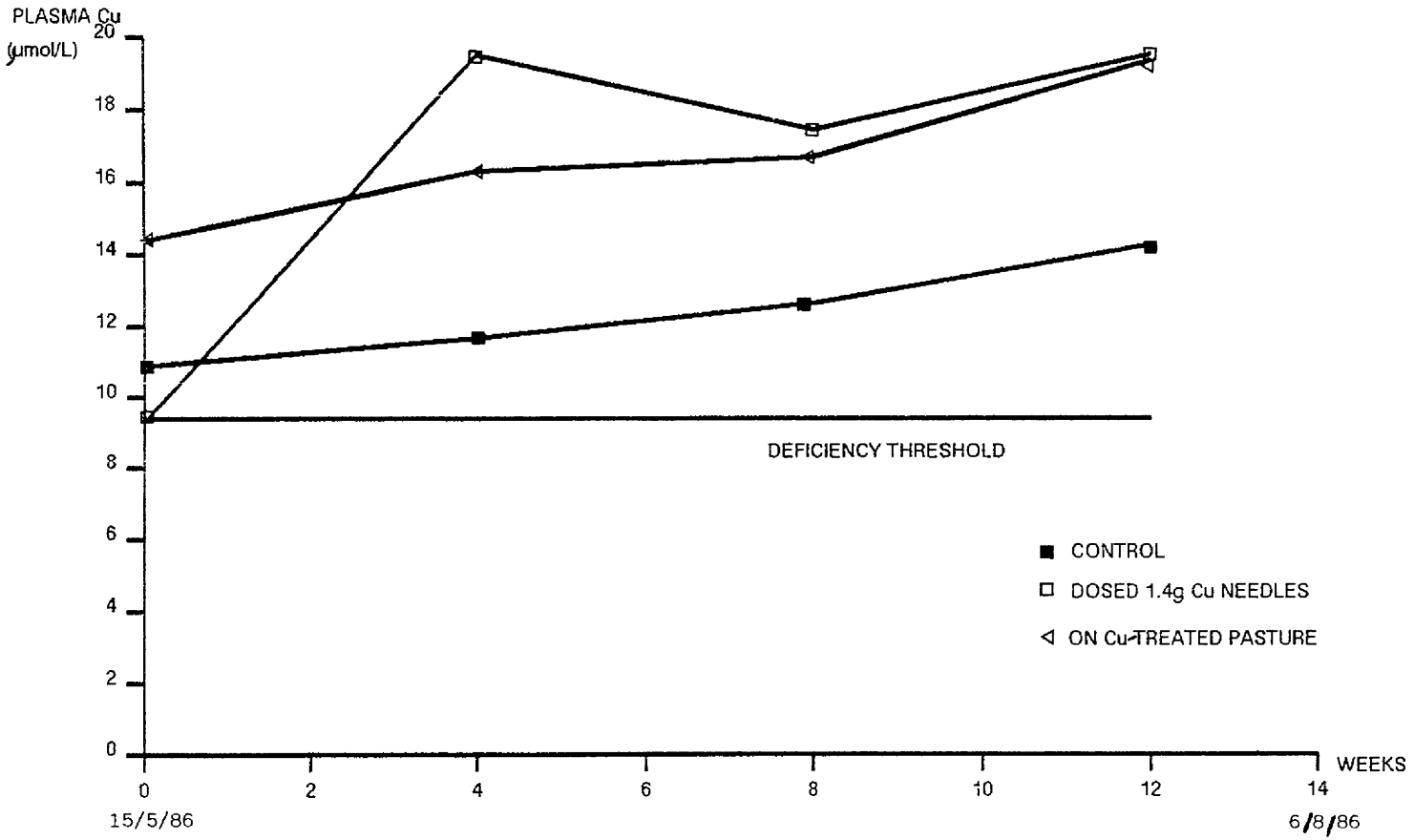
Mean lamb plasma Cu concentrations and numbers of Cu-deficient lambs in 1986

Date	Treatment group:	Mean plasma Cu (μmol/l) Control (n=12)	Cu needles (n=11)	Cu fertiliser (n=26)	S.E.D.
15/05/86		10.88^{a}(4)*	9.33^{a}(5)	14.40^{b}(1)	1.82
11/06/86		11.65^{a}(2)	19.52^{b}(0)	16.30^{c}(0)	1.59
08/07/86		12.65^{a}(0)	17.46^{b}(0)	16.70^{b}(0)	1.11
06/08/86		14.27^{a}(0)	19.54^{b}(0)	19.37^{b}(0)	1.04

Means with different superscripts in any row are significantly different at the $p<0.001$ level (analysis of variance, EDEX, A.F.R.C. Statistics Unit, Edinburgh).

* ( ) - number of Cu deficient lambs.

Figure 5.3. Mean lamb plasma Cu concentrations May 1986 to August 1986.



to a par with those of lambs on Cu-treated pasture and these levels were maintained until the end of the trial period (Figure 5.3). The plasma Cu concentration of the control lambs did rise gradually over the summer but was still significantly lower ($p<0.001$) than those of the Cu needles or Cu fertiliser lambs by the end of the trial.

As in the ewe response trial (5.5.1) there were no significant differences in plasma Mg, Hb, whole blood GSH-Px or Pb concentrations amongst the three lamb treatment groups and so mean values only for each sampling date are given in Table 5.7. All the results were within acceptable limits (Appendix 2). Similarly there were no significant differences in liveweights among the treatment groups and mean values only are given in Table 5.7.

5.5.3 Ewe Response Trial, October 1986 to September 1987

The trial was continued for a second year in order to determine the residual effect of the Cu fertiliser treatment after one year. The loss of six ewes in the first year of the trial, due to death from natural causes and the severe bad weather in the spring, together with the removal of cast ewes (older breeding ewes which are sold from hill to lowland farms where they are used to produce further crops of lambs) meant that the response trial was left with 20 ewes in the treated paddock and 21 in the untreated paddock. Therefore on the 20 October 1986 the numbers in each group were restored to 30 by the introduction of untreated hill ewes.

The first years results (5.5.1) showed that dosing ewes with Cu needles in the Cu-treated paddock had no significant effect on blood Cu status over and above that from the Cu fertiliser. Therefore this

Table 5.7

Mean lamb, plasma Mg, Hb whole blood, GSH-Px and PB concentrations and liveweight, May 1986 to August 1986

Date	Plasma Mg (mg/100 ml)	Hb (g/100 ml)	GSH-Px (U/ml)	Pb (μg/ml)	Liveweight (kg)
15/05/86	2.44	12.98	94.9	0.065	-
11/06/86	2.17	11.80	87.0	0.066	18.8
08/07/86	2.14	13.22	69.1	0.066	25.7
06/08/86	2.14	13.78	77.0	0.065	33.2
S.E.D.	0.06	0.26	8.74	-	0.702

treatment was not repeated in the second year. Consequently on 3 March 1987 only 15 ewes in the untreated paddock were dosed with 5 g Cu needles. The mean plasma Cu concentrations for the resulting three treatment groups are given in Table 5.8 and presented graphically in Figure 5.4. The plasma Cu concentration of control ewes followed a similar pattern to that demonstrated in the first year of the trial (Figure 5.2). The ewes in the untreated paddock but dosed with 5 g Cu needles showed the characteristic increase in their plasma Cu concentration as seen in year one (Figure 5.2). However, this treatment was given in the March of 1987 as opposed to the January of 1986. This later timing of the treatment gave full protection of the ewes from Cu deficiency over the late pregnancy, lambing period and also carried over into the summer until a time when the ewes could obtain sufficient Cu from the herbage. Thus the protection was extended from 12 weeks in 1986 to 20 weeks in 1987 due to the influence of extra Cu from the herbage.

The replacement of 10 ewes in the Cu fertiliser group with new ewes from the hill where ewes are generally of low Cu status resulted in a lowering of the mean plasma Cu concentration from 18 μmol/l on 9 September 1986 to 13 μmol/l on 20 October 1986. Figure 5.4 shows that despite the introduction of these new ewes the mean plasma Cu concentration of the Cu fertiliser group was significantly higher ($p<0.001$) than the control ewes, whose mean was unaffected by the introduction of new ewes, for the whole of the second year of the trial. The mean plasma Cu concentration of the Cu fertiliser ewes was comparable to that obtained by an oral dosing with 5 g Cu needles.

Table 5.8

Mean ewe plasma Cu concentration, October 1986 to September 1987

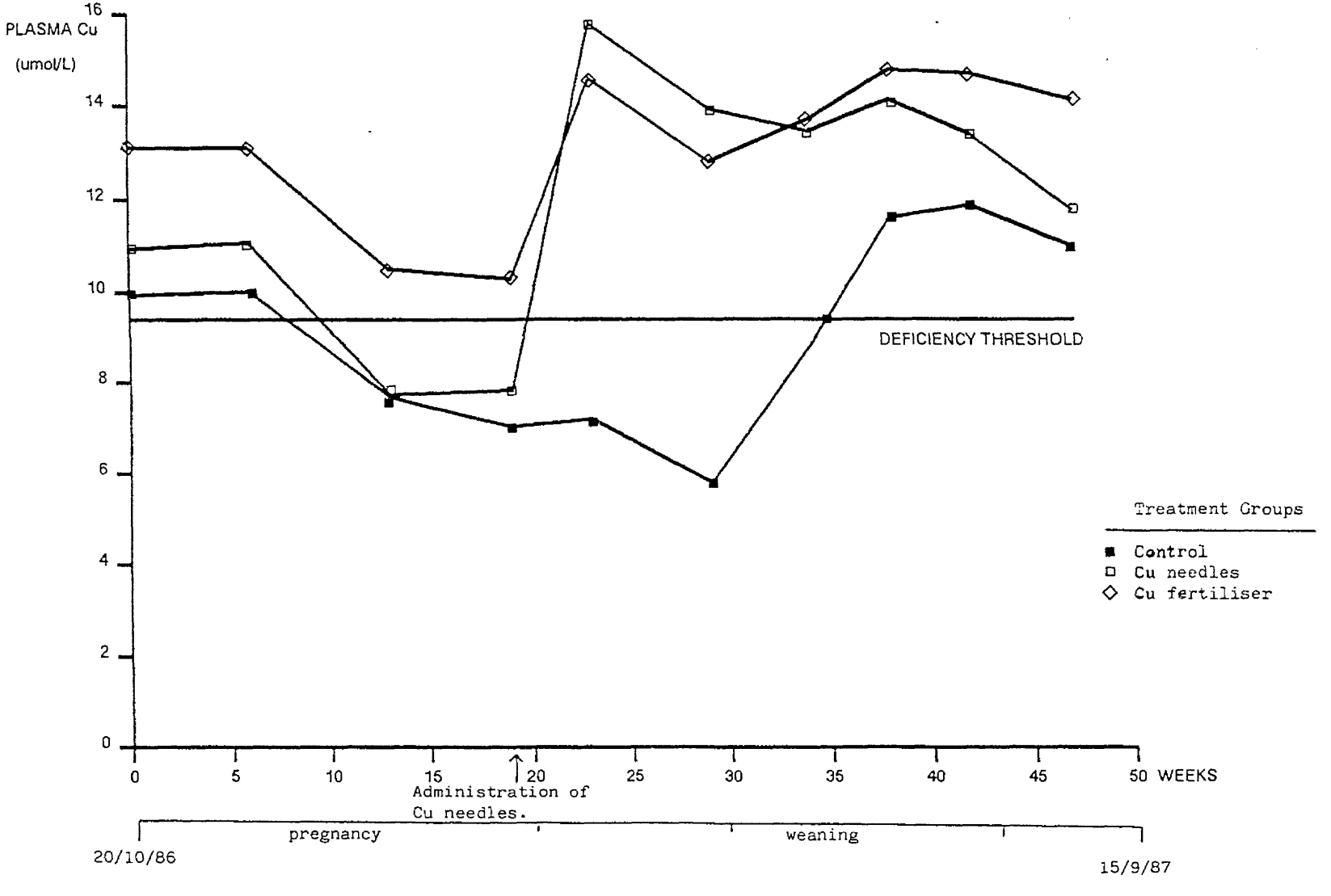
	Mean plasma Cu (μmol/l)			S.E.D.[θ]	
Treatment Group:	Control (A)	Control (B)*	Cu fertiliser (C)	AvC	AvB
Date	(n=15)	(n=15)	(n=30)	BvC	
20/10/86	9.94[a]	10.95[a]	13.12[b]	0.881	1.017
02/12/86	10.02[a]	11.07[a]	13.11[b]	1.041	1.202
27/01/87	7.69[a]	7.74[a]	10.52[b]	0.885	1.022
03/03/87	7.05[a]	7.85[a]	10.29[b]	0.907	1.048
31/03/87	7.21[a]	15.80[b]	14.61[b]	1.293	1.493
12/05/87	5.81[a]	13.95[b]	12.81[b]	1.468	1.695
16/06/87	8.84[a]	13.47[b]	13.72[b]	1.365	1.576
14/07/87	11.61[a]	14.16[ab]	14.81[b]	1.284	1.483
04/08/87	11.90[a]	13.41[ab]	14.74[b]	1.155	1.334
15/09/87	11.70[a]	11.78[a]	14.15[a]	1.666	1.924

*ewes treated 5 g Cu needles on 03/03/87.

[θ]as there were unequal numbers of ewes in the treatment groups, analysis of variance was carried out between groups AvC and BvC (30 ewes by 15 ewes) and AvB (15 ewes by 15 ewes) and so two S.E.D.s were obtained for each date.

Means with different superscripts in any row are significantly different at the $p<0.001$ level (analysis of variance, EDEX, A.F.R.C. Statistics Unit, Edinburgh).

Figure 5.4. Mean ewe plasma Cu concentrations, October 1986 to September 1987 after a single Cu fertiliser application on 19/11/85.



When the new ewes are compared with the original ewes in the Cu fertiliser group, the plasma Cu concentration of the latter are higher throughout the whole of the year (Table 5.9). The mean plasma Cu concentration of the original ewes was above the deficiency threshold at every date whilst that of the ewes introduced on 20 October 1986 fell below this level on two dates (27 January and 3 March 1987). Despite falling below the deficiency threshold the new ewes in the Cu-treated paddock maintained plasma Cu concentrations well above those of the new control ewes. Thus the single Cu fertiliser application in November 1985 has maintained significantly higher ($p<0.001$) plasma Cu concentrations in the Cu fertiliser ewes, than those of the control ewes, for a second successive year.

Table 5.9

Mean ewe plasma Cu concentrations of original ewes and those introduced in October 1986 to the Cu-treated paddock

Date	Original ewes (n=21)	Ewes introduced October 1986 (n=9)
20/10/86	13.9	12.43
02/12/86	14.2	11.10
27/01/87	10.84	8.57
03/03/87	10.94	8.56
31/03/87	16.14	9.93
12/05/87	14.35	9.85
16/06/87	15.05	10.26
14/07/87	15.50	13.0
04/08/87	15.80	12.80
15/09/87	15.32	12.72

The results for plasma Mg, Hb, whole blood GSH-Px and Pb analysis revealed no significant differences between the groups and a trial mean for each sampling date is given in Table 5.10. The majority of results are within the normal ranges found in ovine blood (Appendix 2).

Table 5.10

Mean ewe plasma Mg, Hb whole blood, GSH-Px and Pb concentrations, October 1986 to September 1987

Date	Plasma Mg (mg/100 ml)	Hb (g/100 ml)	GSH-Px (U/ml)	Pb (μg/ml)
20/10/86	1.90	10.66	31.15	0.08
02/12/86	1.88	10.74	65.97	0.09
27/01/87	2.20	12.94	75.14	0.07
03/03/87	2.15	11.60	116.49	0.11
31/03/87	2.09	10.80	96.89	0.09
12/05/87	2.03	10.51	101.59	0.13
16/06/87	2.13	9.27	85.37	0.12
14/07/87	2.10	8.63	65.20	0.07
04/08/87	2.05	11.52	20.10	0.08
15/09/87	1.92	13.08	30.46	0.09
S.E.D.	0.21	0.57	8.34	-

During the second year of the trial 7 ewes died, mainly at lambing time, 4 from the Cu-treated paddock and 3 from the untreated paddock. Post-mortem examination revealed that all the ewes died of natural causes. However analysis of the liver and kidney from each ewe showed large differences in the Cu concentrations (Table 5.11) of ewes from

Table 5.11

Mean tissue analysis results from dead ewes and lambs and the incidence of swayback in 1987

Treatment	Mean tissue analysis (mg/kg D.M.)								Swayback cases
	Liver				Kidney				
	Cu	Zn	Pb	Cd	Cu	Zn	Pb	Cd	
Ewes									
Untreated paddock (n=3)	11.9	186	3.8	2.5	21.5	117.5	2.6	1.8	N.A.
Cu-treated paddock (n=4)	184.3	86.2	1.8	0.6	12.0	79.0	1.5	4.0	N.A.
Lambs									
Untreated paddock	45.1	172.7	5.5	0.15	11.0	70.1	0.9	0.25	2
Cu-treated paddock	102.6	244	4.7	0.15	14.9	100.4	2.0	0.25	2

(N.A. = Not Applicable)

the two paddocks. Ewes in the Cu treated paddock had a much higher mean Cu concentration in their liver (184.3 mg/kg) than those in the untreated paddock (11.9 mg/kg) two of which had concentrations below the deficiency threshold of 10 mg/kg. Thus grazing Cu-treated pasture increased the amount of Cu stored in the liver as well as increasing plasma Cu concentrations. However, the liver Cu concentrations found in the Cu-fertiliser ewes were still within the normal range (Appendix 2) and therefore well below the accepted toxicity threshold of 1000 mg/kg (Grace, 1983).

Zinc, Pb and Cd concentrations in both liver and kidney (Table 5.11) were all within the normal ranges found in sheep (Appendix 2) and were also well below toxic concentrations (Underwood, 1977).

5.5.4 Lamb Response Trial May 1987 to August 1987

Ultrasonic scanning of the ewes suggested that 53 lambs were expected from the ewes in the untreated paddock and 50 from ewes in the Cu-treated paddock. Of the 26 lambs that died during lambing, 15 were obtained for post-mortem, 7 from the untreated paddock and 8 from the treated paddock. The remainder were found to be too decomposed or damaged by predators to enable a post-mortem examination. This left a total of 77 lambs for the response trial, 41 in the untreated paddock and 36 in the Cu-treated paddock.

Post-mortem examination confirmed that there were two cases of "congenital" swayback in lambs born to control ewes. There were also two unconfirmed cases of swayback and one confirmed case of delayed swayback in the untreated paddock. Two confirmed cases of "congenital" swayback lambs were also born to ewes on the Cu-treated

pasture; these lambs were born to ewes introduced to the trial on the 20 October 1986 which were themselves, at lambing time, on the borderline of Cu deficiency, with plasma Cu concentrations below 10 μmol/l. There were no swayback lambs from ewes which had been in the Cu-treated paddock from the start of the trial in 1985. It is possible that there were further cases of swayback in the unrecovered lambs; swayback lambs are weak and therefore likely to die early in life.

Analysis of liver and kidney from the dead lambs showed that lambs born to ewes in the Cu-treated paddock had a much higher liver Cu concentration than those from control ewes, 103 mg/kg compared with 45 mg/kg respectively (Table 5.11). The results of tissue analysis for Zn, Pb and Cd showed no differences between the treatment groups.

On 12 May 1987 half the lambs born to ewes in the untreated paddock were dosed with 1.4 g Cu needles. Blood samples were taken on 12 May, 16 June, 14 July and 4 August and the mean plasma Cu concentrations for each of the three treatment groups on each of these days are given in Table 5.12 and presented graphically in Figure 5.5. Lambs born to Cu fertiliser ewes had significantly higher ($p<0.001$) mean plasma Cu concentrations (12.98 μmol/l) than those born to control ewes (9.92 μmol/l). Table 5.12 also shows that the control, Cu needles and Cu fertiliser groups contained 11, 10 and 6 lambs, respectively, with plasma Cu concentrations below the deficiency threshold on 12 May. Of the six Cu-deficient lambs in the Cu fertiliser group, four of them were from ewes introduced to the trial on the 20 October 1986 (5.5.3) and the other two lambs were borderline cases with plasma Cu concentrations greater than 9.0 μmol/l but below the deficiency

Figure 5.5. Mean lamb plasma Cu concentrations May 1987 to August 1987.

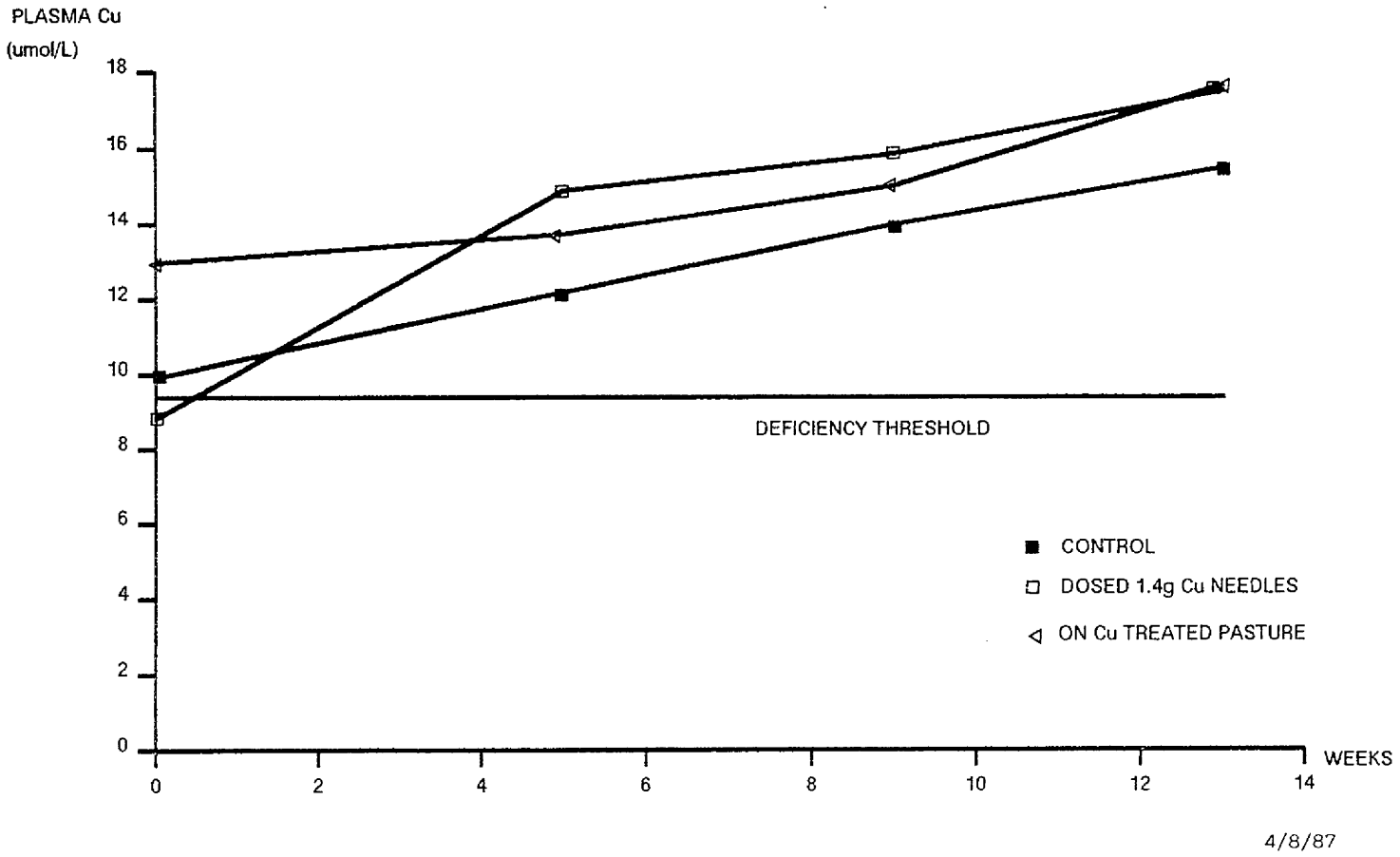


Table 5.12

Mean lamb plasma Cu concentration and numbers of Cu deficient lambs

Date	Treatment group: Control	Cu needles	Cu fertiliser	S.E.D.
12/05/87	9.92^{ab} (11)*	8.82^{a} (10)	12.98^{b} (6)	1.62
16/06/87	12.20^{a} (4)	14.91^{b} (2)	13.76^{ab} (2)	1.11
14/07/87	14.01^{a} (1)	15.88^{b} (0)	15.04^{ab} (1)	0.90
04/08/87	15.52^{a} (1)	17.52^{ab} (0)	17.67^{b} (0)	1.03

Means with different superscripts in any row are significantly different at the $p<0.001$ level (analysis of variance, EDEX, A.F.R.C. Statistics Unit, Edinburgh).

* ( ) - number of Cu deficient lambs

threshold of 9.4 µmol/l. Only 16% of the lambs in the Cu-treated paddock had plasma Cu concentrations below the deficiency threshold compared with 51% of the lambs in the untreated paddock on 12 May.

Figure 5.5 shows that the blood plasma Cu concentration of the control lambs slowly increased over the summer with the number of Cu-deficient lambs declining from 11 on the 12 May to 1 on the 4 August (Table 5.12). The lambs in the Cu-treated paddock maintained plasma Cu concentrations significantly higher ($p<0.001$) than those of the control lambs for the duration of the trial. Lambs in the untreated paddock but dosed with 1.4 g Cu needles had their plasma Cu concentration boosted, within 5 weeks, to equal that achieved by lambs in the Cu-treated paddock. These concentrations were maintained until the end of the trial on the 4 August. The trial was concluded at this date as the lambs were sold on the 10 August.

Although there were differences in mean liveweights, the Cu needles group was heavier than the control group which in turn was heavier than the Cu fertiliser group, the rate of daily liveweights gain for all 3 groups was similar and this despite the fact that the lambs of Cu fertiliser ewes were on average four days younger. Thus any differences were not statistically significant. The mean values for all the lambs on each date are given in Table 5.13.

As in the previous years lamb trial (5.5.2) and both years of the ewe trial (5.5.1 and 5.5.3) there were no significant differences in any other blood parameters analysed, among the three treatment groups, and so mean values for all the lambs on each sampling date are also shown in Table 5.13.

Table 5.13

Mean lamb plasma Mg, Hb whole blood, GSH-Px and PB concentrations and liveweight, May 1987 to August 1987

Date	Plasma Mg (mg/100 ml)	Hb (g/100 ml)	GSH-Px (U/ml)	Pb (μg/ml)	Liveweight (kg)
12/05/87	2.06	11.61	78.20	0.04	10.30
16/06/87	2.09	12.42	67.03	0.03	20.70
14/07/87	2.17	11.52	72.91	0.06	27.80
04/08/87	2.12	12.26	46.42	0.04	32.40
S.E.D.	0.12	0.56	4.05	-	1.13

5.5.5 Ewe Response Trial, October 1987 to May 1988

The loss of seven ewes in the second year of the trial, due to death from natural causes, and the removal of cast ewes meant that the response trial was left with 18 ewes in the treated paddock and 21 in the untreated paddock. Therefore on 27 October 1987 the numbers in each group were restored to 30 by the introduction of untreated hill ewes. On that date 15 of the control ewes were dosed with 5 g Cu needles and this was repeated on 7 March 1988 in accordance with the farmers normal practise for the rest of his flock.

The mean plasma Cu concentrations for the three treatment groups on each of the five sampling dates are given in Table 5.14 and presented graphically in Figure 5.6. The mean plasma Cu concentration of the control ewes followed the same pattern of decline over the winter and into late pregnancy as was shown in the first 2 years of the trial (5.5.1 and 5.5.3). Treatment with Cu needles gave an increase in plasma Cu concentrations within 6 weeks and protection from Cu deficiency for the next 12 weeks after which they began to decline. A second treatment with Cu needles on 7 March gave protection throughout the whole trial period and gave plasma Cu concentrations significantly higher ($p<0.001$) than those of the control group on each sampling date.

The introduction of 12 new ewes to the Cu fertiliser group reduced the mean plasma Cu concentration from 14.15 µmol/l on the 15 September to 11.8 µmol/l on the 27 October. The plasma Cu concentration of the Cu fertiliser group can now be subdivided into 3 groups, i.e. those in the treated paddock from November 1985, those introduced in October

Table 5.14

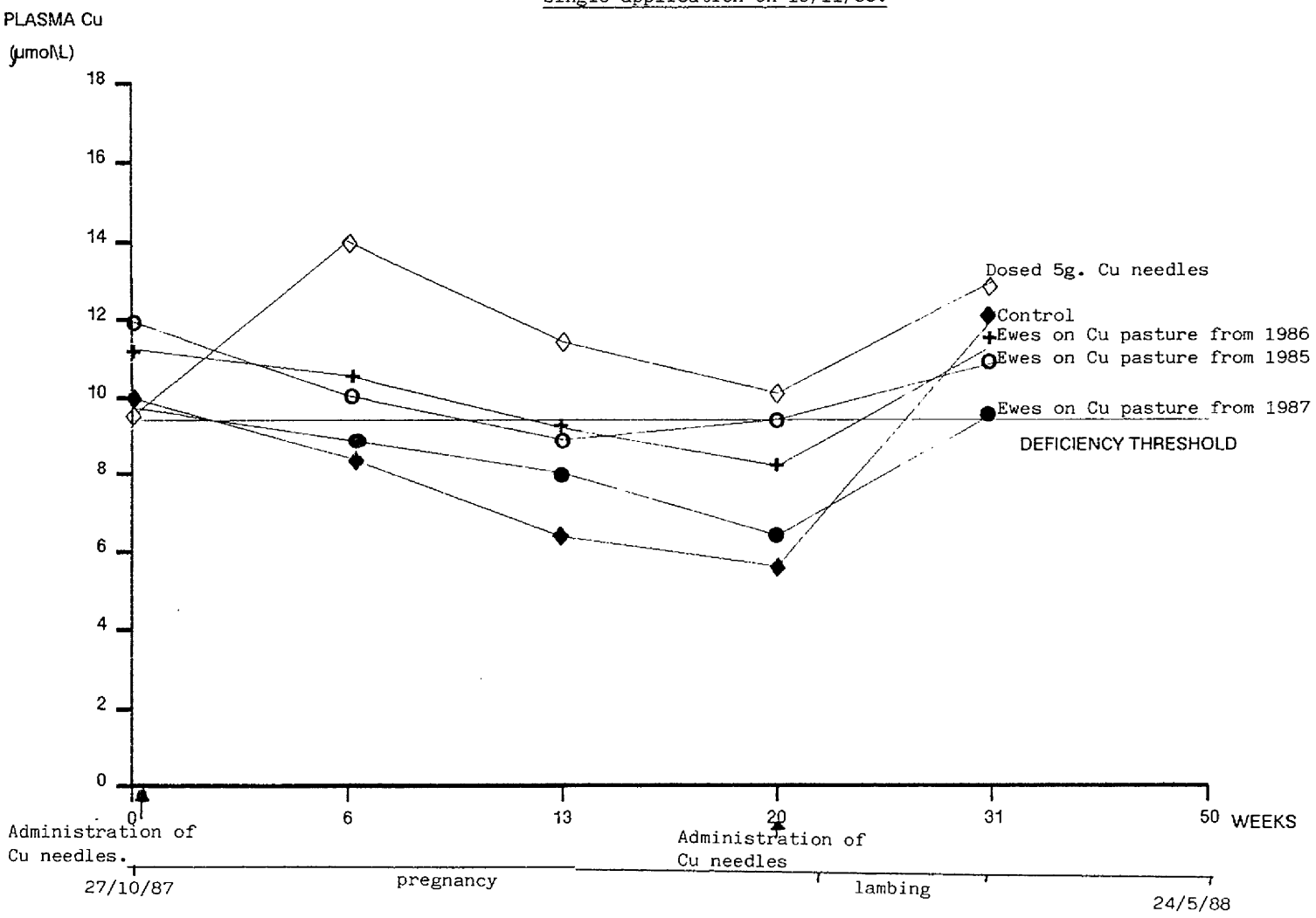
Mean ewe plasma Cu concentration, October 1987 to May 1988

	Mean plasma Cu (µmol/l)			S.E.D.	
Treatment Group:	Control (A)	Cu needles*	Cu fertiliser (C)	AvC	AvB
Date	(n=15)	(n=15)	(n=30)	BvC	
27/10/87	9.99^{a}	9.51^{a}	11.18^{a}	1.27	1.47
08/12/87	8.43^{a}	14.03^{b}	9.79^{a}	1.32	1.52
19/01/88	6.38^{a}	11.42^{b}	8.53^{a}	1.18	1.37
07/03/88	5.60^{a*}	10.09^{b}	8.27^{b}	1.04	1.19
24/05/88	11.99^{ab}	12.97^{a}	10.68^{b}	1.05	1.21

Means with different superscripts in any row are significantly different at the $p<0.05$ level (analysis of variance, EDEX, A.F.R.C. Statistics Unit, Edinburgh).

*Controls treated with Cu needles on 07/03/88.

Figure 5.6. Mean ewe plasma Cu concentrations October 1987 to May 1988 after a single application on 19/11/85.



1986, and those in October 1987. As the data in Figure 5.6 and Table 5.15 demonstrate the plasma Cu concentrations of all three of these groups declined over the winter and into late pregnancy. By the 19 January 1988, 14 ewes in this group were Cu deficient with plasma Cu concentrations less than 9.4 μmol/l. These Cu-deficient ewes were equally spread between the three subgroups. However, the mean plasma Cu concentrations of all three subgroups together was still significantly higher ($p<0.05$) than the plasma Cu concentration of the control ewes.

Table 5.15

Mean plasma Cu concentrations of ewes in the Cu-treated paddock for different periods of time (1987-88)

Date	Original (n=14)	Introduced 1986 (n= 4)	Introduced 1987 (n=12)
27/10/87	11.96	11.25	9.75
08/12/87	10.04	10.56	8.90
19/01/88	8.90	9.21	8.02
07/03/88	9.41	8.19	6.40
24/05/88	10.86	11.31	9.56

By the 7 March 1988, 13 of the control ewes were Cu deficient with 8 of them having plasma Cu concentrations below 5 μmol/l. At these very low Cu concentrations, the risk from Cu deficiency is high and swayback in lambs can be anticipated. To prevent these expected losses all 15 of the ewes were treated with 5 g Cu needles on that date for humanitarian reasons. This action prevented the onset of Cu deficiency in any lamb born to that group in April 1988. It also

boosted the mean plasma Cu concentration of the control group so that it was not significantly different from that of the Cu needles group of ewes which were also treated, for a second time on that date. There were no Cu-deficient lambs born to this group either.

The mean plasma Cu concentration of the ewes in the Cu-treated paddock was still below the deficiency threshold on 7 March 1988 and so the 15 ewes with the lowest Cu concentrations were also treated with 5 g Cu needles. Thus after 7 March there were no Cu-deficient ewes on the response trial and as a result there were no lambs with plasma Cu concentrations below the deficiency threshold in 1988.

The mean plasma Cu concentration of the 15 ewes not treated with Cu needles was 10.3 μmol/l on 7 March 1988. These ewes maintained this level throughout lambing and finished the trial with a mean plasma Cu concentration of 10.5 μmol/l.

As in the previous years no significant differences in plasma Mg, Hb, whole blood GSH-Px or Pb concentrations were found among the treatment groups and all the results were within the normal ranges for these parameters in ewes. Trial mean values for each of these blood parameters on each sampling date are given in Table 5.16.

Table 5.16

Mean ewe plasma Mg, Hb whole blood, GSH-Px and Pb concentrations, October 1987 to May 1988

Date	Plasma Mg (mg/100 ml)	Hb (g/100 ml)	GSH-Px (u/ml)	Pb (μg/ml)
27/10/87	2.20	8.85	27.89	0.09
08/12/87	2.19	9.64	31.68	0.13
19/01/88	2.28	13.05	63.94	0.08
07/03/88	2.22	9.41	75.03	0.08
24/05/88	2.20	13.48	115.10	0.09
S.E.D.	0.18	0.49	10.21	-

5.5.6 Soil and Herbage Analysis

The results of soil analysis (Table 5.17) show that broadcast application of the Cu fertiliser increased the total Cu concentration of the soil when compared with the soil from untreated pasture. The increase from 22.4 mg/kg to 25.1 mg/kg in September 1986, 10 months after the Cu fertiliser application, was maintained at the last sampling on 29 September 1987.

Table 5.17

Total soil Cu concentration in field 1986 and 1987

Treated	Soil Cu concentration (mg/kg)	
	September 1986	September 1987
Untreated paddock	22.4	22.7
Cu treated paddock (370 kg/ha)	25.1	26.2

Climatic conditions at Garmore together with continual grazing produce poor herbage coverage of the field over the winter months. Therefore herbage samples could only be obtained, over the spring and summer without the risk of severe soil contamination. The results of herbage Cu analysis for the summer of 1986 are given in Figure 5.7 and those for 1987 in Figure 5.8. On the first sampling date, six months after the Cu fertiliser application the Cu concentration of herbage from the treated paddock was over 100 mg/kg. This fell gradually over the summer and by September the herbage Cu concentration had levelled out at a consistent 3-4 mg/kg higher than the Cu concentration of herbage from the untreated paddock for the rest of the growing season. The resumption of herbage analysis in 1987 showed that a similar difference was maintained for a second year (Figure 5.8).

On the 25 June 1987 a difference in colour was observed between the sward in the Cu-treated paddock and the untreated paddock. The Cu fertiliser treated sward was a darker green colour. This observation was verified by chlorophyll analysis, 25.1 mg/l chlorophyll was found in the treated sward compared with 18.3 mg/l in the untreated sward. Analysis for a wide range of parameters, to determine why this occurred, was carried out (Table 5.18). Apart from increased Cu and Zn concentrations there were no differences in the herbage content, between untreated and treated sward, for any other parameter (Table 5.18). This difference in colour was observed throughout the rest of the trial until it finished in June 1988.

Figure 5.7 Herbage Cu concentration for treated vs untreated paddocks, 1986.

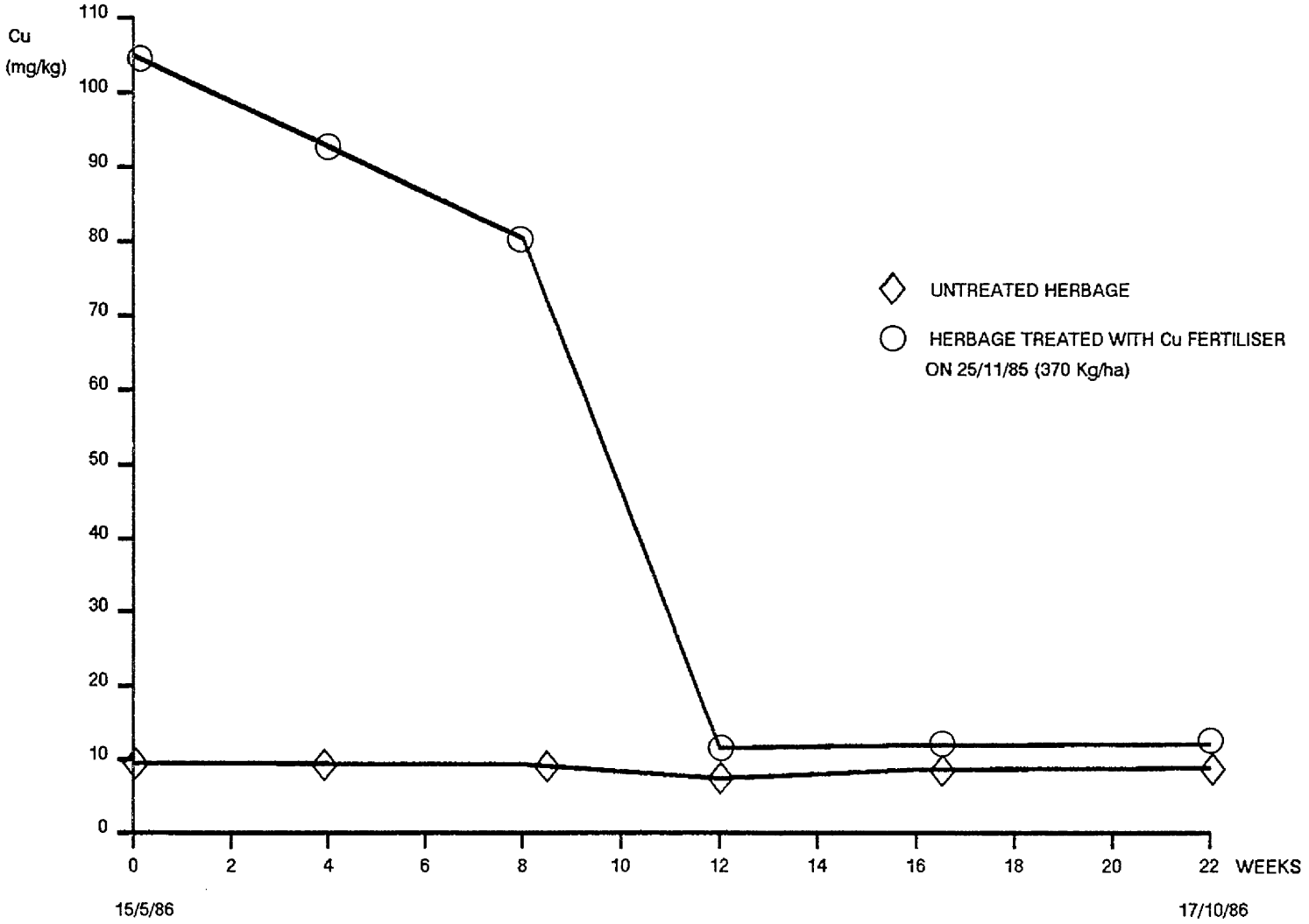


Figure 5.8 Herbage Cu conentration for treated vs untreated paddocks, 1987.

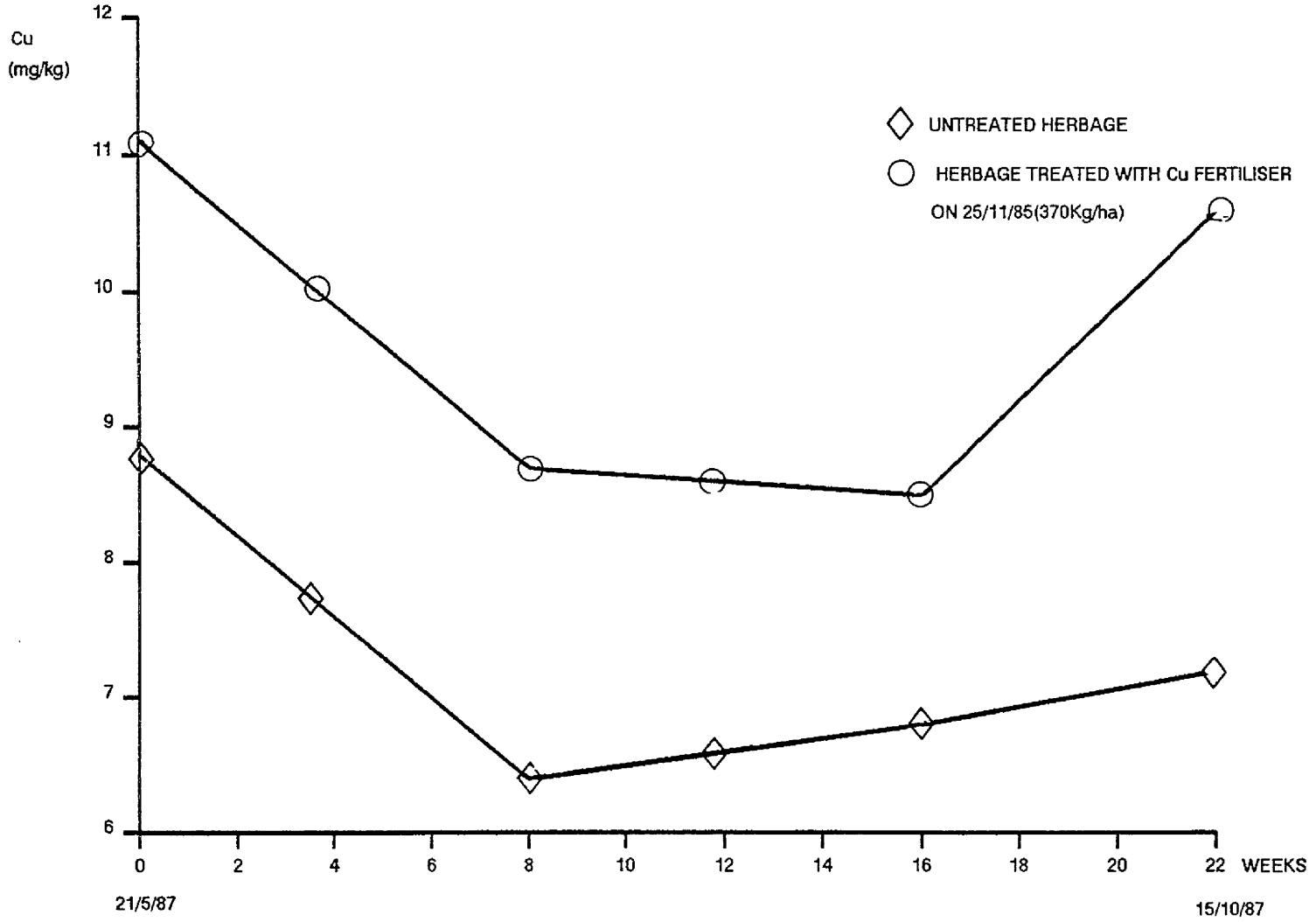


Table 5.18

Herbage analysis results from 25/06/87

Analysis	Untreated paddock	Cu-treated paddock
Chlorophyll (mg/l)	18.3	25.1
Cu (mg/kg)	8.9	11.0
Zn (mg/kg)	51.1	58.9
S (g/kg)*	3.02	3.23
K (g/kg)*	28.3	22.5
Mg (g/kg)*	1.4	1.3
Mo (m/kg)*	0.3	0.3
Dry matter (g/kg)*	264	289
Protein (g/kg)*	168	169
P (g/kg)*	4.4	4.2
Organic matter (g/kg)*	911	918
Cd (mg/kg)	0.01	0.01
Ni (mg/kg)	1.5	1.5
Cr (mg/kg)	0.1	0.1
Pb (mg/kg)	1.3	1.1

*Analysis carried out by S.A.C. Analytical Services Unit, Auchincruive.

In 1988 one bulk herbage sample was taken from each paddock and the Cu concentration determined. This showed that the untreated paddock had a herbage Cu concentration of 5.43 mg/kg compared with 7.75 mg/kg in the treated paddock. Thus the difference was maintained into a third year.

5.6 DISCUSSION

The results show that Cu is the only measured blood parameter where concentrations below the recognised deficiency threshold (Grace, 1983) were found and for which significant differences were shown among the treatment groups. The results for all other analyses revealed very similar means in all the treatment groups over the three years for both ewes and lambs. The majority of these results were well within the normal ranges for the different parameters. Thus it was purely a potential Cu deficiency problem in the sheep that was investigated in this trial.

5.6.1 Control Ewes

The control ewes demonstrated a normal decline (Wiener and Field, 1974; Underwood, 1977) in plasma Cu concentrations with time over the winter. This would be due to foetal Cu demands combined with a low Cu intake over the winter and poor Cu storage in the liver due to low herbage Cu availability in the summer. Plasma Cu concentrations did rise gradually over the summer months as Cu reserves were replenished. These results show that there was a potential Cu deficiency problem on the farm with the possibility of swayback lambs in severe cases. The low ewe plasma Cu concentrations were parallelled by low plasma Cu concentrations in their lambs in 1986 and 1987, with the two cases of swayback lambs in the latter year born to the ewes with the lowest plasma Cu levels. In 1988 the plasma Cu concentrations of several of the control ewes were so low (5.5.5) that the birth of swayback lambs was anticipated. As the potential Cu deficiency problem had already been demonstrated to be severe by blood analysis all 15 ewes were

treated with Cu needles. This action prevented Cu deficiency and low plasma Cu concentrations in any lamb born to this treatment group in the final year.

5.6.2 Cu Needle Treatment

Ewes grazing in the untreated paddock but dosed with 5 g Cu needles in late pregnancy in 1986 and 1987 showed an increase in plasma Cu concentrations within 6 weeks which was sufficient to keep plasma Cu concentrations above the deficiency threshold for 12 to 20 weeks respectively over the critical late pregnancy and lambing period when Cu is in most demand by the ewe and its foetus. In the final year of the trial Cu needle treatment in early and late pregnancy gave complete protection from Cu deficiency throughout the trial period. In all three years the Cu needles prevented swayback and reduced the risk of Cu deficiency in lambs born to this group. The increased plasma Cu concentrations and the longevity of the treatment demonstrated in this trial are similar to those reported by other workers (Ellis, 1980; Whitelaw **et al**, 1983) and confirm that the Cu needles treatment is an effective method of reducing the risk of Cu deficiency in sheep. It was therefore, a suitable choice for comparison with the Cu fertiliser pasture treatment for preventing Cu deficiency.

5.6.3 Pasture Treatment with the Cu Fertiliser

(a) Year 1

The single application of the Cu fertiliser as a top dressing on 25/11/85 raised the mean plasma Cu concentrations of the ewes allowed to graze the treated sward within six weeks and maintained it above

the deficiency threshold of 9.4 μmol/l throughout the remainder of the first year of the trial. The plasma Cu concentrations obtained were generally higher than those obtained using Cu needles. It is unlikely that the initial increase in plasma Cu concentration was a result of increased Cu concentrations in the herbage, as at that time of the year there was very little herbage growth and so little opportunity for Cu uptake by the herbage in sufficient amounts to cause the rapid 5 μmol/l increase in ewe plasma Cu concentrations obtained. Poor herbage growth in the field prevented sampling to determine herbage Cu concentration for confirmation of this hypothesis. In addition the ewes had been grazing the pasture for 7 to 8 weeks before the increased plasma Cu concentrations were found by which time it was too late to try and determine actual herbage Cu concentrations after the Cu fertiliser application in order to establish the reason for the increase. This point, however was noted for investigation in further experiments.

When unwashed herbage samples could eventually be obtained in May 1986, they had Cu concentrations of over 100 mg/kg. As this concentration was over 10 times that found using a similar application rate in the Garmore herbage trial (Chapter 6) and was above the phytotoxic threshold of 30 mg/kg (Davies, 1980) it was improbable that the 100 mg Cu/kg found was obtained through plant uptake. This suggests that Cu fertiliser was adhering to or contaminating the herbage throughout the first six months of the trial. This period of contamination was longer than that found in the leaf adherence trial (Chapter 9) and implies that the extended period of decline in herbage Cu concentrations may be due to the trampling action of the sheep

continually soiling the herbage with the Cu fertiliser which is mixed into the topsoil. Thus it is very probable that some of the Cu fertiliser was ingested by the sheep. Healy **et al** (1974) have proposed that grazing sheep can involuntarily ingest up to 300 g soil/kg herbage D.M.I. when herbage cover is poor over the winter months. Thus it was also likely that Cu fertiliser was ingested along with soil during the winter. It is therefore suggested that the initial increase in ewe plasma Cu concentrations was as a result of Cu fertiliser ingestion by the sheep. If this was the case then herbage Cu concentrations (Figure 5.7) imply that Cu fertiliser ingestion would have continued through to at least July 1986. It is proposed that the effects demonstrated in year one, i.e. the rapid increase and maintenance of plasma Cu concentrations, are almost entirely due to ingestion of the Cu fertiliser. The ewes also probably benefited from grazing herbage of higher Cu concentration over the summer months.

The treatment of 15 ewes in the Cu fertiliser paddock with 5 g Cu needles produced no additional increase in plasma Cu concentrations compared to that obtained by grazing the treated paddock alone (Figure 5.2 and Table 5.2). However, sheep have a homeostatic mechanism for maintaining their plasma Cu concentrations within a range of 9.4 to 18.9 μmol/l, with excess Cu being stored in the liver (Grace, 1983). As Cu treatment of pasture was sufficient to increase plasma Cu concentrations to the top of this range the extra Cu from the Cu needles was probably diverted to the liver for storage. This could not be confirmed because liver sampling requires surgery, which is rarely undertaken in the U.K. especially under field conditions and was not practical in this trial, or the death of an animal whereupon

liver Cu assay can be made. Thus no liver samples were taken to confirm the above. Therefore, it is not impossible that Cu concentrations in the liver of both groups of ewes in the Cu fertiliser paddock could have approached toxic levels, especially since concentrations of Cu in the liver can rise to levels which might be expected to be toxic without the animals appearing to be adversely affected (Ellis, 1980). Therefore it was decided not to administer any Cu needles to ewes in the Cu fertiliser paddock in subsequent years.

(b) Year 2

By the end of the first year the Cu treated herbage was maintaining a 2 to 4 mg Cu/kg advantage over the untreated herbage. The increase was similar to that found in the ungrazed herbage trial at the same site using an identical Cu fertiliser application rate (370 kg/ha) where there was no contamination of the herbage due to the soiling action of grazing sheep. This implies that there was no contamination of the herbage in the Cu fertiliser paddock with Cu fertiliser at the start of the second year.

The ewes which had been in the Cu fertiliser paddock from November 1985 maintained plasma Cu concentrations above the deficiency threshold and significantly greater than those of the control group for the whole of the second year. Although the plasma Cu concentrations of the 9 ewes introduced to the Cu fertiliser paddock in October 1986 did decline to a low of 8.56 μmol/l over the winter, the increase in herbage Cu concentrations was sufficient to prevent them declining as far as they did in the control ewes, which fell to 5.81 μmol/l. However, the new ewes were introduced in October when

herbage coverage was again poor and was not enough to supply sufficient Cu to prevent their mean plasma Cu concentrations dropping below the deficiency threshold on 27 January and 3 March 1987. The increase in plasma Cu concentrations of the new ewes to above the deficiency threshold on 31 March 1987 and subsequent maintenance over lambing and the rest of the summer may have been due to the increased herbage growth in the spring and consequent increased Cu intake. The possibility of Cu fertiliser ingestion along with soil over the winter, however, cannot be ruled out as it is still present in the surface layer of the soil even in year 2 and most probably helped prevent the decline in plasma Cu concentrations of the new ewes and contributed to their subsequent increase.

The plasma Cu concentration of the original ewes in the Cu fertiliser paddock was always above that of the new ewes and the deficiency threshold. In addition, tissue analysis from dead ewes in the second year of the trial showed that the ewes in the Cu fertiliser paddock had a much higher Cu concentration in their liver than those in the untreated paddock. Thus grazing the Cu fertiliser paddock also increased the amount of Cu in the liver and suggests that Cu intake, probably in the form of Cu fertiliser, in year 1 was in excess of blood requirements and homeostatic mechanisms diverted it to the liver for storage. Even on a very Cu-deficient diet, liver Cu concentrations of over 100 mg/kg, as found by tissue analysis, should maintain Cu sufficiency in the whole animal for over 100 days (S.A.C., 1982), a period which was exceeded in this trial. Thus the liver Cu stores accumulated in times when Cu intake was high, should have helped to maintain plasma Cu concentrations significantly higher than

those of the control ewes in the second year of the trial once the Cu intake from herbage declined in the winter. The presence of increased herbage Cu concentrations and the possibility of further Cu fertiliser ingestion along with soil should extend the longevity of the liver Cu stores.

Overall the results demonstrated that the single Cu fertiliser application maintained a significantly higher plasma Cu status in ewes grazing the treated paddock than that of comparable ewes grazing the untreated paddock for a second successive year. The maintenance of plasma Cu concentrations in both the new and original Cu fertiliser ewes above those of the control group suggests that the Cu fertiliser has a residual value of at least two years. However, this is not a fertiliser residual value in the conventional sense in which the term is used, in that here its effects are as a result of continued ingestion of the Cu fertiliser combined with residual Cu in the liver rather than through a long lasting effect on the herbage through the soil.

(c) Year 3

In the third year after application of the Cu fertiliser the ewes in the Cu fertiliser paddock, although still maintaining plasma Cu concentrations higher than those of the control group dropped below the deficiency threshold. The Cu-deficient ewes were spread evenly among the three sub-groups now grazing this paddock and it is therefore assumed that any Cu stores accumulated over the first year had now been depleted and that ingestion of the Cu fertiliser had now stopped. Although there was still a 2 mg Cu/kg advantage in herbage concentrations on Cu fertiliser treated land this was not

sufficient to prevent plasma Cu concentrations dropping below the deficiency threshold. By late pregnancy some of the ewes were well below the deficiency threshold and Cu needles treatment was required to remove the risk of swayback in their lambs.

As the plasma Cu concentration of the Cu fertiliser ewes was still higher than the control ewes, the Cu fertiliser was still showing a residual effect in the animal probably through its effects on both the herbage and the further possibility of ingestion along with soil. However this was not enough to meet the needs of the pregnant ewe. The results imply that after liver Cu stores are depleted and when the possibility of ingestion of large amounts of Cu fertiliser has decreased, then the increased herbage Cu concentrations are not sufficient to meet the Cu requirements of the grazing sheep.

Thus it appears that the single Cu fertiliser application only effectively reduced the risk of Cu deficiency in sheep for two years; in the third year, although there was a residual effect in the herbage it was not sufficient to be of significant value to the grazing animal. Indeed, the results of other workers do suggest that the small increase in herbage Cu concentrations of the levels demonstrated here are unlikely to benefit the grazing animal (Reith, 1975). This further confirms the ingestion effect proposed above.

5.6.4 Lamb Trials

In the first year of the trial lambs of ewes grazing the Cu fertiliser paddock were born with plasma Cu concentrations significantly higher than those found in the untreated paddock. Treatment with 1.4 g Cu needles was required to bring the mean plasma Cu concentration of the

control group up to that found on the treated pasture. No cases of swayback were found in the first year of the trial. However, as swayback lambs are weak they are the ones most likely to succumb in bad weather and so it cannot be guaranteed that none of the many casualties had swayback, nor that any of the treatments tested prevented its occurrence.

The pattern of plasma Cu concentration results were repeated in the second year when, however, swayback lambs were found in both the control and Cu fertiliser paddocks. The two cases of swayback in the Cu fertiliser paddock were from ewes newly introduced to the trial, both of which had plasma Cu concentrations below the deficiency threshold at lambing. Thus the residual value of the Cu fertiliser in the herbage was not sufficient to prevent the birth of Cu-deficient lambs except when the ewes had had time to accumulate liver Cu stores.

Although there were significant differences in plasma Cu concentrations among the different treatment groups, none of the lambs were sufficiently Cu-deficient to make it a growth limiting nutrient. Thus no liveweight differences would be anticipated nor were any obtained.

5.6.5 Herbage

Herbage analysis has shown that application of the Cu fertiliser resulted in a 2 to 4 mg/kg increase in Cu concentrations which agrees with the increases obtained for the 370 kg/ha Cu fertiliser treatment demonstrated in the herbage trial (Chapter 6) in which there was no contamination of the herbage. Thus the Cu fertiliser has produced an increase in herbage Cu uptake and concentrations.

The difference in herbage colour obtained in 1987 is unlikely to be as a result of increased Cu or Zn concentrations in the treated herbage as these elements are only likely to influence chlorophyll production if the grass was actually formerly Cu or Zn-deficient (Bussler, 1981) which it was not as there were no yield response to the applied Cu and Zn (Chapter 6). Thus this application should not have increased chlorophyll production. There were no differences between untreated and treated herbage in the concentration of any other element measured (Table 5.8) thus they should not have affected chlorophyll production in the one paddock only. As there was not sufficient time to investigate this effect further the cause of it remains unknown.

5.6.6 Conclusion

This trial has shown that a single application of the Cu fertiliser (370 kg/ha) was successful in both raising and maintaining plasma Cu concentrations above the deficiency threshold in ewes throughout pregnancy, lambing and weaning and in their lambs. The once only application was effective for two years, in ewes which had grazed the land throughout that time, and results were comparable to those obtained using an annual oral administration of Cu needles. In this respect, therefore, the Cu fertiliser has been more successful in terms of longevity than other soil treatments such as $CuSO_4$ in providing a source of Cu to prevent Cu deficiency in sheep (Reith, 1983). However, closer examination of the results implies that, although the Cu fertiliser did produce increases in herbage Cu concentrations comparable to those found in both the ungrazed herbage trial (Chapter 6) and from copper sulphate treatment (Evans 1983; Reith, 1983), it was more successful than other treatments only

because it was probably ingested by the grazing ewe, via both leaf adherence and along with soil, an effect which is unlikely with water soluble $CuSO_4$. Thus it is unlikely that the Cu fertiliser would have been as effective in the second year of the trial if all the ewes were newly introduced.

It is suggested that the initial increase of plasma Cu concentrations was due to direct ingestion of the Cu fertiliser both from coated herbage and from that mixed with surface soil, whilst the sward was poor. At this time excess Cu from fertiliser ingestion was stored in the liver, and this together with the increased Cu supply from the herbage and additional but more limited ingestion, probably maintained plasma Cu concentrations over the second winter of the trial. Liver Cu stores are only likely to last over one season; therefore in the third year of the trial when the increased herbage Cu concentration as a result of Cu fertiliser application alone was left to fully sustain the animal, the results show that this extra supply of Cu was not sufficient to maintain plasma Cu concentrations above the deficiency threshold.

Although the mean plasma Cu concentration of the Cu fertiliser ewes did fall below the deficiency threshold in the third year it was still higher than that of the control ewes. This was probably due to the increased herbage Cu concentration and the possibility of some further Cu fertiliser ingestion along with soil. Thus the Cu fertiliser is still showing a residual value and such an effect has not been achieved with any other herbage Cu treatment. However, although the Cu fertiliser has demonstrated that it is better than any previous soil or pasture treatment for the prevention of Cu deficiency in sheep

it is felt that it is still not good enough especially when compared to methods for the direct treatment of individual animals.

5.7 SUMMARY

5.7.1 Application of the Cu fertiliser to the sward successfully raised and maintained plasma Cu concentrations above the deficiency threshold in grazing ewes and their lambs throughout the first two years of the trial.

5.7.2 The single application of the Cu fertiliser gave comparable protection from Cu deficiency to that obtained using oral dosing of sheep with Cu needles which had to be repeated annually over a two year period.

5.7.3 After surface adherence of the Cu fertiliser to the grass ceased, the treated herbage maintained a 2-4 mg Cu/kg D.M. advantage throughout the trial period (from November 1985 to June 1988) over the untreated pasture.

5.7.4 In the third year after application of the Cu fertiliser ewes grazing treated pasture, although still maintaining plasma Cu concentrations higher than those of the control group, had dropped below the deficiency threshold. It therefore appears that the effects of the Cu fertiliser only lasted for two years in this trial.

5.7.5 It is proposed that the initial increase in ewe plasma Cu concentrations was due to direct ingestion of the Cu fertiliser, and that subsequent maintenance was due to a

combination of increased Cu supply from the herbage together with storage of Cu in the liver from times when the Cu intake was high and further Cu fertiliser ingestion over the winters.

CHAPTER 6. HERBAGE FIELD TRIAL, GARMORE FARM

6.1 INTRODUCTION

Although various Cu compounds have been used effectively to correct Cu deficiency in plants (section 2.4.1), increased Cu concentration in herbage when they are applied to pasture is not always of significant benefit to the grazing animal (Reith, 1975; Evans, 1983). The most frequently used method of correcting Cu deficiency in plants is to apply copper sulphate to the soil. This is a soluble form of Cu and gives an immediate increase in the plant available Cu concentration of the soil which is enough to overcome plant deficiency. However, the added Cu^{2+} ions are then rapidly immobilised and made plant unavailable in the soil (Gartrell, 1980). The residual effectiveness of this $CuSO_4$ application is high under arable conditions where cultivation in following years improves the mixing of the Cu within the rooting zone and allows general desorption of Cu^{2+} ions to provide an increased Cu supply capacity (Reith, 1975; Gartrell, 1980). This mixing does not occur under conditions of permanent pasture and so the residual effectiveness of $CuSO_4$ application is lowered. To overcome this problem other less soluble compounds such as Cu metal, Cu oxides and Cu slags which dissolve more slowly to release Cu^{2+} ions over a longer time period have been tried on pasture (Follet **et al**, 1981). By the use of these materials, increases of 2-4 mg/kg in the Cu concentration of pasture have been maintained for 2 to 4 years (Evans, 1983; Jost, 1960).

The trial below was initiated both to evaluate the effectiveness of the Cu fertiliser for increasing herbage Cu levels, and to monitor the

movement and behaviour of the applied Cu in the soil under conditions away from the animal where soil contamination was minimised. In addition herbage Zn, Fe, Pb, Cd, Mn, Cr and Ni concentrations were monitored to ensure that their presence in the fertiliser did not result in elevated herbage concentrations to the possible detriment of grazing livestock.

The animal response trial at Garmore farm was initiated to test the effectiveness of applying the Cu fertiliser to grazed sward for the prevention of Cu deficiency in sheep. However, the application rate used, i.e. 370 kg/ha was selected, only as it gave a comparable amount of Cu, 7.5 kg/ha, to that applied in other treatments (Reith, 1983; Evans, 1983). Whether the different forms of Cu would act in the same way in the soil or if the Cu fertiliser would have a slow release effect were unknown. Therefore it was felt that a more detailed study covering a range of application rates, and their effects on the Cu status of soil and herbage was merited.

6.2 AIMS AND OBJECTIVES

The objective of this trial was to investigate the long term (3 years) effect of a single Cu fertiliser application at different rates at two soil pH levels on the yield and Cu concentration of herbage in an established sward. In addition the movement of the applied Cu in the soil was monitored.

6.3 EXPERIMENTAL

6.3.1 Site Description

An area of 30 x 30 m of uniform established sward was fenced off in the centre of the animal response trial site at Garmore (Figure 5.1). Soil and sward conditions were therefore the same as in the animal response trial. Within this area thirty two 3 m by 2 m plots were measured out (Figure 6.1).

Prior to Cu fertiliser or lime application soil samples at 0-10 cm depth representative of each plot were taken (section 3.2.1) to assess the uniformity of plant available Cu in the plots and also to determine the amount of lime required to obtain the desired soil pH levels. The samples were analysed for E.D.T.A. extractable Cu (section 3.2.8), soil pH (section 3.2.5) and lime requirements (section 3.2.5). The results of the analysis are given in Table 6.1. They show the plots to be on marginally Cu-deficient soil (M.I.S.R., 1985) and that there were no significant differences in either extractable Cu concentrations or pH between plots. Therefore the site was acceptably uniform for use in this trial.

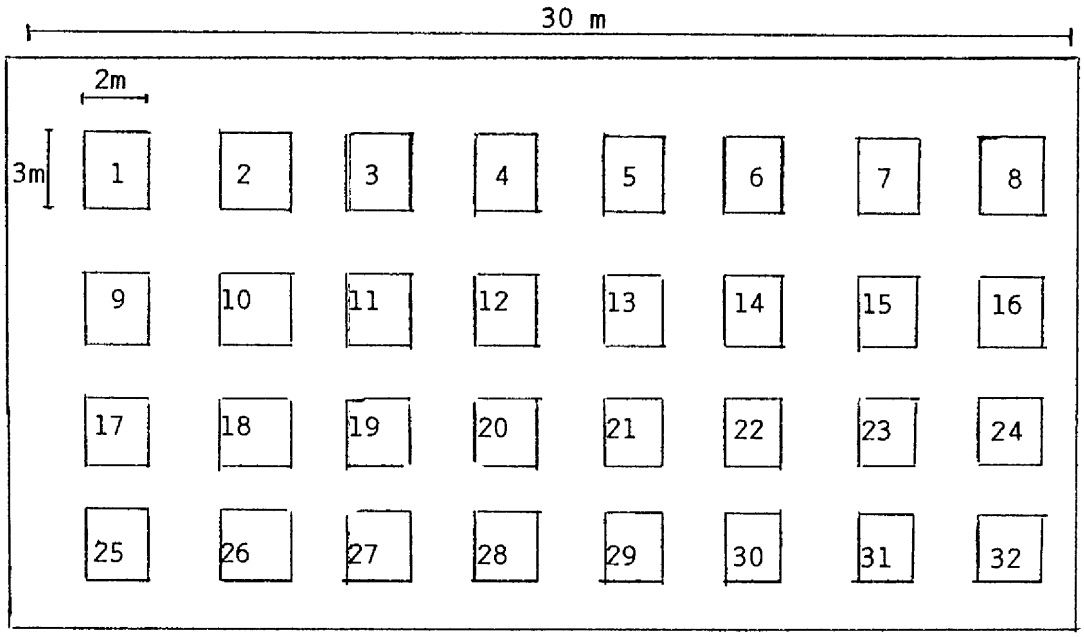
Table 6.1

Soil analysis results, November 1985

	Range of results	Mean	S.E.
E.D.T.A. extractable Cu (mg/kg)	3.21-4.27	3.82	0.36
Soil pH	4.95-5.2	5.1	0.10
Lime requirement (t/ha)		4.8	

Figure 6.1

Arrangement of herbage trial plots



The corresponding treatment for each individual plot is given overleaf:

Treatments

Plot	pH	Application rate (kg/ha)	Plot	pH	Application rate (kg/ha)	Plot	pH	Application rate (kg/ha)	Plot	pH	Application rate (kg/ha)
1	5.8	0	9	5.3	740	17	5.3	370	25	5.3	370
2	5.8	370	10	5.8	0	18	5.8	185	26	5.8	370
3	5.3	0	11	5.8	370	19	5.8	740	27	5.8	0
4	5.8	740	12	5.8	740	20	5.3	185	28	5.3	740
5	5.3	370	13	5.3	185	21	5.8	0	29	5.8	185
6	5.8	185	14	5.3	370	22	5.3	740	30	5.8	740
7	5.3	740	15	5.3	0	23	5.3	0	31	5.3	185
8	5.3	185	16	5.8	185	24	5.8	370	32	5.3	0

6.3.2 Treatments

Four rates of Cu fertiliser application were selected for study in this trial:

(i) 0 kg/ha, the control treatment.
(ii) 185 kg/ha, half the rate used in the animal response trial.
(iii) 370 kg/ha, the rate used in the animal response trial.
(iv) 740 kg/ha, double the rate used in the animal response trial.

These rates represent 0, 3.7, 7.5 and 15.0 kg Cu/ha respectively.

To determine whether pH has an effect on the availability of Cu in Cu fertiliser supplemented soil, the soil was limed to increase the pH to that recommended by M.I.S.R. and S.A.C. (1985) for optimum grassland production. Thus each application rate was applied at the following pH values.

(i) pH 5.3 the inherent pH of the site.
(ii) pH 5.8 the pH for grassland on mineral soil, recommended by M.I.S.R. and S.A.C. (1985).

Each of the eight treatments were replicated four times in a randomised block design of 32 plots (Figure 6.1).

6.3.3 Fertiliser and Lime Application

The Cu fertiliser was broadcast on to the plots on 20 December 1985. Wooden boards were erected around each plot prior to application to block out wind and prevent any drifting of the fertiliser. The correct amount of Cu fertiliser for each plot was placed in a 500 ml plastic bottle, the lid of which contained eight 5 mm holes. A good

even spread of the fertiliser was obtained by shaking the bottle approximately 3 ft from the ground.

The plots were limed with $CaCO_3$ on 18 February 1986. The lime was applied by hand.

A compound fertiliser (17% N, 17% P_2O_5, 17% K_2O) at a rate of 185 kg. N/ha was applied to each plot in April of 1986, 1987 and 1989 in accordance with normal management practice for the field. A further 185 kg N/ha was applied to each plot after the first cut of each year.

6.3.4 Herbage Harvesting

Two cuts per year were taken in 1986 and 1987 but only one in 1988, on the following dates:

8 July 1986;

3 September 1986;

24 June 1987;

26 August, 1987;

30 June 1988.

Slow growth due to the cold, wet climate of the area prevented any further cuts being obtained in the first 2 years.

Prior to harvesting each plot a subsample of herbage was taken from its centre using steel sheep shears. Herbage was cut approximately 3 cm from the soil surface to prevent trace element contamination from soil. This sample was weighed and retained for dry matter and trace elements analysis.

The remaining herbage on the plot was cut using an autosythe (Agria 450). The herbage was collected and weighed. The fresh weight yield of herbage for each plot was then determined by adding the weight of the subsample to that of the cut herbage. The cut herbage was then discarded. In 1988 the plots were only subsampled for analysis and no total yield was measured.

6.3.5 Herbage Analysis

A dry matter yield for each plot was determined first (section 3.3.2). The subsamples obtained in 1986 and 1987 were then analysed for total Cu, Zn, Fe, Pb, Cd, Cr and Ni (section 3.3.4). Eight samples from each cut were also analysed for Mo (section 3.3.5) and S concentrations (section 3.3.6) so that the amount of Cu in the herbage, which might be available to the animal could be determined (section 2.3.6). In 1988 samples were analysed for total Cu only.

6.3.6 Soil Sampling and Analysis

Two years after the Cu fertiliser had been applied representative bulk soil samples (section 3.2.1) from 0-5 cm and 5-10 cm depth were obtained from each plot. The samples were analysed for E.D.T.A. extractable Cu (section 3.2.8) and total soil Cu (section 3.2.9).

6.4 RESULTS AND DISCUSSION

6.4.1 Yield

The application of the Cu fertiliser at rates up to 740 kg/ha had no significant effect on herbage yields in any cut in 1986 or 1987. Therefore mean herbage yields for all treatments are given in Table

6.2. Although growing on marginally Cu-deficient soil, the herbage was not found to be Cu-deficient, less than 4 mg Cu/kg (M.I.S.R., 1985), at any time (Tables 6.3, 6.4, 6.5). Consequently Cu was not a rate limiting element in the growth of this herbage and so yield differences would not be expected.

Table 6.2

Mean herbage yields

	Fresh wt (kg)	Dry matter (g/kg)	Dry matter yield (kg)
Cut 1 1986	12.95	163	2.0
Cut 2 1986	7.2	174	1.2
Cut 1 1987	14.1	175	2.5
Cut 2 1987	13.1	173	2.3
S.E.D.	-	-	0.42

Table 6.3

Mean herbage Cu concentration 1986 (mg/kg)

Cut	Soil pH	Cu fertiliser applicaton rate (kg/ha)			
		0	185	370	740
1	5.3	8.70	9.03	9.77	9.89
	5.8	9.00	9.34	9.93	10.09
2	5.3	7.65	8.14	9.55	9.92
	5.8	7.36	8.27	8.85	9.55

S.E.D. = 0.46

Table 6.4

Mean herbage Cu concentrations 1987 (mg/kg)

Cut	Soil pH	Cu fertiliser application rate (kg/ha)			
		0	185	370	740
1	5.3	6.70	8.02	8.73	9.71
	5.8	6.88	7.62	8.46	9.47
2	5.3	7.70	8.89	9.32	10.95
	5.8	7.89	8.65	9.47	10.41

S.E.D. = 0.36

Table 6.5

Mean herbage Cu concentrations 1988 (mg/kg)

Cut	Soil pH	Cu fertiliser application rate (kg/ha)			
		0	185	370	740
1	5.3	5.95	6.09	6.62	9.41
	5.8	5.25	5.63	6.70	8.90

S.E.D. = 0.55

6.4.2 Cu Concentration of Herbage

The herbage Cu concentrations for each treatment in years 1986, 1987 and 1988 are given in Tables 6.3, 6.4, 6.5, respectively, and summarised graphically in Figures 6.2, 6.3 and 6.4. The results show that a single broadcast application of the Cu fertiliser to the sward, at all three application rates, produced significant increases ($p<0.01$ for cut 1 1986, $p<0.001$ for all other cuts) in herbage Cu

Figure 6.2. Herbage Cu concentrations in 1986 after a single application of Cu fertiliser in December 1985.

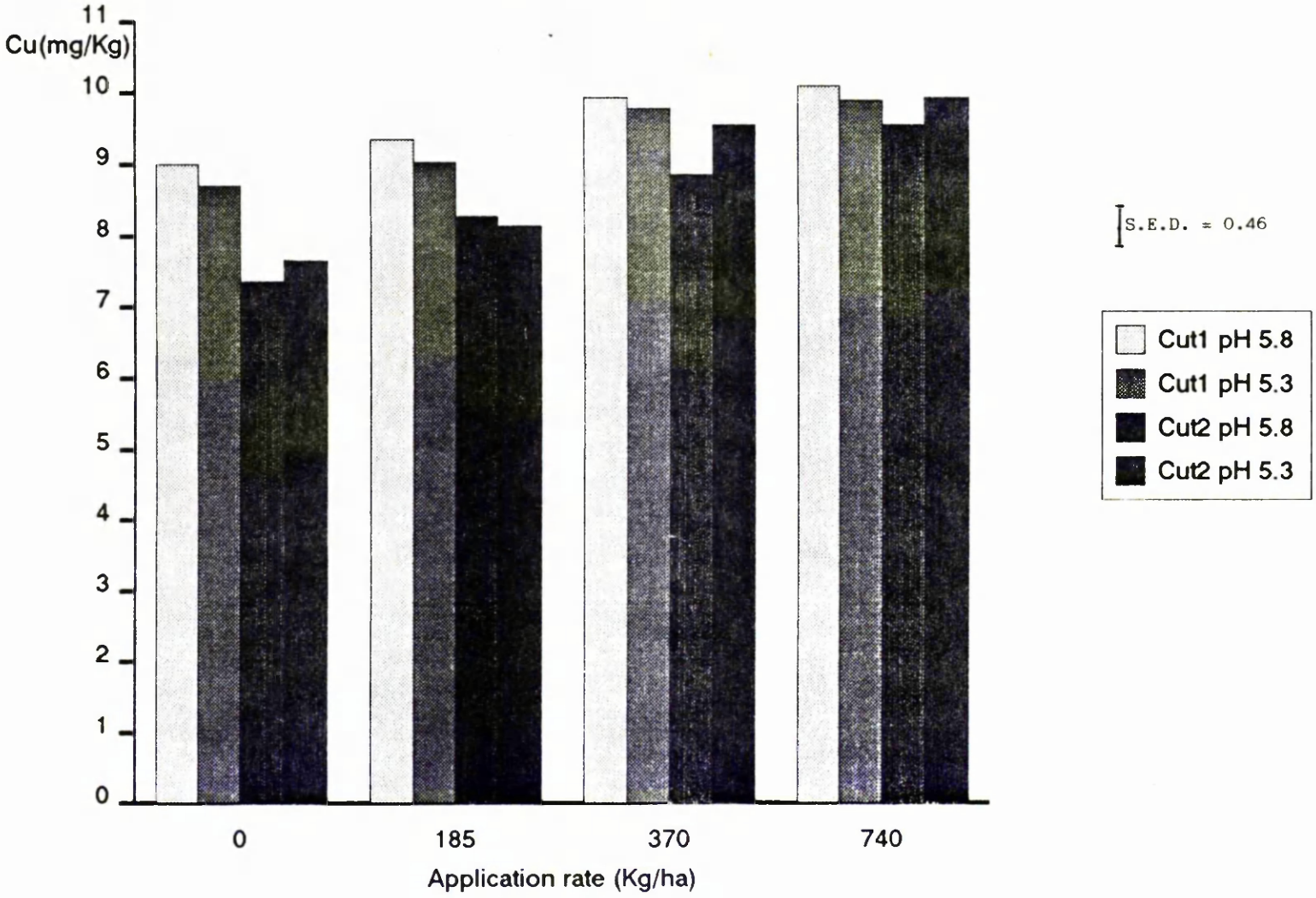


Figure 6.3. Herbage Cu concentrations in 1987 after a single application of Cu fertiliser in December 1985.

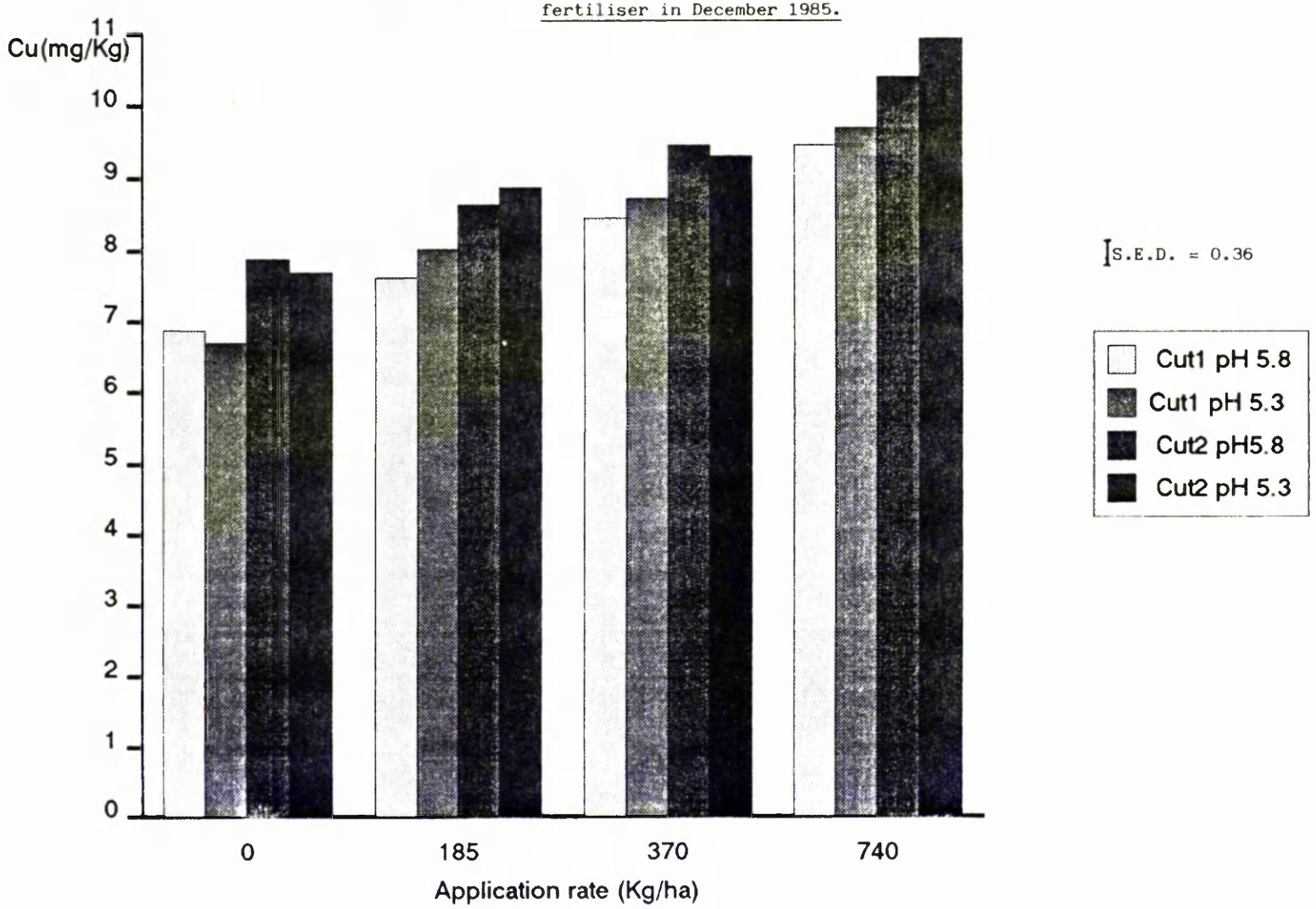
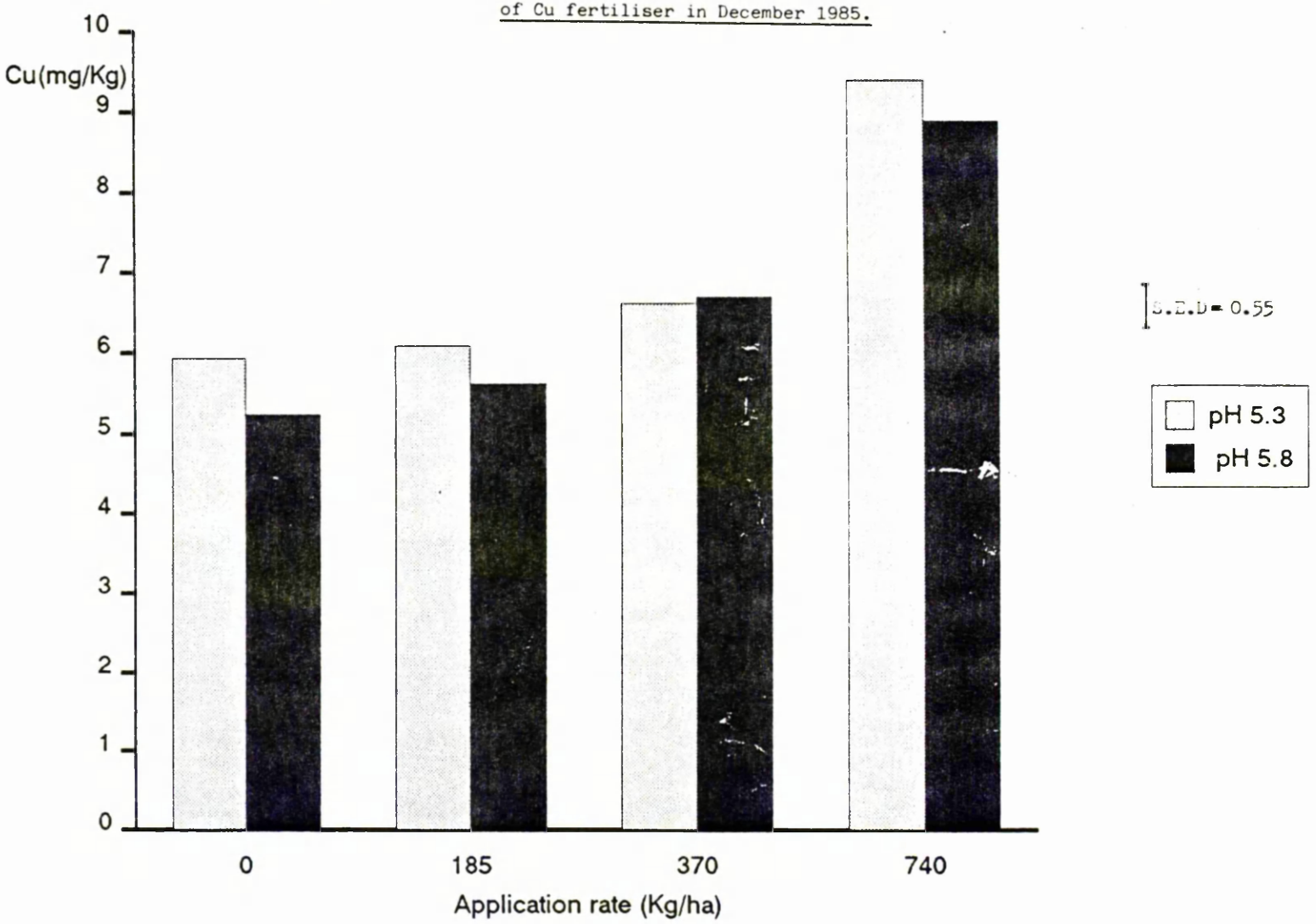


Figure 6.4. Herbage Cu concentrations in 1988 after a single application of Cu fertiliser in December 1985.



concentrations compared to control in all five cuts taken over a period of 31 months. There were no significant differences in herbage Cu concentrations between limed (pH 5.8) and unlimed (pH 5.3) plots at any application rate in any cut. As the lime was broadcast onto an established sward and would take a considerable time, up to one year, to fully affect the soil pH, differences might not be expected in the first year. In subsequent years the relatively small difference in soil pH between the two treatments, 5.3 and 5.8 was probably not enough to cause any significant effect on herbage Cu concentrations in the field. The 370 kg/ha Cu fertiliser application rate, as used in other experimental work, gave a 20% increase in herbage Cu concentrations.

6.4.3 Concentrations of other elements in the herbage

Significant differences ($p<0.001$) in herbage Zn concentrations were found between treatments. Zinc, therefore, showed similar effects to that of Cu; increasing fertiliser application rates resulted in significant increases in herbage Zn concentrations in both cuts in 1986 and 1987 (Table 6.6) when it was measured. A 370 kg/ha (7.5 kg Zn/ha) Cu fertiliser application rate gave a 20% increase in herbage Zn concentrations. Herbage Zn was not analysed in the 1988 samples, it can be assumed to have exhibited a similar effect to that of Cu based on the previous two years results and the fact that the same plant processes are used for Cu and Zn uptake (Bowen, 1981).

Table 6.6

Mean herbage Zn concentration (mg/kg)

Year	Cut	Cu fertiliser application rate (kg/ha)				S.E.D.
		0	185	370	740	
1986	1	41.16	42.53	47.70	48.31	2.15
	2	31.35	34.91	38.56	40.63	
1987	1	31.29	34.40	38.12	41.07	1.91
	2	32.36	36.85	41.77	46.45	

No significant differences in herbage Fe, Pb, Cd, Cr, Mn or Ni concentrations among treatments were found in either 1986 or 1987; trial mean concentrations of these elements for both years are given in Table 6.7. The concentrations of all the elements were well within acceptable levels (Table 6.7). The Cu fertiliser caused no toxicity problems in this trial. Based on these results it was assumed that these elements would not cause any problem in the future and so analysis for Fe, Pb, Cd, Cr, Mn or Ni concentrations were not carried out in 1988. As only 0.2% of the applied Cu was actually taken up by the plants it is suggested that if a similar ratio is used for Pb, Cd, Cr, Mn or Ni uptake then the increases would be undetectable as they are present in the Cu fertiliser at much smaller concentrations.

6.4.4 Available Cu in the Herbage

The amount of Cu in the herbage available to sheep when the herbage Mo and S concentrations were taken into consideration (section 2.3.6) increased with increasing Cu fertiliser application rates (Table 6.8) in years 1986 and 1987. As only eight samples from each cut, one per

Table 6.7

Mean trace element concentration of herbage

							Mean concentration (mg/kg)	
Element	1986	S.E.D.	1987	S.E.D.	1988	S.E.D.	Phytotoxic threshold*	Zootoxic threshold*
Cu	9.04	0.46	8.68	0.36	6.82	0.55	20	30
Zn	40.47	2.15	37.79	1.19	-	-	200	500
Fe	165.63	18.45	156.44	18.87	-	-	500	-
Mn	200.83	21.80	212.91	18.13	-	-	-	-
Cd	<0.01	-	<0.01	-	-	-	10	3
Pb	1.14	0.15	1.03	0.14	-	-	35	15
Ni	1.28	0.24	1.25	0.09	-	-	11	50
Cr	0.1	0.02	0.1	0.02	-	-	10	50

* Davies (1980)

treatment, were analysed for Mo and S the above statement is only a trend and cannot be said to be significant. However, as the concentrations of Mo and S in the herbage remained relatively consistent throughout the monitoring period it is suggested that since the Cu fertiliser did cause significant increases in herbage Cu concentrations it is likely that if all 32 samples had been analysed for Mo and S a significant increase in the concentration of available Cu would have been recorded as the Cu:Mo ratio would have been altered (section 2.3.6).

Table 6.8

Available Cu content of herbage after Mo and S are taken into consideration (mg/kg) (Suttle, 1981c)

Year	Cut	Soil pH	Cu fertiliser application rate (kg/ha)			
			0	185	370	740
1986	1	5.3	0.59	0.53	0.59	0.62
		5.8	0.48	0.55	0.57	0.59
	2	5.3	0.38	0.40	0.39	0.51
		5.8	0.40	0.39	0.61	0.67
1987	1	5.3	0.32	0.36	0.46	0.46
		5.8	0.21	0.53	0.46	0.52
	2	5.3	0.34	0.41	0.45	0.48
		5.8	0.26	0.43	0.45	0.51

6.4.5 Effects of Time on Herbage Cu Concentrations

The mean herbage Cu concentrations for each Cu fertiliser application rate in all five cuts are given in Table 6.9 and presented against

time in Figure 6.5. They show that there is an increase in herbage Cu concentrations for all three application rates over the control between the first and second cut. This is probably due to the distribution of the Cu fertiliser within the rooting zone improving over the summer to allow better uptake of Cu by the plant. The elevated herbage Cu concentrations, over the control, were maintained throughout the trial period for the 370 kg/ha Cu fertiliser application rate which implies that it is providing a constant supply of Cu to the rooting zone. The 185 kg/ha Cu fertiliser treatment also maintained a higher herbage Cu concentration than the control; however the herbage Cu concentrations for the control and 185 kg/ha treatment start to converge in the fifth cut suggesting that the effectiveness of this treatment is now diminishing. The interesting point is that there is a dramatic increase in the herbage Cu concentration of the 740 kg/ha treatment, compared to the control, between the first and last cuts. Thus the effectiveness of the 740 kg/ha Cu fertiliser application rate is increasing with time. The reason for this and how long the effectiveness of the treatment will last is unknown. It is possible that mixing of the soil by soil organisms has continued to improve the distribution of the Cu fertiliser within the soil over time with the largest application rate, which should release a greater amount of Cu, becoming the most closely integrated with the plant roots. The effectiveness of other Cu sources in the arable situation has been found to improve with time due to the constant mixing of the soil (Graham and Nambier, 1981). The results do however imply that the Cu fertiliser at both the 370 and 740 kg/ha application rate will continue to supply Cu to the plant into the future.

Figure 6.5. Mean herbage Cu concentration for each Cu fertiliser application rate in all five cuts versus time.

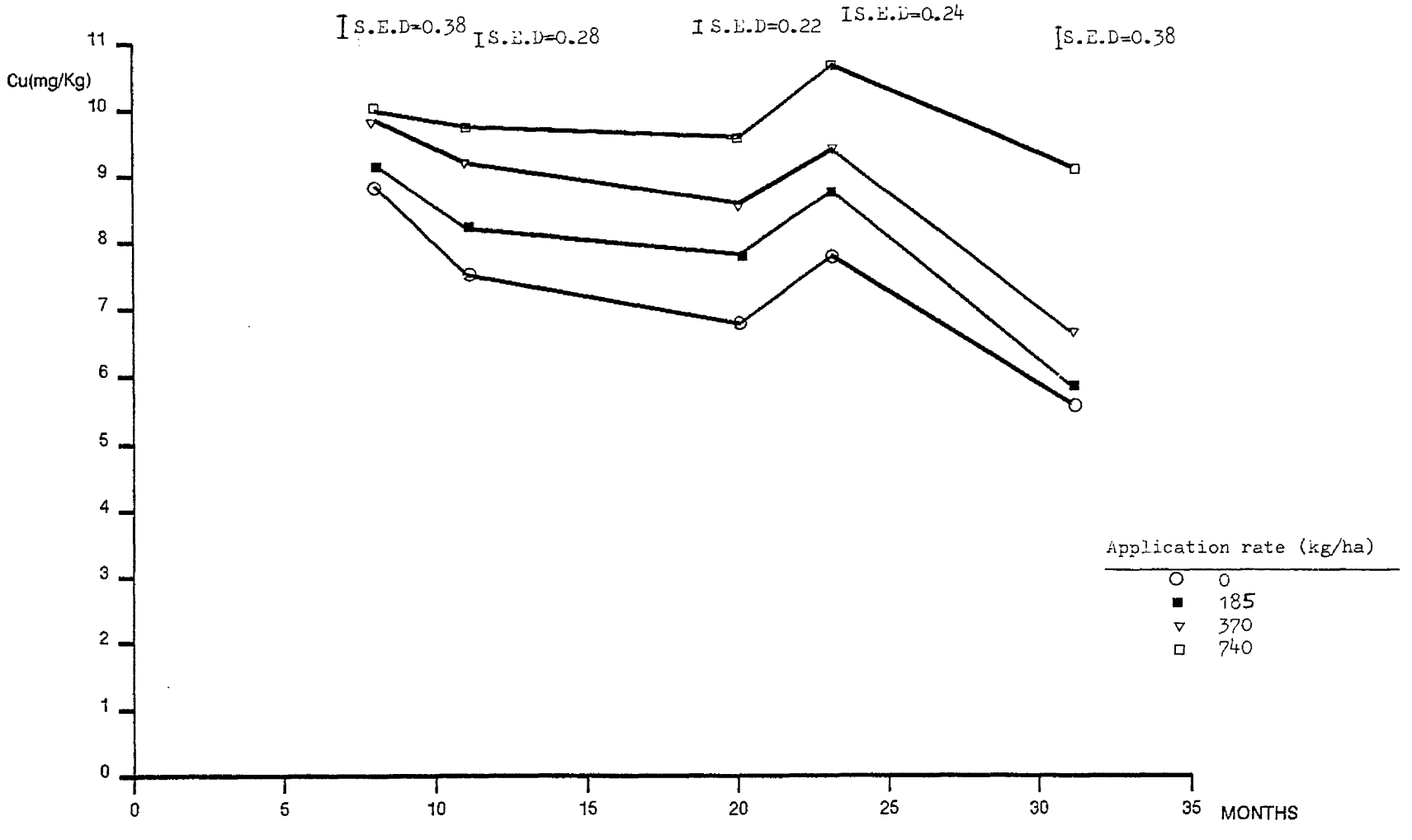


Table 6.9

Mean herbage Cu concentration for each Cu fertiliser application rate for all cuts

Year	Cut	Cu fertiliser application rate (kg/ha)				S.E.D.
		0	185	370	740	
1986	1	8.85	9.18	9.85	9.99	0.38
1986	2	7.51	8.21	9.20	9.74	0.23
1987	1	6.79	7.82	8.60	9.59	0.22
1987	2	7.80	8.77	9.40	10.68	0.24
1988	1	5.60	5.86	6.66	9.15	0.38

6.4.6 Soil Cu Concentrations

Soil sampling of plots, two years after the initial application revealed a thin grey line in the soil cores approximately 2 cm from the surface. As the thickness of the line appeared to increase in the plots with the highest application rates, it was examined and found to be the Cu fertiliser still present in an observable form. Soil analysis showed elevated soil Cu concentrations in the top 5 cm of the soil (Table 6.9). The 370 kg/ha Cu fertiliser application rate increased the total Cu concentration in the top 5 cm of the soil by an average of 7 mg/kg. This is very close to the 7.5 mg/kg increase in soil Cu concentration that should in theory be obtained by a 370 kg/ha Cu fertiliser application rate. Increasing application rates resulted in increased total soil Cu concentrations in the top 5 cm only (Figure 6.5 and Table 6.9). This increasing soil Cu concentration in the top 5 cm with each application rate increment is emphasised more by the E.D.T.A. extractable soil Cu concentration results (Figure 6.7). It

Table 6.10

Mean E.D.T.A. extractable and total soil copper concentratons at 0.5 cm and 5-10 cm depth

	Limed				Unlimed			
Cu fertiliser application rate (kg/ha):	**0**	**185**	**370**	**740**	**0**	**185**	**370**	**740**
E.D.T.A. extractable Cu, 0-5 cm depth (mg/kg)	4.1	6.6	10.2	10.3	3.8	7.4	10.5	11.9
E.D.T.A. extractable Cu, 5-10 cm depth (mg/kg)	3.6	3.9	3.9	3.9	3.6	3.9	3.5	3.8
Total Cu, 0-5 cm depth (mg/kg)	24.5	27.7	31.0	31.0	24.7	33.4	31.3	40.9
Total Cu, 5-10 cm depth (mg/kg)	22.9	23.9	22.7	23.6	24.1	21.0	22.2	23.5

Figure 6.6. Total soil Cu concentration at 0-5 and 5-10 cm depth.

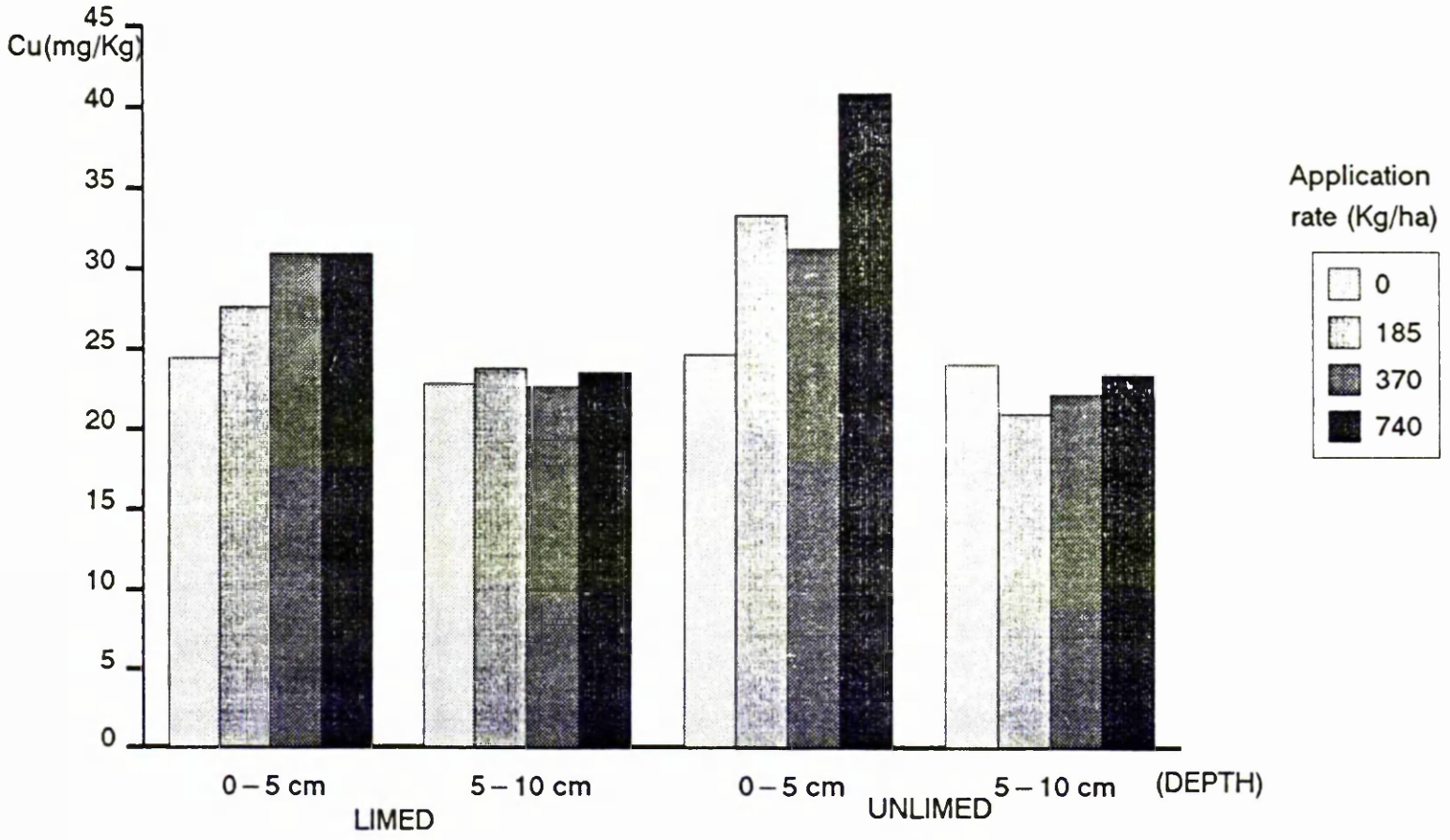
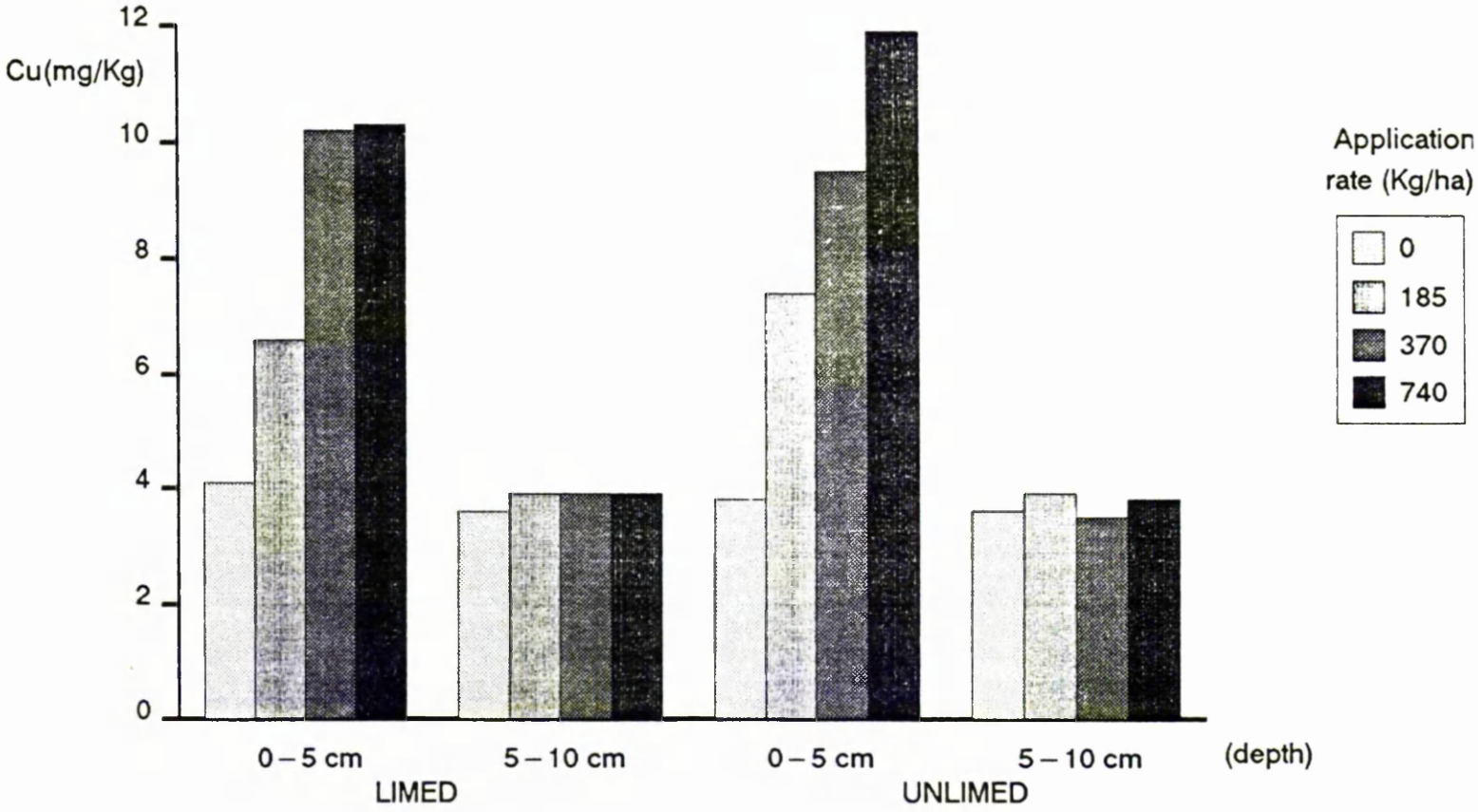


Figure 6.7. E.D.T.A. extractable soil Cu concentration at 0-5 and 5-10 cm depth.



can be concluded that the Cu fertiliser does increase soil Cu concentrations; it is immobile in the soil and remains in the top 5 cm of the soil.

6.4.7 Conclusions

This new slow release Cu fertiliser when applied to the soil does increase the uptake of Cu by herbage. After application it appears to degrade very slowly and is still present in an identifiable form in the soil two years later. During this time it has released enough Cu to significantly increase herbage Cu concentrations and this effect was carried over into a third year. A similar effect was shown for Zn. These increases were not accompanied by increases of Fe, Pb, Cd, Cr, Mn or Ni concentrations in the herbage. The concentrations of all the elements monitored in the herbage were within acceptable toxicity limits and Cu fertiliser application had no positive or deleterious effects on herbage yields at rates of up to 740 kg/ha (15.0 kg Cu/ha). The Cu fertiliser has provided a source of Cu to herbage over the three year monitoring period with the possibility of similar provision in the future as it is still present in the soil.

Although the Cu fertiliser had a significant effect on the Cu content of the herbage it appears to be no more effective than copper sulphate or other less soluble Cu forms would have been under similar conditions. The 2-4 mg/kg increase in herbage Cu concentrations obtained by use of the Cu fertiliser is equivalent to those found elsewhere using similar Cu application rates in the form of $CuSO_4.5H_2O$ or other soil applied Cu treatments (Reith, 1983; Evans, 1983). All of these have been found to be effective for 2-4 years in the soil,

which the Cu fertiliser has at least matched and may exceed in future years.

The 7.5 kg/ha (370 kg/ha) Cu fertiliser application rate used in the Garmore ewe response trial (Chapter 5) has been shown by these results to be an effective Cu fertiliser for herbage. This application rate gave an mean 20% increase in herbage Cu concentrations for three successive years in this herbage trial. The possible use of other Cu fertiliser application rates for grazed sward will be discussed later.

6.5 SUMMARY

6.5.1 Broadcast application of the Cu fertiliser to established sward had no beneficial or deleterious effects on herbage yields at rates of up to 740 kg/ha.

6.5.2 Observation and analysis showed it to be present in the top 5 cm of the soil two years after surface application. The Cu fertiliser appears to be relatively immobile and only sparingly soluble in the soil.

6.5.3 Application of the Cu fertiliser increased herbage Cu concentrations. Increasing application rates produced significant increases in herbage Cu concentrations in three successive years after a single application.

6.5.4 Liming of the soil from pH 5.3 to 5.8 had no significant effect on herbage Cu concentrations at any application rate.

6.5.5 Increasing fertiliser application rates also produced significant increases in herbage Zn concentrations. There were

no corresponding increases in herbage Pb, Fe, Ni, Mn, Cr or Cd concentrations.

CHAPTER 7. ANIMAL RESPONSE TRIAL, PINMACHER FARM

7.1 INTRODUCTION

The first years results from the ewe response trial at Garmore Farm demonstrated that application of the Cu fertiliser at 370 kg/ha, as a top dressing to grassland significantly raised plasma Cu concentrations of ewes grazing the treated pasture above those of control ewes where plasma Cu concentrations actually declined over the winter and spring months. However, it was thought that ingestion of the Cu fertiliser by the ewes contributed towards this effect (Chapter 5). A second field trial was therefore initiated to determine if a lower application rate, with the consequent reduced potential for surface contamination and ingestion, would result in comparable increases and maintenance of sheep plasma Cu concentrations to those found in the first animal trial. The application rate chosen was 250 kg/ha Cu fertiliser. This was thought to be a sufficient reduction to reduce surface contamination whilst still high enough to raise herbage Cu concentrations. The herbage trial at Garmore (Chapter 6) had previously demonstrated that an application rate of 185 kg/ha was sufficient to raise herbage Cu concentrations by approximately 1 mg/kg. In addition it was hoped that this trial would confirm the results of the Garmore trial, i.e. that the Cu fertiliser was an effective source of Cu for the grazing sheep.

At Pinmacher the milder climate helped give sufficient herbage growth to allow sampling at any time of the year. Thus samples could be taken before and after Cu fertiliser application in order to monitor the longevity of surface contamination. This was not possible in the

Garmore trial as herbage samples could not be obtained until spring growth occurred.

Incorporation of a Cu needle treatment into the trial allowed comparison of sheep grazing pasture naturally low in Cu, with both those treated with a recognised method of preventing Cu deficiency (section 2.4.2) and with those grazing pasture treated with the Cu fertiliser at 250 kg/ha.

7.2 EXPERIMENTAL

7.2.1 Site Description

The field site was situated at Pinmacher Farm near Girvan in South West Scotland (O.S. map reference NX235888). The farm has a history of Cu deficiency problems due to low soil Cu concentrations and was severely affected in the Spring of 1986 with many swayback lambs.

The area used was a recently reseeded pasture (1984) on a free draining brown forest soil of the Benan series. It is at an elevation of 100-120 m and receives an average annual rainfall of 900 mm. Within this area a 3 ha paddock had been fenced off for silage production in previous years but was now used for grazing; it was to this paddock that the Cu fertiliser was applied.

Soil samples were taken at 0-10 cm depth from the paddock and surrounding hillside to assess the uniformity of fertility and Cu status of the two areas. The samples were then analysed for E.D.T.A. extractable Cu (section 3.2.8), pH (section 3.2.4), % loss on ignition (section 3.2.6), lime requirments (section 3.2.5), available K

(section 3.2.7), and available P (section 3.2.7). The results listed in Table 7.1 show that the site does have a low soil Cu concentration (M.I.S.R., 1985) and that the two areas were acceptably uniform for use in this trial.

Table 7.1

Pinmacher response trial, soil analysis results

Analysis	Paddock	Surrounding hillside
% loss on ignition	15.5	15.5
pH	5.54	5.55
Available P (mg/l)	16	9.1
Available K (mg/l)	139	100
E.D.T.A. extractable Cu (mg/kg)	1.61	1.43

7.7.2 Cu fertiliser application

The Cu fertiliser was applied to the paddock as a single broadcast application to the existing sward, at a rate of 250 kg/ha, which is equivalent to 5 kg Cu/ha, on 24 January 1987. A Vicon Varispreader was used to apply the fertiliser as in section 5.3.2. A wind free day prevented drifting and a visibly even spread of the fertiliser was obtained.

7.2.3 Sheep Selection and Treatment

On 28 October 1986 sixty Suffolk x Scottish Blackface hoggs* were selected and randomly divided into 3 groups of 20. Each hogg was then

*female sheep from weaning to first shearing

tagged for identification. One group was then allowed to graze the 3 ha paddock from 7 February, 2 weeks after Cu fertiliser application. The two other groups, one of which was dosed with 5 g Cu needles on 28 October 1986 and again on 11 March 1987, were released onto the surrounding pasture. Thus there were three treatment groups:

(a) Control group grazing untreated sward (n=20).

(b) Grazing untreated sward but dosed 5 g Cu needles (n=20).

(c) Grazing Cu fertiliser treated sward from 7 February 1987 (n=20).

As the hoggs were sold after shearing the trial was repeated with 60 new hoggs in 1987-88; the initial selection and Cu needle treatment of the new sheep occurred on 8 October 1987 in the second year of the trial.

7.2.4 Blood Sampling and Analysis

At intervals of approximately every six weeks throughout the winter and spring, blood samples were taken from all animals in the trial. The blood was analysed for the parameters listed below using the procedures given in section 3.4.

(i) Plasma Cu - to assess the Cu status of the hogg.

(ii) Haemoglobin - to check for anaemia which is a symptom of Cu deficiency in animals.

(iii) Plasma Mg - to monitor for hypomagnesaemia problems

(iv) Glutathione peroxidase - to monitor Se status.

7.2.5 Herbage Sampling and Analysis

Herbage samples were obtained at regular intervals between December 1986, before Cu fertiliser applicaton, and April 1988 when the trial was completed. Samples were taken as described in section 5.3.7 and were analysed for total Cu concentration only.

7.3 RESULTS

7.3.1 Plasma Cu Concentrations 1986 to 1987

Mean plasma Cu concentrations for each treatment group of hoggs in the first year of the trial are given in Table 7.2 and presented graphically in Figure 7.1. Administration of Cu needles on 28 October 1986 and 11 March 1987 produced plasma Cu concentrations which were maintained significantly above ($p<0.001$) those of the control group for the duration of the trial. The two doses of 5 g Cu needles were sufficient to prevent Cu deficiency for the full 35 weeks of the trial. These results are similar to those obtained in the Garmore ewe response trial (Chapter 5) and for sheep by other workers (Suttle, 1981; Whitelaw **et al**, 1983) and demonstrate that Cu needles are an effective method of preventing Cu deficiency in hoggs on this farm. Therefore the Cu needle treatment was again a suitable yardstick against which to compare the effectiveness of the new Cu fertiliser.

Figure 7.1 also shows that prior to the application of the Cu fertiliser, the mean plasma Cu concentration of the hoggs grazing both the paddock and the control area, declined at a similar rate with no significant differences from October until the end of January. The control and Cu fertiliser groups had six and five hoggs,

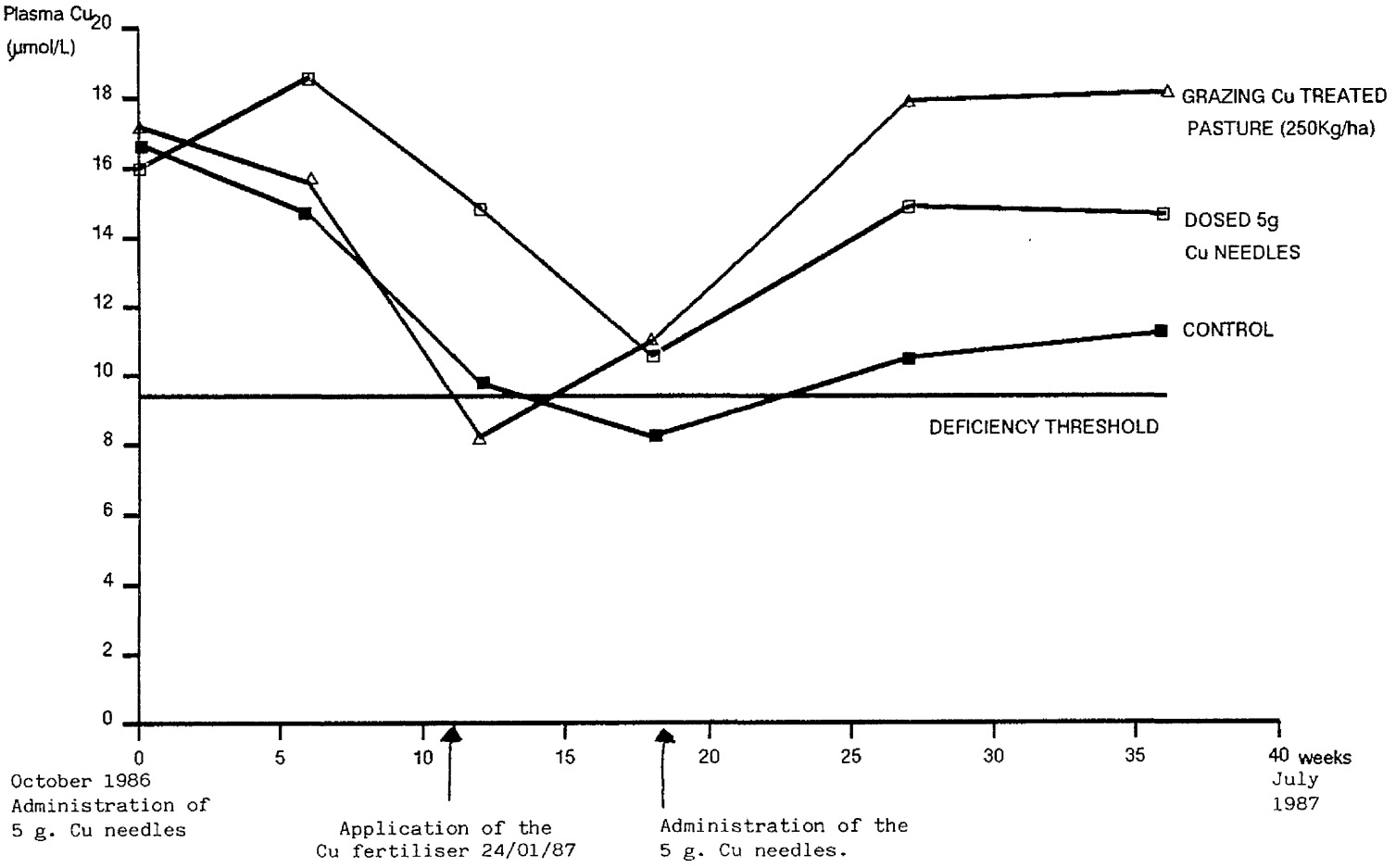
Table 7.2

Mean hogg plasma Cu concentrations October 1986 to July 1987

	Plasma Cu concentration (μmol/l)			
Date of sampling	**Control**	**Cu needles**	**Grazing Cu treated pasture**	**S.E.D.**
28/10/86	16.7^{a}	16.0^{a}	17.2^{a}	0.95
14/12/86	14.7^{a}	18.6^{b}	15.6^{a}	1.36
29/01/87	9.8^{a}	14.8^{b}	8.2^{a}	0.94
11/03/87	8.2^{a}	10.6^{b}	11.0^{b}	0.84
12/05/87	10.5^{a}	14.9^{b}	17.9^{c}	0.94
01/07/87	11.2^{a}	14.7^{b}	18.2^{c}	0.89

Means with different superscripts in any row are significant at $p<0.001$ (analysis of variance, EDEX, A.F.R.C., Statistics Unit, Edinburgh).

Figure 7.1: Mean hogg plasma Cu Concentrations October 1986 to July 1987.



respectively, with plasma Cu concentrations below the deficiency threshold on 29 January 1987. However, after application of the Cu fertiliser, and the reintroduction of the 20 hoggs to the treated pasture 14 days later, the mean plasma Cu concentration of the Cu fertiliser hoggs increased to be significantly ($p<0.001$) above that of the control hoggs which remained below the deficiency threshold for a further 10 weeks. By 11 March 1987 the Cu fertiliser treatment had produced plasma Cu concentrations which were equivalent to those obtained by administering Cu needles and 10 weeks later they were significantly higher ($p<0.001$) than those of the Cu needle treatment and both were significantly higher than the mean plasma Cu concentration of the control group. The elevation in the plasma Cu concentration, from 8.2 to 18.2 µmol/l, of the hoggs grazing the Cu fertiliser treatment was maintained until the end of the trial on the sale of the hoggs in July 1987. Thus a similar effect to that at Garmore (Chapter 5) was achieved but with the lower application rate, the increase in Cu concentration took longer, 12 weeks compared with less than seven weeks at Garmore. In addition the results for Cu fertiliser treatment were again comparable to those obtained using oral dosing with Cu needles.

7.3.2 Haemoglobin, Plasma Mg and Gluthathione Peroxidase Concentrations

No significant differences in haemoglobin, plasma Mg or gluthathione peroxidase concentrations in the blood were found among the three treatment groups; trial mean concentrations for each of these parameters on each sampling date are given in Table 7.3. The majority of individual results were within the normal range for each of these

parameters in sheep (Appendix 2), any other result was only marginally outside its normal range. Therefore as only plasma Cu concentrations showed significant differences or deficiencies among the groups it was purely a potential Cu deficiency problem on the farm. Thus analysis for these other parameters was not carried out in the second year of the trial.

Table 7.3

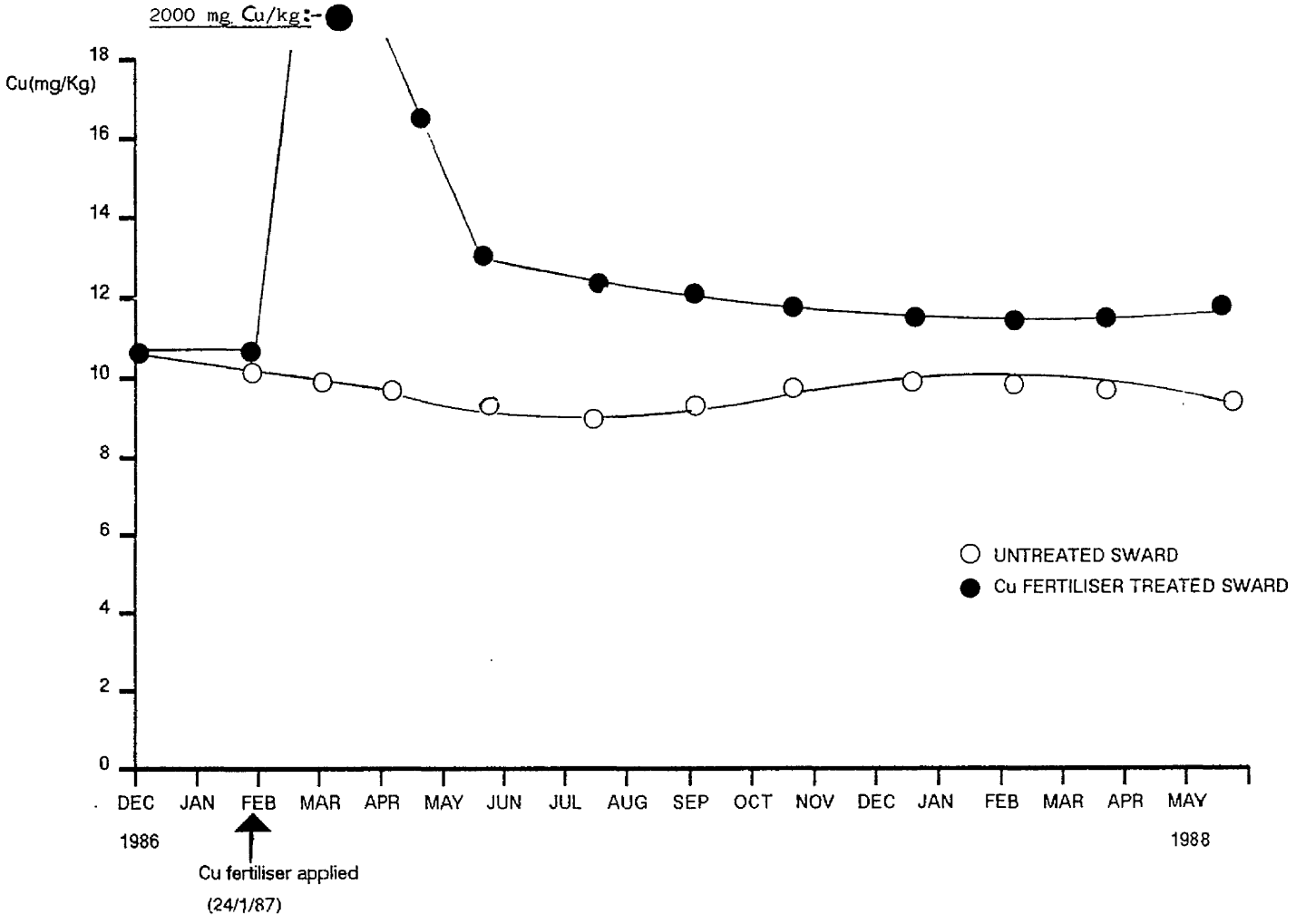
Mean Hb, Plasma Mg and GSH-Px Concentrations on each Sampling Date

Date of sampling	Haemoglobin	Plasma Mg	Gluthathione peroxidase
28/10/86	12.7	2.1	32.1
14/12/86	10.1	1.9	49.1
29/01/87	13.0	1.8	80.8
11/03/87	11.6	1.9	62.3
12/05/87	11.4	1.9	81.9
01/07/87	8.7	2.2	60.4
S.E.D.	0.57	0.16	8.56

7.3.3 Herbage Cu Concentrations

Herbage Cu concentrations for the Cu fertiliser treated paddock and the surrounding pasture are shown in Figure 7.2; they show a dramatic increase in the herbage Cu concentration from 11 mg Cu/kg to over 2000 mg Cu/kg one week after application of the Cu fertiliser. As at Garmore (section 5.5.6) this very high Cu concentration can only be due to the Cu fertiliser coating the surface of the herbage. This adherence to the herbage slowly declined over the next 10 weeks,

Figure 7.2: Herbage Cu Concentrations for Cu Treated and Untreated Sward at Pinmacher.

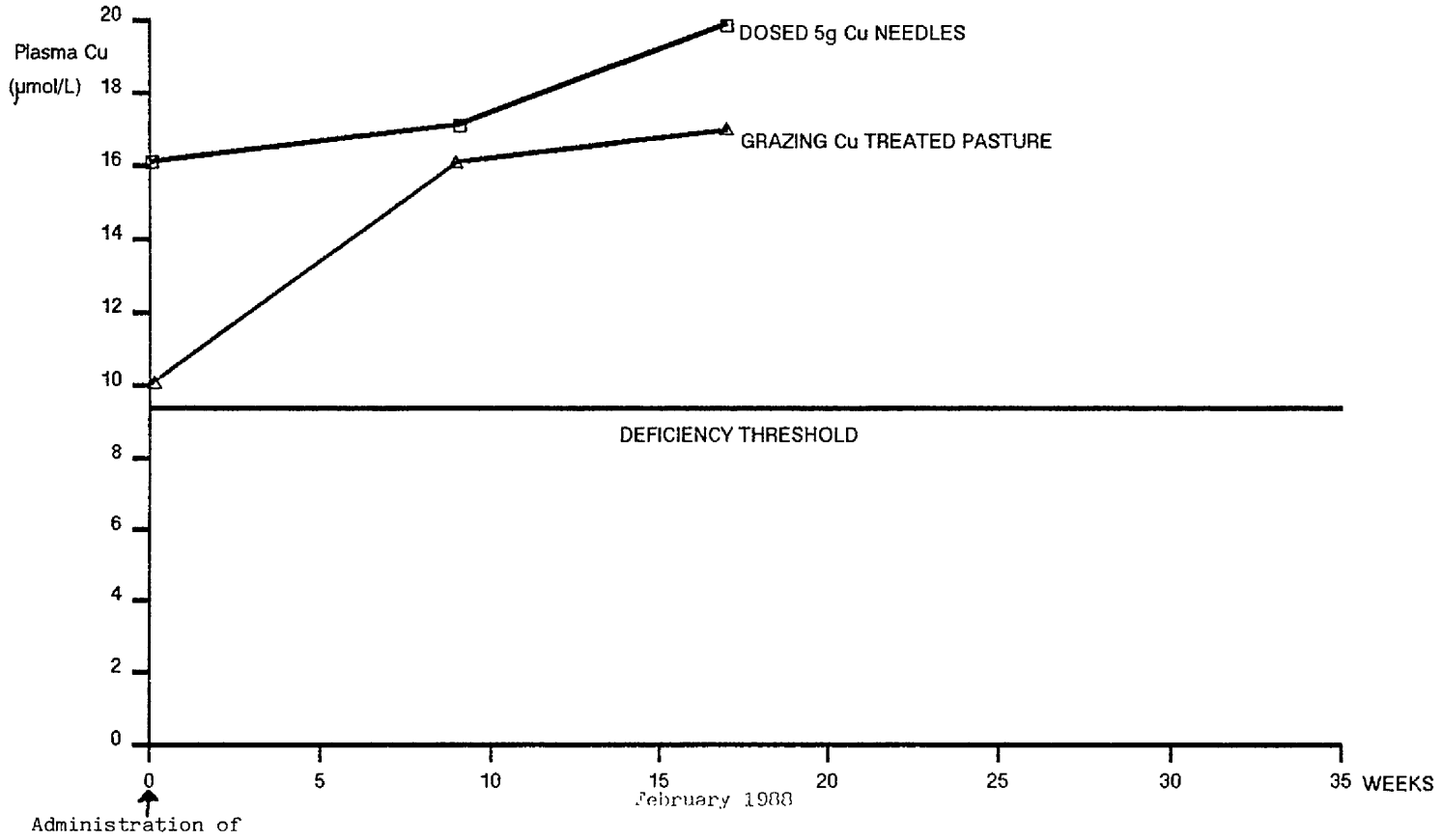


probably due to a combination of herbage growth, rainfall and grazing. By early May the treated herbage had a Cu concentration only 3 mg/kg higher than that of the surrounding hillside. The Cu fertiliser treated herbage maintained a 2 to 3 mg Cu/kg advantage in Cu throughout the 12 remaining months of the monitoring period, to May 1988. This differential is probably due to absorption by the plant. As at Garmore these results imply that the increase in plasma Cu concentrations of the hoggs (7.3.1) was probably due to ingestion of the Cu fertiliser when it was adhering to herbage rather than to the increased herbage Cu concentrations found after May 1987.

7.3.4 Plasma Cu Concentrations 1987 to 1988

Sixty new hoggs were introduced to the trial site on 8 October 1987. Twenty were allowed to graze the Cu treated pasture, twenty were dosed on that date with 5 g Cu needles and twenty kept for control animals. The mean plasma Cu concentration for each group of hoggs on each sampling date are given in Table 7.4 and presented graphically in Figure 7.3. Unfortunately all twenty control hoggs were accidentally dosed with Cu needles by the farmer between the first and second sampling date. However, the trial was continued although the significance of the second years results cannot be determined due to the lack of a suitable control group for comparison. The hoggs treated with Cu needles demonstrated the expected increase in plasma Cu concentration after administration and this was maintained until the trial was terminated seventeen weeks later. Introduction of the new hoggs to the Cu fertiliser treated paddock resulted in an increase in their mean plasma Cu concentration from 10.05 to 16.1 µmol/l within ten weeks at a time of year when, based on the first years control

Figure 7.3: Mean Hogg Plasma Cu Concentrations October 1987 to 1988.



group and general experience, plasma Cu concentrations usually decline. This increased plasma Cu concentration was maintained until 22 February 1988. The results had demonstrated by this date that the low application rate used here continued to have a residual effect on the animal one year after application. However, because of the lack of a suitable control group the trial was brought to a close as reliance could not be put upon the findings.

Table 7.4

Mean hogg plasma Cu concentrations October 1987 to February 1988

	Plasma Cu concentration (µmol/l)		
Date of sampling	**Control**	**Cu needles**	**Grazing Cu treated pasture**
08/10/87	12.8	16.1	10.1
18/12/87	-	17.2	16.1
22/02/88	-	19.9	17.0

Figure 7.2 suggests that by the start of the second year of the trial on 8 October 1987 there was little or no surface contamination of the herbage with the Cu fertiliser and as the sward cover was very good, even over the winter, it is unlikely that the sheep ingested much soil and associated Cu fertiliser. Therefore as the hoggs were new to the paddock the increased herbage Cu concentration was probably the major contributor to the increase in plasma Cu concentrations although the possibility of some ingestion of the Cu fertiliser along with soil in this second year cannot be discounted.

7.3.5 Conclusions

The results demonstrate that the lower Cu fertiliser application rate has resulted in a comparable increase in and maintenance of the plasma Cu concentration of sheep grazing the Cu treated sward to that found at Garmore.

In the first year the main reason for the initial increase and maintenance of plasma Cu concentrations was probably due to direct ingestion of the Cu fertiliser as it coated the herbage in the first eleven weeks after application. This supports the hypothesis put forward in Chapter 5 that the initial increase of plasma Cu concentrations in the ewe response trial were due to direct ingestion of the Cu fertiliser from coated herbage. However, the increase in plasma Cu concentrations took longer than at Garmore. This is probably due to the lower application rate producing less surface adherence than at Garmore. The surface contamination in this trial lasted only eleven weeks compared with over thirty weeks at Garmore although at Garmore the period was probably extended by the ewes trampling the ground and re-contaminating the herbage over the summer months. At Pinmacher the hoggs were sold at the start of the summer and this coincides with reduction in adherence. Hence the lower Cu fertiliser application rate appears to reduce slightly the possibility of direct ingestion of the Cu fertiliser, but was probably still the cause of the increased plasma Cu concentration in the first year.

When twenty new hoggs were introduced to the Cu fertiliser treated paddock one year after the application of the Cu fertiliser, there was also, within ten weeks, a significant increase in their plasma Cu

concentrations. Levels were then maintained above the deficiency threshold over the subsequent winter. It appears that the 2 to 3 mg/kg increase in herbage Cu concentrations obtained by use of the Cu fertiliser was sufficient to raise the level of Cu intake by the grazing animal and prevent Cu deficiency. The validity of this result cannot be guaranteed due to the lack of a suitable control group. However, if they are compared with the first years control group results or to the seasonal changes in plasma Cu concentration found in the control group at Garmore (section 5.6.1) then it appears that the Cu fertiliser has shown residual effects in the second year of the trial. The fact that this occurs is promising as at Garmore the plasma Cu of ewes newly introduced to the treated paddock did not increase until new herbage growth occurred in the spring and so the result was not so clear cut.

The single Cu fertiliser application was effective for at least two years and results were comparable to those obtained using oral dosing of sheep with Cu needles. However, over the two year period the sheep were dosed with Cu needles on 3 occasions compared with the single Cu fertiliser application. Application of the Cu fertiliser could offer a substantial saving in time and labour costs, as each Cu needle treatment involves collection of the sheep, dosing and then returning the sheep to the pasture. In this trial the Cu fertiliser has produced results better than those obtained using other soil Cu treatments by other workers (2.4.2); it has provided protection from Cu deficiency for a longer time period. However it is only in the second year that the results could be due to increased herbage Cu concentrations and the trial would have had to continue for at least

one more year to confirm this.

In summary this trial has again shown that application of the Cu fertiliser is an effective method of preventing Cu deficiency in sheep. The lower Cu fertiliser application rate, 250 kg/ha compared with 370 kg/ha at Garmore, has reduced the potential for surface contamination of the herbage but has still produced comparable increase in and maintenance of plasma Cu concentrations of sheep grazing the treated sward. The results support the hypothesis put forward in Chapter 5 that the initial increase in plasma Cu concentrations was due to direct ingestion of the Cu fertiliser. However, the Cu fertiliser also produced increased herbage Cu concentrations which were sufficient to maintain plasma Cu concentrations above the deficiency threshold in twenty new sheep introduced in the second year which could not have directly ingested the Cu fertiliser coated onto herbage. The Cu fertiliser also still offered the potential of a similar response in subsequent years as elevated herbage Cu concentrations were maintained up to the end of the monitoring period.

7.4 SUMMARY

7.4.1 The lower application rate does reduce the potential for surface adherence of the Cu fertiliser. It does not, however, remove it and the results demonstrated that the Cu fertiliser was available for ingestion by the grazing sheep.

7.4.2 The Cu fertiliser has produced comparable increases in and maintenance of sheep plasma Cu concentrations to those found at

Garmore (Chapter 5) despite a lower application rate.

7.4.3 The increased plasma Cu concentrations of twenty new sheep introduced to the Cu fertiliser treated sward in the second year of the trial showed that the Cu fertiliser has a residual effect of at least two years.

7.4.4 The results obtained using the Cu fertiliser were comparable to those obtained using Cu needles but probably involved less time and energy. Thus the trial has demonstrated that the Cu fertiliser treatment is an effective method of preventing Cu deficiency in sheep.

7.4.5 The results support the hypopthesis (Chapter 5) that the initial increase in plasma Cu concentration was due to direct ingestion of the Cu fertiliser, with subsequent maintenance being due to the increased Cu concentration of the herbage.

CHAPTER 8. LEAF ADHERENCE TRIAL

8.1 INTRODUCTION

Results from the animal response trial (Chapter 5) showed elevations in herbage Cu concentrations of over 100 mg/kg in the first six months after the Cu fertiliser application. These increases cannot be entirely due to plant uptake of Cu, as concentrations above 30 mg/kg in herbage are phytotoxic (Davies, 1980). In addition the herbage field trial (Chapter 6) showed that a Cu fertiliser application rate of 370 kg/ha only gave a 2-4 mg/kg increase in herbage Cu concentrations. This suggests that some of the Cu fertiliser is adhering to the herbage, especially in the first six months after application. If this is the case then the grazing animals are actually ingesting Cu fertiliser which has either coated the herbage on application or has soiled the herbage due to the trampling action of the sheeps feet. However, herbage sampling at the two sites, especially at Garmore, was irregular in the period immediately after Cu fertiliser application as the sward was short and not suitable for cutting without contamination from soil at the time of sampling. Therefore, in order to obtain evidence on which to base advice for the control of direct ingestion of the Cu fertiliser by the grazing animal and thus minimise the risk of any potentially harmful effects, a more detailed study of its persistence on herbage after a broadcast application was required. This experiment may also provide evidence as to whether surface adherence or soiling was the main cause of the elevated Cu concentrations.

8.2 OBJECTIVES

The aim of this trial was to:

(a) Measure the longevity of adherence of the Cu fertiliser to herbage after a broadcast application.

(b) Estimate the interval required between broadcast application and the introduction of animals to the treated sward, by correlating the results of (a) with daily rainfall data.

8.3 EXPERIMENTAL

In the two field trials (Chapter 5 and 7), for experimental expediency, the Cu fertiliser was applied in the late autumn/winter period. Thus, this trial was carried out from January to March 1988 for comparison. As herbage growth is minimal at this time it is fair to assume that little dilution of adherence due to herbage growth would occur. This should ensure that any reduction in herbage Cu concentration was due to the action of the weather and physical characteristics of the material only.

The Cu fertiliser was broadcast, to two 16 m^2 areas of established herbage, at an application rate of 370 kg/ha as used in both Garmore trials (Chapter 5 and 6). The herbage was sampled at weekly intervals and additionally after periods of heavy rainfall. Analysis of herbage Cu concentration was carried out and the results correlated with daily rainfall data.

8.3.1 Site Description

Two neighbouring 4 x 4 m areas of uniform, established sward within the West of Scotland College estate. Sixteen 1 x 1 m plots were marked out within each area, thus creating 32 individual plots. Each plot was then subdivided into quarters to create four subplots (Figure 8.1). One 4 x 4 m area was used for weekly herbage sampling and the other kept for sampling after periods of heavy rainfall.

8.3.2 Fertiliser Application

The Cu fertiliser was broadcast at a rate of 370 kg/ha to alternate plots using the procedures described in section 6.3.3 on 18 January 1988. Thus 16 plots were treated with Cu fertiliser and 16 retained as controls (Figure 8.1).

8.3.3 Herbage Sampling and Analysis

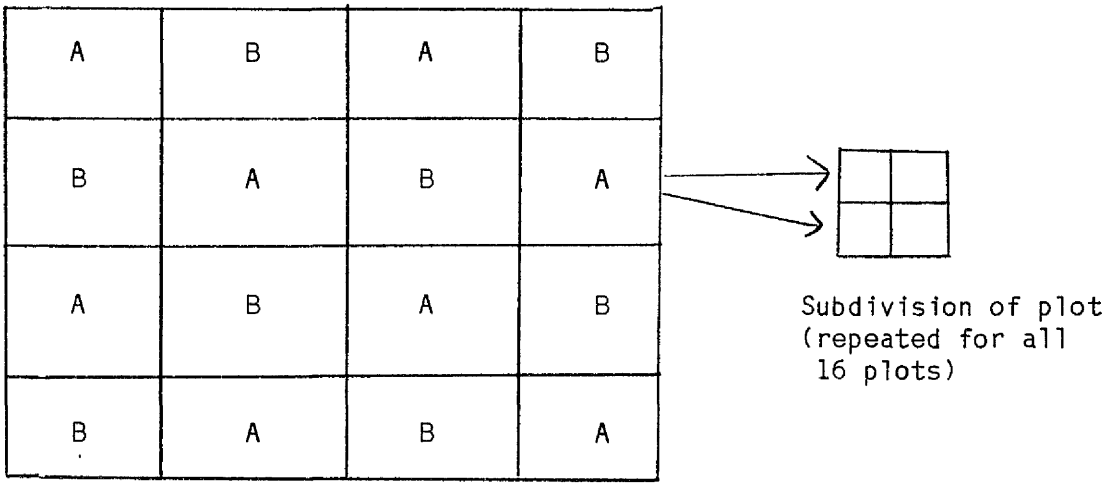
The herbage was sampled immediately after application of the Cu fertiliser and at weekly intervals thereafter for a total of 9 weeks. Four subplots from the control and Cu fertiliser treatments were cut at random on each sampling day. In addition samples were cut on days following periods of heavy or prolonged rainfall. The herbage was cut using steel shears. The samples were then dried, milled and the Cu concentration of the unwashed herbage determined (section 3.3.4) as a measure of adherence.

8.3.4 Rainfall Data

Daily rainfall data was obtained from the West of Scotland College meteorological station which is situated within 100 m of the plots.

Figure 8.1

Plan of experimental plots for the leaf adherence trial



A = Control

B = 370 kg/ha Cu fertiliser

This layout was repeated two metres away on the same type of herbage.

8.4 RESULTS AND DISCUSSION

Figure 8.2 shows the herbage Cu concentration of samples obtained from both control and Cu fertiliser plots. The Cu concentration of the control herbage remained consistently between 8 and 10 mg/kg for the duration of the trial. The differences in Cu concentrations of the control plots were presumed to be due to biological and experimental variation. The herbage Cu concentration of the Cu fertiliser plots, which was used as a measure of adherence, showed that despite its inorganic and powdery physical characteristics the Cu fertiliser can adhere to foliage.

On application of the Cu fertiliser, the Cu concentrations both in and on the herbage rose from 8 mg/kg to nearly 2000 mg/kg. Within 7 days this fell to 250 mg/kg Cu, which implies that not all of the Cu fertiliser was actually adhering to the herbage, it was just a surface coating which was quickly washed off by rain or fell off due to its own weight. After fourteen days, herbage Cu concentrations had dropped well below 100 mg/kg and then continued to decline more slowly over the subsequent seven weeks. Thus after fourteen days the wash off appears to be more gradual. The small blip at day 32 coincides with a rainstorm which may have caused recontamination due to the rain splashing Cu fertiliser back on to the herbage. After nine weeks the Cu concentration of the treated herbage was still 6 mg/kg higher than the 8 mg/kg Cu concentration of the control herbage. As the trial was carried out in the winter when there was little herbage growth, uptake of this amount of Cu by the herbage was unlikely. Therefore the gradual decline in Cu concentration over the last six weeks suggests that the Cu fertiliser does adhere to foliage and is slowly washed off

Figure 8.2. Herbage Cu concentration versus time.

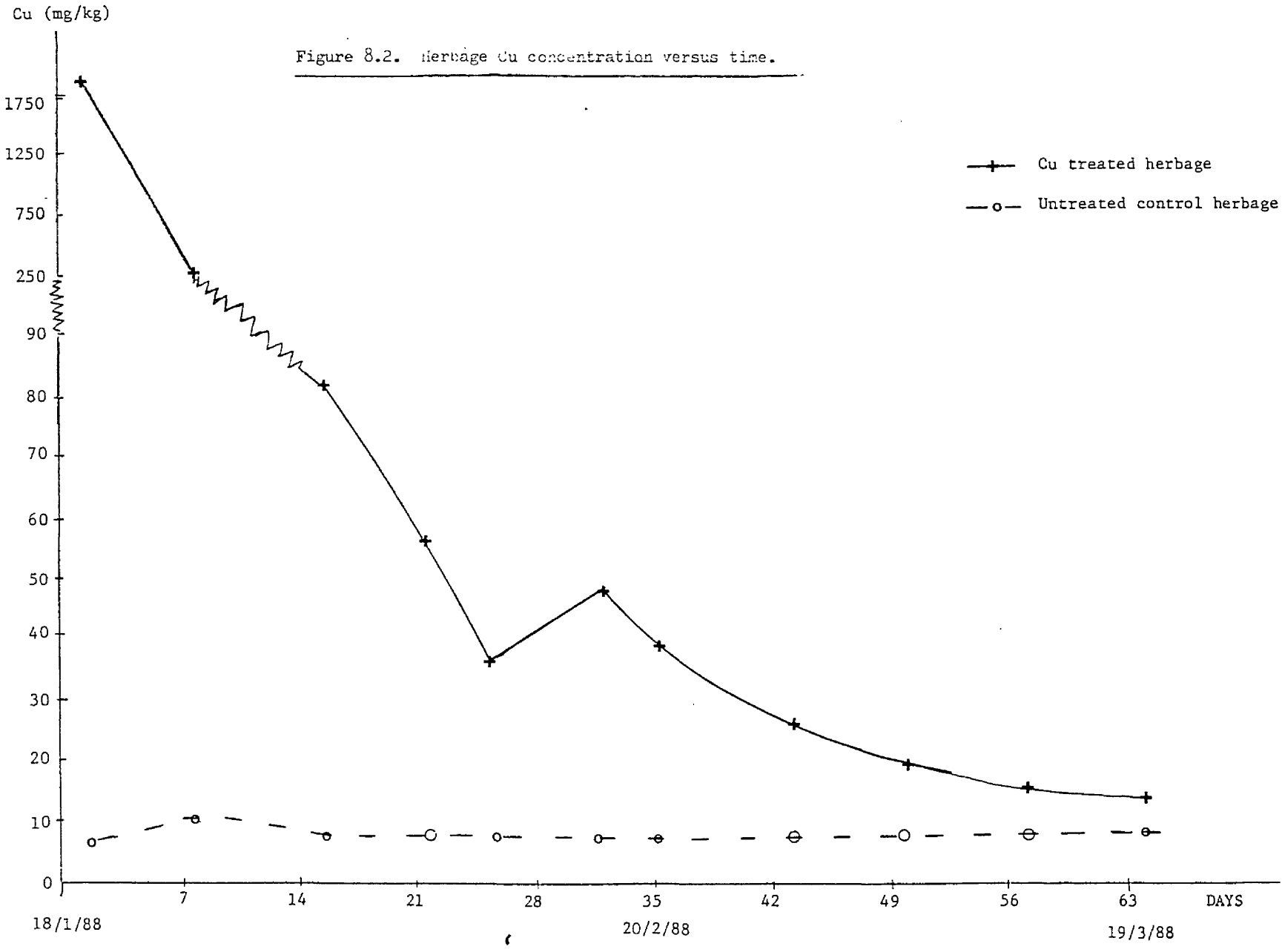
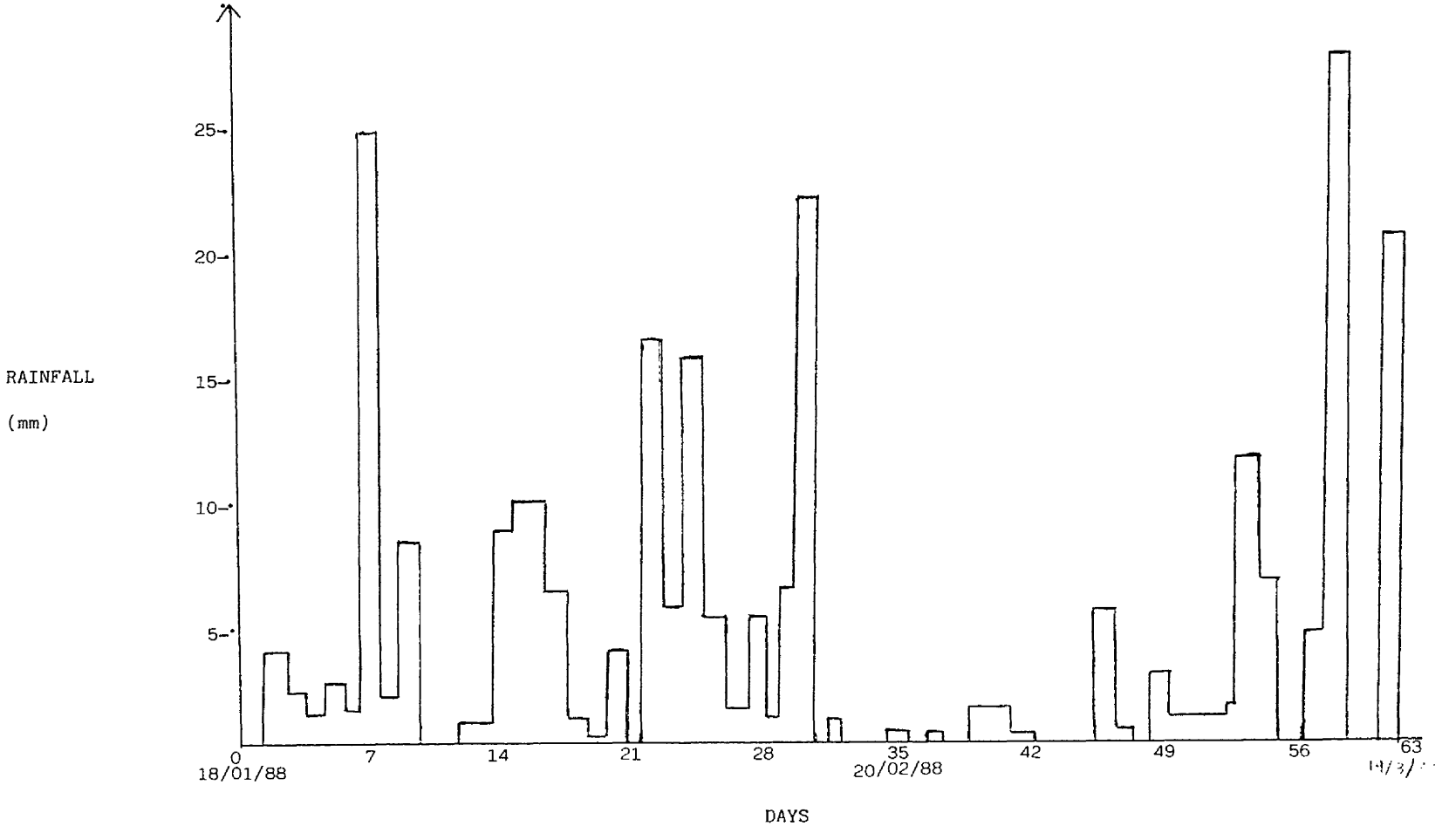


Figure 8.3: Daily rainfall levels for duration of leaf adherence trial.



by rain or blown off in the wind.

Daily rainfall data are given in Figure 8.3. No relationship was found between Cu adherence and rainfall data on either a daily or cummulative basis. However it is possible that if the trial was repeated several times under different rainfall regimes or using artificial systems some relationship may become clear.

Thus the trial did show that the Cu fertiliser adhered to herbage and was therefore available for ingestion by grazing animals. However, as there is no apparent relationship between Cu fertiliser adherence and rainfall in this trial, an estimate of how long animals should be kept off treated ground after a broadcast application of the Cu fertiliser, based on rainfall, cannot be made from these results. It is therefore proposed that, since the greatest amount of leaf adherence occurs in the first week after Cu fertiliser application and this declines rapidly, to below potentially zootoxic levels (Davies, 1980), over the next two weeks, a period of three weeks be allowed between broadcast application and the resumption of grazing.

8.5 SUMMARY

8.5.1 The trial showed that the copper fertiliser can adhere to herbage and is therefore available for ingestion by grazing animals.

8.5.2 No relationship was demonstrated between Cu fertiliser adherence and rainfall levels.

8.5.3 Initial high herbage Cu concentrations due to coating with the Cu fertiliser are quickly reduced and it is therefore proposed that a three week period be allowed between broadcast application and the resumption of grazing by animals.

CHAPTER 9. INGESTION TRIAL

9.1 INTRODUCTION

Results from the animal response trials at both Garmore and Pinmacher farms have shown that the treatment of an established sward with the Cu fertiliser increased plasma Cu concentrations in grazing ewes within a very short period of time after its application. This increase could not be as a result of increased herbage Cu concentrations as the Cu fertiliser was applied in the late autumn/winter months when there was little herbage growth and so there was little possibility of Cu uptake in sufficient amounts by the herbage to cause the rapid increases in plasma Cu concentrations recorded. Therefore, it has been proposed (section 5.6.6) that the initial increase in ewe plasma Cu concentrations was due to ingestion of the Cu fertiliser by the sheep. The leaf adherence trial (Chapter 8) and the herbage samples taken at Pinmacher (Chapter 7) demonstrated that the Cu fertiliser can adhere to herbage for periods of up to twelve weeks. It is also probable that the Cu fertiliser was ingested along with soil during the winter. Healy **et al** (1974) suggested that grazing sheep can involuntarily ingest up to 300 g soil/kg herbage D.M.I. when herbage cover is poor over the winter months.

It is likely that the grazing sheep did ingest the Cu fertiliser, from both leaf adherence and mixed in the topsoil, particularly over the first winter following its application. This is thought to play an important role in the initial raising of ewe plasma Cu concentrations, especially since the Cu fertiliser becomes more soluble under acidic conditions (Chapter 4) such as are found in the ovine gastro

intestinal tract. Thus a more detailed study of the Cu fertiliser's effects on the animal was required.

Animal copper intake in excess of immediate requirements is stored in the liver (Underwood, 1977). Thus it is possible that at the time of Cu fertiliser ingestion a substantial amount of Cu was stored in the liver, and critical levels of Cu in the liver and tissue damage could have occurred. However, this was not monitored in either field trial except in the second year at Garmore when the death of eight ewes allowed determination of liver Cu concentrations. These showed significantly higher liver Cu concentrations in ewes from Cu fertiliser treated pasture. These concentrations were probably even higher in the first year of the trial and liver damage may have occurred without outward clinical symptoms. When liver stores reach a critical level there may be a sudden increase in blood plasma Cu concentrations of 20 to 30 fold. Haemolysis occurs 24 to 48 hours later and usually results in death. Six to eight weeks before this haemolytic crisis there is a marked increase in the activity of blood serum glutamic-oxalacetic transaminase (S.G.O.T.) enzyme. S.G.O.T. is a liver enzyme which is released when liver necrosis due to Cu loading occurs and its assay in blood has been shown to give an effective early diagnosis of chronic Cu poisoning in sheep (MacPherson and Hemingway, 1969; Gracey **et al**, 1976). Thus by feeding Cu fertiliser to sheep and measuring S.G.O.T. concentrations, the development of elevated liver Cu concentrations and the risk of Cu toxicity should be monitored.

Of the heavy metals present in the Cu fertiliser Pb and Cd are probably the most likely to cause concern as they may be zootoxic at

low concentrations. Thus, this trial will also offer the opportunity for monitoring blood Pb and Cd concentrations to see if their presence in the fertiliser when it is ingested would result in elevated blood concentrations, to the possible detriment of the animal.

9.2 OBJECTIVE

The objectives of this trial were:

(a) To determine if ingestion of the Cu fertiliser alone by sheep would result in increased plasma Cu concentrations and thus confirm the ingestion hypothesis above (section 5.6.6).

(b) To look for any deleterious effects resulting from ingestion of the Cu fertiliser. In particular to monitor liver function and any elevations in blood Cu, Pb and Cd concentrations.

9.3 EXPERIMENTAL

An estimation of the daily intake of the Cu fertiliser by ewes on the Garmore trial and was made in conjunction with results obtained from the leaf adherence trial (section 9.3.1). Sixteen housed hoggs were then dosed daily with the Cu fertiliser over a ten week period. Ten weeks being the time interval over which the initial increase in plasma Cu concentrations occurred in the grazing trial. The trial was monitored by weekly blood sampling and liveweight measurement.

9.3.1 Estimation of the Daily Intake of the Cu Fertiliser

At Garmore 30 ewes grazed an area of 4 ha which gives a stocking rate of 1 ewe per 0.13 ha (7.5 ewes/ha).

Using an application rate of 370 kg/ha, each 0.13 ha area was covered by 49.3 kg Cu fertiliser.

Assuming each ewe grazes one 0.13 ha only it has access to 49.3 kg Cu fertiliser.

The leaf adherence trial suggests that over the first ten weeks approximately 5% of this is available to the sheep, i.e. 2.46 kg.

Assuming 10% of this is consumed by the sheep over a ten week period, the average daily intake is 3.51 g/day or 4.92 g/day using a five day week. Dosage rates were then chosen to span this value.

9.3.2 Treatments

Sixteen housed hoggs were divided into four equal groups and tagged for identification. They were then dosed orally with the copper fertiliser once per day, five days a week, at the following rates:

Group 1 0 g Cu fertiliser/day (0 g Cu/day).
Group 2 1 g Cu fertiliser/day (0.02 g Cu/day).
Group 3 5 g Cu fertiliser/day (0.1 g Cu/day).
Group 4 10 g Cu fertiliser/day (0.2 g Cu/day).

The Cu fertiliser was suspended in 50 ml distilled water and administered by means of a plastic dosing bottle which was emptied by placing it over the sheeps tongue and allowing the contents to run out

down the oesophagus.

9.3.3 Blood Sampling and Analysis

Blood samples were taken from the 16 hoggs on the day before dosing began and at weekly intervals thereafter.

The blood was analysed for the following parameters:

(a) Weekly - plasma Cu (section 3.4.2)
haemoglobin (section 3.4.4).

(b) Fortnightly - lead (section 3.4.5)
S.G.O.T. (section 3.4.8)
groups 1 and 4 only.

After ten weeks an extra sample was obtained from groups one and four and analysed for total Cd concentration*.

9.3.4 Liveweight

All sixteen hoggs were weighed weekly.

9.3.5 Diet

The hoggs were each fed 200 g flaked maize per day and ad lib urea treated hay.

*Analysis was by flameless ionisation atomic absorption spectroscopy and was conducted by Dr. G. S. Fell, Department of Pathology, The Royal Infirmary, Glasgow.

9.4 RESULTS AND DISCUSSION

The plasma Cu concentrations for all four groups over the ten week trial period are shown in Table 9.1 and presented graphically in Figure 9.1. At no time did any of the animals have a plasma Cu concentration below the deficiency threshold. However, their blood Cu concentrations were at the lower end range, of 9.4 to 18.9 μmol/l (Grace, 1983), on day 1 and so there was still the possibility of obtaining increases.

The results for the 1 and 5 g/day treatment show no significant increase in plasma Cu concentrations at any time during the ten week period compared to the 0 g/day treatment. The mean plasma Cu concentration of the hoggs dosed with 10 g Cu fertiliser per day shows a gradual rise from 12.29 μmol/l in week 0 to 17.02 μmol/l in week 8 after which it levels out and ends the trial at 16.98 μmol/l. At this plateau stage the extra Cu intake was probably stored in the liver. Although the 10 g/day treatment shows an immediate increase in plasma Cu concentration it is not until the end of week three that it is significantly higher ($p<0.05$) than the control group. By weeks seven and nine this difference was increased to even greater significance at $p<0.01$ and $p<0.001$ respectively. Therefore direct ingestion of the Cu fertiliser at levels of 10 g/day or more will significantly increase plasma Cu concentrations.

Although the 10 g/day treatment is twice the estimated daily intake of the sheep in the Garmore field trial it is a relatively crude estimate of the daily Cu fertiliser intake. However, if the estimate is close to the actual level of intake then a possible reason for the lower

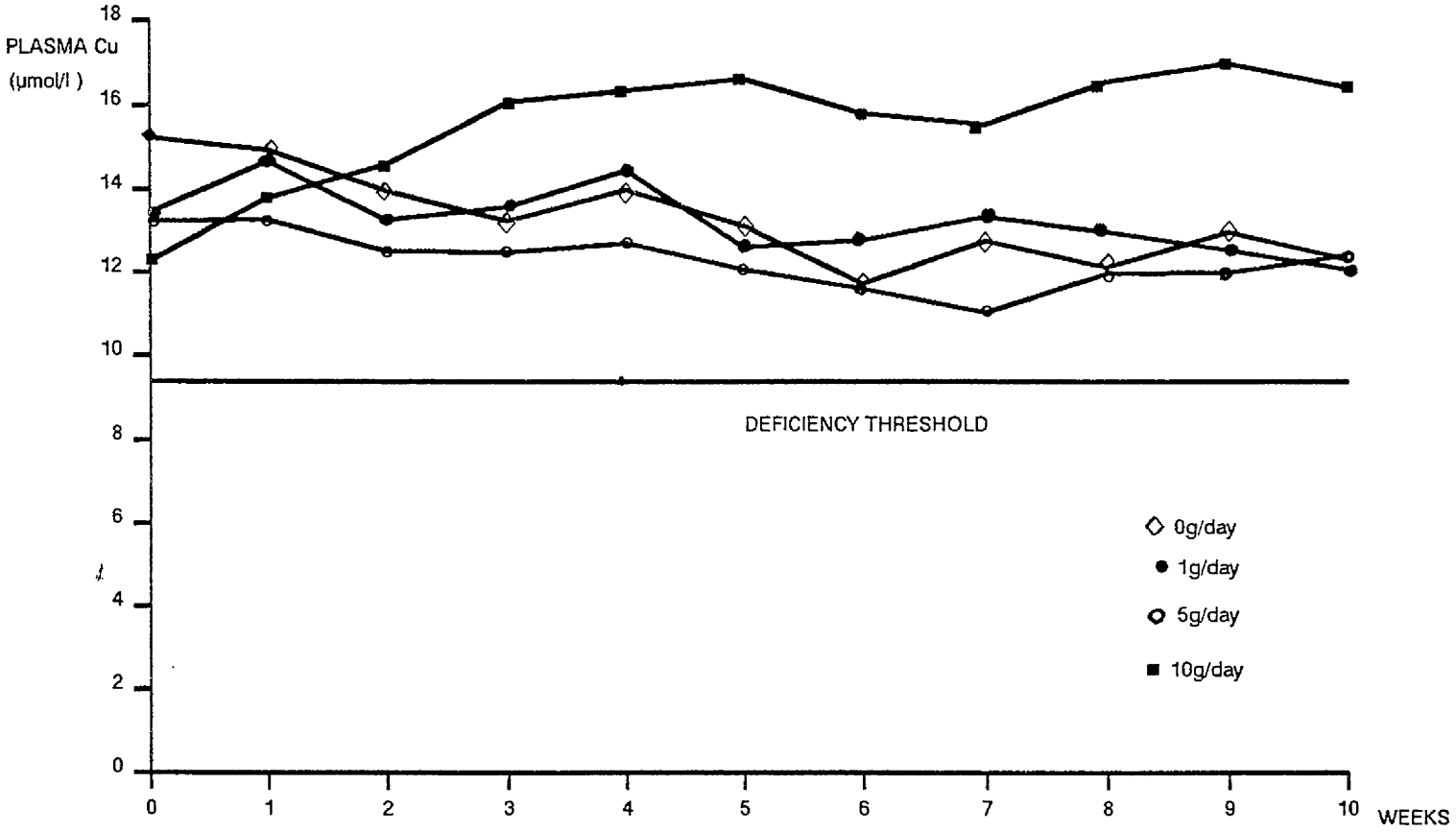
Table 9.1

Ingestion trial mean plasma Cu concentration versus time

Week of sampling	Treatment group: 0 g/day (n=4)	1 g/day (n=4)	5 g/day (n=4)	10 g/day (n=4)	S.E.D.
0	14.71	13.78	13.45	12.29	1.82
1	14.36	13.60	14.71	13.82	1.45
2	13.79	12.38	13.27	14.63	1.08
3	13.09	12.53	13.59	16.11[a]	0.98
4	13.73	12.77	14.50	16.38	0.95
5	12.75	12.37	12.63	16.65[a]	1.39
6	11.53	11.91	12.80	15.85[a]	1.25
7	11.78	12.28	12.94	16.61[b]	1.07
8	12.34	12.61	13.44	17.02[b]	0.88
9	12.28	12.15	13.39	16.99[c]	0.76
10	12.66	12.14	13.49	16.98[c]	0.92

Means with superscripts are significantly different from control at the following levels: [a]$p<0.05$; [b]$p<0.01$, [c]$=0.001$, analysis of variance (EDEX, A.F.R.C. Statistics Unit, Edinburgh).

Figure 9.1: Mean Plasma Cu Concentrations Versus Time of Hoggs Dosed Orally With Cu Fertiliser at Four Different Rates.



dosing rates having no effect is that the estimated 5 g/day intake would occur over a whole day in the field situation, whereas in the housed situation it was given in one dose and it is possible that a large proportion of this was quickly excreted by the animal. No faecal samples were collected to substantiate this theory. In addition, in the field the Cu fertiliser ingested would be mixed with soil and/or herbage as opposed to on its own in the housed situation and this may influence the rate of Cu release or adsorption in the gut.

Tables 9.2 and 9.3 show that the presence of Pb or Cd in the fertiliser had no potentially toxic effect as none of the blood samples analysed contained significant amounts of either Pb or Cd. There were no deleterious effects on the liver, as monitored by S.G.O.T. concentrations in groups 1 and 4 (Table 9.2), during the ten week trial period due to the increased Cu intake. Thus neither Cu nor any other element in the Cu fertiliser were stored in the liver at levels likely to cause liver necrosis or toxicity.

There were no significant differences in either liveweight or haemoglobin concentration between treatments throughout the trial (Table 9.4). However as there was no actual Cu deficiency or toxicity problem and no toxicity due to either Pb or Cd, any differences in haemoglobin concentration or liveweight among the groups would not have been expected.

Table 9.2

Ingestion trial mean whole blood lead and S.G.O.T. concentrations versus time for groups 1 and 4

Week of sampling	Group 1 (0 g/day)		Group 4 (10 g/day)	
	Lead (mg/ml)	S.G.O.T. (S.F./ml)	Lead (μg/ml)	S.G.O.T. (S.F./ml)
0	-	80.0	0.13	83.0
2	0.11	81.3	0.16	76.0
4	0.14	69.2	0.12	83.7
6	0.15	81.2	0.12	82.2
8	0.17	81.9	0.18	82.1
10	0.13	84.2	0.16	83.4

Table 9.3

Ingestion trial mean whole blood Cd concentrations for groups 2 and 4 at week 10

Treatment	Whole blood Cd (nmol/l)
0 g/day (group 1)	1
10 g/day (group 2)	1

Table 9.4

Ingestion trial mean liveweight and haemoglobin concentration at each sampling date

Week of sampling	Mean liveweight (kg) (n=16)	Mean haemoglobin (g/100 ml) (n=16)
0	35.1	9.64
1	35.9	10.21
2	36.5	13.84
3	36.8	11.97
4	36.6	9.27
5	36.9	9.59
6	36.9	9.63
7	37.0	10.11
8	36.6	10.41
9	37.0	9.69
10	37.2	9.73
	S.E.D. = 1.23	S.E.D. = 0.59

9.5 SUMMARY

9.5.1 Ingestion of Cu fertiliser by sheep can increase plasma Cu concentrations. An intake of 10 g Cu/fertiliser per day increased mean plasma Cu concentration from 12.3 µmol/l to 16.6 µmol/l within five weeks and maintained it at, or just above this level until the end of the trial.

9.5.2 The increase in plasma Cu concentrations was not accompanied by increases in whole blood Pb or Cd concentrations.

9.5.3 Ingestion of the Cu fertiliser at rates of up to 10 g/day for a ten week period had no deleterious effect on the sheeps liver, as monitored by S.G.O.T. concentrations, in this trial.

CHAPTER 10. GLASSHOUSE POT TRIAL 1

10.1 INTRODUCTION

When the two field trials at Garmore were initiated in 1985 there were no published results on the effects of different rates and methods of application of the Cu fertiliser on herbage Cu concentrations. Glasshouse pot trials gave a quick turnover of results as well as allowing greater replication of treatments and more control over growing conditions than is possible in field trials. In March 1986, therefore, a pot trial was initiated to determine if the Cu fertiliser application rates used in the field trials were likely to increase herbage Cu concentrations.

Broadcast application of the Cu fertiliser does not allow immediate availability of Cu to plant roots as the Cu is water insoluble (Chapter 4) and therefore not readily leached into the soil. Hence different methods of application of the Cu fertiliser and their effects on herbage Cu concentrations were also studied. Quicker and more efficient uptake of Cu from the fertiliser is likely if it is incorporated into the rooting zone. This method of application may be particularly important as Cu deficiency in livestock often occurs after reseeding soils of low or marginal Cu concentration when the increased herbage production depresses herbage Cu concentrations through a dilution effect (section 2.3.3).

Herbage Zn, Fe, Pb, Cd, Cr, Mn and Ni concentrations were also monitored to see if their presence in the Cu fertiliser resulted in zootoxic herbage concentrations. As soil pH markedly affects the

availability of trace elements (Lucas and Knezek, 1972) the above rates and methods of application were applied to a range of pHs, which were typical of those in Scottish soils.

10.2 EXPERIMENTAL

10.2.1 Soil for Pot Trial

A Cu-deficient, sandy loam soil was collected from the Kirkbean area on the Solway Firth coast (O.S. map reference NX953560). Samples were obtained from the B horizon of the soil profile, to minimise the amount of organic matter present which reduces Cu uptake by plants. The soil has a low E.D.T.A.-extractable Cu concentration (section 3.2.8) of 1.25 mg/kg (Table 10.1). Soil analysis was also carried out to determine:

(a) Available P and K (section 3.2.7) so that necessary fertiliser treatments could be calculated.

(b) pH and lime requirements (section 3.2.4 and 5) so that soil pH could be adjusted.

(c) Percentage loss on ignition (section 3.2.6) to ensure that this was not high and thus affect the availability of any added Cu (section 2.2.6).

The results are listed in Table 10.1.

Moist soil was used after being passed through a 5 mm sieve to remove any large stones and break up any aggregates.

Table 10.1

Analysis of pot trial soil

% loss on ignition	5.4
pH	5.3
Lime requirement	3.6
Available P (mg/l)	102
Available K (mg/l)	166
E.D.T.A. extractable Cu (mg/kg)	1.25

10.2.2 Experimental Treatments

(a) Method of Cu fertiliser application:

(i) Broadcast.
(ii) Deep soil incorporation.
(iii) Seed bed incorporation.

(b) Application rate (kg/ha):

(i) 0.
(ii) 370.
(iii) 740.

(c) Soil pH:

(i) 5.5.
(ii) 6.0.
(iii) 6.5.

These methods of application were chosen to simulate surface application to an established sward, incorporation into the plough

layer at reseeding and incorporation into the top 2 cm of soil in a minimum cultivation system respectively.

The application rates represented a control with no added Cu, and two application rates as used in the Garmore herbage trial.

The three soil pHs selected for study here are typical of those found in Scottish mineral soils.

Each of the treatments was replicated four times giving a total of 108 pots arranged in a randomised block design on glasshouse benches.

10.2.3 Preparation and Ryegrass Seeding

Four-litre polythene pots were filled with 3 kg of soil which had been limed to the desired pH with calcium hydroxide, and fertilised with ammonium nitrate, potassium nitrate and triple superphosphate as recommended by the Scottish Agricultural Colleges (1985) for grass establishment.

For deep soil-incorporation of the Cu fertiliser, the appropriate application rate was mixed throughout the 15 cm depth of soil. To simulate seedbed incorporation, the top 2 cm of soil in each pot was removed, mixed with the Cu fertiliser and returned to the pot. For the broadcast application, the Cu fertiliser was surface applied after the pots had been sown with ryegrass.

Each pot was then transferred from the laboratory to the glasshouse. The moisture content of the soil was kept constant by standing the pots on a 10 cm deep capillary bed of moist perlite.

The pots were sown with two grams of Springfield perennial ryegrass on 6 March 1986. Deionised water and liquid feed (section 3.5.3) were applied when required.

10.2.4 Grass Cutting and Analysis

Cuts were taken from the pots after 72 and 149 days (on 17 May 1986 and 2 September 1986). To prevent any soil contamination the grass was cut 2 cm above the soil surface of each pot using steel shears. The fresh weight yield of grass was determined and the samples retained for dry matter and trace element analysis. After determination of the dry matter yield of the grass from each pot, a subsample was taken and analysed for total Cu, Zn, Fe, Pb, Cd, Cr, Mn and Ni (section 3.3.4).

10.2.5 Soil pH

At the end of the pot trial 25 soil samples were taken at random from the total of 108 pots and the soil pH determined to see if it had changed over the duration of the trial. There was no significant change in soil pH over the trial period.

10.3 RESULTS AND DISCUSSION

10.3.1 Germination and Yields

Germination of ryegrass seed was not prevented by any of the treatments. Mean grass yields for both first and second cuts are given in Table 10.2. There were no significant differences in grass yields among the treatment groups at either cut. Thus Cu fertiliser application had no positive or deleterious effects on grass growth.

Despite growing in soil of low available Cu concentration, no grass sample (Table 10.4) was found to be Cu-deficient, i.e. containing less than 4 mg Cu/kg (M.I.S.R., 1985). Consequently, Cu was not a growth limiting element and so yield differences would not be expected.

Table 10.2

Mean grass yields from herbage pot trial cuts 1 and 2

	Cut 1	S.E.D.	Cut 2	S.E.D.
Fresh weight (g)	205	-	193	-
Dry matter (g/kg)	103	-	102	-
Dry matter yield (g)	21.3	0.81	19.7	1.27

Table 10.3

Mean trace element concentrations of grass cuts 1 and 2

	Concentration in grass (mg/kg)			
Element	**Cut 1**	**S.E.D.**	**Cut 2**	**S.E.D.**
Cu	16.7	0.86	10.2	1.08
Zn	80.6	7.62	78.8	9.66
Fe	172.1	23.34	146.4	28.6
Pb	0.90	0.11	0.78	0.24
Cd	0.08	0.01	0.06	0.01
Cr	<0.01	-	<0.01	-
Mn	308.5	48.2	292.1	57.6
Ni	1.0	0.25	1.06	0.19

10.3.2 Cut 1, Trace Element Concentration

Table 10.3 shows the mean Cu, Zn, Pb, Cd, Ni, Fe, Cr and Mn concentrations of the grass in cut 1. There were no significant differences in concentrations among the different treatments. Thus, application of the Cu fertiliser by any of the three methods had no effect on the trace element concentration of the grass in the first sixty days after sowing.

This may have been due to the roots not having been well-enough distributed throughout the pot to have had access to all the Cu fertiliser added and as such would have had to obtain their Cu requirements directly from the small amount available in the adjacent soil. Alternatively, there may not have been a sufficient time interval between seeding of the grass and the first cut for the Cu fertiliser to release enough Cu to have had a significant effect on the size of the plant-available Cu pool in the soil.

10.3.3 Cut 2, Copper Concentration

The grass Cu concentration for each treatment in the second cut is given in Table 10.4 and presented graphically in Figure 10.1. The results show that increasing rates of Cu fertiliser application led to significant increases ($p<0.001$) in the Cu concentration of the grass. The 370 kg/ha rate gave an average rise in the Cu concentration of 2.75 mg/kg and the 740 kg/ha a 3.95 mg/kg rise. Of the extra Cu taken up by the grass, these increases represent 0.22% and 0.15% of the added Cu from the 370 kg/ha and 740 kg/ha treatments respectively.

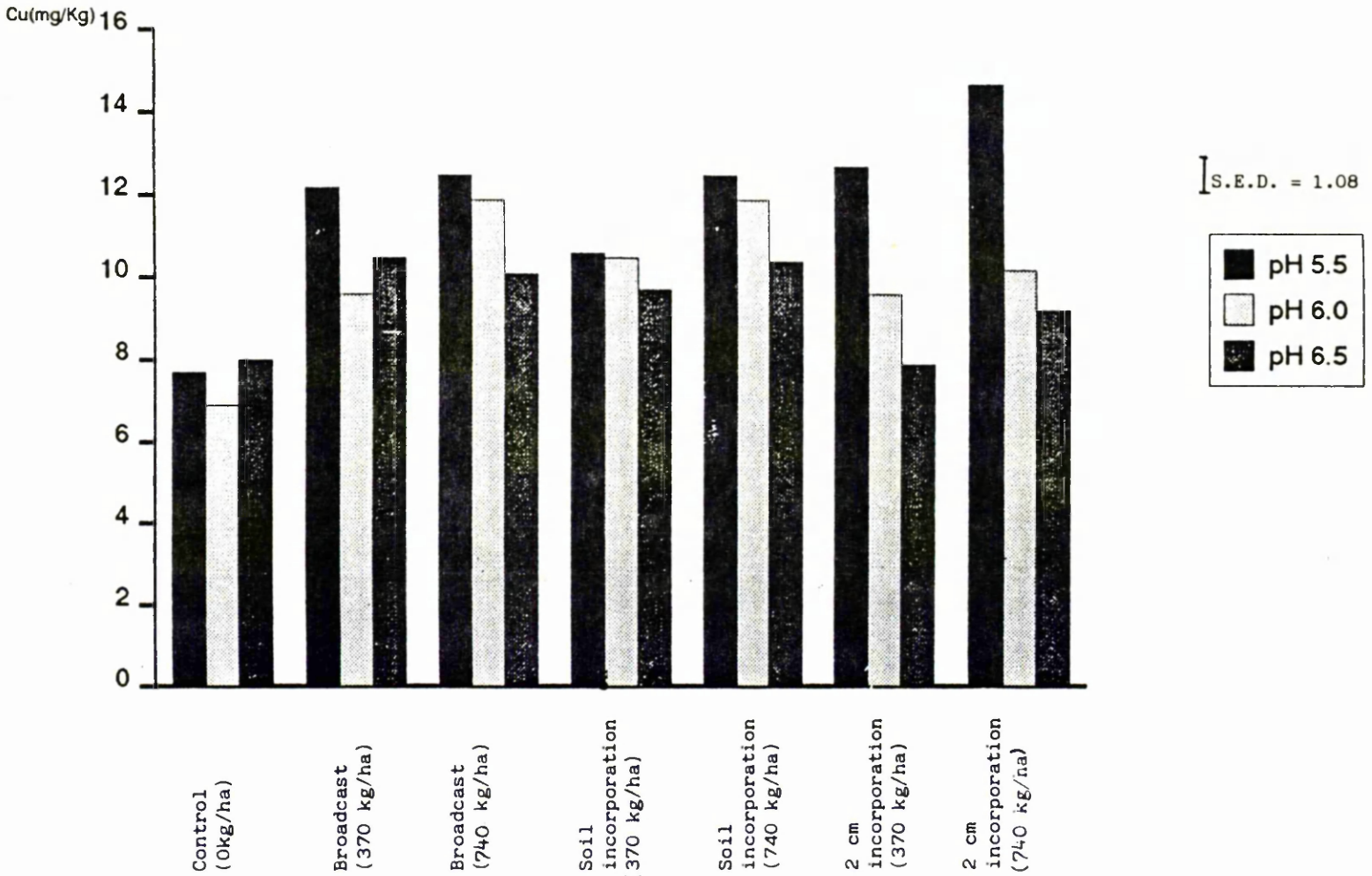
Table 10.4

Mean grass Cu concentrations in cut 2

Application method	Application rate (kg/ha)	Cu concentration pH: 5.5	6.0	6.5
Control	0	7.7	6.9	8.0
Broadcast	370	12.2	9.6	10.5
Broadcast	740	12.5	11.9	10.1
Soil incorporation	370	9.7	10.5	9.7
Soil incorporation	740	12.5	11.9	10.4
2 cm incorporation	370	12.7	9.6	7.9
2 cm incorporation	740	14.7	10.2	9.2

S.E.D. = 1.08

Figure 10.1: Grass Cu Concentrations at Cut 2 on 2nd September 1988.



The results also showed that lowering soil pH raised the Cu concentration in the grass with the effect being highly significant ($p<0.001$). This effect was probably due to the effects of pH on the solubility of Cu from the fertiliser rather than on the availability of Cu from the soil which is thought to be little affected by soil pH (section 2.2.6).

There were, however, no significant differences in grass Cu concentrations due to the different application methods. This may be because the grasses were well-rooted and the roots had access to all the Cu fertiliser in the confined space of the pot. Thus, regardless of how the Cu fertiliser was applied any Cu released could be taken up by the grass. Therefore, the rate rather than the method of application of the Cu fertiliser was likely to play the more important role.

10.3.4 Cut 2, Zn Concentration

The grass Zn concentratons for each treatment at the second cut are given in Table 10.5 and in Figure 10.2. These show that Zn followed a similar pattern to Cu, increasing the application rate and lowering the soil pH gave significant increases ($p<0.001$) in the Zn concentration of the grass. Again, there were no significant differences in grass Zn concentrations as a result of different application methods. As the same plant processes are thought to be involved in both Cu and Zn uptake (Bowen, 1981) the discussion in 10.3.3 applies to Zn as well as Cu.

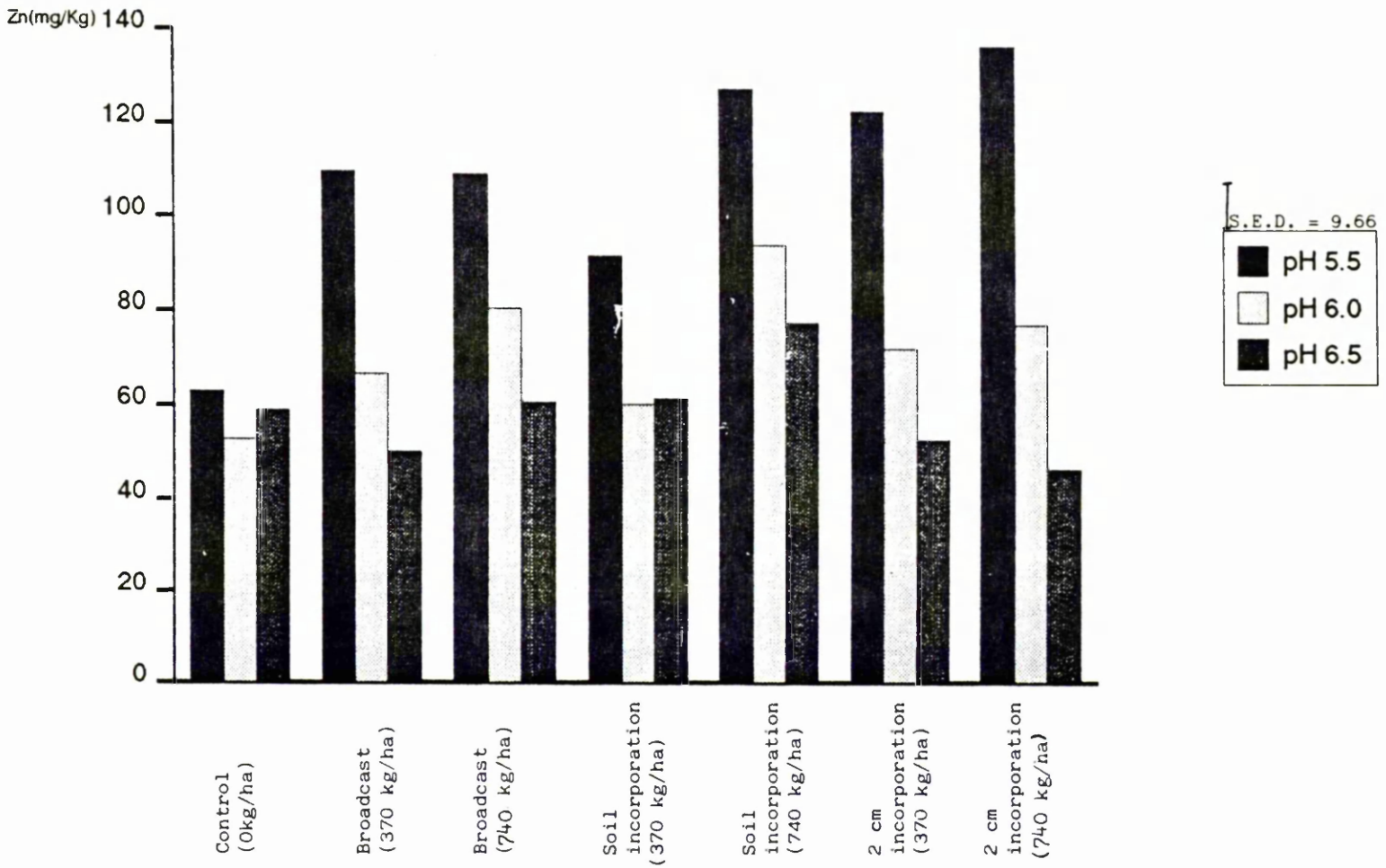
Table 10.5

Mean grass Zn concentrations in cut 2

Application method	Application rate (kg/ha)	Cu concentration pH: 5.5	6.0	6.5
Control	0	63.1	52.9	59.0
Broadcast	370	109.6	66.7	50.3
Broadcast	740	109.1	80.5	60.8
Soil incorporation	370	91.6	60.4	61.5
Soil incorporation	740	127.4	93.8	77.4
2 cm incorporation	370	122.6	71.9	52.6
2 cm incorporation	740	136.5	77.1	46.4

S.E.D. = 9.66

Figure 10.2: Grass Zn Concentrations at Cut 2 on 2nd September 1988.



10.3.5 Cut 2, Fe, Pb, Cd, Cr, Mn and Ni Herbage Concentrations

Table 10.3 gives the mean Fe, Pb, Cd, Cr, Mn and Ni concentrations of the grass from cut 2. Application of the Cu fertiliser did not elevate the concentration of these elements above the concentration found in the controls. There were no significant differences in herbage concentrations among the different treatments, which were well below phytotoxic or zootoxic levels (Table 6.7). It can therefore be assumed that the Cu fertiliser treatments posed no toxicity problems in this trial. As in the herbage field trial any possible uptake of these elements was probably reduced by the competitive uptake of the much larger concentrations of Cu and Zn present in the fertiliser. In addition, as approximately only 0.25% of the added Cu was removed in the second cut and assuming similar rates of uptake by the plant, any increases in Pb, Cd, Cr, Mn or Ni concentrations would be negligible.

10.3.6 General Discussion

This experiment showed that application of the Cu fertiliser caused significant increases in grass Cu and Zn concentrations. These increases were not accompanied by increased Fe, Pb, Cd, Cr, Mn and Ni concentrations. The results are comparable to those obtained with mixed herbage in the field trial at Garmore (Chapter 6) and show that application of this Cu fertiliser is a method of increasing herbage Cu concentrations.

The average 4 mg/kg increase in the ryegrass Cu concentration obtained in this pot experiment is much larger than that obtained by several other workers using $CuSO_4$ in the field situation where increases of only 1-2 mg/kg were recorded using similar Cu application rates

(Reith, 1983; Burridge **et al**, 1983; Reith **et al**, 1984). However, the results are comparable to those from a pot trial by McGrath **et al** (1982a) who demonstrated that $CuSO_4$ (6 kg Cu/ha) can give 4 mg/kg increases in grass Cu concentrations. Therefore, the new fertiliser was shown to be equally as effective as $CuSO_4$ in increasing grass Cu concentrations when used in pots.

Although Zn deficiency in crops or animals is rare in Great Britain, the increased Zn concentrations obtained using this fertiliser were significant and may prove useful in other areas of the world especially South America and Africa where combined Cu and Zn deficiencies are common (McDowell, private communication).

The different methods of Cu fertiliser application used in this trial had no significant effects on grass Cu concentrations.

However, any effects due to the different application methods may have been limited by the confined volume of the pots. Thus, a field-scale trial in which roots have access to a much greater volume of soil is necessary to determine if the method of application would affect the rate of Cu uptake by the plant following application of the Cu fertiliser.

10.4 SUMMARY

10.4.1 Application of the Cu fertiliser at rates of up to 740 kg/ha had no beneficial or deleterious effects on ryegrass germination or yields.

10.4.2 Application of the Cu fertiliser increased grass Cu and Zn concentrations. Increasing application rates produced significant increases in herbage Cu and Zn concentrations.

10.4.3 Lowering the soil pH resulted in significant increases in ryegrass Cn and Zn concentrations when grown in soil supplemented with the Cu fertiliser.

10.4.4 There were no significant differences in ryegrass Cu concentrations due to the different application methods employed.

10.4.5 The increase in grass Cu concentrations obtained were similar to those obtained by some other workers using $CuSO_4$.

CHAPTER 11. GLASSHOUSE POT TRIAL 2

11.1 INTRODUCTION

In order to look in more detail at soil factors which might influence the plant availability of Cu from Cu fertiliser supplemented soil, a second pot trial was conducted using ryegrass as the Cu extractant, because no chemical soil Cu extractant has been found suitable for use in assessing plant available Cu in the soil when the Cu fertiliser is present (Chapter 4) which meant that soil incubation experiments were not feasible.

Probably the principal factor which affects Cu availability in soil is its organic matter content (sections 2.1.2 and 2.1.6). In general the more organic matter a soil contains the less available Cu is to the plant and the more quickly any added Cu is adsorbed by the soil and rendered plant unavailable. The drainage status of the soil also affects Cu availability, the concentration of plant available Cu in a soil is usually higher in poorly drained soils (section 2.2.6). Drainage conditions may also affect the solubility of the Cu fertiliser. Although the evidence for soil pH affecting the availability of Cu to plants is conflicting (section 2.2.6), the first pot trial (Chapter 10) showed that soil pH had an effect on the Cu concentration of ryegrass and this, therefore, warranted further investigation.

The following pot trial was, therefore, conducted to investigate the effects of soil pH, waterlogging and organic matter content on the plant availability of Cu from Cu fertiliser supplemented soil. The

results should help in determining what combination of soil types, drainage status and pH is most favourable for Cu fertiliser supplementation.

By designing the pot trial to last for up to one year any residual value of the Cu fertiliser, for supplying Cu to the plant, could be monitored. Herbage Zn, Fe, Pb, Cd, Cr, Mn and Ni concentrations were also monitored in order to determine if their release from the Cu fertiliser and plant availability were affected by the different soil factors.

11.2 EXPERIMENTAL

11.2.1 Soils and Preparation

Three soils with varying organic matter contents were collected. The soils include one from the Garmore response trial site as the moderate organic matter content soil and this may enable a better understanding of what is happening to the Cu fertiliser in the field. The others used were a peaty gley topsoil (high organic matter) a sandy subsoil (low organic matter).

The soils were riddled through a 5 mm sieve to remove any large stones and break up any aggregates. Analysis was also carried out to determine:

(a) Percentage loss on ignition (section 3.2.6) to determine the percentage organic matter in each soil.

(b) pH and lime requirement (section 3.2.4 and 5) so that the pH of the soil could be suitably adjusted.

(c) Available P and K (section 3.2.7) so that fertiliser recommendations could be determined.

(d) E.D.T.A. extractable Cu (section 3.2.8) for background information on the three soils.

The results in Table 10.1 show that the soils all have a low E.D.T.A. extractable Cu concentration, as defined by M.I.S.R. (1985) and do encompass a wide range of organic matter contents.

Table 11.1

Soil analysis results for soils used in pot trial

	Soil organic mattent content		
Analysis	**Low**	**Moderate**	**High**
Organic matter content (%LOI)	5.5	15.6	57.9
E.D.T.A. Extractable Cu (mg/kg)	2.75	3.68	2.69
pH	5.54	5.03	4.33
Lime requirement (t/ha)	1.7	6.7	17.8
Available P (mg/l)	1.5	8.1	2.5
Available K (mg/l)	60	66	100

The soils were packed moist into 17.5 cm plastic pots with a base dressing of 3 g of 20 N; 10P; 10K ground fertiliser incorporated into each pot. Target pHs for each pot were obtained by incorporation of calcium hydroxide throughout the soil by hand.

11.2.2 Treatments

Three soil factors were studied: soil organic matter content, soil pH and waterlogging, each of them with or without Cu fertiliser addition. The treatments were:

(a) Soil organic matter content

The three soils used in this pot trial were selected on the basis of their different organic matter contents which were represented by loss on ignition of 5.5%, 15.6% and 59.6% respectively. The soils were therefore classified as:

(i) Low organic matter content - sandy loam subsoil.

(ii) Moderate organic matter content - sandy loam.

(iii) High organic matter content - peaty gley (Scottish Agricultural Colleges, 1985).

(b) Soil pH

Each of the above soils were all limed with Ca (OH_2) to obtain pHs of:

(i) 4.5.

(ii) 5.5.

(iii) 6.5.

This range of pHs is wider than that used in the first pot trial (Chapter 10) and is more likely to emphasise any pH effects.

(c) Waterlogging

Two moisture contents were compared by creating a low and high water table environment for the pots as follows:

(i) aerated - low water table.

(ii) waterlogged - high water table.

The high watertable was obtained by sitting the soil filled pots in trays containing 5 cm depth of water. The low water table comparison was obtained by sitting the pots on trays of perlite. This allowed the latter to be well aerated and free draining with the perlite offering a source of water when required by the plant.

(d) Copper fertiliser supplementation

The Cu fertiliser was applied at:

(i) 0 kg/ha.

(ii) 370 kg/ha.

The Cu fertiliser was mixed throughout the 12 cm depth of the pot.

(e) Replication

Each of the 36 treatments above was replicated four times giving a total of 144 pots arranged in a randomised block design.

11.2.3 Sowing of Seed and Feeding

After treatment two grams of Italian ryegrass was sown into the top of each pot on 25 March 1987. The pots were watered with deionised water and fed 5 ml of liquid feed (section 3.5.3) as required.

11.2.4 Grass Cutting and Analysis

Five cuts were taken from all the pots on 3 June 1987, 12 August 1987, 14 October 1987, 21 January 1988 and 29 March 1988. The grass was cut 2 cm from the soil surface of each pot using steel shears to prevent

any soil contamination. The grass samples were then weighed and retained for dry matter determination and trace element analysis. After dry matter determination, a subsample was removed for total Cu and Zn analysis (section 3.3.4). The grass samples obtained from the three cuts on 3 June 1987, 12 August 1987 and 21 October 1987 were also analysed for Fe, Mn, Ni, Pb, Cr and Cd (section 3.3.4).

Due to the detrimental effects of mildew infection and thrip infestation samples taken at the fourth cut had to be discarded.

11.2.5 Soil pH

At the end of the pot trial, the soil of 27 pots were sampled at random from the total of 144 pots and the soil pH determined (section 3.2.4) to see if it had changed over the duration of the trial. This showed that the target pHs were maintained throughout the trial period.

11.3 RESULTS AND DISCUSSION

11.3.1 Yield

There were no significant differences in grass yields among the treatment groups at any cut (Table 11.2). Thus Cu fertiliser application had no positive or deleterious effects on grass growth. Despite growing in soils of low available Cu concentration no grass samples were found to be Cu-deficient (less than 4 mg Cu/kg (M.I.S.R., 1985)), consequently as in the first pot trial (Chapter 10) no yield difference would have been expected.

Table 11.2

Mean grass yields

Date	Yield			S.E.D.
	Fresh wgt (g)	Dry matter (g/kg)	Dry matter (g)	
03/06/87	41.5	255	10.5	0.72
12/08/87	63.1	191	12.1	1.32
14/10/87	47.2	167	7.9	0.98
21/01/88	39.2	-	-	-
24/03/88	46.6	161	7.5	0.86

11.3.2 Grass Cu Concentration

The mean grass Cu concentrations for each treatment in the four cuts in which they are measured are shown in Table 11.3. Irrespective of treatment, application of the Cu fertiliser produced significant increases ($p<0.001$) in grass Cu concentrations over the control treatments, which were maintained until the end of the trial.

The increase in grass Cu concentration due to the application of the Cu fertiliser compared to the control declines with successive cuts (Figures 11.5 and 11.6). In cut 1 there was an average increase of 8.0 mg/kg in grass Cu concentrations which dropped to 2.4 mg/kg by the third cut but plateaued out at this level and was 2.2 mg/kg at the final cut. These increases represent removal of 0.21% and 0.06% of the added Cu by the grass in the first and last cuts respectively. These results imply that an initial flush of Cu was released from the Cu fertiliser following application, which was reflected in higher Cu concentrations at the first sampling. In subsequent cuts the soil may have removed any excess Cu released from the Cu fertiliser or alternatively its rate of release may have slowed down, the Cu now coming from larger particles that degrade more slowly. Another possibility is that the Cu reserves in the soil and from the Cu fertiliser may simply have declined with time. Thus the elevation in Cu concentrations begins to decline until an equilibrium position is reached after which the difference from controls remains relatively constant. These results are similar to those observed by other workers who have shown in pot experiments that after soil Cu supplementation, elevation of the Cu content of grasses decreased with successive cuts (McGrath **et al**, 1982a) as the added Cu is immobilised

Table 11.3

Mean grass Cu concentration for each treatment

Treatment									
Organic matter content:	**Low**			**Moderate**			**High**		
pH:	**4.5**	**5.5**	**6.5**	**4.5**	**5.5**	**6.5**	**4.5**	**5.5**	**6.5**
Cut 1									
Control	5.2	4.4	5.3	7.3	6.2	6.0	9.0	3.8	7.9
+ Cu fertiliser	19.9	15.4	9.6	16.2	23.7	17.0	15.4	14.8	7.9
Waterlogged control	7.4	6.4	8.7	6.1	4.8	5.3	8.1	6.0	7.6
Waterlogged + Cu	12.2	13.7	9.4	20.4	12.7	11.8	13.5	13.7	9.4
				S.E.D. = 2.27					
Cut 2									
Control	7.9	7.2	6.9	7.9	5.7	6.7	5.2	7.3	7.4
+ Cu fertiliser	13.3	12.2	11.8	13.7	12.3	8.5	14.3	17.9	13.2
Waterlogged control	8.1	7.4	6.8	6.5	5.5	7.2	7.7	7.6	6.2
Waterlogged + Cu	15.2	13.8	9.7	14.5	15.2	12.2	13.1	13.5	10.6
				S.E.D. = 1.803					

Table 11.3 cont.

Treatment									
Organic matter content:	**Low**			**Moderate**			**High**		
pH:	**4.5**	**5.5**	**6.5**	**4.5**	**5.5**	**6.5**	**4.5**	**5.5**	**6.5**
Cut 3									
Control	6.6	6.2	5.8	7.2	8.4	6.9	8.1	7.5	5.7
+ Cu fertiliser	8.5	7.7	8.7	11.0	9.5	7.9	9.4	7.9	7.9
Waterlogged control	8.8	6.6	6.6	8.1	8.0	7.6	11.1	6.8	7.4
Waterlogged + Cu fertiliser	10.9	10.5	9.4	10.9	13.0	9.6	12.4	11.6	9.7
				S.E.D. = 0.82					
Cut 5									
Control	9.3	9.4	9.2	7.9	9.6	8.4	5.3	6.5	5.8
+ Cu fertiliser	11.7	10.1	10.5	11.8	10.8	10.6	10.2	7.9	6.7
Waterlogged control	11.0	8.6	9.2	8.4	10.9	5.8	5.4	6.5	4.1
Waterlogged + Cu fertiliser	10.3	11.6	11.4	13.5	12.5	10.8	8.4	6.3	4.9
				S.E.D. = 0.96					

by the soil.

(a) Effects of soil pH on Cu concentrations

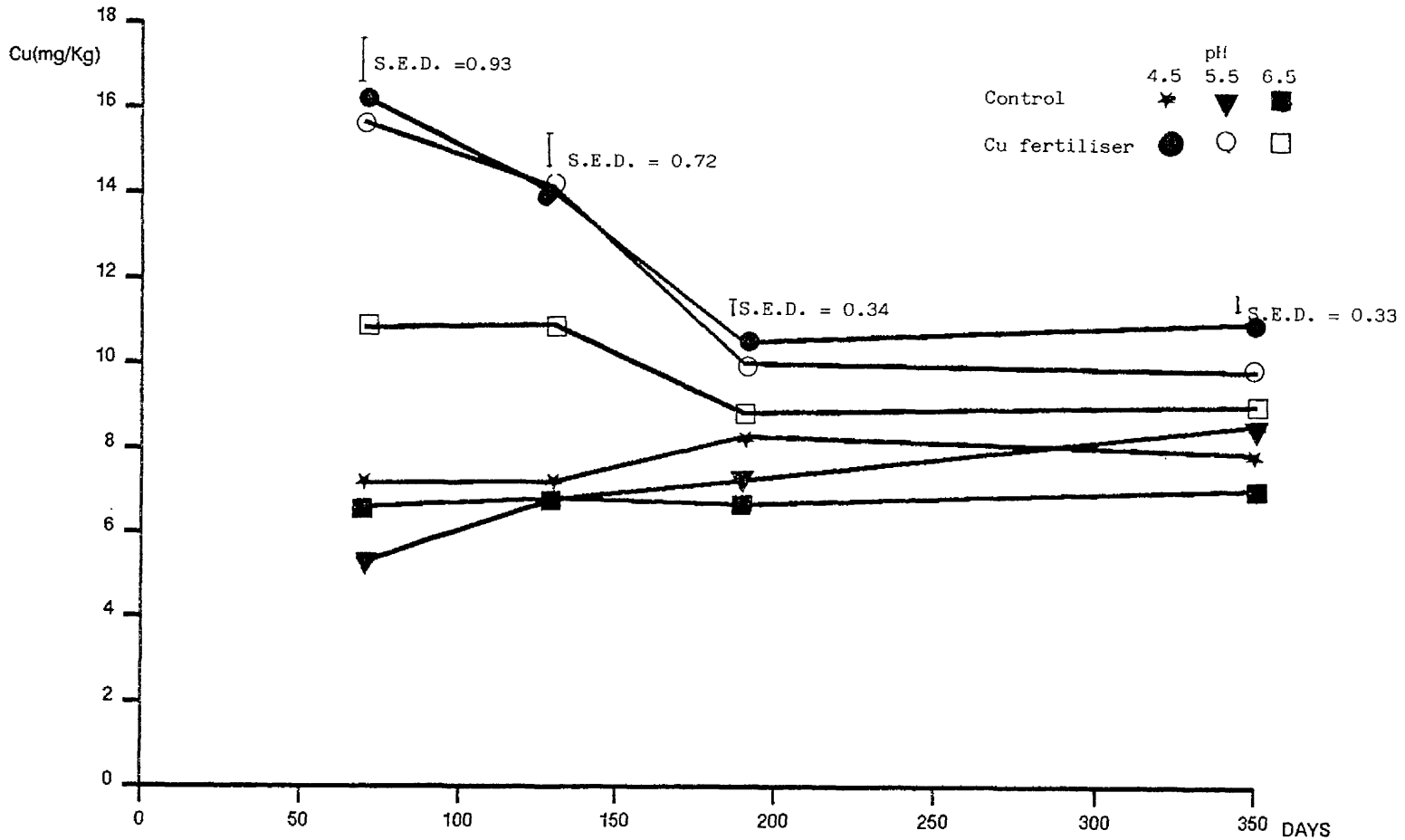
The mean Cu concentrations for the control and Cu fertiliser treatments at each pH level in all four cuts are given in Table 11.4 and presented against time in Figure 11.5. They show that there is no significant effect of soil pH on the control grasses. However, when Cu fertiliser was applied, lowering the pH from 6.5 to 5.5 caused significant increases (cut 1 $p<0.001$, cut 2 $p<0.01$, cut 3 $p<0.001$, cut 5 $p<0.001$) in the Cu concentration of ryegrass. However there was only a significant difference ($p<0.001$) between grass Cu concentrations for pH 4.5 and 5.5 at cut 5. Although at pH 4.5 the grass Cu concentrations tended to be higher than those at pH 5.5. In addition the lowest pH level maintained the greatest elevation in grass Cu concentration throughout the trial period. Therefore it appears that soil pH is influencing the concentration and rate of release of Cu from the Cu fertiliser to both the soil and plant. As in the first pot trial (Chapter 10) the evidence therefore suggests that the more acidic soil allows the Cu fertiliser to degrade more rapidly and provide a higher concentration of Cu available to the plant. The lower soil pHs may also allow this more rapid rate of release to be maintained for a longer period of time.

Table 11.4

Mean Cu concentrations for the control and Cu fertiliser treatments at each pH level in each cut

Cut	Cu fertiliser (kg/ha)	pH		
		4.5	5.5	6.5
1	0	7.20	5.29	6.63
	370	16.26	15.67	10.86
		S.E.D. = 0.93		
2	0	7.22	6.80	6.83
	370	14.01	14.14	10.92
		S.E.D. = 0.72		
3	0	7.32	7.26	6.69
	370	10.53	10.04	8.87
		S.E.D. = 0.34		
5	0	7.91	8.60	7.07
	370	10.99	9.86	9.06
		S.E.D. = 0.33		

Figure 11.5: Comparison of mean grass Cu concentrations for control and fertiliser treatments at each pH level against time.



(b) Effects of organic matter content on Cu concentrations

The mean Cu concentration for the control and Cu fertiliser treatments at each organic matter level in all four cuts are given in Table 11.5 and presented against time in Figure 11.6. Application of the Cu fertiliser caused significant increases ($p<0.001$) in ryegrass Cu concentrations on all three soil types in each cut. However, there were no significant differences in the Cu concentration of grass on fertilised soil between soil types. Therefore, organic matter content does not appear to influence initial release of Cu by the fertiliser. At the first three cuts there were no significant differences in the Cu concentration of grass among control treatments. All three soil types had similar E.D.T.A. extractable Cu concentrations (Table 11.1) and so these results for the controls were not unexpected. In the final cut the Cu concentrations of the ryegrass from the control, high organic matter treatment dropped significantly below that of the other control treatments. This could be due to depletion of its own inherent soil Cu reserves by the continual removal of Cu in previous cuts. The Cu fertiliser, high organic matter treatment followed a similar parallel decline but still maintained a 2 mg Cu/kg D.M. advantage over the control throughout this period (Figure 11.6). This implies that although the soils own Cu content is decreasing the Cu fertiliser is still supplying sufficient Cu to significantly increase grass Cu concentrations.

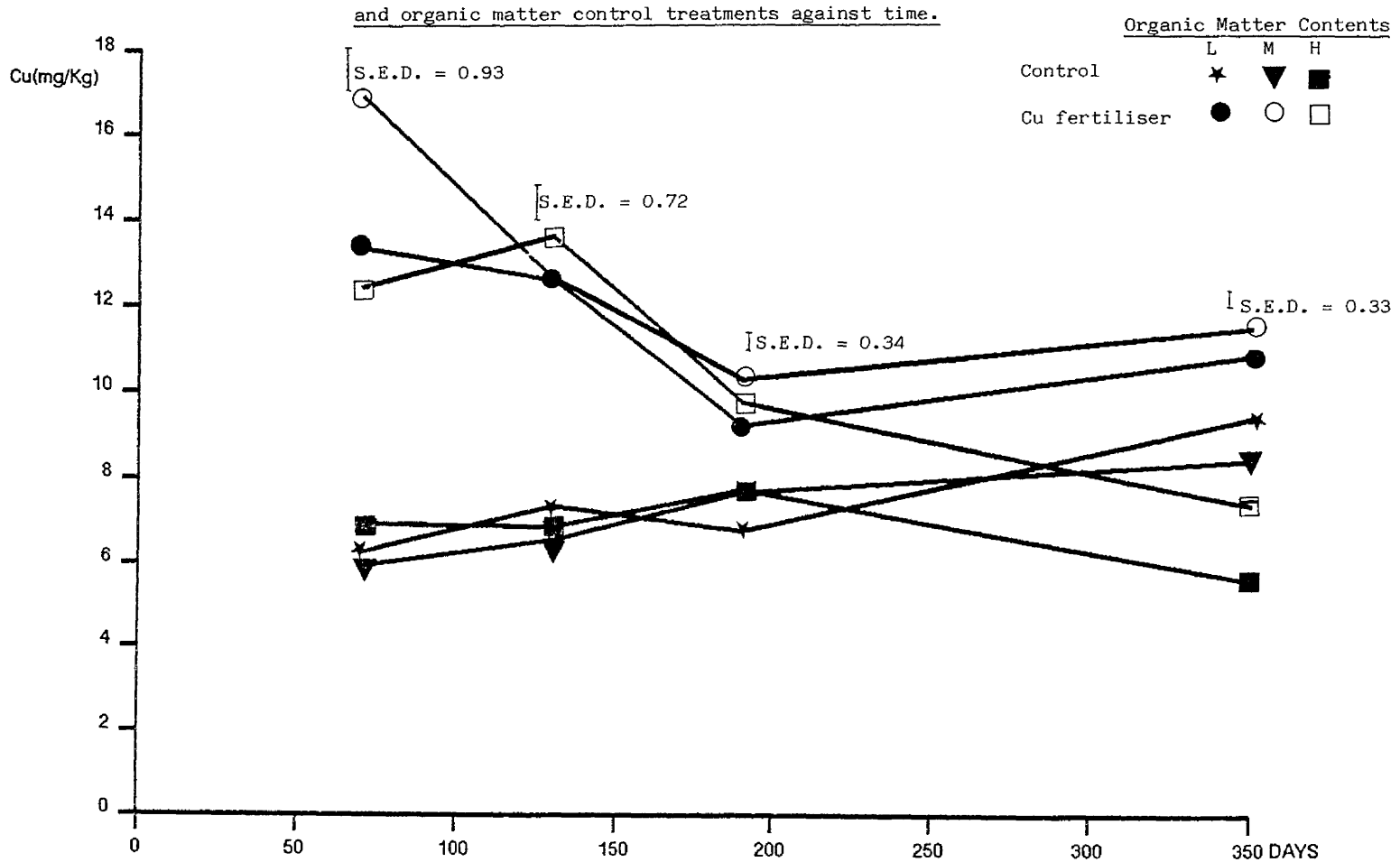
The above results demonstrate that organic matter had no significant effect on the uptake of Cu fertiliser-derived Cu by ryegrass. This suggests that the grass was obtaining most of its Cu directly from the Cu fertiliser which was released at a similar rate in all three soil

Table 11.5

Mean Cu concentration for the control and Cu fertiliser treatments at each organic matter content for all four cuts

Cut	Cu fertiliser (kg/ha)	Organic matter content		
		Low	Moderate	High
1	0	6.26	5.94	6.92
	370	13.39	16.96	12.45
			S.E.D. = 0.93	
2	0	7.38	6.58	6.89
	370	12.66	12.72	13.69
			S.E.D. = 0.72	
3	0	6.79	7.71	7.78
	370	9.28	10.34	9.82
			S.E.D. = 0.34	
5	0	9.47	8.50	5.61
	370	10.92	11.57	7.42
			S.E.D. = 0.33	

Figure 11.6: Comparison of mean grass Cu concentration for the extractions between Cu fertiliser and organic matter control treatments against time.



types, after the initial higher rates of release on application. Whereas, if the Cu fertiliser Cu was first released to the soil and from there to the plant the high organic matter soil should adsorb this extra Cu quickly and render it unavailable to the plant and so the grass Cu concentration of the control and Cu fertiliser treatments would converge more quickly as the organic matter content increases. As Figure 11.6 shows, this did not occur. This premise could be tested by use of a comparable $CuSO_4$ treatment in all three soils. In the higher organic matter soil, Cu from $CuSO_4$ would probably be quickly immobilised by soil adsorption processes and thus less Cu would be available for plant uptake than when the Cu fertiliser is used.

(c) Effects of waterlogging on Cu concentration

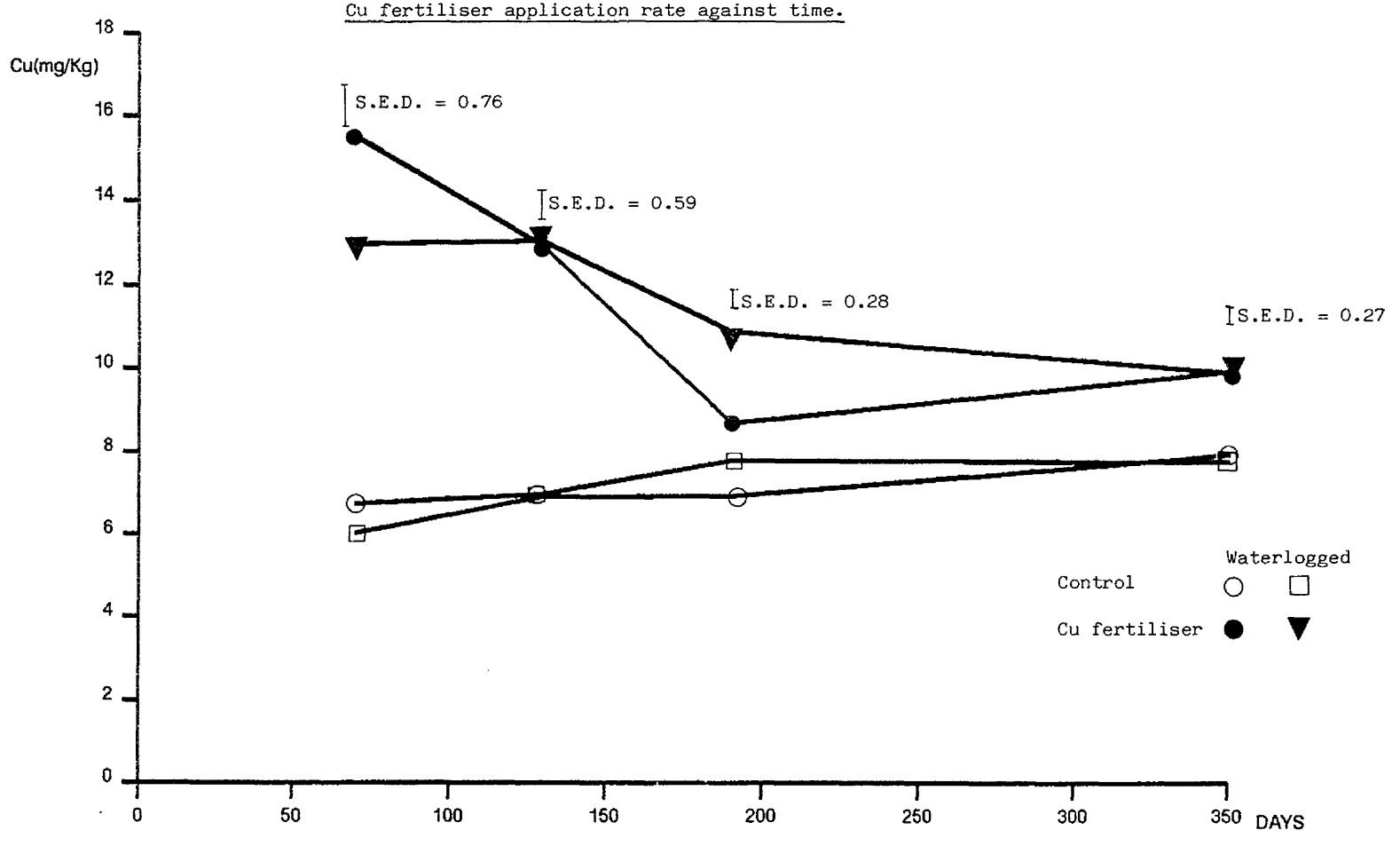
The mean Cu concentrations of the ryegrass for the control and Cu fertiliser treatments in both aerated and waterlogged soils are given in Table 11.6 and presented against time in Figure 11.7. There was no consistent significant difference in Cu uptake by grasses grown in waterlogged or aerated treatments in either 0 or 370 kg/ha Cu fertiliser treatments. Watertable height has apparently had no effect on the solubility of Cu from the Cu fertiliser. This was probably because, despite the different water tables the pots were in an enclosed system where there was not a constant through flow of water to remove any Cu from the soil solution and even if any Cu was leached out of the soil it only went as far as the tray in which the pot was sitting from where it could be reabsorbed by plant roots.

Table 11.6

Mean grass Cu concentrations for aerated and waterlogged treatments at each Cu fertiliser application rate in each cut

Cut	Cu fertiliser (kg/ha)	Aerated	Waterlogged
1	0	6.01	6.74
	370	15.55	12.89
		S.E.D. = 0.76	
2	0	6.91	6.99
	370	12.97	13.08
		S.E.D. = 0.59	
3	0	6.94	7.81
	370	8.72	10.90
		S.E.D. = 0.28	
5	0	7.95	7.77
	370	9.98	9.95
		S.E.D. = 0.27	

Figure 11.7: Comparison of mean Cu concentration for waterlogged and not waterlogged treatment at each Cu fertiliser application rate against time.



11.3.3 Grass Zn Concentrations

Herbage Zn concentrations followed a similar pattern to those of Cu (Table 11.7). Application of the Cu fertiliser resulted in significant increases ($p<0.001$) in grass Zn concentrations. Waterlogging or organic matter content had no significant effect on grass Zn concentrations. These results are consistent with those of previous work (Chapters 6 and 10) in which Cu and Zn followed similar uptake patterns.

11.3.4 Grass Fe, Pb, Cd, Cr, Mn and Ni Concentrations

Application of the Cu fertiliser had no significant effect on the Cu concentration of any of these elements (Table 11.8). Thus the rate of release of these elements from the Cu fertiliser or their plant availability were not affected by the different soil factors. Consequently they were not analysed in the grass from the final cut on 29 March 1988. The concentrations of these elements were well below both phytotoxic and zootoxic thresholds (Table 6.7). These results are consistent with those of previous work (Chapters 6 and 10) and imply that the presence of these elements in the Cu fertiliser is unlikely to have any detrimental or toxic effect in either the plant or animal.

11.3.5 Conclusions

Results from this pot trial have further confirmed that the Cu fertiliser is a method of increasing grass Cu and Zn concentrations. The availability of Cu from the Cu fertiliser does not appear to be affected by the organic matter content or water table of a soil. This

Table 11.7

Mean grass Zn concentration for each treatment

Treatment									
Organic matter content:	**Low**			**Moderate**			**High**		
pH:	**4.5**	**5.5**	**6.5**	**4.5**	**5.5**	**6.5**	**4.5**	**5.5**	**6.5**
Cut 1									
Control	34.2	30.5	29.5	39.4	28.3	35.1	29.1	28.6	29.3
+ Cu fertiliser	39.6	36.7	35.9	38.1	37.9	35.2	58.6	62.0	51.8
Waterlogged control	31.0	28.7	29.1	37.8	32.9	32.3	24.2	25.9	22.1
Waterlogged + Cu fertiliser	48.9	43.3	43.1	42.9	39.2	32.6	61.6	43.3	36.9
				S.E.D. = 2.62					
Cut 2									
Control	35.6	30.5	27.2	38.7	19.4	36.2	28.9	29.7	26.7
+ Cu fertiliser	40.4	37.7	37.7	42.7	39.6	45.5	40.6	53.1	47.8
Waterlogged control	42.0	29.1	29.7	36.9	32.6	35.1	26.7	35.7	32.0
Waterlogged + Cu fertiliser	53.9	44.9	58.6	50.5	49.5	40.9	49.3	40.0	38.6
				S.E.D. = 2.68					

Table 11.7 cont.

Treatment									
Organic matter content:	**Low**			**Moderate**			**High**		
pH:	**4.5**	**5.5**	**6.5**	**4.5**	**5.5**	**6.5**	**4.5**	**5.5**	**6.5**
Cut 3									
Control	34.9	31.1	29.6	36.7	22.1	34.8	30.6	30.9	30.3
+ Cu fertiliser	39.9	37.2	35.9	43.9	39.9	37.6	51.2	48.9	49.4
Waterlogged control	29.1	28.4	30.0	34.6	31.3	24.0	29.9	32.2	33.5
Waterlogged + Cu fertiliser	47.3	42.2	41.3	40.9	41.6	38.7	56.7	50.3	48.7
				S.E.D. = 2.16					
Cut 5									
Control	35.1	33.6	35.2	35.2	29.2	33.8	27.2	26.3	27.5
+ Cu fertiliser	39.3	38.4	39.5	39.8	37.6	34.6	43.6	39.4	37.5
Waterlogged control	33.2	41.0	35.6	32.1	31.6	29.4	26.2	29.1	29.6
Waterlogged + Cu fertiliser	46.4	44.2	41.6	37.2	38.1	32.6	52.1	43.8	41.7
				S.E.D. = 2.32					

Table 11.8

Mean elemental concentration of grass

Element	Date of cutting							
	03/06/87	S.E.D.	12/08/87	S.E.D.	14/10/87	S.E.D.	29/03/88	S.E.D.
Cu	10.32	2.27	9.99	1.80	8.62	0.82	8.91	0.96
Zn	37.05	2.62	38.08	2.68	37.15	2.16	35.01	2.32
Fe	132.6	19.3	142.0	18.9	119.9	16.7	-	-
Pb	0.5	0.18	0.87	0.22	0.82	0.21	-	-
Cd	0.04	0.01	0.03	0.01	0.03	0.01	-	-
Cr	0.2	0.04	0.3	0.06	0.2	0.06	-	-
Mn	171.8	22.4	204.5	29.5	189.0	24.1	-	-
Ni	4.77	0.52	6.01	0.46	5.12	0.96	-	-

implies that the Cu fertiliser could be effectively used on a wide range of soil types. However, soil pH does influence the availability of Cu from the Cu fertiliser; lowering the pH caused significant increases in the Cu concentration of ryegrass. It is probable that pH affects the solubility of the Cu fertiliser. This compares well with the characterisation work (Chapter 4) which showed that the solubility of the Cu fertiliser increased with increasing acidity. The Cu fertiliser would probably be more effective on acid soils in which Cu from $CuSO_4$ would probably be quickly immobilised by adsorption in the soil (section 2.4.1).

Under the intensive growing conditions of the glasshouse this work has demonstrated that the Cu fertiliser could provide a long term source of Cu for plants. The elevated grass Cu concentrations were maintained throughout the duration of the trial which was 350 days. As in the previous trials the Cu and Zn increases were not accompanied by increased Fe, Pb, Cd, Cr, Mn or Ni concentrations.

These results suggest that the Cu fertiliser could act as a long term source of Cu for grass particularly on the acidic soils of Great Britain where $CuSO_4$ cannot be effectively used as a soil applied Cu fertiliser, although comparative trials would be necessary to confirm this. In addition it has potential for use as a Zn fertiliser. Its ability to supply either Cu or Zn does not appear to be affected by organic matter content or waterlogging as described in this chapter.

11.4 SUMMARY

11.4.1 Application of the Cu fertiliser had no positive or deleterious effects on grass yields.

11.4.2 Application of the Cu fertiliser maintained a significant increase in grass Cu concentrations in all three soil types throughout the duration of the trial.

11.4.3 Organic matter content of the soil did not adversely affect the availability of Cu to grass in soils supplemented with Cu fertiliser.

11.4.4 Soil pH is the main factor which influences the plant availability of Cu from Cu fertiliser supplemented soil.

11.4.5 Lowering the soil pH resulted in significant increases in the Cu concentrations of Cu fertiliser treated ryegrass.

11.4.6 Waterlogging had no consistently significant effect on the Cu concentrations of Cu fertiliser treated soil.

CHAPTER 12. SOIL INCORPORATION FIELD TRIAL

12.1 INTRODUCTION

Preceding pot and field trials demonstrated that application of the Cu fertiliser increased the Cu concentration of both ryegrass or mixed herbage. Although broadcast application to grassland is preferable to soil incorporation from a practical point of view as it is the simplest and most convenient method, some toxicity problems may arise due to direct ingestion of the Cu fertiliser adhering to the surface of herbage by sheep susceptible to Cu poisoning, such as the North Ronaldsay, or if high application rates are used. This problem may be overcome by incorporation of the Cu fertiliser into the soil. Copper deficiency in sheep occurs particularly often after pasture improvement (see section 2.3.3). If incorporation of the Cu fertiliser into the soil at reseeding increases herbage Cu concentrations then it may prevent any subsequent Cu deficiency in livestock.

The first glasshouse pot trial (Chapter 10) showed no significant differences in Cu uptake by ryegrass when surface application and soil incorporation of the Cu fertiliser were compared. However, the limited confines of the pot probably meant that the plant roots had access to all the Cu fertiliser in the pot regardless of application method (section 10.3.3). In the field situation although broadcast application of the Cu fertiliser produced increased herbage Cu concentrations (Chapter 6) this method of application does not allow immediate access of the roots to the applied Cu. Quicker and more efficient uptake of Cu from the fertiliser may occur if it is

incorporated into the soil.

Therefore a field trial was set up to investigate the effect of incorporation of the Cu fertiliser into the seedbed at reseeding on herbage Cu concentrations. Due to dilution effects and because the risk of ingestion would be reduced it was felt that a high Cu fertiliser application rate could be used in this trial as the increase in herbage Cu after broadcasting at Garmore had not been as high as might have been preferred. Therefore in addition to using the 370 kg/ha Cu fertiliser application rate, for direct comparison with broadcast application (Chapters 5, 6 and 8), a treatment of 1000 kg/ha Cu fertiliser incorporated into the soil was included.

12.2 EXPERIMENTAL

12.2.1 Site Description and Preparation

An area 15 m x 8 m on a uniform established sward at Temple Field on the West of Scotland College estate (O.S. map reference NS382238) was prepared as follows to simulate an autumn reseed:

(a) The existing herbage was destroyed using the herbicide glyphosate.

(b) The dead herbage was then cut and removed.

(c) The area was ploughed to break up and invert the soil.

(d) The area was rotivated, then raked to break up any clods and prepare a seedbed.

(e) Twelve 3 m x 2 m plots were marked out.

(f) The Cu fertiliser was then applied, as described in 6.3.3, to the soil surface at the following rates:

(i) 0 kg/ha.

(ii) 370 kg/ha.

(iii) 1000 kg/ha.

Each treatment was then replicated four times in a single randomised block (Figure 12.1).

(g) The Cu fertiliser was incorporated into the top 5 cm of the soil by means of a rotaspike.

(h) On 10 August 1987 the area was seeded with ryegrass at a rate of 10 g seed/m^2.

12.2.2 N.P.K. Fertiliser Application

After germination, triple-17 N.P.K. fertiliser at a rate of 185 kg/ha was applied to each plot by hand. This application was repeated in April 1988.

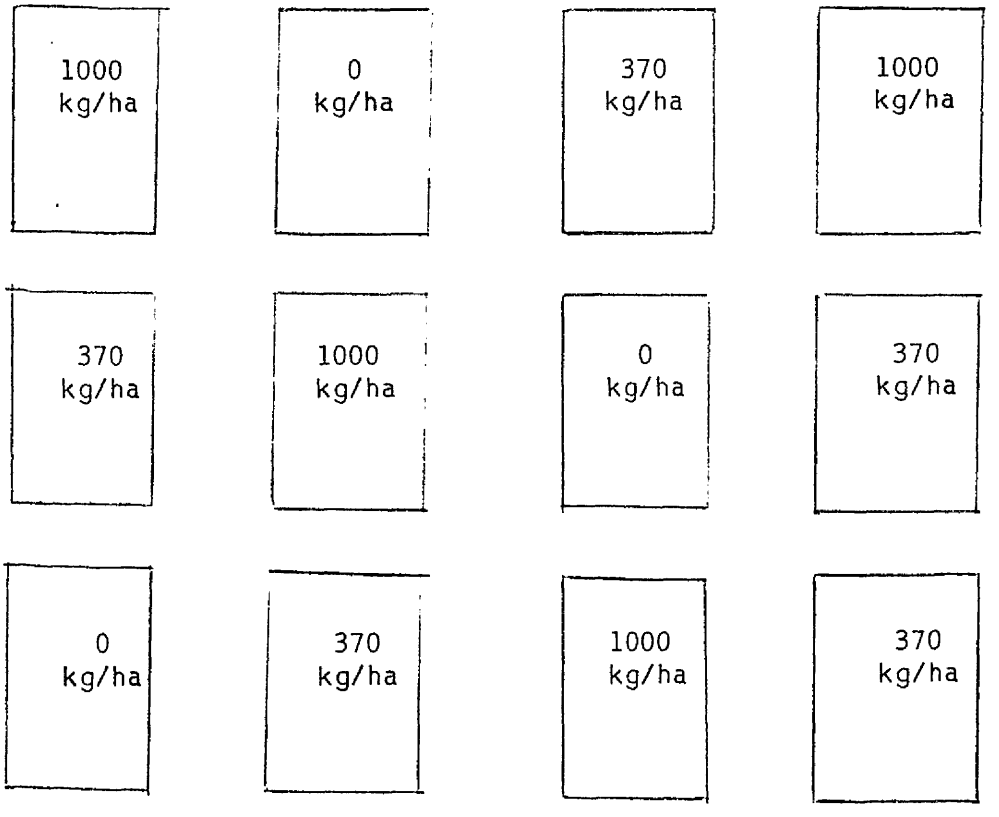
12.2.3 Herbage Sampling and Analysis

As no yield responses were found in previous trials (Chapters 6, 10 and 11) and no Cu deficiency was expected in this trial, no measurements of grass yields were made.

The ryegrass in the plots was sampled on 29 October 1987 and 2 June 1988. The samples were taken up the centre strip of each plot using steel sheep shears. The grass was cut approximately 3 cm from the soil surface. As there was still only limited growth the replicate

Figure 12.1

Arrangement of trial plots



samples collected on 29 October 1987 for each treatment were bulked together in order to obtain sufficient grass for analysis. The samples collected from each plot on 2 June 1988 were analysed individually.

Each sample was analysed for total Cu (3.3.4) only.

12.3 RESULTS AND DISCUSSION

Incorporation of the Cu fertiliser into the seedbed before reseeding was observed to have no deleterious effects on grass seed germination. Incorporation of the Cu fertiliser also produced increases in grass Cu concentrations (Table 12.1). The increases at the first cut were comparable to those at the second cut which were significant at $p<0.001$. Increasing the Cu fertiliser incorporation rate from 370 kg/ha to 1000 kg/ha had no significant effect on grass Cu concentration.

Table 12.1

Mean ryegrass Cu concentrations

Cu fertiliser application rate (kg/ha)	Cu concentration (mg/kg)	
	29/10/87	02/06/88
0	11.0	6.9[a]
370	14.9	9.9[b]
1000	15.0	9.8[b]
S.E.D.	N/A	0.54

*Means with different superscripts in any column are significant at the $p<0.001$ level.

The increases of 3-4 mg/kg in the grass Cu concentrations produced using 370 kg/ha Cu fertiliser are greater than those obtained using the same rate broadcast in the Garmore herbage trial. This could reflect the different compositions of the two swards rather than the effectiveness of the Cu fertiliser. However as the Cu fertiliser is not very soluble and as Cu is not very mobile in the soil the incorporation should give more immediate access of Cu to the roots. Thus quicker and more efficient uptake of Cu from the Cu fertiliser should occur when it is mixed into the rooting zone rather than broadcast to the soil surface. This possibility could only be confirmed by a direct comparison of incorporation and broadcast applications under identical field conditions.

The increases in grass Cu concentrations are very similar to those obtained in the two pot trials when 370 kg/ha Cu fertiliser was used. They are however larger than those obtained by several workers using $CuSO_4$, at comparable Cu application rates, in the field situation where increases of only 1-2 mg/kg in ryegrass were recorded (Burridge **et al**, 1983; Reith **et al**, 1984).

Without further investigation the reasons for the two different application rates providing similar increases in grass Cu concentrations cannot be explained. Although the two Cu fertiliser application rates gave similar increases in grass Cu concentrations, in the first year of the trial, it is possible that the 1000 kg/ha Cu fertiliser application rate may provide greater long term residual effects than the 370 kg/ha rate. This possibility could not be followed up within the time constraints of the project.

Incorporation of the Cu fertiliser into the soil at reseeding thus provides increased grass Cu concentrations without any surface contamination and potential problems this may cause. It, therefore could be used at reseeding to overcome or reduce the incidence of Cu deficiency in sheep which is often associated with upland pasture improvements. The increase would probably be sufficient to prevent Cu deficiency in grazing livestock, especially where this has arisen purely as a result of land improvement processes (section 2.3.3). However a large scale incorporation trial using animals would be necessary to confirm this.

These results show that the Cu fertiliser incorporated into the seedbed can provide Cu to a new crop. These results with ryegrass suggest that the Cu fertiliser could be used as an immediate source of Cu to arable crops with possible long term residual effects. As copper deficiency in arable crops is a worldwide problem (Alloway and Tills, 1984) this is a use for the Cu fertiliser worthy of further study.

12.4 SUMMARY

12.4.1 Application of the Cu fertiliser to the seedbed had no deleterious effect on grass seed germination at reseeding.

12.4.2 Incorporation of the Cu fertiliser into the soil at reseeding produced significant increases in grass Cu concentrations.

12.4.3 There were no significant differences in grass Cu concentrations between a 370 and a 1000 kg/ha Cu fertiliser application rate.

CHAPTER 13. GENERAL DISCUSSION

Copper is an essential nutrient for both crops and animals. Its deficiency in sheep continues to cause problems throughout Great Britain and many other parts of the world. The importance and extent of Cu deficiency is increasing as agricultural land is improved to increase production. Direct treatment of the animal is demanding and the convenience and simplicity of a soil treatment to raise herbage Cu concentrations is therefore an attractive proposition for the prevention of Cu deficiency in sheep. Previous studies have indicated that soil application of Cu compounds increased plant uptake by small amounts which were seldom dependable in overcoming livestock deficiencies.

The objective of this project was to evaluate the potential of a Cu rich material, a largely unrefined and unprocessed by-product of the brass manufacturing industry, which may have properties that make it suitable for use as a slow release Cu fertiliser, for the prevention of Cu deficiency in grazing sheep. No previous studies of this type had been carried out using this material. The investigations included laboratory and glasshouse studies to characterise the nature of the Cu fertiliser and its behaviour in the soil. In addition the work included field trials to assess the effects of the Cu fertiliser on the Cu status of herbage and a comparison of the performance of sheep grazing pasture naturally low in available Cu with that of sheep grazing comparable Cu fertiliser treated pasture.

Time constraints on the project meant that field trials had to begin immediately so that the residual value or longevity of the Cu

fertiliser treatment could be measured. Ideally field trials would have been preceded by laboratory and glasshouse studies. However initial results from the field trials showed increased sheep plasma Cu concentrations that could not be explained on the basis of the information available at that time. This led to more trials and additional work to look at the leaf adherence characteristics of the Cu fertiliser and its effects when ingested by sheep, in addition to laboratory and glasshouse studies.

Application of the Cu fertiliser both incorporated into the soil at reseeding and broadcast onto an established sward, increased herbage Cu concentrations by 2 to 4 mg/kg. Both pot and field trials showed that increased Cu fertiliser application rates produced significant increases in herbage Cu concentrations. In addition the pot trials demonstrated that increasing soil acidity caused significant increases in Cu concentrations of herbage grown on supplemented soils, which was probably due to it increasing the rate of release of Cu from the fertiliser. Characterisation studies supported this by demonstrating that the solubility of, and rate of release of Cu from the Cu fertiliser increased, as pH decreased. The pH effects were not supported in the field.

The water insolubility of the Cu fertiliser (Chapter 4) means that it overcomes the problem of Cu immobilisation by soil adsorption processes associated with $CuSO_4$ on acidic soils. The increase in herbage Cu concentrations occurred on several different soil types including one of high organic matter content which was likely to immobolise any added Cu. Thus the Cu fertiliser should be able to supply Cu to herbage on most soil types.

In the case of the Garmore herbage trial (Chapter 6) the elevated Cu concentrations were maintained for three successive years after a single Cu fertiliser application. As the Cu fertiliser is still visibly present in the soil, the possibility exists for the continued maintenance of such values in the future. Under the intense growing conditions of the glasshouse, increased grass Cu concentrations were maintained in 5 cuts over a year long period.

It has thus been demonstrated that the Cu fertiliser does supply Cu to plants and also appears to act as a long-term source of Cu. The increased herbage Cu concentrations are similar to those reported using other soil applied Cu treatments but as the Cu fertiliser is still present in the topsoil there is the possibility that it may have a greater residual value. However as no direct comparisons were made it cannot be categorically stated that the Cu fertiliser would or could have a longevity greater than other Cu treatments if compared under identical conditions. In addition further monitoring is required to establish the actual residual value of the Cu fertiliser in the field.

Broadcast application of the Cu fertiliser (370 kg/ha) to a sward with known low Cu content raised and maintained the plasma Cu concentration of grazing ewes above the deficiency threshold for two years (Chapter 5). During this time plasma Cu concentrations were significantly higher than those found in control animals and were comparable to those obtained using annual oral administration of Cu needles to ewes, which is a well recognised method of preventing Cu deficiency (section 2.4.2). In the third year ewes on the treated pasture, although still maintaining plasma Cu concentrations higher than those of the control

group, dropped below the deficiency threshold and were significantly lower than those of ewes treated with Cu needles.

The initial increase in plasma Cu concentrations occurred within six weeks of introducing the ewes to the treated pasture. This was unlikely to be due to increased Cu concentrations of the herbage as it happened at a time of year when little herbage growth occurs and it is proposed that direct ingestion of the Cu fertiliser by the sheep was taking place. It is known that sheep can eat large quantities of soil when herbage growth is poor over the winter (section 2.3.6). As there was insufficient herbage to obtain samples free from soil contamination in the winter months it is likely that ingestion of the Cu fertiliser along with soil occurred. In addition analysis of unwashed spring herbage following autumn application revealed Cu concentrations above the phytotoxic threshold and up to 10 times those found in pot and herbage trials. These data suggest that the Cu fertiliser was also adhering to the herbage and was thus available for ingestion along with it.

The hypothesis that ingestion of the Cu fertiliser by the sheep was the cause of their increased plasma Cu concentrations was supported by results from leaf adherence (Chapter 8) and ingestion (Chapter 9) trials. These showed that the Cu fertiliser can adhere to foliage and this when ingested does increase plasma Cu concentrations. The surface contamination is at its highest in the first few days after application and declines rapidly over the next week. A period of three weeks is proposed between application of the Cu fertiliser and allowing sheep to graze treated pasture. However, further investigations under more controlled conditions are required with

regard to leaf adherence and ingestion, to look at the effect of leaf adherence when applied to wet or dry swards and to look at the percentage uptake of Cu from the Cu fertiliser in the sheep's gastrointestinal tract if these proposals are to be confirmed.

To try and reduce the amount of Cu fertiliser available for ingestion by sheep a second field trial (Chapter 7) was carried out using a lower application rate, 250 kg/ha compared to 370 kg/ha, as it was anticipated that this should reduce the potential for surface contamination. Comparable increases in and maintenance of plasma Cu concentrations of sheep grazing the treated sward to those found in the first trial were obtained. However, high herbage Cu concentrations were again probably due to surface contamination with the Cu fertiliser. The lower application rate reduced the period of contamination, the period between time of application and when the herbage Cu concentration declines to a consistent level over the control, from 10 months in the first field trial to 4 months in the second trial.

When herbage analysis results for the adherence trial are compared to those from the animal response trial, a large difference in the period of contamination is shown, 8 weeks compared with 10 months respectively. This suggests that under the grazing situation, trampling and to a lesser extent passage of the Cu fertiliser through the animal extends the period of surface contamination. Thus the initial increases in plasma Cu concentrations in both trials were likely to be due to ingestion of the Cu fertiliser despite the lower application rate used in the second trial.

Excess Cu absorbed from the sheep's abomasum is mainly stored in the liver (Underwood, 1981). Tissue analysis from dead ewes, in the second year of the Garmore ewe response trial, showed that ewes on Cu-treated pasture had a much higher Cu concentration in their liver than those on untreated pasture. Thus it appears that high amounts of Cu intake due to ingestion of the Cu fertiliser resulted in storage in the animal's liver. Copper so stored would contribute to subsequent maintenance of plasma Cu concentrations. Sheep on the Garmore trial where the potential for ingestion was highest immediately following the Cu fertiliser application, maintained plasma Cu concentrations above the deficiency threshold better than those newly introduced to the trial in a second year when the potential for Cu fertiliser ingestion was reduced. Therefore the subsequent maintenance of sheep plasma Cu concentrations was probably due to a combination of liver Cu stores and the increased Cu concentrations of herbage after the high levels of contamination had been reduced. In addition as the Cu fertiliser evidently remained in the topsoil there was the possibility of further Cu ingestion along with soil at times of poor sward growth in subsequent winters.

In the third year of the Garmore animal response trial, the extra 2 to 3 mg/kg advantage in herbage Cu concentrations as a result of Cu fertiliser application was sufficient to maintain plasma Cu concentrations of sheep grazing treated sward above those of the control sheep. It is also possible that further ingestion of Cu fertiliser mixed with soil could have contributed to this. However, the extra Cu intake was not sufficient to maintain all of the sheep on the treated sward above the deficiency threshold, below which the risk

of swayback in lambs is increased.

Lambs born to ewes grazing Cu-treated pasture had plasma Cu concentrations significantly higher than those found for lambs on the untreated pasture. Treatment with 1.4 g Cu needles was required to bring the mean plasma Cu concentration of the control group up to that of the lambs on the treated pasture. However, there were insufficient swayback cases in the trial to determine if use of the Cu fertiliser would prevent this form of Cu deficiency.

In the second animal response trial (Chapter 7) the replacement of the original sheep by new animals one year after the Cu fertiliser application also resulted in an increase in plasma Cu concentrations. This occurred within 10 weeks and was maintained above the deficiency threshold over the subsequent winter. Herbage analysis showed a 2 to 4 mg Cu/kg advantage over the untreated sward. Thus there was no contamination of the herbage by the Cu fertiliser and as sward cover was very good, even over the winter it is unlikely that the sheep ingested much soil and the associated Cu fertiliser. This result implies that under certain circumstances, such as where the deficiency is marginal or due to land improvement, the Cu fertiliser might be successful in preventing livestock deficiencies. However, the significance of these second year data cannot be relied upon due to the lack of a suitable control group.

Incorporation of the Cu fertiliser into the soil at reseeding was also investigated. This method greatly reduces the problem of surface contamination and allows an evaluation of its potential to overcome the Cu deficiency which is often associated with reseeding (section

2.3.3). The fertiliser when incorporated at 370 kg/ha in to the soil produced a 3 to 4 mg/kg increase in grass Cu concentrations. Thus incorporation is equally as effective as broadcast application in increasing grass Cu concentrations but involves no surface contamination. This increase should be sufficient to prevent Cu deficiency in livestock grazing reseeded land where the Cu deficiency is purely as a result of the land improvement processes. However, a large scale field trial using animals would be necessary to confirm this.

Application of the Cu fertiliser at rates of up to 1000 kg/ha had no positive or deleterious effects on grass seed germination or herbage yields in this work. However, no herbage or grass sample was ever found to be actually Cu deficient and consequently Cu was not a growth limiting element. Analysis of the Cu fertiliser showed that in addition to Cu (2%) it contained 2% Zn and smaller concentrations of Pb, Cd, Cr, Ni and Mn. Although the amounts of these elements applied to the soil were well below the maximum concentrations recommended for application in sewage sludge - the nearest comparable recommendations to inorganic fertilisers - it was felt necessary to monitor their concentrations in herbage to ensure there would be no toxicity problems in grazing livestock. Results showed that the Cu fertiliser supplied Zn to herbage as effectively as it did Cu. Even with the increased Zn uptake total herbage Zn concentrations were well below toxicity thresholds. No increases in the concentration of any of the other elements in herbage were found. The competitive uptake of Cu and Zn from the Cu fertiliser probably prevented the uptake of these elements by the plant. In addition, as approximately only 0.2% of the

added Cu was removed in each cut, if Pb, Cd, Cr, Ni or Mn uptake did occur at a similar rate any increase in plant concentrations of these elements would be too small to measure and thus its effects would probably be negligible.

A greater threat from the heavy metal content of the material was likely when it was directly ingested by the animal. Of the potentially hazardous elements Pb was present in the largest amounts, and therefore its concentration in blood was also measured in both field and ingestion trials. No elevated blood Pb concentrations due to ingestion of the Cu fertiliser were detected. Also Cd is probably considered to be the most zootoxic, thus its concentration in blood was monitored in the ingestion trial. Ingestion of the Cu fertiliser did not cause elevated blood Cd concentrations. Tissue analysis for Cu, Cd or Pb supported the view that ingestion of the Cu fertiliser at the rates used presented no risk to the animal from Cu, Cd or Pb toxicity. As there were no effects due to Cu, Cd or Pb it is unlikely that Cr, Ni or Mn would cause any problem. Although the Cu fertiliser is an effective method of increasing herbage Cu concentrations, the increases have been no better than those obtained using other soil applied treatments reported by other workers. However, the three year monitoring period of the work was insufficient to say if the persistence of the treatment will be significantly better than those of other treatments, although as the Cu fertiliser is still evident in the soil there is a strong possibility that the Cu fertiliser will continue to supply Cu to the plant in future years.

The similarity in increases in herbage Cu concentrations reported here for the Cu fertiliser and elsewhere for $CuSO_4$ (section 2.4.1) suggests

that although the Cu is applied in different forms it may have a similar mode of release to the plant. In the case of $CuSO_4$ the added Cu is adsorbed on to the soil which may then act as a slow release source of Cu, whereas in this work the Cu fertiliser is probably acting as the Cu source.

The protection from Cu deficiency in sheep afforded by the Cu fertiliser is probably due to ingestion of the Cu fertiliser. The 2 to 3 mg/kg increase in herbage Cu concentrations would probably be significant on marginally deficient or reclaimed land. In the Garmore trial the increase was insufficient to be of benefit to the grazing sheep, for the prevention of Cu deficiency, once the Cu fertiliser ingestion and liver storage effects had worn off. The second year results from the Pinmacher animal response trial (Chapter 7) suggest in certain circumstances the increase may be of longer term benefit to the animal. However, the latter results were inconclusive as there was no control group in the second year and so the emphasis must be put on the results of the initial trial, at Garmore, where the increase was not sufficient to provide adequate long-term protection from Cu deficiency.

Despite the problem mentioned above the Cu fertiliser did give two years protection from Cu deficiency. This is longer protection than that provided by oral administration or injection of Cu to sheep. The results of a single Cu fertiliser application were comparable to those obtained using annual dosing of sheep with Cu needles over two years. However, Cu needles only gave protection over the critical late pregnancy and lambing period whereas the Cu fertiliser consistently maintained plasma Cu concentrations above the deficiency threshold for

2 years. As the Cu fertiliser appears to be most effective when ingested by sheep its effectiveness may be affected by other dietary and genetic factors that affect Cu absorption rates, e.g. Mo and S; thus further work is required in this area.

Although both Cu fertiliser application rates used in the animal response trials produced comparable increases in plasma Cu concentrations this was probably due to ingestion of the Cu fertilisers. However, as sheep have a homeostatic mechanism for maintaining plasma Cu concentrations within a set range and as the Cu fertiliser increased it to the top of this range in both groups of sheep, the difference between the two application rates was not manifested in blood sampling. It may, however, have been in liver Cu concentrations. Thus if any long-term benefit from the Cu fertiliser is to be obtained, especially in marginally deficient areas, then the higher rate of 370 kg/ha should prove more successful.

The Cu fertiliser supplied Zn to herbage as effectively as Cu. Zinc showed similar effects to Cu. Increasing Cu fertiliser application rates produced significant increases in herbage and grass Zn concentrations. Although Zn deficiency is not a problem in Great Britain it is common in several areas of the world and is often associated with Cu deficiency (McDowell, private communication). Thus the Cu fertiliser could have a potential for use as a Zn or combined Cu/Zn fertiliser for crops and livestock and this is worthy of further investigation.

Although the Cu fertiliser prevented Cu deficiency in sheep for two years this was due to its ingestion rather than through an increased

herbage Cu concentration effect. This is not the way in which a Cu fertiliser should work as it appears to depend on having situations where grass growth is poor during winter or where animals are introduced to pasture immediately after application. This is not a very controlled method of getting Cu into the animal as it does not guarantee an adequate or even any consumption of Cu by each individual animal. Neither is it a well regulated method of Cu intake from day-to-day or month-to-month. This means that although the Cu fertiliser has been shown to be better than any previous soil or pasture treatment for raising sheep Cu status it is still not good enough.

Oral administration of the Cu fertiliser to sheep has demonstrated that this is a method of increasing plasma Cu concentrations. However, feeding the Cu fertiliser directly to the animal is not practical as there are already specially formulated Cu compounds, such as Cu needles, for this purpose on the market. In addition, this would also require repeated use which creates extra work for the farmer and was one of the problems that pasture treatment was intended to overcome. For these reasons the use of the Cu fertiliser, either as a pasture treatment or as a direct animal treatment, for the long term prevention of Cu deficiency in sheep could not be recommended. In addition, it is felt that there are no further experiments that could be carried out which could lead to a reversal of this recommendation. Thus farmers should continue to treat animals directly for the prevention of Cu deficiency.

The Cu fertiliser does, however, have a possible role in the long term prevention of Cu deficiency in crops. Copper deficiency in arable crops is a worldwide and increasing problem (Graham and Nambier, 1981;

Alloway and Tills, 1984). The Cu fertiliser provided a supply of Cu to grassland for at least three years and possibly more. The incorporation trial also showed that it could provide Cu to a new crop. Although the new crop here was ryegrass the results imply that the Cu fertiliser could also be a source of Cu to arable crops grown on Cu-deficient land which at present are usually sprayed annually with a Cu source. In addition, annual ploughing of arable land supplemented with the Cu fertiliser would repeatedly mix it throughout the rooting zone and would probably increase its effectiveness in the years after application. Therefore if the Cu fertiliser is to have a future use in agriculture then its potential as a long-term source of Cu to arable crops is the major area worth investigating.

APPENDIX I. MATERIALS AND METHODS

A. DETERMINATION OF LIME REQUIREMENT OF SOILS BY A MODIFIED ELECTROMETRIC TITRATION METHOD

1. Reagents

(a) 0.01 M Calcium chloride

Weight out 54.75 g (AR) $CaCl_2$, dissolve in deionised water and make up to 25 litres.

(b) 0.014 M calcium chloride

Weigh out 76.75 g (AR) $CaCl_2$, dissolve in deionised water and make up to 25 litres.

(c) Saturated lime water (approx. 0.04 N)

Ignite approximately 40 g calcium oxide (selected lump) at 900°C for 1 hour; cool and shake. Transfer to a Winchester and fill this with distilled water. Mix thoroughly. To prepare for use, syphon the supernatant lime water through a No. 2 filter paper into a suitable container. Top up Winchester after use.

Maintain a check on the strength of the lime water by titrating 50 ml + 100 ml 0.014 $CaCl_2$ with 0.1 N HCl. Only use the lime water when this titration is 20 ml, + 0.2 ml 0.1 N HCl (Indicator Bromo-Thymol Blue).

2. Method

Measure out duplicate volumes of soil, one for pH and one for L.R. (this volume is equivalent to 40 g of an average soil) into 12 oz shaking bottles in racks.

(a) pH

To each pH bottle add 100 ml 0.01 M calcium chloride. Place the racks on the end over end shaker and shake for 30 minutes. Remove from shaker and read immediately.

(b) L.R.

To the L.R. bottle add 100 ml of 0.14 M calcium chloride and 40 ml saturated lime water (by automatic pipette). Place the racks on end over end shaker and shake for 30 minutes. Remove from shaker and leave to stand overnight before reading.

3. Calculation

(i) "Water" pH = pH in 0.01 M calcium chloride + 0 unit.

(ii) Lime requirement (tons/acre) =

$$2 \times \frac{5.70-\text{pH}(CaCl)\times 20}{(\text{pH}(CaCl_2)+LW-\text{pH}(CaCl_2))} \times \frac{20}{200} = 4 \times \frac{(5.70-\text{pH}(CaCl_2))}{\text{pH}(CaCl_2+LW-\text{pH}\ (CaCl_2)}$$

This calculation makes the following assumptions:

(a) Titration of 50 ml lime water (LW), 20 ml 0.1 N HCl.

(b) Weight of 1 acre of soil to 9" deep, 2.25×10^6 lb.

(c) A field factor of approx. 2 exists, i.e. the lime requirement as estimated by this method is about half the actual lime requirement in the field.

B. DETERMINATION OF MOLYBDENUM IN PLANT MATERIAL

1. Apparatus

Muffle furnace.

Hotplate.

100 ml beakers and watchglasses.

50 ml beakers and watchglasses.

4 oz jars.

150 ml quickfit conical flasks with stoppers.

Filter funnels; 5 mm and 120 mm.

Filter papers; No. 541, 11 cm and 1 P/S 18.5 cm.

2 ml and 10 ml syringe.

Polystyrene tubes 13 mm x 100 mm (I.C.P. tube).

pH meter.

2. Reagents

Distilled water.

6 N HCl.

Redistilled HNO_3.

Chloroform-analar.

4% 8-hydroxyquinoline.

0.125% Triton X.

4 ppm molybdenum standard.

Ammonia; 1:1.

3. Procedure

Weigh 5 g dried milled herbage into a 100 ml beaker, cover with watchglass and place overnight in the muffle furnace at 470°C. When

cool add 10 ml of water and 10 ml 6 N HCl and reflux on a hotplate for 30 minutes. Filter through a No. 541 filter paper into a 4 oz jar. Wash residue with water. Re-ash filter paper, add 5 ml H_2O and 2 ml HCl, reflux for 30 minutes and filter into the appropriate bottle. Add 5 ml of ammonia to filtrate, allow to cool and adjust pH to 0.90 using ammonia. Add 35 ml 8-hydroxyquinoline.

Wash resultant solution into a 150 ml flask and add 25 ml chloroform, stopper and shake for 2 minutes. Transfer mixture into a phase separating paper and collect the chloroform in a 50 ml beaker. Allow solvent to evaporate.

When dry, weigh beaker and residue, add 2 ml nitric acid, cover with watchglass and reflux gently for 30 minutes. Remove from hotplate and rinse down watchglass and beaker with 8 ml of 0.125% "Triton X". Weigh beaker and liquid and then transfer to ICP tube.

An internal standard containing 5 ml of 4 ppm Mo, 10 ml 6 N HCl and 50 ml H_2O and also a blank of 10 ml HCl and 55 ml water should be taken through the pH and solvent extraction stages.

Prepare a 2 ppm Mo standard and blank containing 20% HNO_3 and 0.1% "Triton X" for standardisation of the ICP.

The Mo concentration is then determined by inductively coupled plasma.

APPENDIX 2. NORMAL BLOOD LIVER AND KIDNEY CONCENTRATIONS

Parameter	Blood	Liver (mg/kg)	Kidney (mg/kg)	
Cu (umol/l)	9.4-18.9	30-1000	N/A	Grace (1983)
Mg (mg/100 ml)	1.8-3.0	N/A	N/A	Grace (1983)
GSH-Px (u/ml)	>27	N/A	N/A	S.A.C. (1982)
Hb (g/100 ml)	8-13	N/D	N/A	Underwood (1977)
Ca (mg/100 ml)	8-12	N/A	N/A	Grace (1983)
Vit B_{12} (ng/l)	>400	N/A	N/A	S.A.C. (1982)
Pb (μg/ml)	<0.4	<10	<10	Underwood (1977)
Cd (nmol/l)	<5	<0.2	0.5-1.6	
Zn (umol/l)	9-18.5	15-300	N/A	Grace (1983)

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