FACTORS AFFECTING THE CONCENTRATIONS OF COPPER MOLYBDENUM AND SULPHUR IN PASTURE PLANTS

A thesis submitted
by

GOGU DAMODAR REDDY, M.Sc.Ag. (A.P.A.U. Hyderabad, India)

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of the
University of Adelaide

Department of Agronomy,
Waite Agricultural Research Institute,
Glen Osmond, Adelaide,
South Australia.

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SUMMARY

The effect of fertilizer application (P,S,Cu,Mo and $CaCO_3$) and soil temperature and water content on the concentrations of Cu, Mo and S in subterranean clover have been investigated in a series of glasshouse experiments with several soil types. In addition, seasonal variation in the concentrations of these elements in subterranean clover, silver-grass, Wimmera ryegrass and capeweed has been examined both in the glasshouse and in field sampling studies.

In a pot experiment, application of Mo to a lateritic podzolic soil increased the dry matter production of subterranean clover only when applied in conjunction with P. In other pot experiments, application of high amounts of S to lateritic podzolic soils and a red-brown earth tended to decrease the dry matter production of subterranean clover with a concomitant decrease in the concentration of Mo in the plant to near or below the critical limit for subterranean clover (about 0.10 ppm in the shoot). At low P,Cu and Mo supply, application of $CaCO_3$ to the lateritic podzolic soil increased the dry matter production of subterranean clover but at higher applications of P,Cu and Mo, $CaCO_3$ had no effect. The dry matter production of subterranean clover was decreased by soil water stress and increased by increasing the temperature of the lateritic podzolic soil and calcareous sand from 12 to 22°C. On the lateritic podzolic soils and a red-brown earth, the two major constituents of superphosphate (<u>viz</u> P and S) produced opposite effects on the concentrations of Cu,Mo and S in subterranean clover. Application of P decreased Cu and S but increased Mo concentration in the plant, while application of S increased Cu and S but markedly decreased Mo concentration. The extent to which P application affected the concentrations of Cu,Mo and S in the plant depended on the amounts of Cu,Mo and S applied to the soil. The decreases in the concentrations of Cu and S in subterranean clover where P was applied were greater at low Cu and S supply than at high Cu and S supply to the soil. Application of P increased the concentration of Mo in subterranean clover to an increasing extent as Mo supply in the soil increased.

On the calcareous sand, application of P decreased the concentration of Cu but had no effect on Mo and S in subterranean clover, while application of S had no effect on the concentration of Cu but markedly decreased the concentration of Mo and increased that of S in the plant.

When equal amounts of P and S were applied together to the soils, the effect of S on the concentration of Mo in the plant was greater than that of P; the net result was a marked decrease in the concentration of Mo.

Increasing soil temperature from 12 to 22°C significantly increased the concentrations of Cu and Mo, but not S, in subterranean clover grown on a lateritic podzolic soil and calcareous sand. Soil water stress slightly decreased the concentration of Mo but had no effect on Cu and S in subterranean clover.

Subterranean clover contained higher concentrations of Cu and S but lower Mo than silvergrass and Wimmera ryegrass. Capeweed contained higher concentration of Cu and Mo but lower S than subterranean clover. In general, the concentrations of Mo and S in capeweed were similar to that found in the grasses. The concentration of Cu and S in all genotypes declined as the season advanced (autumn to spring): the decline was faster in the grasses than in subterranean clover and capeweed. The concentration of Mo in plants tended to remain constant throughout the season.

The results indicate the factors that need to be taken into account when regulating the concentrations of Cu, Mo and S in pasture plants. Results also show that care must be taken not to impair the quality of the forage in terms of the Cu nutrition of grazing ruminants in an effort to maximize the yield of pastures by the application of fertilizers. Testing of the effects of fertilizers on the composition in pasture plants must be done under field conditions for each soil type and genotype, and under different environmental conditions.

STATEMENT

This thesis contains no material that has been accepted for the award of any other degree or diploma in any University, and, to the best of my knowledge and belief, it contains no material previously published or written by another person, except when due reference is made in the text.

Gogu Damodar Reddy

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1.0 INTRODUCTION

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The occurrence of deficiencies and toxicities of Cu in grazing animals and their relationship to the intake of not only Cu but other elements in the diet such as Mo and S is well recognised in many parts of the world (Dick, 1956; Underwood, 1971; Suttle, 1975). The incidence and severity of Cu toxicity and Cu deficiency vary from season to season and from year to year. This may be due largely to changes in the concentrations of Cu, Mo and S in the forage brought about by seasonal changes in soil, plant and environmental conditions (Bull, 1951; Cunningham, 1951).

The concentrations of elements in plants are likely to be influenced not only by their chemical availability in the soil, by soil temperature and soil water content, but also by management practices such as fertilizer application and grazing management. Soil conditions and management practices can have a marked influence on species composition of pastures and chemical composition of the pasture as a whole may thus be affected. The role of these factors in influencing the concentrations of Cu, Mo and S in pastures is not fully understood. Therefore, some factors that may affect the concentrations of Cu, Mo and S in pasture plants were examined in glasshouse experiments, supported by a limited sampling program in the field. The experiments were conducted on a range of soils that had a known history of Cu or Mo deficiency or toxicity problems in plants or grazing animals. Emphasis was placed on lateritic podzolic soils (Anderson, 1946 and 1956).

The effect of fertilizer application (P,S, Cu, Mo and $CaCO_3$) and soil environment (water and temperature) on the concentrations of Cu, Mo and S in subterranean clover were studied in a series of glasshouse experiments using lateritic podzolic soils as well as red-brown earth and calcareous sand. In another glasshouse experiment, the seasonal variation in the concentrations of Cu, Mo and S in four common pasture species was studied. In addition, the concentrations of these elements were determined in samples of the same genotypes collected from six field sites on lateritic podzolic soils over a twelve month period.

It is hoped that the results of this study will help in understanding some of the most important factors affecting the concentrations of Cu, Mo and S in pasture plants and perhaps indicate means for their regulation so that both pasture production and animal health can be maintained at high standards.

2.0 REVIEW OF THE LITERATURE

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It is well recognised that the interaction of Cu, Mo and S in grazing ruminants poses significant nutritional problems in many parts of the world including Australia, Great Britain, India, Japan and U.S.A. (Underwood, 1971). The objective of the present review is to report the literature pertaining to factors affecting the concentrations of Cu, Mo and S in pasture plants.

2.1 Distribution of Cu, Mo and S in rocks, minerals and soils

2.1.1 Total concentrations of Cu, Mo and S in rocks and minerals

Elements tend to accumulate in certain minerals, depending upon their ionic radii and charge (Goldschmidt, 1954). The most important mineral forms of Cu, Mo and S that exist in rocks are presented in Table 1.

Because of the strong covalent bond that Cu forms with S, it occurs mainly as submicroscopic grains of sulphide between crystals of silicate minerals. Cu also substitutes for more abundant elements such as Ca, Mg and Fe and thus tends to concentrate more in basalt than in granite. Cu also occurs in shale, sandstone and limestone as shown in Table 2. The average concentrations of Mo and S in rocks are also presented in Table 2.

Mo in igneous rocks occurs mainly in association with granite and syenite, as molybdenite (MoS_2). Mo contrasts with Cu in that it occurs in higher concentrations in granites than in basalts (Goldschmidt, 1954; Taylor, 1964). In sedimentary rocks, both Cu and Mo are more concentrated in shales than in sandstone and limestone (Table 2). Some black shales in U.S.A. and Great Britain have been reported to contain concentrations of Mo as high as 300 ppm (Robinson, 1948; Thomson *et al.*, 1972).

Table 1. The most important mineral forms of Cu, Mo and S that exist in rocks.[+]

Element	Minerals	Formula
Cu	Valerite	$Cu_2Fe_4S_7$ = $2CuS.3FeS.FeS_2$
	Cubanite	$CuFe_2S_3$ = $CuS.2FeS$
	Chalcopyrite	$CuFeS_2$
	Bornite	Cu_5FeS_4 = $2Cu_2S.CuS.FeS$
	Covellite	CuS
	Chalcocite	Cu_2S
	Malachite	$Cu_2(OH)_2CO_3$
	Cuprite	Cu_2O
	Chrysocolla	$CuSiO_3.2H_2O$
Mo	Molybdenite	MoS_2
	Powellite	$CaMoO_4$
	Wulfenite	$PbMoO_4$
	Chillagite	$Pb(Mo,W)O_4$
	Ferrimolybdite	$Fe_2(MoO_4)_3.8H_2O$
S	Pyrite	FeS_2
	Pyrrhotite	$FeS.Fe_5S_6$
	Chalcopyrite	$CuFeS_2$
	Pentlandite	$(Fe,Ni)_9S_8$
	Bornite	Cu_5FeS_4
	Anglesite	$SrSO_4$
	Barite	$BaSO_4$
	Gypsum	$CaSO_4.2H_2O$

[+] Combined data from Goldschmidt (1954) and Krauskopf (1972).

Table 2. Mean concentrations of Cu, Mo and S in rocks.+

Rocks	Cu	Mo	S
		(ppm)	
Earth's crust	70	2	2100
Basalt	100	1	250
Granite	10	2	270
Shale	50	3	2600
Sandstone	30	1	300
Limestone	4	0.4	1100

+ Combined data from Goldschmidt (1954), Turekian and Wedepohl (1961), Taylor (1964), Krauskopf (1967 and 1972).

S in rocks is present mainly in the form of sulphides of Fe, Ni, Cu and other heavy metal cations. A very small fraction of the S is present as sulphate (Rankama and Sahama, 1950). Although S associates with Mo, the amount present in this form is negligible when compared to that associated with Cu and other metals.

Table 2 indicates that the concentration of S is slightly higher in granite than in basalt, while shale contains more S than limestone or sandstone. Williams and Steinbergs (1962) reported that 18 samples of Australian limestones had total S concentrations ranging between 25 and 445 ppm.

2.1.2 Total concentrations of Cu, Mo and S in soils

The rangesin concentrations of total Cu, Mo and S in agricultural soils are presented in Table 3.

Table 3. Total concentrations of Cu, Mo and S in agricultural soils.

Soils	Cu	Mo	S
		(ppm)	
Common range	1 to 70	0.4 to 4	20 to 2,000
Exceptional range	0.1 to 150	0.1 to 300	2 to 10,000

The concentration of total Cu in soils commonly ranges from 1 to 70 ppm depending upon the parent material and on pedogenic factors (Holmes 1943; Mitchell, 1948; Swaine, 1955; Kishk et al., 1973). Concentrations up to 150 ppm have also been reported (Shiha, 1951; Oertel, 1961; Oertel and Giles, 1963).

The concentration of total Mo in soils has been reported to range between 0.4 and 4.0 ppm with the average being about 2 ppm (Walsh et al., 1952; Giles et al., 1962; Sequi, 1972; Cheng and Ouellette, 1973). Exceptionally large amounts of Mo (up to 100 ppm) have been found in certain soils of Great Britain derived from shales containing abundant Mo (Lewis, 1943; Thomson et al., 1972).

Soils vary greatly in their total S concentration; the values quoted by different workers range between 20 and several thousand ppm. In aerated and leached soils such as podzols, the average concentration of total S is relatively low, 20 - 260 ppm (Mann, 1955; Little, 1958; Williams and Steinbergs, 1958). Concentrations of S ranging between 90 and 1990 ppm has been reported for calcareous soils (Williams and Steinbergs, 1962). Williams and Steinbergs (1958) and Freney (1961) found that the concentration of total S in a wide range of Australian soils varied between 40 and 370 ppm. Exceptionally high concentrations of total S of above

10,000 ppm have been reported in soils developed in tidal marsh areas where sulphide has accumulated, and in saline soils in arid areas that have accumulated sulphate (Harmsen et al., 1954; Fleming and Alexander, 1961; Delver, 1962).

2.1.3 Relationship between total concentrations of Cu, Mo and S in soils and parent material

McKenzie (1957 and 1959), Tiller (1959 and 1963) and Oertel (1961), in their studies on the soil-forming processes in Australia, presented strong evidence that parent material had little influence on the content of trace elements, including Cu and Mo, in most soils. On the other hand, Swaine and Mitchell (1960) and Thomson et al. (1972) in Great Britain and Nair and Cottenie (1971) in Belgium reported a close relationship between the total content of trace elements in soils and that in parent materials. These contrasting results may be explained in terms of the degree of weathering of soils in Northern Europe and those in Australia. Therefore, it is hard to generalise on the quantitative relationships for Cu and Mo that exist between parent materials and the soils derived from them without an understanding of the pedogenic history of the soils.

During the processes of the weathering of S bearing minerals, sulphides are oxidised to sulphate and the retention of S in the soil depends upon the factors affecting the leaching of sulphates (Evans and Rost, 1945; Whitehead, 1964). For many soils little or no quantitative relationship for S exists between the parent material and the soil derived from it (Goldschmidt, 1954), except when sulphate gets precipitated as sparingly soluble gypsum, barite and anglesite under conditions such as those leading to the formation of saline soils in arid regions (Rankama and Sahama, 1950).

2.1.4 Distribution of Cu, Mo and S in the soil profile

Knowledge of the distribution of elements in the soil is of great value in understanding their availability to plants. Hodgson (1963) and Mitchell (1964) have described in detail the soil forming processes which influence micronutrient distribution in soils.

In general, Cu tends to accumulate in the clay and organic matter fractions of the soil (McKenzie,1957 and 1959; Mitchell, 1964; Reith, 1968). On the other hand, Mitchell (1955), Oertel (1961) and Tiller (1963) have observed that the total Cu in podzolic soils changed little with depth, despite the fact that there was a large increase in the clay content. They attributed this to the formation of clay by weathering *in situ* rather than to accumulation of clay by illuviation.

Most of the Mo in soils has been reported to associate with clay, Fe oxides and ironstone nodules (Wells, 1956; Tiller, 1963).

Up to 80 per cent of the total S in most agricultural soils is associated with the organic matter fraction (Evans and Rost, 1945). Distribution of inorganic sulphate in the soil profile is influenced to a large extent by the pedogenic factors. Under extreme arid conditions accumulation of "white alkali" containing K, Ca, Mg and Na sulphates occur at or near the soil surface. As leaching increases, accumulation of sulphates occurs at progressively lower depths in the soil, and in humid regions all free sulphates, except possibly the infrequent Ba and Sr sulphates, may be completely leached from the soil. Some sulphate is adsorbed by clays and sesquioxides in the soil (Harward and Reisenauer, 1966).

2.2 Reactions of Cu, Mo and S in soils

According to the "solution" and "contact exchange" theories, only nutrient elements present in the soil solution and on the normal exchange sites in the soil are readily available to plant roots (Jenny and Overstreet, 1939; Viets, 1962). Cu present in the soil solution and on the normal exchange sites accounts for only one or two per cent of the total Cu content in most soils, yet is sufficient for normal growth of plants (Mitchell et al., 1957; McLaren and Crawford, 1973a). The readily available fraction of the total Cu is determined by factors affecting the equilibrium between the different forms of Cu in the soil (Hodgson, 1963).

Robinson et al. (1951), Walsh et al. (1952) and McKenzie (1966) have reported the occurrence of healthy and Mo-deficient plants on soils with similar total contents of Mo indicating that the total content of Mo in the soil, similar to that of Cu, is not a good indicator of availability to plants.

Where most of the S is associated with the organic matter fraction of the soil (Evans and Rost, 1945; Lowe and DeLong, 1963; Lowe, 1964), availability depends largely on factors affecting the decomposition of the soil organic matter (Nicolson, 1970). The availability of sulphate, depends largely on the adsorption and desorption characteristics of the soil (Williams and Steinbergs, 1958; Chao et al., 1962).

Thus, it is evident that the concentrations of total Cu, Mo and S in the soil are of little value in predicting their availability to plants. The reactions of these elements in the soil must be considered. Reactions with the inorganic and organic constituents of the soil are discussed separately for convenience.

Reactions of nutrient elements with the inorganic soil constituents include adsorption and precipitation. Generally, adsorption isotherms appear to be more useful than solubility products in dealing with the reactions in the soil from the point of view of plant nutrition (Fried and Broeshart, 1967; Quirk and Posner, 1975). Adsorption reactions may be specific or non-specific. Non-specific adsorption is the term used to describe reversible bonding of ions by electrostatic forces at the surface of clay minerals and oxides. At constant pH and ionic strength, the surface charge is constant and exchange of cations can occur at the negatively charged sites and anions at the positively charged sites. Equations for these reactions have been considered in detail for anions by Hingston (1970) and for cations by Padmanabham (1975). Certain ions, such as Na^+, K^+, Ca^{2+}, Cl^-, NO_3^-, and ClO_4^- appear to participate only in non-specific adsorption reactions, while other ions including Cu^{2+}, HPO_4^{2-}, MoO_4^{2-} and SO_4^{2-} also take part in specific adsorption reactions at the surface of clays and oxides.

Specific adsorption takes place irrespective of the electrical charge on the surface, and the presence of a large excess of non-specifically adsorbed ions has little effect on the amount of specific adsorption that occurs. The specifically adsorbed ions are located at the inner Helmholtz plane rather than at the outer plane, where the sites for non-specific adsorption are located. Specific adsorption exhibits a degree of irreversibility when desorption is attempted at the pH at which the ions were adsorbed. The irreversibility may be due in part to conversion from monodentate to multidentate ligands at the clays and oxides surfaces (Muljadi et al., 1966; Kafkafi et al., 1967; Aylmore et al., 1967; Hingston et al., 1974; Padmanabham, 1975).

2.2.1 Reactions of inorganic constituents with Cu

Bower and Truog (1941) originally suggested that hydrolysis is a factor in reactions that result in bonding of metal ions to clay surfaces. Hodgson, Tiller and Fellows (1964) suggested that an exchange mechanism could also be involved. The possible reactions are shown below, for hydrolysis of Cu^{2+} and adsorption on the exchanger (X^-):

$$Cu^{2+} + H_2O \rightleftharpoons CuOH^+ + H^+$$

$$CuOH^+ + X^- \rightleftharpoons X\ CuOH$$

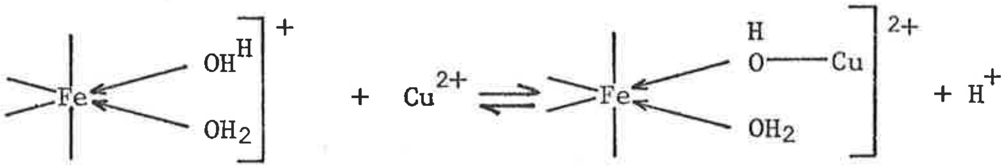
and for surface exchange:

$$Cu^{2+} + HX \rightleftharpoons \overset{+}{Cu}X + H^+$$

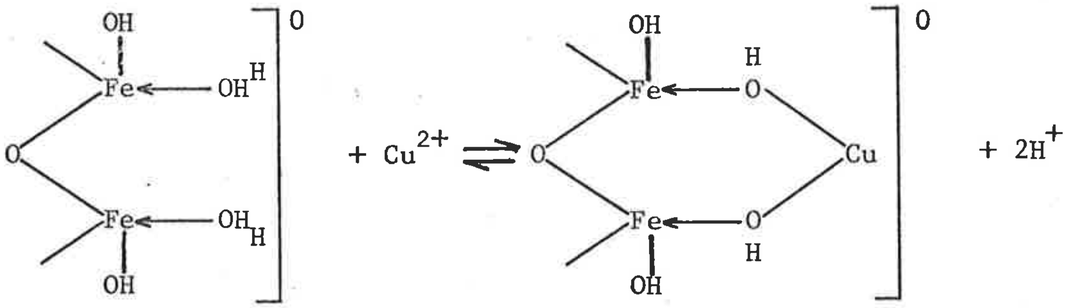
Forbes (1973) and Padmanabham (1975) showed that specific adsorption of Cu and other heavy metal cations by Fe oxides was generally accompanied by the release of one to two moles of H^+ per mole of the metal ion adsorbed. The adsorption can be viewed as a hydrolysis promoted by the surface of clay and oxides.

Padmanabham (1975) identified two types of sites on the surface of goethite that were capable of adsorbing Cu, namely, one of low bonding energy at high Cu concentrations (easily desorbable) and the other of high bonding energy at low Cu concentrations (difficultly desorbable). He suggested that the differences in bonding energy were related to the number of -OH moieties with which the Cu was co-ordinated:

Cu adsorbed by goethite with low bonding energy



Cu adsorbed by geothite with high bonding energy



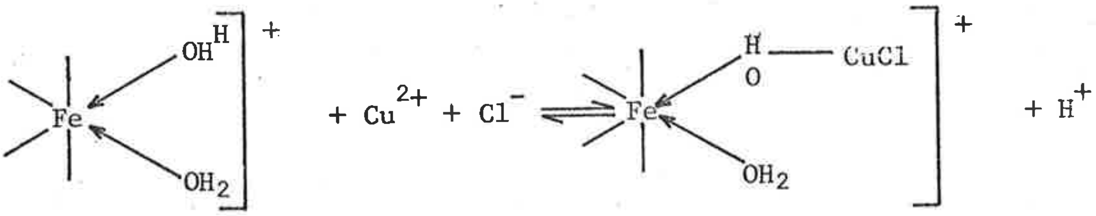
Adsorption of Cu by soil constituents such as clays and oxides increases with increase in solution concentration and as pH increases above 5.5 (Peech, 1941; Lucas, 1948; Menzel and Jackson, 1951; Ellis and Knezek, 1972).

The amount of Cu adsorbed depends on the nature of the colloid. For example, McLaren and Crawford (1973b) found that at pH 5.5 adsorption of Cu by different soil constituents followed the order Mn oxides>organic matter>Fe oxides>secondary clay minerals. Among the clay minerals, it has been reported that montmorillonite adsorbs more Cu than illite or kaolinite under similar conditions (Menzel and Jackson, 1950; Heydemann, 1959). In the above studies, variation in the specific surface area of different soil colloids and the charge per unit area were not taken into account: all results were expressed on a per unit mass basis. Differences in specific surface area and the surface charge of different soil constituents could at least be partly responsible for the observed differences in their capacities to adsorb Cu.

The presence of other ions in the bulk solution also influences the adsorption of Cu by clays. For example, under acid conditions, H^+ may compete with Cu^{2+} and decrease its adsorption by clays. Ions such as Mg, Fe and Al released from octahedral positions in the clay lattice at low pH, may compete with Cu for the adsorption sites, thereby decreasing adsorption (Elgabaly, 1950; Heydemann, 1959).

At neutral or slightly alkaline pH, retention of Cu by soils and soil constituents could be due to precipitation of $Cu(OH)_2$ (Bingham _et al._, 1964; Forbes, 1973). Mishra and Tiwari (1966) suggested that Cu could also be retained in soils by the precipitation of hydroxy-carbonates under certain conditions.

The nature of the anion also plays an important role in the adsorption reactions of Cu. Padmanabham (1975) found that, at a given pH, more Zn and Co were adsorbed in the presence of Cl^- than NO_3^- and suggested that transition metal ions may form cationic chloro-complexes such as $CoCl^+$, $ZnCl^+$ or $CuCl^+$ which could be adsorbed on the inner Helmholtz plane at the mineral surface as shown below:



Information on the effect of phosphate and sulphate on the adsorption of Cu by soils and soil constituents is lacking.

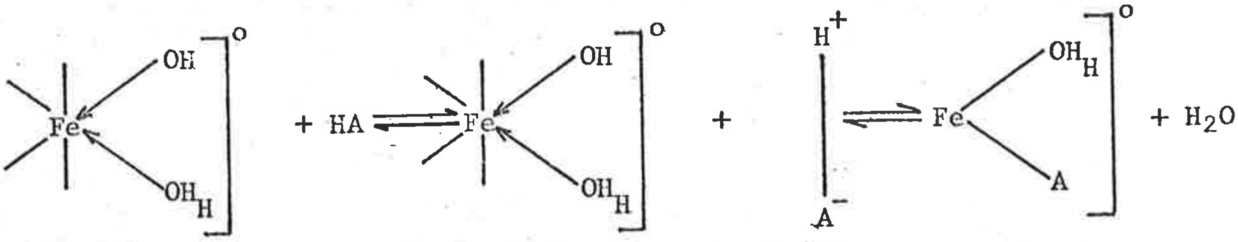
2.2.2 Reactions of inorganic soil constituents with Mo

Mo reacts in soils predominantly as molybdate. Non-specific adsorption of molybdate by soil constituents occurs through anion exchange, primarily with the surface hydroxyl groups of soil minerals and is accompanied by release of OH^- (Barshad, 1951). Specific adsorption on oxide surfaces involves ligand exchange, with displacement of water molecules (Hingston et al., 1967).

Reisenauer et al. (1962) described the reaction of molybdate with ferric hydroxide at a constant pH 4.0 by the following equation, suggesting the chemisorption of molybdate on complex iron-water-hydroxyl solid phase materials:

$$2Fe(OH)_3 + 3Na_2MoO_4 + 6HCl \longrightarrow Fe_2(MoO_4)_3 + 6NaCl + 6H_2O$$

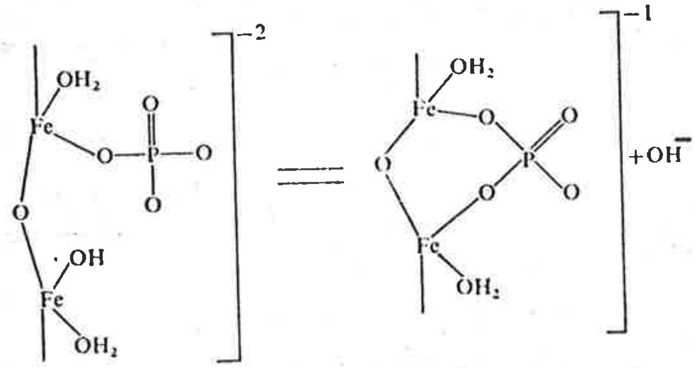
This reaction may also be explained in terms of molybdate entering the co-ordination layer of the surface of the oxide by affecting ligand exchange with displacement of OH^- and H_2O (Hingston, 1970). According to Hingston et al. (1971) specific adsorption of anions, including molybdate, depends on the ease of dissociation of the conjugate acids at the mineral surface. At pH values corresponding to the pK_a of the conjugate acid of the anion, the anion can co-ordinate with the metal ion by ligand exchange. The anion and the proton reacts with surface hydroxyls to form co-ordinated water as shown below (ligand exchange):



The pK_a of molybdic acid is 4.5, which provides an explanation for the observed high adsorption of molybdate by soils and soil clays at about pH 4.0 (Reisenauer et al., 1962; Reyes and Jurinak, 1967).

The pH dependent adsorption of specifically adsorbed anions on oxide surfaces has been explained by Hingston et al. (1967 and 1972) as follows: The undissociated free acid and the most highly charged form of the anion are not adsorbed when present alone, at about pH 7 in case of molybdate, but that the presence of both forms of the anion is necessary before adsorption can occur i.e. at about pH 4.5 in the case of molybdate where both $HMoO_4^-$ and MoO_4^{2-} species are present in the solution. Therefore, the requirement for adsorption of an anion of a weak monobasic acid such as molybdate is the presence of both a proton donor (acid) and an acceptor (base). The decrease in the adsorption of molybdate by soil constituents with increasing pH from about 4 to 8 reported by Jones (1957), Reisenauer et al. (1962) Reyes and Jurinak (1967) are in accordance with this explanation.

Reyes and Jurinak (1967) studied the adsorption of molybdate on Fe_2O_3 (hematite) at pH 4.0 and 7.8. At pH 4.0, two distinct absorption reactions were observed, which depended on the concentration of molybdate in the solution. The first reaction was insensitive to changes in temperature and occurred up to an equilibrium concentration of 10 ppm whereas the second reaction, which was endothermic, occurred at higher concentrations. These reactions were ascribed to the adsorption of molybdate at two different sites on the iron oxide. Possibly the differences in adsorption of molybdate may be due to the formation of mono- and multi-dentate ligands at the surface of the oxide as suggested by Kafkafi <u>et al</u>. (1967) and Hingston <u>et al</u>. (1974) for the adsorption of phosphate by goethite and gibbsite as follows:



(a) Labile or reversibly adsorbed P, probably adsorbed by the formation of monodentate ligands at the surface of geothite

(b) Inert or irreversibly adsorbed P, probably adsorbed by the formation of a ring structure at the surface of goethite due to bridging or multidentate ligands

As is the case with Cu, the adsorption of molybdate depends on the nature of the adsorbent. For example, Jones (1957) reported that the adsorption of Mo was greatest on Fe-oxide followed by Al-oxide, halloysite, nontronite and kaolinite. Once again, the comparison has been made on a unit mass basis ignoring the specific surface area and charge per unit surface of the colloids, so that the above order does not necessarily reflect the relative affinity of molybdate for these adsorbents.

Adsorption of molybdate by soils and soil constituents depends on the presence of other ions. The effects of phosphate and sulphate on the adsorption of molybdate by soils are of particular interest.

Wells (1956) studied the adsorption of 1.25 mg Mo by 1 g soil at pH 5.7 in the presence and absence of an equal amount of phosphate and found that phosphate completely prevented the adsorption of molybdate by the soil. More recently, Gorlach _et al_. (1969) reported that the amount of molybdate adsorbed by the soil decreased as the concentration of phosphate in solution increased. This was attributed to the competition of phosphate with molybdate for the adsorption sites in the soil. Gonzalez _et al_. (1974) also reported that the effect of phosphate in decreasing the adsorption of molybdate by volcanic ash soils in Chile increased with increasing phosphate to molybdate ratio in the soil solution.

The effect of phosphate on the adsorption of molybdate by soils depends upon the pH of the soil suspension. For example, Gorlach _et al_. (1969) reported that the effect of phosphate in decreasing the adsorption of molybdate by soils was maximum at about pH 6.0 and decreasing in more acid medium as well as in alkaline medium. Barrow (1970) found that as the pH of the soil suspension decreased from 6 to 4 the ratio of molybdate to phosphate adsorption increased by 20-fold. This was attributed to increased ability of molybdate to dissociate at about pH 4.5 (pK_a of molybdic acid) and therefore its greater adsorption over phosphate (Hingston _et al_., 1967 and 1972).

Barrow (1974) studied the ability of phosphate to displace previously adsorbed molybdate and found that the displacement of molybdate increased with increasing phosphate concentration. The effect of phosphate in displacing the adsorbed molybdate was maximum near pH 7.0 which is near the second pK_a of phosphoric acid. At pH 7.0 phosphate would be expected to be adsorbed preferentially to molybdate, thereby displacing molybdate previously adsorbed by the soil (Hingston et al., 1967 and 1972).

Another factor influencing the effect of phosphate on molybdate is the time of adsorption of molybdate with the soils. Gorlach et al. (1969) found that when molybdate had been adsorbed by the soil for 84 days, phosphate displaced only a small proportion of the adsorbed molybdate from the soil when compared to the molybdate displaced by phosphate when molybdate had been adsorbed for only one day. This may be due to fixation of molybdate after long periods of reaction, possibly by precipitation or occlusion (Smith and Leeper, 1969). It is clear from the above literature that the effect of phosphate on the adsorption of molybdate by soils depends on the concentrations of P and Mo, on pH and on the time of the reaction of Mo with soil.

Various effects of sulphate on the adsorption of molybdate by soils and soil constituents have been reported. For example, Stout et al. (1951) found that adsorption of molybdate by halloysite was decreased in the presence of $MgSO_4$ (1M) and attributed this to the competitive effect of sulphate. On the other hand, Wells (1956) found that the adsorption of molybdate by soil was increased in the presence of an equal amount of sulphate, while Gorlach et al. (1969) and Gonzalez et al. (1974) found that sulphate had no effect on the adsorption of molybdate, irrespective of the ratio of sulphate to molybdate. Thus it is hard to generalise on the effects of sulphate, but they appear to be small compared to those of phosphate on the adsorption of molybdate.

Many experiments concerning the competition between phosphate, sulphate and molybdate for the adsorption sites on soils and soil constituents involved the use of concentrations much higher than normally found in soils. Therefore, systematic studies are required on the competitive adsorption of phosphate, sulphate and molybdate at concentrations normally found in soils, taking into account pH, specific surface area and surface charge per unit area of the adsorbent, time of reaction and other important factors that influence the adsorption reactions.

2.2.3 Reactions of inorganic soil constituents with S

Inorganic S in most well drained soils is in the form of sulphate. Under anaerobic conditions, sulphate is reduced to sulphide which may react with heavy metal cations to form sparingly soluble sulphides. Similar to molybdate, sulphate undergoes both non-specific and specific adsorption on soil colloids. Adsorption of sulphate by soils and soil constituents decreases with increasing solution pH (Williams and Steinbergs, 1962; Chao et al., 1964; Harward and Reisenauer, 1966; Barrow et al., 1969). Adsorption of sulphate by soil colloids may be expected to be maximum at about pH 2.0 and to decrease with increasing pH, as the pK_a for sulphuric acid is 2.0 (Hingston et al., 1967 and 1972).

Aylmore et al. (1967) showed that there is a considerable hysteresis in the isotherms for sulphate adsorption on Fe and Al oxides.

The adsorption of sulphate by soils depends on the type of the clay mineral. For example, Kamprath et al. (1956) showed that 1:1 clay minerals adsorbed more sulphate than 2:1 clay minerals, and Chao et al. (1962) reported that the amount of sulphate retained by clays on a per unit mass basis was in the order: kaolinite > illite > bentonite.

The adsorption of sulphate is decreased in the presence of phosphate (Kamprath et al., 1956; Chao et al., 1962). This may be due to the competition of phosphate with sulphate ions for the adsorption sites in the soil. As the pK_a values of phosphate and sulphate are different from each other, the competitive effects of phosphate on sulphate adsorption are pH-dependent in a manner similar to that already described for the competitive adsorption of phosphate and molybdate.

2.2.4 Reactions of organic matter with Cu

The association of Cu with the organic matter fraction of soils is well known (Hodgson et al., 1966; Geering and Hodgson, 1969; McLaren and Crawford, 1973a, b and 1974). Cu forms both water soluble and water insoluble complexes with organic matter. For example, complexes of Cu with organic acids, polyphenols, amino acids and peptides have been reported to be water soluble, whereas Cu complexed with humic and fulvic acids is water insoluble (Stevenson and Ardakani, 1972). The most important functional groups in the humic and fulvic acid fraction of soil organic matter are the carboxyl and phenolic groups (Broadbent and Bradford, 1952; Randhawa and Broadbent, 1965; Schnitzer and Skinner, 1966; Schnitzer, 1969). Other functional groups that form stable complexes with Cu are OH, enolic OH and C=O (Stevenson and Ardakani, 1972). Functional groups reported to complex Cu in peats include OH and sulphydryl (Dawson and Nair, 1950; Himes and Barber, 1957).

Lewis and Broadbent (1961) using a series of phenolic and carboxylic acids as model compounds showed that Cu was adsorbed as $Cu2^+$ by certain sites and as $CuOH^+$ by others, depending upon the pH of the adsorption. Gamble et al. (1970) showed that pH decreases on addition of Cu to fulvic acids, suggesting that protons were released on adsorption of Cu. Mechanisms by which fulvic and humic acids complex heavy metals are discussed by Schnitzer (1969).

Recently, McLaren and Crawford (1974) reported that the largest proportion of labile Cu in soils exists as organically bound forms.

More than 90 per cent of Cu in the solution phase of the soil has been found to occur as metallo-organic complexes (Hodgson et al., 1965, 1966; Geering and Hodgson, 1969). Such Cu complexes play an important role in the availability of Cu in the soil and its transport to the plant roots, particularly under alkaline conditions (Elgawhary et al., 1970).

In addition to exchange reactions, Cu associated with organic matter in the soil will be released when these materials decompose. Release of Cu in this manner will be affected by factors influencing the decomposition of organic matter such as, temperature, aeration, pH and microbial activity (Stevenson and Ardakani, 1972).

2.2.5 Reactions of organic matter with Mo

A significant positive correlation between the total Mo and the organic matter content of soils has frequently been reported, indicating a possible association of Mo with organic compounds (Hodgson, 1963; Sharova et al., 1970; Jaakkola, 1972).

Lindoy et al. (1965) characterized some of the naturally occurring Mo-complexes with several organic ligands and Tuev et al. (1965) reported that the complexes of Mo with soil organic matter are of heteropoly acid and phenolic types. Szilagyi (1967) who studied the adsorption of Mo by humus, reported that MoO_4^{2-} was first reduced by the humic acids to Mo^{5+} and then fixed in the cationic form. However, the reduction of MoO_4^{2-} to Mo^{5+} occurs readily only at pH 1.0 and therefore this reaction is very unlikely to occur in soils (Szalay and Szilagyi, 1968).

The role of organic matter in controlling the availability of Mo in soils is uncertain. Mulder (1954) and Davies (1956) have reported the occurrence of Mo deficiency in acid organic soils and Hodgson (1963) suggested that this might be due to the formation of insoluble Mo-organic matter complexes. However, acid conditions favour the adsorption of Mo by clays and oxides in the soil resulting in low availability to plants (Jones, 1957; Reisenauer *et al.*, 1962). That some organic soils produce forage with high concentrations of Mo may be attributed mainly to their alkaline pH rather than to their organic matter content (Cunningham, 1950; Reuther and Labanauskas, 1966; Caldwell, 1971).

Gupta (1971) found that addition of organic matter along with Mo to the soil resulted in low recovery of Mo with 0.2 M ammonium oxalate solution at pH 3.3, and claimed that more Mo was fixed by the soil in the presence of organic matter than in its absence. This may have resulted from the formation of Fe molybdate-humic acid complexes analogous to the Fe phosphate -humic acid complexes reported by Weir and Soper (1963). On the other hand, reaction of organic matter with Al and Fe oxides in the soil may decrease the ability of the minerals to adsorb Mo, thereby increasing the availability of Mo (Selevlsova, 1969; Lal *et al.*, 1971).

2.2.6 Reactions of organic matter with S

Organic S is deposited in soils as plant, animal and microbial residues and accumulates in various humus fractions. Evans and Rost (1945) showed that up to 80 per cent of the total S present in 39 Minnesota soils of various types was associated with organic matter. Most of the soils contained more than 45 per cent of their S in organic form. In general, the proportion of organic S was higher in chernozems and black prairie soils than in podzols.

It is well known that the amount of S in a soil is closely related to the amounts of C and N. Swanson and Miller (1917) and Swanson and Latshaw

(1922) were some of the earliest workers who reported that the mean ratio of C:N:S for certain soils was 220:10:2. Tables showing the remarkable similarity of C:N:S from soils in various parts of the world have been compiled by Whitehead (1964) and Freney and Stevenson (1966).

A large proportion of organic S in soils may exist as organic sulphates due to the adsorption of sulphate by organic colloids (Freney, 1957 and 1961; Spencer and Harada, 1959; Nissen and Benson, 1961).

Organic S in soils also exists directly bonded to C in compounds like amino acids (Putman and Schmidt, 1959; Gilbert and Altman, 1966). The amount of free amino acids in soils is likely to be small since they provide a readily available substrate for micro-organisms. Whitehead (1964) and Freney and Stevenson (1966) have reported that about 11 to 19 per cent of the total S present in a range of soil types was in amino acid form. S-containing amino acids were liberated by acid hydrolysis of soil (Sowden, 1955; Stevenson, 1956). Lowe and DeLong (1963) and Lowe (1964) estimated that organic S bonded to C was as high as 58 per cent suggesting that much of the organic S in soils occurred in compounds other than amino acids. S in soil organic matter occurs incorporated into the humus in the form of SH groups (Mason, 1955; DeLong and Lowe, 1962; Whitehead and Tinsley, 1963). Apart from amino acids, only a limited number of organic S compounds have been isolated from soils and identified. These have included trithiobenzaldehyde, that accounted for approximately one third of the total S present in the California soil from which it was recovered (Shorey, 1913).

Based on biochemical reactions known to occur in the plant tissues, Whitehead (1964) and Freney and Stevenson (1966) suggested that organic S compounds may include dihydrothiazine and thio - derivatives of aldehydes, including reducing sugars. The compounds formed part of the S fraction of soil humus which was stable and resistant to microbial attack.

Much of the organic S in soil is not readily available to plants. Estimates of total S are not well correlated with plant growth (Williams and Steinbergs, 1959). It is evident that the value of organic S for plant nutrition depends on factors that affect the decomposition of soil organic matter and the resultant mineralization of S compounds.

2.3 Availability of soil Cu, Mo and S to plants

2.3.1 Soil conditions and occurrence of deficiencies and toxicities of Cu, Mo and S in plants

In the preceding sections the important reactions of Cu,Mo and S in soils have been discussed. The implications of these reactions on the availability of Cu, Mo and S in soils with special reference to the soil factors affecting the deficiency or toxicity of these elements in plants are described hereunder.

Peech (1941), Piper (1942), Ellis and Knezek (1972) and McLaren and Crawford (1973b) reported that the availability of Cu in soils is slightly more in acid than in alkaline medium, but does not appreciably increase until the pH falls below 5.0. McLaren and Crawford (1973b) proposed a relationship of the type shown below operating between the three most important fractions of Cu in the soil that govern the availability of Cu to plant roots.

$$\begin{array}{c} \text{Solution and} \\ \text{exchangeable Cu} \end{array} \rightleftharpoons \begin{array}{c} \text{Cu adsorbed on} \\ \text{inorganic sites} \end{array} \rightleftharpoons \begin{array}{c} \text{Organically} \\ \text{bound Cu} \end{array}$$

Here, with a decrease in pH, the equilibrium shifts to the left and thus can increase the availability of Cu in the soil for plant roots.

However, Cu deficiency in plants has been reported more often on both mineral and organic acid soils than on alkaline soils (Lees, 1950; Cunningham, 1950; Kubota and Allaway, 1972). Of the mineral soils, acid podzolic and leached sandy soils are frequently Cu deficient (Reuther and Labanauskas, 1966). The occurrence of Cu deficiency in plants growing on acid and leached soils may be due to their low Cu retention capacity resulting in loss of Cu by leaching and thus to low total Cu content in the soil. High degrees of complexing of Cu with organic ligands in soil solution may be associated with the lower incidence of the occurrence of Cu deficiency in plants on alkaline soils (Hodgson _et al._, 1966). However, there is no clear understanding of how Cu deficiency occurs in plants in some highly organic soils (Caldwell, 1971). Cu deficiencies reported on certain alkaline soils, particularly calcareous soils, have been attributed to the fixation of Cu by $CaCO_3$ in addition to other reactions that might result from the high pH (Donald _et al._, 1952; Brown and Jurinak, 1964).

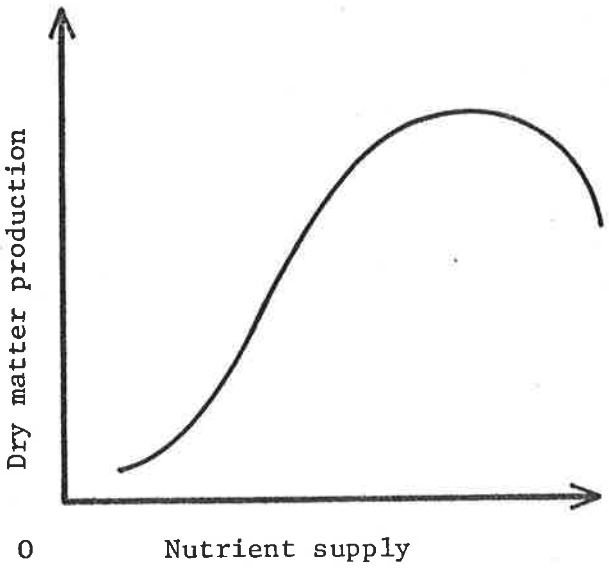
The availability of Mo and S in soils, on the other hand, is less in acid than in alkaline soils and raising the soil pH has been found to increase the availability of these nutrients to the plants (Anderson and Moye, 1952; Anderson, 1956; Williams and Steinbergs, 1964; Harward and Reisenauer, 1966). This results from the higher adsorption of Mo and S by soil constituents in acid than in alkaline pH medium as discussed earlier. Throughout the world, widespread areas of Mo and S deficiencies are known to exist mostly on acid soils that are rich in clay and oxides of Al and Fe (Anderson 1952, 1956; Dawes, 1956; Coleman, 1966; Johnson, 1966). Although toxicity of Mo and S in plants has not been found, luxury uptake of these nutrients by plants on alkaline soils has been reported (Lewis , 1943; Barshad, 1948, 1951; Eaton, 1966).

In general, availability of Cu and S in soils is associated more with the organic than with the inorganic fraction of the soil; it depends to a large extent on the factors affecting the decomposition of organic matter. On the other hand, Mo availability in soils is influenced to a large extent by the soil pH and adsorption by the inorganic constituents of soils.

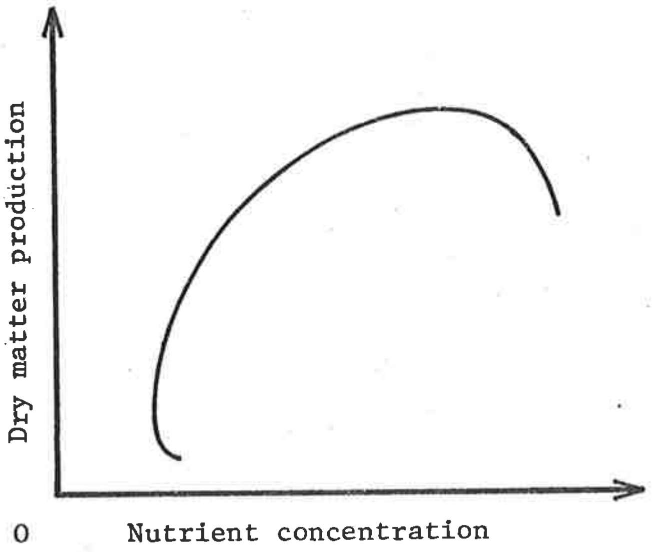
2.3.2 Relationship between the nutrient supply in the soil and its concentration in the plant

Although nutrient supply in the soil affects the concentration of the nutrient in the plant, the relationship is not linear. It depends, among other factors, on the plant's growth response to the nutrient.

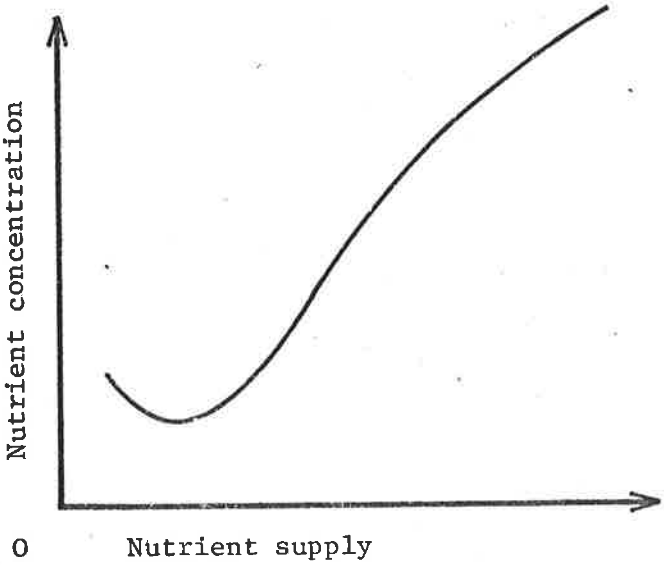
The relationship between the dry matter production of plants to increasing supply of the nutrients can be represented by a sigmoidal curve as shown below (Hellriegel and Wilfarth, 1889; Steenbjerg, 1952, 1954; Steenbjerg and Jacobson, 1963).



Steenbjerg (1950 and 1954) and Friis-Nielsen (1969) obtained the following relationship between the dry matter production and nutrient concentration in the plant.



The relationship between the nutrient supply and the concentration of the element in the plant is as follows.



This indicates that concentration of element in the most deficient plant is higher than that in the less severely deficient plant. This may be explained in terms of the rapid increase in the plant growth to the addition of the initial amounts of the nutrient in minimum resulting in the dilution of it in the plant. This may be called "growth dilution". When the element ceases to limit the growth of the plant, further addition of the nutrient increases the concentration in the plant without increasing the yield. Excessive additions of the nutrient supply may lead to the luxury uptake of the element by the plant. At this stage addition of further amounts of the nutrient may be toxic to the plant and may decrease the yield with slight or no effects on the concentration of the element in the plant.

The luxury uptake of a nutrient by the plant depends, among other factors, upon the element and the genotype. According to Reuther and Labanauskas (1966), a concentration of less than 4 ppm Cu in the plant tops is considered to be deficient, between 5 and 20 ppm is considered normal, while higher concentrations may cause Cu toxicity in plants. Less than 3 ppm Cu in the tops of subterranean clover produced Cu deficiency symptoms but no Cu toxicity symptoms were noted in plants containing up to 32 ppm Cu (Teakle, 1942; Teakle and Turton, 1943).

The minimum concentration of Mo in the plant that is needed for the normal growth of plants has been reported differently by different workers but usually varies between 0.1 and 0.5 ppm (Stout <u>et al.</u>, 1951; Anderson, 1955, 1956; Reisenauer, 1956; Stout and Johnson, 1956; Anderson, 1956). Although the concentration of Mo in plant tops can accumulate up to as high as 300 ppm, Mo toxicity in plants has not been reported (Dye and O'Harra, 1959; Kubota <u>et al.</u>, 1961, 1967).

The critical concentration of S in plants has been reported differently by different workers depending upon genotype and the stage of maturity of plants. For example, Bear and Wallace (1950), Harward _et_ _al_. (1962) and Adams and Sheard (1966) reported a critical value of 0.20 per cent S for the normal growth of lucerne at the early flowering stage whereas Martin and Walker (1966) have reported a rather higher value of about 0.26 per cent S for non-flowering white clover. The normal range of S concentration in most plants varies from about 0.20 to 0.40 per cent (Rendig, 1956; Martin and Walker, 1966). In a S-rich environment excessively high concentrations of S up to 1.5 per cent in lucerne plants have been reported (Thomas _et_ _al_., 1950).

2.4 Genotypic differences in the concentrations of Cu, Mo and S in plants

The greatest differences in the mineral concentrations among the important forage species has been reported to occur between legumes and grasses (Thomas _et_ _al_., 1952; Williams, 1963; Fleming, 1965 and 1973; Reid _et_ _al_., 1970). However, significant differences in the mineral composition also exist between classes, species and varieties (Lane and Fleming, 1966; Gladstones _et_ _al_., 1975). Vose (1963) has described the possible mechanisms which may lead to the differential uptake of nutrients by different plant species.

Adams and Elphick (1956), Fleming (1965) and Gladstones _et_ _al_. (1975) reported that leguminous plants and herbs had Cu concentrations higher than those of grasses. For example, Gladstones _et_ _al_. (1975) found that subterranean clover and capeweed had greater than 10 ppm Cu whereas Wimmera ryegrass grown under similar conditions had about 5 ppm Cu. On the other hand, Beck (1941 and 1962), in field sampling studies in Western Australia found that legumes, herbs and grasses growing under similar conditions had equal concentrations of Cu of about 10 ppm. These differences cannot be explained without the knowledge of the Cu supply in the soil. Mitchell et al. (1957) found that the concentrations of Cu in different plants respond differently to variations in the soil supply. Beck (1962) suggested, that at low Cu supply, grasses may have similar concentration to that of legumes and herbs but at high Cu supply legumes and herbs tend to have higher con- concentration of Cu than that of grasses.

Dick et al. (1953), Beck (1962), Gladstones (1962) and Fleming (1963) reported that grasses had higher concentrations of Mo than when legumes grown on soils with low Mo supply. On the other hand, Lewis (1943) in England, and Barshad (1948 and 1951) in U.S.A. reported that leguminous plants contained higher Mo concentration than did grasses on high Mo supplying soils. The relative Mo concentrations of grasses and legumes appear to depend upon the soil supply of Mo. Dick (1953) from a field experiment with legumes and grasses growing on a Mo responsive soil, showed that under conditions of low Mo supply/addition (40 g ha^{-1} Mo), silver-grass and subterranean clover contained equal concentrations of Mo (2 ppm) but at a higher Mo supply (1 kg ha^{-1} Mo) subterranean clover contained a significantly higher concentration of Mo (31 ppm) than did silvergrass (23 ppm).

In general, the differences in the concentration of S in legumes and grasses were found to be small. For example Walker et al. (1956), and Walker and Adams (1958) reported that generally grasses and subterranean clover contained similar concentrations of S (from 0.1 to 0.4 per cent S in the plant) over a range of S applications of up to 40 kg ha^{-1} S. However, Martin and Walker (1966) suggested that competition between species and time of sampling can influence the size of the differences observed in the concentration of S in different genotypes.

2.5 Effects of soil environment on the concentrations of Cu, Mo and S in plants

2.5.1 Effects of soil water content

The available evidence suggests that soil water content has little or no effect on the availability of Cu in the soil (Ng Siew Kee and Bloomfield, 1962),and its uptake by the plant (Kubota *et al.*, 1963). Nambiar (1975) found that soil water content had no significant effect on the concentration of Cu in the ryegrass and noted that plant roots absorbed significant amounts of Cu from even nearly air dry top soil when the roots had access to subsoil water. Adams and Honeysett (1964) observed that the concentration of Cu in subterranean clover and ryegrass plants increased slightly when plant growth had been severely stunted by waterlogging. These effects cannot be attributed to soil water *per se* but are probably due to changes associated with anaerobic conditions in the waterlogged soil.

Kubota *et al.* (1963) reported that increasing soil water content increased the concentration of Mo in the plant. High soil water content can favour increased mass flow and diffusion of Mo in the soil thereby leading to high accumulation of Mo at the root surface, and increased concentration of Mo in the plant (Lavy and Barber, 1964). Kubota *et al.* (1961 and 1967) found that the Mo concentration in plants growing on poorly drained soils was significantly higher than that in plants growing on well drained soils which had similar pH and water soluble Mo content. Lewis (1943) and Barshad (1948) found that when waterlogged soils were drained, the concentration of Mo in plants decreased, and claimed that soil water content influenced the uptake of Mo by plants. However, the physical, chemical and biological properties of waterlogged soils change when they are drained. Drainage may increase the losses of Mo from the soil solution by leaching. It also provides oxidising conditions in the soil. Sulphide may be oxidised to sulphate which can compete with molybdate and thereby decrease Mo concentration in the plant. Oxidation of Fe and Mn in the soil promote the adsorption of Mo. Also, when Fe-oxides precipitate, Mo could either co-precipitate with Fe-oxide or occlusion of Mo can take place (Sequi, 1972). Thus, the decreased concentration of Mo in plants on drainage of waterlogged soils may be due to decreased availability of Mo in the soil.

In the case of S, Williams (1967) and Nicolson (1970) showed that both low and high soil water content restricted the mineralization of organic matter and thereby decreased the availability of S in soils. Effects of soil water content on the concentration of S in plants are not well documented. However, it may be said that soil water content through its effects on mineralization of S in the soils and its effects on plant growth can influence the concentration of S in plants.

From the above literature it is apparent that soil water content has only a small effect on the concentration of Cu in the plant but large effects on that of Mo. Soil water content may have effects on S concentration in the plant.

2.5.2 Effects of soil temperature

Smith (1969 and 1971) grew lucerne under three temperature regimes; 15 to 10^{o}C (day/night), 21 to 15^{o}C and 27 to 21^{o}C, and found that concentration of Cu in the plant increased from 9 to 15 ppm as temperature increased.

Follet and Barber (1967) reported that increasing soil temperature increased water soluble Mo in the soil. Effects of soil temperature on the concentration of Mo in the pasture plants is not well documented.

Nielsen and Cunningham (1964) reported that increasing soil temperature from 11 to 28°C had little effect on the concentration of S in rye grass. Nicolson (1970) reported that increasing soil temperature from 10 to 24°C increased the water soluble sulphate in the soil, but S concentration in subterranean clover was slightly decreased from 0.2 to 0.1 per cent when S was not added to the soil and from 0.5 to 0.4 per cent when 80 kg ha^{-1} S was added. The decreased concentration of S in the plant with increased soil temperature was a result of the increased plant growth.

2.6.0 Effects of fertilizer application on the concentrations of Cu, Mo and S in pasture plants

Concentrations of Cu, Mo and S in pasture plants can be increased by the application of fertilizers containing these elements. The effects of fertilizer application on the growth and nutrient concentration in plants depend to a large extent on the supply of available nutrient in the soil, and on the reactions of the fertilizer with the soil constituents. The most important reactions have already been discussed. A brief discussion follows of how Cu, Mo and S fertilizers may effect the concentration of these elements in the plant. As concentrations of these elements may also be affected by application of other fertilizers, the effects of nitrogen and phosphorus fertilizers are also considered.

2.6.1 Effects of Cu fertilizers

Gladstones et al. (1975) reported no increase in dry matter production in a range of plant species in a field experiment, where up to 7 kg ha^{-1} Cu was applied to a lateritic podzolic soil. They found that application of 2.5 kg ha^{-1} Cu had generally no effect on the concentration of Cu in 13 leguminous species whereas 7 kg ha^{-1} Cu increased the concentration from about 8.5 to 10 ppm. On the other hand, application of 2.5 kg ha^{-1} Cu increased the concentrations of Cu in grasses and herbs from 8 to 10 ppm and from 12 to 13 ppm respectively, but 7 kg ha^{-1} Cu had no further effect. Gladstones et al. (1975) also observed that the effects of Cu fertilization on the concentration of Cu in plants were generally more pronounced at early stages of plant growth (3 months age) than at later stages (4 and 5 months age).

According to Reuther and Labanauskas (1966) normal rates of Cu applications on many soils is 1 to 5 kg ha^{-1}. However, it is well known that organic soils need higher amounts of Cu than mineral soils to produce similar effects on yield and concentrations of Cu in plants, as organic soils fix more Cu than mineral soils (Murphy and Walsh, 1972).

Problems of fixation of Cu in the soil may be overcome if the fertilizer is directly applied to the plant. For example Reith et al. (1968) found that foliar application of 0.25 kg ha^{-1} Cu increased the concentration of Cu in plants from 1.6 to 2.7 ppm where soil applications of 4 kg ha^{-1} Cu had no effect.

It is evident that the effects of Cu fertilization on the concentration of Cu in the plant depend, among other factors, on genotype, soil type and method of Cu application.

The effects of Cu fertilization on the concentration of Mo and S in plants are not well documented, but if plant growth increases in response to the application of Cu, concentrations of other elements, including Mo and S may change. The sulphate content of copper sulphate could conceivably decrease the concentration of Mo in the plant (see section 2.6.3) and increase that of S but such effects are likely to be small at normal rates of application (1 to 20 kg ha^{-1} $CuSO_4.5H_2O$).

2.6.2 Effects of Mo fertilizers

Stout *et al*, (1951), James *et al*. (1968), James and Ruckman (1973) and Gupta and MacLeod (1975) reported that application of Mo to soils increased the concentration of Mo in pasture plants. Soil type, plant species and interacting elements are the important factors that influence the effects of Mo fertilization on the concentration of Mo in the plant.

Stout *et al*. (1951) found that application of 1 kg ha^{-1} Mo to five soils increased the concentration of Mo in subterranean clover from the range 0.1 to 4.3 ppm to the range 1.8 to 92.0 ppm depending upon the soil. Similarly, James *et al*. (1968) reported that application of 0.4 and 0.8 kg ha^{-1} Mo to eight soils increased the concentration of Mo in lucerne from the range of 0.12 to 0.8 to the ranges 0.18 to 3.35 and 0.65 to 5.8 ppm respectively. Gupta and MacLeod (1975) showed that application of 4 kg ha^{-1} Mo increased the concentration of Mo from 0.2 to 5.7 ppm in timothy, 0.4 to 22.2 in lucerne and 0.2 to 33.8 in red clover.

Effects of Mo fertilization on the concentration of Mo in the plant increases in the presence of P and decreases in the presence of S. For example, Stout *et al*. (1951) found that application of 1 kg ha^{-1} Mo increased the concentration of Mo in subterranean clover from 0.2 to 4.3 ppm in the absence of P, but when applied with 200 kg ha^{-1} P it increased the concentration of Mo from 0.2 to 79 ppm. Gupta and MacLeod (1975) reported that application of 4 kg ha^{-1} Mo increased the concentration of Mo in forage plants from 0.2 to 14.8 ppm in the absence of added S, whereas when 400 kg ha^{-1} S was also added it increased Mo concentration from 0.2 to 2.3 ppm.

Because of the small amounts of Mo applied, concentrations of Cu and S are unlikely to be changed by Mo fertilizers unless plant growth is substantially increased.

2.6.3 Effects of S fertilizers

Jones (1964), Martin and Walker (1966) reported that application of 80 kg ha^{-1} S increased the concentration of S in forage plants from 0.1 to 0.5 per cent.

It has been reported by several workers that application of S fertilizers to the soil decreased the concentration of Mo in the plant (Stout *et al.*, 1951; Jones and Ruckmann, 1973; Gupta and MacLeod, 1975). For example, Gupta and MacLeod (1975) reported that application of 400 kg ha^{-1} S decreased the concentration of Mo in the plant from 15 to 2 ppm in timothy, 41 to 16 ppm in lucerne and 78 to 16 ppm in red clover. Thus, S has an appreciable effect on the concentration of Mo in the plant. Stout *et al.* (1951) suggested that sulphate competes with molybdate for the absorption sites on the plant root thereby decreasing the concentration of Mo in plants.

Effects of S on the concentration of Cu in plants may depend upon the genotype. For example, Gupta and MacLeod (1975) reported that application of 400 kg ha^{-1} S increased the concentration of Cu in timothy from 8.7 to 9.9 ppm whereas it had no effect in lucerne and red clover.

2.6.4 Effects of N fertilizers

N fertilization can influence the concentrations of Cu, Mo and S in plants or the requirement of plants for these elements, primarily through the effects of N on plant growth.

Experimental evidence concerning the effects of N fertilization on the concentration of Cu in pasture plants is lacking. However, the available evidence with other crops suggests that N fertilization can decrease the concentration of Cu in plants by increasing the plant growth (Steenbjerg, 1954, Chaudhry and Loneragan, 1970). Rasheed and Sheeley (1966) reported a positive correlation between Cu and N concentrations in a wide range of species, whereas Gladstones et al. (1975) found a positive correlation between Cu and N in cereals, grasses and herbs but not in legumes. The reasons for the association of Cu with N are not clear. Rasheed and Sheeley (1966) suggested that Cu forms protein complexes in the plant which influence the concentration and behaviour of Cu in plants. Gladstones et al. (1975) suggested that absorption of Cu by plant roots may be as soluble N containing complexes and therefore directly dependent on N uptake by the plant; alternatively that N status determines the size of the plant's complexing sink for Cu in the soil.

In the case of Mo, Barshad (1951) reported that application of 200 kg ha^{-1} N as ammonium sulphate decreased the concentration of Mo in pasture plants from about 120 to 20 ppm and attributed this to the cumulative effect of sulphate and decreased soil pH both of which decrease the concentration of Mo in the plants.

High applications of N can decrease the concentration of S in pasture plants through its effects on increasing the growth. Havre and Dishington (1962) reported that application of calcium ammonium nitrate decreased the concentration of S in pasture plants from about 0.3 to 0.2 per cent, whereas application of ammonium sulphate increased it from 0.3 to 0.4 per cent. The latter effect was due to the sulphate content of the ammonium sulphate. Steward (1966) suggested that S should be applied along with N fertilizers to maintain the N:S ratio in the plant of 15:1 otherwise N-induced S deficiency in plants might arise.

2.6.5 Effects of P fertilizers

Application of P fertilizers may decrease the concentration of Cu in plants by increasing the plant growth (Greenwood and Hallsworth, 1960; Spencer, 1966).

Effects of P on the concentration of Mo in plants depends upon the supply of Mo. For example, Stout et al. (1951) reported that application of 200 kg ha^{-1} P, without added Mo, slightly increased the concentration of Mo in subterranean clover from 0.2 to 0.8 ppm but when applied along with 1 kg ha^{-1} Mo, Mo concentration in the plant was markedly increased from 0.2 to 79 ppm. On the other hand, Jones and Ruckman (1973) reported that application of 112 kg ha^{-1} P both without added Mo and with 0.3 kg ha^{-1} Mo decreased the concentration of Mo in subterranean clover from 0.6 to 0.5 ppm and 6.7 to 4.8 ppm respectively. As discussed earlier, application of P can increase the availability of Mo in the soil by decreasing the adsorption of Mo by soil constituents. However, Stout et al. (1951) suggested that P may assume a dominant role in releasing Mo from root cells into the translocation system of the plant. Barshad (1951) speculated that P may enhance the uptake of Mo by the plant by the formation of a phosphomolybdate complex, which is more readily absorbed by the plant root than molybdate. However, there was no evidence produced in support of the suggestion.

Application of superphosphate has been found to decrease the concentration of Mo in pasture plants (Ouellette, 1963; Widdowson, 1966). The effect of superphosphate in decreasing the concentration of Mo in plants could be due to increased plant growth and/or the effects of the sulphate content of superphosphate which can decrease the concentration of Mo in plants. This point needs further investigation.

Robertson and Yuan (1973) reported that application of 150 kg ha^{-1} P did not affect the yield of white clover, but S concentration in the plant decreased from 0.21 to 0.19 per cent on one soil and increased from 0.20 to 0.23 per cent on the other. They also found that application of 150 kg ha^{-1} P increased the yield of soya bean and decreased the concentration of S in the plant from 0.4 to 0.3 per cent. Thus,the effects of P on the concentration of S in plants appear to depend upon the soil type, plant species and plant growth.

2.7 Seasonal variation in the concentrations of Cu, Mo and S in pasture plants

Seasonal effects on the concentrations of nutrients in pasture plants have been studied by collecting forage samples at certain intervals during the growth period and by defoliation where plants are repeatedly harvested after reaching the same stage of maturity and analysing the samples to determine changes in the concentrations of nutrients in plants.

Beeson and McDonald (1951), Thomas *et al.* (1952), Gladstones *et al.* (1975), by collecting plant samples in field studies, reported that concentration of Cu in grasses decreased with the advancing age. On the other hand, Lane and Fleming (1966) both from field sampling studies and by repeatedly harvesting when plants had reached the same stage of maturity, observed no significant changes in the concentration of Cu in perennial ryegrass with advancing season.

Piper and Beckwith (1951), by collecting the plant samples from field study,noted that Cu concentration in subterranean clover increased to the mid flowering stage and then gradually decreased, whereas Gladstones *et al.* (1975) showed that Cu concentration in a number of leguminous plants including subterranean clover decreased progressively with advancing age.

Hemingway (1962), by defoliating the plants, observed that concentration of Mo in grasses was increased during the season from 1.2 to 2.3 ppm whereas in clover no change in Mo concentration was observed. On the other hand, Lane and Fleming (1966) from similar studies of defoliating plants found that Mo concentration in perennial ryegrass was decreased from 10 to 6 ppm with advancing season whereas in another study where plants were harvested at certain intervals during the growth period, they observed no change in the concentration of Mo with advancing age. Fleming and Murphy (1968) while studying the effects of the stage of maturity on Mo concentration in perennial ryegrass observed that concentration of Mo in the plant slightly decreased from 0.8 to 0.5 ppm until flowering and then a slight increase from 0.5 to 0.8 ppm in concentration was apparent. Whitehead and Jones (1969) observed that Mo concentration in red clover increased while Piper and Beckwith (1950), Williams (1956), Reid <u>et al</u>. (1970) reported that Mo concentration in subterranean clover decreased with advancing age.

Several workers have reported that the concentration of S in pasture plants was high in the young plants and declined with advancing age (Pumphrey and Moore, 1965; Whitehead and Jones, 1969). For example, Pumphrey and Moore (1965) reported that the concentration of S in lucerne decreased from 0.4 to 0.2 per cent with advancing age.

Most of the differences in the observations concerning seasonal variation in the concentrations of Cu and Mo in pasture plants are difficult to interpret without the adequate information regarding soil supply of these nutrients, competition with other plant species, climatic factors and other details of growth conditions. Different plant parts contain different amounts and concentrations of elements and changes in leaf to stem ratio and the ratio between different plant parts can bring about seasonal variations in the concentration of elements in plants.

It is often difficult to distinguish the changes in the concentrations of elements in plants due to maturation of the plant from that of the effects of seasonal and climatic factors.

2.8 The interaction of Cu, Mo and S in grazing ruminants

Deficiencies and toxicities of Cu in animals may be simple i.e. associated solely with low or high intake of Cu, or they may be induced as a result of an imbalance of other elements in the diet. The available evidence suggests that an imbalance of Cu, Mo and S in the nutrition of animals profoundly influences their metabolism and may produce either Cu deficiency or toxicity (Underwood, 1971; Suttle, 1975).

2.8.1 Cu deficiency in ruminants

The occurrence of simple Cu deficiency was reported by Beck (1941 and 1962) to have occurred in animals grazing on pastures containing less than 4 ppm Cu.

A high Mo content in the diet of animals decreases absorption and utilization of Cu, and may produce Cu deficiency even where the diet has a concentration of Cu that might be considered adequate (Ferguson et al., 1940, 1943; Cunningham, 1950; Dick, 1956). This type of deficiency is commonly referred to as Mo toxicity, molybdenosis or Mo-induced Cu deficiency. Concentrations of Mo in the forage considered potentially harmful to grazing ruminants range from 3 to 10 ppm or more (Cunningham, 1950; Wynne and McClymont, 1955; Underwood, 1971). The effect of Mo on the availability of Cu to the animal depends on the concentration of Cu in the forage. For example, Cunningham (1950) reported that New Zealand pastures containing about 10 ppm Mo were toxic to grazing animals (due to induced Cu deficiency) if the concentration of Cu was greater than 10 ppm, while about 3 ppm Mo in the forage was toxic when the concentration of Cu in the forage was less than 10 ppm.

Dick (1953), Cunningham and Hogan (1959), Vanderveen and Keener (1964) reported that high intake of S by ruminants enhanced Cu deficiency in the animal. Lessard et al. (1970) suggested that S concentrations in the forage higher than about 0.4 per cent could induce Cu deficiency. Suttle (1974) demonstrated that an increase in the concentration of dietary S from 0.2 to 0.4 per cent or Mo from 2.5 to 4.5 ppm had similar effects in decreasing the availability of Cu to the animal. The decreased availability may be due to the formation in the rumen of relatively insoluble compounds such as CuS or $CuSMoO_4$ (Tridot and Bernard, 1962; Suttle, 1974).

In addition to the direct effects of S on Cu availability in animals S also influences Mo induced Cu deficiency, the effects of which depend upon Cu supply. Gray and Daniel (1964) and Underwood (1971) have reported that at low Cu supply (below 10 ppm in the diet), high amounts of S (above 0.4 per cent) in the diet can intensify Cu deficiency in the animal, whereas at high levels of Cu (above 10 ppm in the diet) S may alleviate the Cu deficiency. The beneficial effects of S on Mo induced Cu deficiency in animals at high Cu supply may be due to the competition of sulphate with molybdate at absorption sites in the animal, which results in an increased urinary excretion of molybdate (Dick et al.,1953; Huisingh et al., 1973).

2.8.2 Cu toxicity in ruminants

Albiston et al. (1940) and Bull (1951) reported Cu toxicity in animals that was associated with the ingestion of very high amounts of Cu; this usually occurred in animals grazing pastures near Cu mining areas that contained from about 30 to 70 ppm Cu or more. Cu toxicity in animals has been also reported when soils and pastures have been sprayed with fungicides containing Cu (Schaper and Luitje, 1931; Lafenetre et al., 1935; Gray and Todd, 1960). At normal Cu supply, low intakes of Mo and S in the diet (less than 0.4 ppm Mo and 0.2 per cent S) can lead to the accumulation of toxic amounts of Cu in the liver of sheep (Dick and Bull, 1945; Dick et al., 1953). Occurrence of such Cu toxicity has been reported in sheep grazing on pastures that contained about 10 to 19 ppm Cu, but less than 0.4 ppm Mo and 0.2 per cent S (T.J.I.C., 1947; Bull, 1951).

2.8.3 Seasonal variation in the occurrence of Cu deficiency and toxicity in ruminants

Seasonal variation in the incidence and severity of Cu deficiency and toxicity in grazing animals has been reported by several workers (Cunningham, 1950; Marston, 1951; Russell and Duncan, 1956; Hartmans and Van Der Grift 1964). Cu deficiency in animals grazing pastures on alkaline peat soils in New Zealand increased in severity as the concentration of Mo in the forage increased from 5 to 15 ppm during the season. However, Russell and Duncan (1956) stated that seasonal variation in Cu deficiency in grazing animals has seldom been correlated with seasonal differences in the concentrations of Cu or Mo, or with the Cu:Mo ratio in the forage. Cunningham (1950) reported the occurrence of Cu deficiency of sheep in areas of the Canterbury district of New Zealand where the Cu and Mo concentrations of the forage were similar to other areas where no deficiency was observed.

The occurrence and severity of Cu toxicity in sheep has been reported to vary from year to year and from season to season within any year (Russell and Duncan, 1956). For example, severe outbreaks of Cu toxicity in sheep occurred when sheep grazed on pastures predominant in subterranean clover on Adelaide Hills during early autumn (T.J.I.C. 1947). Here, subterranean clover had Cu concentrations ranging from 11 to 18 ppm and Mo concentrations from 0.01 to 0.40 ppm. The Cu:Mo ratio varied from 45 to 1800. Unfortunately, seasonal variation of concentrations of Cu and Mo was not studied. Sheep grazed on these pastures showed a steady increase in Cu content of their livers to toxic amounts. On the other hand, no incidence of Cu toxicity in sheep has been reported on other subterranean clover pastures with concentrations of Cu and Mo and Cu:Mo ratio similar to those just mentioned above (Piper and Beckwith, 1951).

In general, use of Cu or Mo concentrations or Cu:Mo ratio in the forage has not been particularly successful in predicting the occurrence of Cu deficiency or toxicity in grazing animals (Suttle, 1975). This is not really surprising in view of the role of S in moderating the absorption of Cu in the rumen. It is clearly necessary to take concentration of S into account when studying deficiency and toxicity of Cu in animals.

Suttle and McLauchlan (1975) recently derived the following equation to predict the availability of Cu to sheep

$$\text{Log Cu availability} = 1.153 - 0.07555\,(S) - 0.0131\,(Mo \times S)$$

where S = concentration of S (per cent) in the diet and Mo = concentration of Mo (ppm). Log Cu availability is in arbitrary units only.

In their investigations, the sheep were fed semi-purified diets which contained a constant supply of Cu (of about 7 ppm) but varying amounts of S (0.1 to 0.4 per cent) and Mo (0.5 to 4.5 ppm). The equation implies that S exerts a predominant and independent effect on the availability of Cu in the diet to the animal, whereas Mo has a lesser and S-dependent effect. According to the above equation cereal diets fed by Todd (1972) which were Cu toxic to sheep and that of Thornton _et al_. (1972) which were Cu deficient gave log Cu values of 0.059 and 0.019 respectively.

A similar approach could be used to predict the availability to animals of Cu in the pasture plants. However, selective grazing by animals i.e., preference for different plants or different plant parts can affect the total intake of Cu, Mo and S by animals. Moreover, Pritchard et al. (1964), Terry and Tilley (1964) and Williamson and McCarrick (1967) have shown that the availability of a nutrient in plants to the animal depends to a certain extent on the part of the plant in which the element is located as this in turn has a bearing on the form of organic complexation of the element and its digestibility. Consequently, selective grazing by animals is of obvious importance which makes it difficult to assess the availability of Cu, Mo and S in pasture.

3.0 EXPERIMENTAL MATERIALS AND METHODS

3.0 EXPERIMENTAL MATERIALS AND METHODS

3.1 Pot Experiments

3.1.1 Pot Experiment 1. Effects of fertilizers on dry matter production and concentrations of Cu and Mo in subterranean clover on a lateritic podzolic soil.

This experiment was conducted in the glasshouse in three parts to study the effects of the application of P, S, $CaCO_3$, Cu and Mo on the concentrations of Cu and Mo in subterranean clover. A lateritic podzolic soil (Soil 1) was used. The origin of the soil (and that of other soils used in subsequent pot experiments) is given in Table 4. Physical and chemical properties of the 0-10 cm layer of the soils are described in Appendix 1.

Table 4. Soils used in the pot experiments.

Soil No	Great Soil Group+	Principal Profile Form#	Soil Association or Family	Location in South Australia	Reference
1	Lateritic podzolic	Dy5.42	Birdwood	Mt.Torrens	Jackson (1957)
2	Lateritic podzolic	Dy5.42	Birdwood	Mt.Torrens	Jackson (1957)
3	Red-brown earth	Dr2.23	Dale	Turretfield	Northcote (1959)
4	Calcareous sand	Uc1.11	Beachport	Robe	Blackburn (1964)

+Stace *et al.*, 1968.

#Northcote, 1971.

Experiment 1a had a 3^3 factorial randomised block design with three replications. The treatments were:

P : 0, 67.5 and 135 mg P per pot as KH_2PO_4
Cu : 0, 7.5 and 30 mg Cu per pot as $CuSO_4.5H_2O$
Mo : 0, 0.3 and 3 mg Mo per pot as $Na_2MoO_4.2H_2O$

Application of 15 mg per pot corresponds to 10 kg ha^{-1}: the conversion is based on the area of the soil surface in the pot.

Experiment 1b had a 3 x 2 factorial randomised block design with three replications. The treatments were:

S : 0, 90 and 180 mg S per pot as $CaSO_4.2H_2O$
P, Cu and Mo : (0) and (135, 7.5, 3) mg per pot

P, Cu and Mo were not varied independently and the salts used were the same as in Experiment 1a. The treatments are later referred to as $(PCuMo)_0$ and $(PCuMo)_1$.

Experiment 1c also had a 3 x 2 factorial randomised block design with three replications. The treatments were:

$CaCO_3$: 0, 4.5 and 13.5 g per pot
P, Cu and Mo : (0) and (135, 7.5, 3) mg per pot, as in Experiment 1b.

Procedure: The soil was collected from the 0-10 cm layers, air dried and passed through a 4 mm sieve to remove stones and plant debris before use.

Three kg of soil was added to 3 litre plastic pots (14.5 cm deep and 16.5 cm diameter) lined with polyethylene bags and packed to a depth of 10 cm.

The fertilizer treatments, and a basal fertilizer application (see Appendix 2.1) were added to the soil in solution, except $CaCO_3$ which was added in solid form, and thoroughly mixed with the soil. Analytical grade chemicals were used.

Experiments 1a, 1b and 1c were conducted concurrently in the same glasshouse. All pots were randomised within each replication. The pots were re-randomised every two weeks. The temperature of the glasshouse was maintained at 20 ± 2°C constantly throughout the period of the experiment.

Graded seeds (2.00 - 2.25 mm diameter) of subterranean clover, Trifolium subterraneum (cv Mt.Barker), were germinated on filter paper wetted with double deionised water. They were inoculated with Rhizobium when the radicles emerged, and were sown 10 to each pot on 31 December 1973 at a depth of 1 cm. The plants were thinned to 6 per pot after 10 days.

All pots were watered with double deionised water to a constant weight corresponding to 0.3 bar suction and subsequently rewatered when they lost water to the weight corresponding to 1 bar suction.

The plants were harvested 8 weeks after sowing, on 25 February 1974, by cutting them off 1 cm above the soil level. They were rapidly washed with double deionised water to remove adhering soil particles and dried at 80°C for 72 hours. The plants were weighed, ground in a Wiley mill fitted with stainless steel blades and retained for the determination of Cu and Mo.

In Experiment 1c about 15g soil was collected from the pots at 14 day intervals using a stainless steel core sampler and the pH of the soil was determined in a 1:5 soil:water suspension.

3.1.2 Pot Experiment 2. Effects of P, S and Cu application on dry matter production and concentrations of Cu, Mo and S in subterranean clover on a range of soil types.

This experiment was conducted to study the effects of the application of P, S and Cu to a range of soils on the growth and concentrations of Cu, Mo and S in subterranean clover. It investigated in more detail some of the more significant interactions observed in Experiment 1. The procedures used were the same as for Experiment 1 except where otherwise stated.

This experiment had 4 x 3 x 3 x 3 factorial randomised block design with three replications. The treatments were:

Soils	:	Four soils as described in Table 4 and Appendix 1
P	:	0, 45 and 135 mg P per pot as KH_2PO_4
S	:	0, 45 and 135 mg S per pot as $CaSO_4.2H_2O$
Cu	:	0 and 7.5 mg Cu per pot as $CuSO_4.5H_2O$

Procedure: Each pot received basal fertilizers as shown in Appendix 2.2. In addition, Soils 1 and 2 received 0.15 mg Mo, and Soil 3 received 0.075 mg Mo per pot as $Na_2MoO_4.2H_2O$. Mo was not applied to Soil 4. These fertilizer applications were decided upon after consideration of soil pH and the readily extractable Mo contents of the soil.

The experiment was conducted in a glasshouse during the winter and spring of 1974. The mean daily minimum and maximum temperatures ranged from 5 and 20°C respectively for June to 15 and 30°C for September. The mean daily light radiation ranged from 7.7 to 13.3 mega joules per meter^{-2} day^{-1}.

Subterranean clover was sown on 11 June and harvested on 10 September. The plants were analysed for Cu, Mo, S and P.

Plants growing on the calcareous sand showed interveinal chlorosis 20 days after sowing and were suspected to be either Fe and/or Mn deficient. The symptoms disappeared when the plants were sprayed with a solution containing 0.05% $FeSO_4.7H_2O$ and 0.05% $MnCl_2 4H_2O$. The plants were sprayed with this solution on three further occasions during the season when chlorosis reappeared.

3.1.3 Pot Experiment 3. Effects of genotype and age of the plant on dry matter production and concentrations of Cu, Mo and S in pasture plants on a lateritic podzolic soil.

This experiment was designed to investigate genotypic differences in growth and concentrations of Cu, Mo and S in plants at different stages of growth on a lateritic podzolic soil (Soil 1).

The experiment had a 4^2 factorial randomised block design with three replications. The genotypes used were subterranean clover (*Trifolium subterraneum*, cv Mt.Barker), silvergrass (*Vulpia* sp.), Wimmera ryegrass (*Lolium rigidum*) and capeweed (*Arctothaeca calendula*). There were four series of pots, one for each of the four plant sampling occasions.

In addition to the basal fertilizer shown in Appendix 2.1, P, S, Cu and Mo were added to the soil in each pot at 45, 45, 7.5 and 0.15 mg respectively per pot. The amounts of P and S correspond to the intermediate rates of application used in Pot Experiment 2.

This experiment was conducted concurrently with and in the same glasshouse as Experiment 2 and the procedures used were the same as those described earlier except where otherwise stated.

Procedure: On June 11, subterranean clover, silvergrass and Wimmera ryegrass were sown 10, 15 and 15 seeds respectively per pot at a depth of 1 cm. In case of capeweed, uniform seedlings collected at the site from which Soil 1 was obtained were transplanted, 4 per pot. Ten days later the subterranean clover was thinned to 6 plants per pot, silvergrass and Wimmera ryegrass to 12 plants per pot, and capeweed to 3 plants per pot.

Plants were harvested on 10 August, 25 August, 9 September and 24 September 1974 (i.e. 60, 75, 90 and 105 days after sowing), and analysed for Cu, Mo and S.

3.1.4 Pot Experiment 4. Effects of soil temperature and water content on the dry matter production and concentrations of Cu, Mo and S in subterranean clover on a lateritic podzolic soil and a calcareous sand.

This experiment was designed to study the effect of soil water and soil temperature on the growth and concentrations of Cu, Mo and S in subterranean clover on the lateritic podzolic soil (Soil 1) and the calcareous sand (Soil 4).

This experiment had a split-plot design with two replications. The treatments were:

Main plot (2 replications)	Soil temperature:	12 and 22°C	
Sub plots (2 replications) within each main plot	Soils	Lateritic podzolic soil (Soil 1) Calcareous sand (Soil 4)	
	Soil water:	High	(0.1 to 0.3 bar)
		Medium	(0.1 to 1.0 bar)
		Low	(0.1 to 10.0 bar)

Procedure: The experiment was conducted in a manner similar to that described for the previous experiments. Pots were filled with 2.5 kg sieved airdry soil. Basal fertilizer was added as in Experiment 3 and the seeds of subterranean clover were sown on 22 July 1974.

All pots were initially watered to the weight corresponding to 0.1 bar soil water suction and were subsequently watered when pots lost water to a weight corresponding to 0.3, 1.0 and 10.0 bar for the high, medium and low water supply treatments respectively. The weight of pots was checked daily.

Soil temperatures were controlled by immersing the pots in thermostatically controlled and insulated water baths.

The experiment was conducted in a glasshouse similar and adjacent to that of Experiments 2 and 3 during the same season. The air temperature measured about 2 cm above the soil surface within the clover canopy was independent of the water bath temperatures.

Plants growing on the calcareous sand were sprayed with Fe and Mn as described for Experiment 2.

Plants were harvested on 7 October 1974. Dry weight was measured and the plants were analysed for Cu, Mo and S.

Immediately after the harvest of the plants, the soil was extracted with $CaCl_2$ and analysed for extractable Cu.

3.2 Field Sampling Study

Seasonal variation in the concentrations of Cu, Mo and S in four pasture species at six field locations (Figure 1) was examined. Soils at all sites were lateritic podzolic soils (Dy5.42, Northcote, 1971) of the Birdwood Association (Jackson, 1957) that had been in pasture for over 25 years. Superphosphate, Cu, Mo and Zn fertilizers had been applied to the pastures, but the amounts are not known.

Samples of prominent species at all locations viz, subterranean clover (cv Mt. Barker), silvergrass, Wimmera ryegrass and capeweed were collected at monthly intervals during the spring of 1973 and during the winter and spring of 1974. There was no plant material to sample during the summer-autumn period 1973-74. Plants were sampled by cutting them off 1 cm above the ground level. They were rapidly washed with double deionised water and dried at 80°C for 72 hours before grinding. The ground plant material was analysed for Cu, Mo and S.

Fig. 1. Locations of field sampling sites.

The map is part of the "Soil Association Map Portions of the Mt. Lofty Ranges, Hundred of Talunga and Part Para Wirra, County Adelaide, South Australia" reproduced, with permission, from Jackson (1957).

The locations of the field sampling sites are shown by stars.

Scale
Km 0 1 2 3 4 5
RDWOOD
MT TORRENS
M^t Torrens
DONNYBROOK?
To WOODSIDE
MP
McV
B
T
N G A T U N G K
N

3.3 Analytical methods

Plant material dried at 80°C was digested with a 15:4:2 mixture of HNO_3, $HClO_4$ and H_2SO_4 as described by Piper (1942), using a sample to acid ratio of 1:10 (w/v). Cu in the plant digest was extracted with ammonium pyrrolidine dithiocarbamate into methyl isobutyl ketone (Allan, 1961) and determined by atomic absorption spectroscopy. Mo was determined by complexing with dithiol and extracting into isoamyl acetate. The intensity of the green Mo-dithiol complex was measured by spectrophotometry as described by Bingley (1957, 1963). P was determined by the Mo blue method as described by Twine and Williams (1971).

For the determination of total S, the plant material was fused with a mixture of $NaHCO_3$ and AgO_2 at 460°C for 3 hours before measuring S by the methylene blue method described by Johnson and Ulrich (1959).

Extractable Cu in the soil at the end of Experiment 4 was determined as follows: Fifty gram soil was shaken with 200 ml of 0.05 M $CaCl_2$ solution in polyethylene bottles on an end-over-end shaker at either 12 or 22°C, that is, at the same temperature as that of the treatments. The suspensions were filtered through Whatman No.42 filter papers and analysed for Cu by atomic absorption spectroscopy as described above for plant digests.

4.0 RESULTS

4.0 RESULTS

4.1 Pot Experiments

4.1.1 Pot Experiment 1

Effect of fertilizers on the dry matter production and concentrations of Cu and Mo in subterranean clover on a lateritic podzolic soil (Soil 1).

(a) Dry matter production

The results in Table 5 show that application of P to the soil increased dry matter production irrespective of the amount of Mo applied. Table 5 also shows that application of Mo increased the dry matter but only when applied in conjunction with P. Dry matter production was unaffected by the application of Cu.

Table 5. Effect of the application of P and Mo to a lateritic podzolic soil (Soil 1) on the dry matter production of subterranean clover.

P applied (mg per pot)	Mo applied (mg per pot) 0	0.3	3.0
	Dry matter production (g per pot)		
0	6.4	6.5	6.0
67.5	10.2	10.7	11.7
135.0	13.4	15.7	15.2
L.S.D. (P = 0.05)		1.3	

The effects of S on the growth of subterranean clover were small. The application of 180 mg S per pot tended to decrease the dry matter in the absence of applied P, Cu and Mo, and to increase it slightly when these fertilizers were added (Table 6).

Table 6. Effect of the application of S and $CaCO_3$ to a lateritic podzolic soil (Soil 1) on the dry matter production of subterranean clover.

Treatments applied		P, Cu and Mo applied (mg per pot) 0, 0 and 0	 135, 7.5 and 3	L.S.D. (P=0.05)
		Dry matter production (g per pot)		
S	0	8.1	14.0	
(mg per pot)	90	9.1	14.6	1.7
	180	7.4	15.9	
$CaCO_3$	0	8.5	14.4	
(g per pot)	4.5	11.8	14.9	1.4
	13.5	6.9	14.0	

As with S, the effects of $CaCO_3$ depended on the availability of other nutrients. In the control (no P, Cu and Mo added), the dry matter was significantly increased by application of 4.5 g $CaCO_3$ per pot but decreased by application of 13.5 g $CaCO_3$ per pot. Where P, Cu and Mo were applied dry matter production was unaffected by the application of $CaCO_3$.

(b) Concentrations of Cu and Mo in the plant

The data presented in Table 7 indicate that application of Cu to the soil invariably increased the concentration of Cu in the plant. P significantly decreased the concentration of Cu in the plant at low levels of Cu application (0 and 7.5 mg per pot), but had no effect when 30 mg Cu per pot was applied. Application of S at both levels of P, Cu and Mo increased the concentration of Cu in the plant (Table 8). The concentration of Cu in the plant was decreased by the application of

Table 7. Effect of the application of P and Cu to a lateritic podzolic soil (Soil 1) on the concentration of Cu in subterranean clover.

P applied (mg per pot)	Cu applied (mg per pot) 0	7.5	30.0
	Concentration of Cu (ppm)		
0	11.4	13.6	18.0
67.5	6.7	11.1	17.6
135	4.9	10.7	18.5
L.S.D. (P = 0.05)		0.9	

Table 8. Effect of the application of S to a lateritic podzolic soil (Soil 1) on the concentrations of Cu and Mo in subterranean clover.

P, Cu and Mo applied (mg per pot)	S applied (mg per pot)	Concentrations (ppm) Cu	Mo
0, 0 and 0	0	10.0	0.15 d
	90	10.2	0.10 e
	180	11.1	0.06 f
135, 7.5 and 3	0	12.3	33.9 a
	90	14.7	6.8 b
	180	14.7	2.1 c
L.S.D. (P = 0.05)		0.6	

Statistical analyses were performed on log-transformed data. Concentrations followed by different letters are significantly different at P = 0.05. See Appendix 3 for further details.

$CaCO_3$ (Table 9). On the other hand, application of Mo to the soil had no effect on the concentration of Cu in the plant.

Table 9. Effect of the application of $CaCO_3$ to a lateritic podzolic soil (Soil 1) on the concentrations of Cu and Mo in subterranean clover.

P, Cu and Mo applied (mg per pot)	$CaCO_3$ applied (g per pot)	Concentrations (ppm)	
		Cu	Mo
0, 0 and 0	0	11.9	0.12 a
	4.5	9.0	0.27 b
	13.5	6.7	0.60 c
135, 7.5 and 3	0	18.9	25.0 d
	4.5	14.6	30.4 e
	13.5	10.0	41.8 f
L.S.D. (P=0.05)		0.4	

Statistical analyses were performed on log-transformed data. Concentrations of Mo followed by different letters are significantly different at P = 0.05. See Appendix 3 for further details.

Application of Mo consistently increased the concentration of Mo in the plant particularly when applied with P (Table 10). The concentration of Mo in the plant was also influenced by the application of P, Cu, S and $CaCO_3$ to the soil (Tables 8,9,10). The effects of P on the concentration of Mo in the plant were dependent on the levels of Mo applied at low levels of Mo application (0 and 0.3 mg per pot), P had only a small effect on the concentration of Mo in the plant, but when 3 mg Mo per pot was applied, P markedly increased the concentration of Mo in the plant (Table 10). The results in Table 10 also show that application of high amounts of Cu (30 mg per pot) decreased the concentration of

Mo in the plant where Mo was applied at 0.3 and 3.0 mg per pot. Mo concentration in the plants was increased by $CaCO_3$ and decreased by S (as $CaSO_4$) at both levels of P, Cu and Mo application (Tables 8, 9).

Table 10. Effects of the application of P, Cu and Mo to a lateritic podzolic soil (Soil 1) on the concentration of Mo in subterranean clover.

P applied (mg per pot)	Cu applied (mg per pot)	Mo applied (mg per pot) 0	0.3	3.0
		Concentration of Mo (ppm)		
0	0	0.14 a	1.33 d	3.44 g
	7.5	0.13 a	1.31 d	3.51 g
	30.0	0.10 b	1.20 c	2.20 g
67.5	0	0.11 b	1.85 f	7.77 k
	7.5	0.10 b	1.39 c	7.51 i
	30.0	0.10 b	1.58 e	5.42 h
135	0	0.09 b	1.54 e	29.23 p
	7.5	0.09 b	1.67 e	23.55 o
	30.0	0.10 b	1.32 d	16.77 n

Statistical analyses were performed on log-transformed data. Concentrations of Mo followed by the same letter are not significantly different at $P = 0.05$. See Appendix 3 for further details.

4.1.2 Pot Experiment 2

Effects of fertilizers on the dry matter production and concentrations of Cu, Mo and S in subterranean clover on a range of soil types.

(a) Dry matter production

Results in Table 11 show that application of P increased the dry matter production on the lateritic podzolic soils (Soils 1 and 2) and the red-brown earth (Soil 3), but not on the calcareous sand (Soil 4), whereas S had little effect on the dry matter production on all soils. Dry matter production was unaffected by the application of Cu.

Table 11. Effect of the application of P and S to different soils on the dry matter production of subterranean clover.

Soils	P applied (mg per pot)	S applied (mg per pot) 0	 45	 135
			Dry matter production (g per pot)	
Lateritic podzolic (Soil 1)	0	2.2	2.4	1.9
	45	4.5	4.7	4.4
	135	6.7	7.5	6.5
Lateritic podzolic (Soil 2)	0	5.5	5.2	4.9
	45	6.9	6.7	6.0
	135	8.5	9.3	8.6
Red-brown earth (Soil 3)	0	9.1	9.2	9.1
	45	9.8	9.9	9.7
	135	13.6	13.9	12.6
Calcareous sand (Soil 4)	0	3.6	3.5	3.7
	45	3.6	3.6	3.6
	135	4.0	3.9	3.8
L.S.D. (P = 0.05)			0.5	

(b) Concentrations of Cu, Mo and S in the plant

Results in Table 12 show that application of Cu invariably increased the concentration of Cu in the plant. The concentration of Cu in the plant was also affected by soil type and the amounts of P and S applied. Application of P decreased the concentration of Cu in the plant on all soils and at both levels of Cu applied. Application of S, on the other hand, increased the concentration of Cu in the plant on the lateritic podzolic soils (Soils 1 and 2) but not on the red-brown earth (Soil 3) and the calcareous sand (Soil 4), as shown in Table 13.

Table 12. Effect of the application of P and Cu to different soils on the concentration of Cu in subterranean clover.

Soils	P applied (mg per pot)	Cu applied (mg per pot) 0	7.5
		Concentration of Cu (ppm)	
Lateritic podzolic (Soil 1)	0	17.5	18.7
	45	15.1	15.6
	135	12.1	13.6
Lateritic podzolic (Soil 2)	0	15.0	15.7
	45	13.2	14.2
	135	11.1	12.0
Red-brown earth (Soil 3)	0	7.7	9.7
	45	8.2	9.8
	135	7.4	8.8
Calcareous sand (Soil 4)	0	2.8	4.8
	45	2.7	3.6
	135	2.1	3.3
L.S.D. (P = 0.05)		0.7	

Table 13. Effect of the application of S to different soils on the concentration of Cu in subterranean clover.

Soils		S applied (mg per pot) 0	45	135
		Concentration of Cu (ppm)		
Lateritic podzolic	(Soil 1)	15.0	15.4	15.9
Lateritic podzolic	(Soil 2)	12.8	13.7	14.0
Red-brown earth	(Soil 3)	8.4	8.9	8.5
Calcareous sand	(Soil 4)	3.3	3.1	3.2
L.S.D. (P = 0.05)			0.7	

The concentration of Mo in the plant, was also influenced by the soil type and the amounts of P and S applied. The data presented in Table 14 indicate that application of P at 45 mg per pot and low amounts of S increased the concentration of Mo in the plant on the lateritic podzolic soils (Soils 1 and 2) and the red-brown earth (Soil 3), but higher application at 135 mg P per pot had no further effect on the Mo concentration. On the calcareous soil (Soil 4), the concentration of Mo in the plant was unaffected by the application of P.

Table 14 also shows that application of S at both levels (45 and 135 mg per pot) decreased the concentration of Mo in the plant on the lateritic podzolic soils (Soils 1 and 2) and the red-brown earth (Soil 3). On the calcareous soil (Soil 4) application of S only at 135 mg per pot decreased the concentration of Mo in the plant. Furthermore, on the lateritic podzolic soils (Soils 1 and 2) and the red-brown earth (Soil 3), S counteracted the effect of P in increasing the concentration of Mo in the plant.

Table 14. Effect of the application of P and S to soils on the concentration of Mo in subterranean clover.

Soils	P applied (mg per pot)	S applied (mg per pot) 0	45	135
		Concentration of Mo (ppm)		
Lateritic podzolic (Soil 1)	0	0.17 e	0.12 f	0.08 g
	45	0.41 c	0.20 e	0.08 gh
	135	0.36 c	0.22 e	0.07 gh
Lateritic podzolic (Soil 2)	0	0.29 d	0.22 e	0.09 g
	45	0.39 c	0.23 de	0.08 g
	135	0.39 c	0.26 d	0.08 g
Red-brown earth (Soil 3)	0	0.23 de	0.17 e	0.08 gh
	45	0.36 c	0.28 d	0.09 g
	135	0.39 c	0.25 d	0.07 gh
Calcareous sand (Soil 4)	0	2.87 a	2.76 a	2.35 b
	45	2.84 a	2.62 a	2.20 b
	135	2.80 a	2.49 a	2.12 b

Statistical analyses were performed on log-transformed data. Concentration of Mo followed by similar letters are not significantly different at P = 0.05. See Appendix 3 for further details.

The data presented in Table 15 show that application of S to the soil invariably increased the concentration of S in the plant. Where S was applied, P decreased the concentration of S in the plants grown on the lateritic podzolic soil (Soil 1) and had a negligible effect on other soils.

Table 15. Effect of the application of P and S to soils on the concentration of total S in subterranean clover.

Soils	P applied (mg per pot)	S applied (mg per pot) 0	45	135
		Concentration of S in the shoot (%)		
Lateritic podzolic (Soil 1)	0	0.23	0.61	0.73
	45	0.26	0.43	0.67
	135	0.27	0.40	0.51
Lateritic podzolic (Soil 2)	0	0.20	0.33	0.42
	45	0.18	0.32	0.45
	135	0.17	0.32	0.40
Red-brown earth (Soil 3)	0	0.20	0.37	0.46
	45	0.18	0.36	0.45
	135	0.18	0.34	0.45
Calcareous sand (Soil 4)	0	0.23	0.35	0.46
	45	0.25	0.36	0.48
	135	0.26	0.37	0.47
L.S.D. ($P = 0.05$)			0.04	

4.1.3 Pot Experiment 3

Effect of genotype and age of the plant on dry matter production and concentrations of Cu, Mo and S in pasture plants.

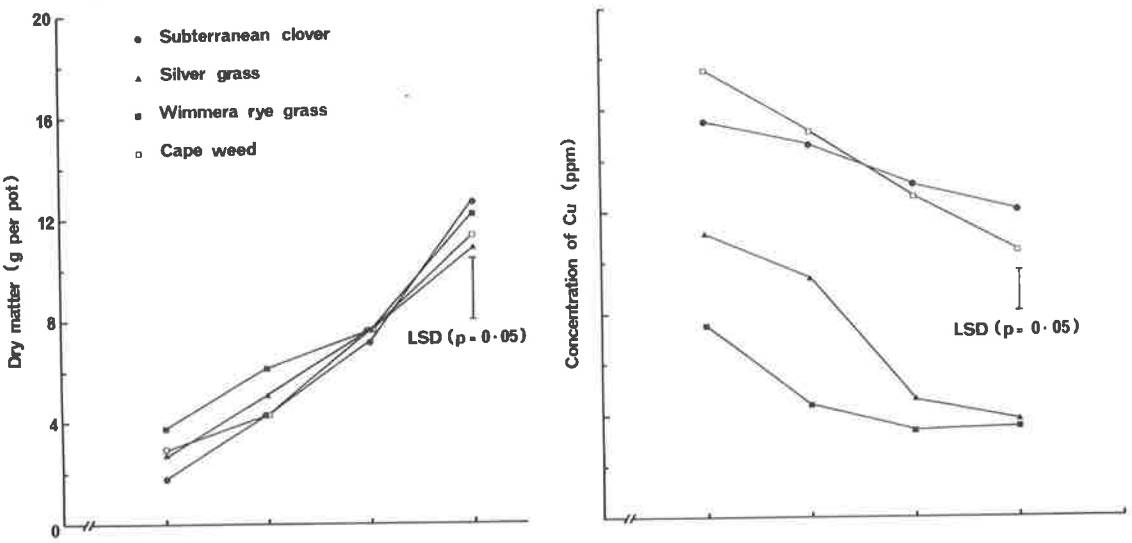
The four species had similar amounts of dry matter at all stages of growth but differed in their concentrations of Cu, Mo and S and in the extent to which these concentrations in the plant changed with the advancement in season (Figure 2 and Table 16).

Figure 2

Dry matter production and concentrations of Cu, Mo and S in pasture plants at different stages of growth on a lateritic podzolic soil (Soil 1)

(a) Dry matter production (g/pot)

(b) Concentration of Cu in the plant (ppm)



(c) Concentration of Mo in the plant (ppm)

(d) Concentration of S in the plant (%)

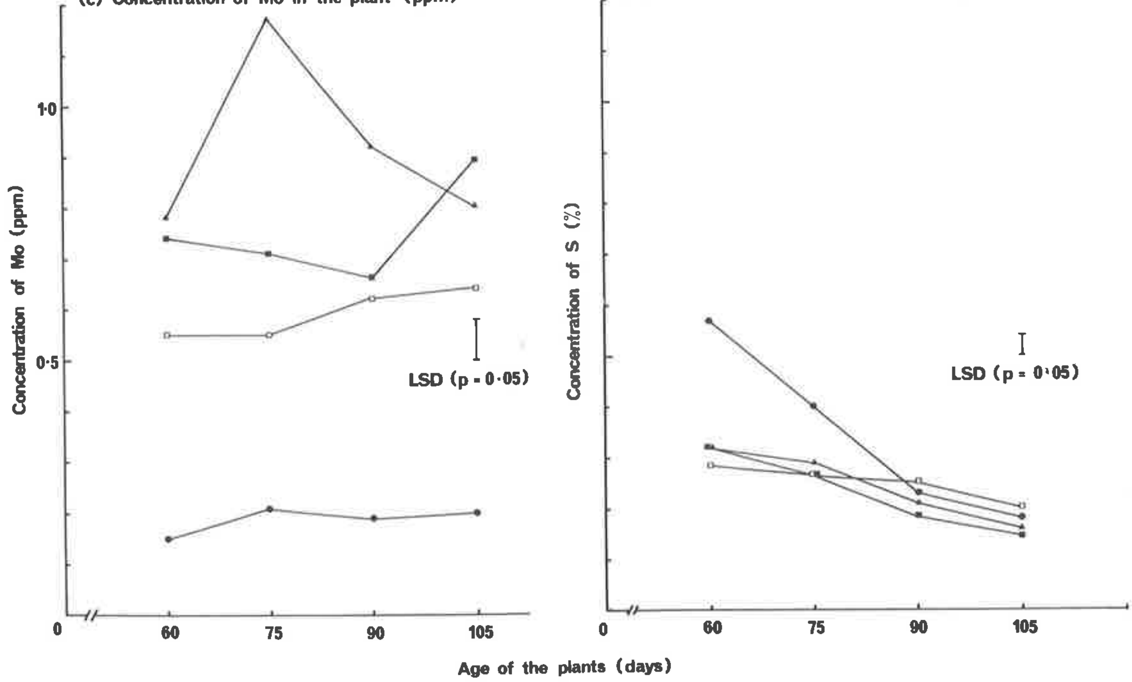


Table 16. Effect of the genotype and age of the plant on the dry matter production, concentrations of Cu, Mo and S in the shoot and time of flowering of pasture plants on a lateritic podzolic soil (Soil 1).

Treatments Genotype	Age (days)	Dry matter production (g per pot)	Concentration of nutrients in the shoot			Age at flowering (days)
			Cu (ppm)	Mo (ppm)	S (%)	
Subterranean clover	60	1.8	15.5	0.15	0.57	94
	75	4.3	14.6	0.21	0.40	
	90	7.2	13.0	0.19	0.23	
	105	12.6	12.2	0.20	0.18	
Silvergrass	60	2.7	11.1	0.78	0.32	80
	75	5.1	9.4	1.17	0.29	
	90	7.6	4.6	0.92	0.21	
	105	10.9	3.8	0.80	0.16	
Wimmera ryegrass	60	3.8	7.5	0.74	0.32	77
	75	6.1	4.4	0.71	0.27	
	90	7.6	3.4	0.66	0.19	
	105	12.1	3.5	0.89	0.15	
Capeweed	60	2.9	17.5	0.55	0.29	87
	75	4.3	15.2	0.55	0.28	
	90	7.6	12.5	0.62	0.25	
	105	11.3	10.4	0.64	0.20	
L.S.D. (= 0.05) Genotype		N.S.	0.8	0.04	0.02	
Age		1.2	0.8	0.04	0.02	
Genotype x Age interaction		N.S.	1.6	0.08	0.04	

The data presented show that the concentrations of Cu in subterranean clover and capeweed were significantly higher than those in the grasses. The concentration of Cu decreased with the advancement in season in all species, the proportional decline being greater in the grasses than in the other species.

All species had similar concentrations of S except for subterranean clover which had significantly higher concentrations than other species at the early stages of growth. The concentration of S decreased with the advancement in season in all genotypes, the decline being greater in subterranean clover than in other genotypes.

The grasses had higher concentrations of Mo than capeweed and subterranean clover. The concentration of Mo tended to remain constant in subterranean clover throughout the season and to increase slightly during the latter part of the season in capeweed and Wimmera ryegrass while the maximum concentration of Mo in silvergrass occurred in mid-season.

4.1.4 Pot Experiment 4

Effects of soil temperature and water content on dry matter production and concentrations of Cu, Mo and S in subterranean clover grown on a lateritic podzolic soil and a calcareous sand.

(a) Dry matter production

Increase in the soil temperature from 12 to 22°C increased the dry matter on both soils, the increase being slightly greater on the calcareous sand than that on the lateritic podzolic soil (Table 17). Results in Table 17 show that low soil water content decreased the dry matter production on both the soils and at both the soil temperatures. In general, the dry matter production of subterranean clover was greater on the lateritic podzolic soil than on the calcareous sand at both soil temperatures and at all levels of soil water supply.

Table 17. Dry matter and concentrations of Cu, Mo and S in subterranean clover grown on a lateritic podzolic soil (Soil 1) and a calcareous sand (Soil 4) as affected by soil temperature and soil water content.+

Soil	Temperature 12°C			Temperature 22°C			L.S.D.
	Low	Medium	High	Low	Medium	High	(P = 0.05)
			Dry matter production (g per pot)				
Lateritic podzolic soil	5.4	7.0	8.4	15.7	17.6	20.5	1.0
Calcareous sand	3.7	4.3	4.7	14.8	16.4	18.4	
			Concentration of Cu (ppm)				
Lateritic podzolic soil	11.4	12.1	11.7	13.7	14.3	14.0	1.2
Calcareous sand	4.1	3.7	4.4	6.9	8.3	8.6	
			Concentration of Mo (ppm)				
Lateritic podzolic soil	0.49	0.49	0.43	1.68	2.32	2.26	0.43
Calcareous sand	4.93	5.10	5.73	10.44	11.58	11.81	
			Concentration of S (%)				
Lateritic podzolic soil	0.40	0.40	0.39	0.35	0.35	0.34	0.02
Calcareous sand	0.39	0.40	0.38	0.35	0.35	0.37	

+Water treatments:

Low = (-0.1 to -10.0 bar)

Medium = (-0.1 to -1.0 bar)

High = (-0.1 to -0.3 bar)

(b) Concentrations of Cu, Mo and S in the plant

The data presented in Table 17 show that the concentration of Cu in subterranean clover was increased by increasing soil temperature from 12 to 22°C at all water treatments, particularly on the calcareous sand. Concentrations of Cu in the plant were not significantly affected by soil water content.

The concentration of Mo in the plant increased as soil temperature increased from 12 to 22°C, and as soil water content increased except on the lateritic podzolic soil (Soil 1) at 12°C.

The concentration of S in the plant was slightly decreased by increasing the soil temperature from 12 to 22°C but was unaffected by soil water content.

Subterranean clover grown on the lateritic podzolic soil had higher concentration of Cu and lower concentration of Mo than that on the calcareous sand. The concentration of S in plants grown on both soils was similar.

4.2 Field sampling study

Seasonal variation in the concentrations of Cu, Mo and S in pasture plants.

The results of the field sampling program are summarised in Table 18. As similar trends in the concentrations of Cu, Mo and S in plants were observed at all locations, only the mean values are given in Table 18. The data are presented in full in Appendix 4.

Table 18. Seasonal changes in Cu, Mo and S concentrations in four pasture species on lateritic podzolic soils of the Birdwood Association.

Genotype	1973	Concentration Cu (ppm)	Mo (ppm)	S (%)	1974	Concentration Cu (ppm)	Mo (ppm)	S (%)
Subterranean clover	Oct.	7.5	0.13	0.20	June	11.4	0.12	0.23
	Nov.	6.2	0.13	0.18	July	10.2	0.13	0.23
	Dec.	5.6	0.14	0.17	Aug.	7.9	0.14	0.23
					Sept.	7.1	0.12	0.20
Silvergrass	Oct.	4.3	0.12	0.15	June	6.4	0.14	0.19
	Nov.	3.9	0.14	0.14	July	6.5	0.12	0.19
	Dec.	4.7	0.14	0.13	Aug.	6.0	0.16	0.17
					Sept.	4.1	0.12	0.16
Wimmera ryegrass	Oct.	5.0	0.13	0.11	June	6.1	0.13	0.13
	Nov.	4.9	0.15	0.11	July	6.5	0.11	0.12
	Dec.	3.9	0.12	0.09	Aug.	5.6	0.11	0.12
					Sept.	5.6	0.14	0.11
Capeweed	Oct.	9.1	0.13	0.15	June	12.7	0.13	0.16
	Nov.	9.5	0.15	0.12	July	12.6	0.14	0.16
	Dec.	8.0	0.13	0.10	Aug.	11.6	0.13	0.14
					Sept.	10.7	0.14	0.13
L.S.D. (P = 0.05)		1.4	N.S.	0.02		1.7	N.S.	0.02

The important features of these results are as follows:

The concentrations of Mo were similar in all genotypes and showed little seasonal changes.

Subterranean clover and capeweed had higher concentrations of Cu than the grasses, but all genotypes showed similar seasonal trends: the concentrations of Cu were low in the spring and early summer of 1973, and much higher values were obtained when the plants were sampled in the winter of 1974.

Subterranean clover had higher concentrations of S than the other genotypes. The concentration of S in all genotypes followed a seasonal pattern similar to that of Cu, i.e. concentrations were high during the winter, and decreased as the season advanced.

5.0 DISCUSSION

5.0 DISCUSSION

5.1 Effects of fertilizer application and soil type on the dry matter production and concentrations of Cu, Mo and S in subterranean clover.

5.1.1 Dry matter production

Application of P increased the dry matter production of subterranean clover on the two lateritic podzolic soils, both of which had low contents (5 and 7 ppm) of sodium bicarbonate-extractable P (Olsen *et al.*, 1954). Yield was also increased on the red-brown earth despite a high extractable P content (22 ppm). Although the calcareous sand had only 7 ppm P, application of P failed to increase the dry matter production of subterranean clover on that soil. Also, subterranean clover on the calcareous sand did not respond to the application of Cu even although the plants contained less than 4 ppm Cu, which is considered to be the critical concentration of Cu for the normal growth of subterranean clover (Teakle and Turton, 1943). Plants growing on the calcareous sand had showed interveinal chlorosis at times during the season in spite of foliar sprays of Fe and Mn. Preliminary experiments had indicated that the chlorosis could be overcome by foliar application of Fe and Mn. The lack of response of subterranean clover on calcareous soil to the application of P and Cu must be due to other factors.

Application of Cu also had no effect on the dry matter production of subterranean clover on the other soils. Plants on these soils contained more than 7 ppm Cu (Table 12).

Application of Mo increased the dry matter production of subterranean clover on the lateritic podzolic soil (Soil 1) only when applied in conjunction with P (Table 5). This type of P x Mo interaction on the dry matter production of subterranean clover has been reported by Mulder (1954) and McLachlan (1955) in glasshouse experiments and by Jones and Ruckman (1973) in field experiments, and it is commonly observed in practice in Australian agriculture on this type of soil (Anderson, 1956).

The results in Table 6 show that application of S tended to increase the dry matter production when P,Cu and Mo were added, and to decrease dry matter when P,Cu and Mo were not added. Of these two effects, only the former was statistically significant at $P = 0.05$. These results corroborate the findings of Reisenauer (1963) who found that 180 kg ha^{-1} S decreased the yield of pea vines when 1 kg ha^{-1} Mo was applied, but increased it at higher levels of Mo application (2 kg ha^{-1}). The results in Tables 8 and 14 show that S application decreased the concentration of Mo in subterranean clover. Where the supply of Mo was low, plants that received the highest S application had Mo concentration of less than 0.10 ppm. This value falls within the range of critical concentrations (0.03 to 0.35 ppm Mo) reported by Stout et al. (1951) and Rubins (1956) for a number of crop plants. Results of the present investigation are consistent with a critical concentration of Mo for the normal growth of subterranean clover of about 0.1 ppm.

The depressing effect of S on Mo concentration emphasizes the need for caution when applying S to soils of marginally adequate Mo status. Mo deficiency, induced by high S application, could result in decreased yield of subterranean clover.

It is well known that application of $CaCO_3$ to acid soils increased the dry matter production of subterranean clover (Anderson and Moye, 1952; Anderson, 1956). One of the direct benefits is to increase the availability of Mo to the plant by raising soil pH. Thus, the increase in dry matter production of subterranean clover where 4.5 g per pot (0.15 per cent) $CaCO_3$ was added to the lateritic podzolic soil without other fertilizers (Table 6) may have resulted from the increased pH (Figure 3) enhancing Mo availability in the soil, as evidenced by the increased Mo concentration in the plant (Table 9). The decrease in dry matter production at the higher application of $CaCO_3$, 13.5 g per pot, (0.45 per cent) despite an increase in the concentration of Mo in the plant, could be due to decreased availability of other nutrients in the soil. For example, both the concentration and total uptake of P by subterranean clover were decreased by the higher application of $CaCO_3$ (Appendix 5). The higher $CaCO_3$ application might also have decreased the availability of micronutrients in the soil (Hodgson, 1963). Unfortunately, insufficient plant samples remained for further analysis to check this.

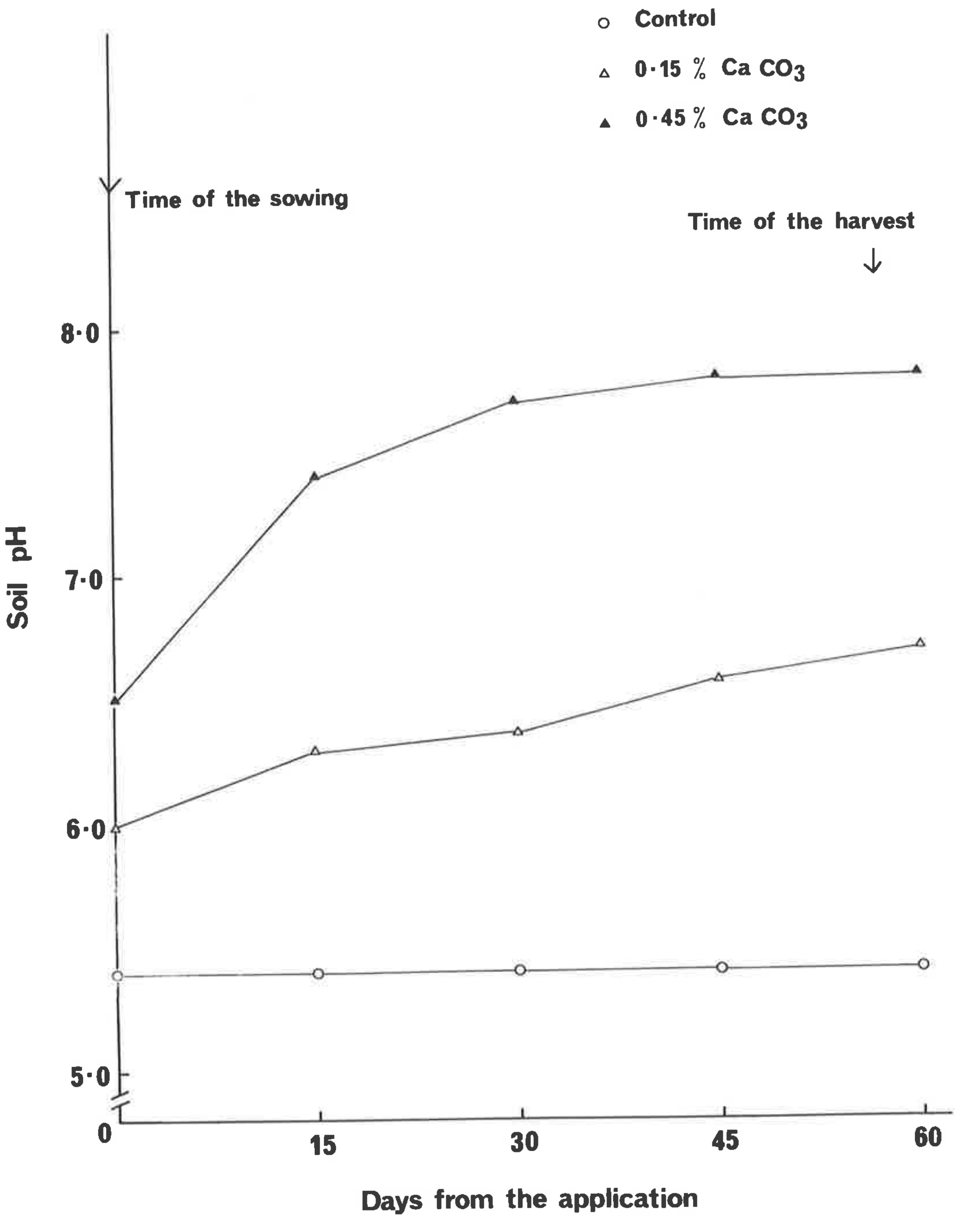
The lack of response of subterranean clover to the application of $CaCO_3$ to the lateritic podzolic soil with P,Cu and Mo supplied also suggests that the effect of $CaCO_3$ at $(P,Cu,MO)_0$ resulted from changes in the availability of P,Cu or Mo in the soil.

5.1.2 Concentrations of Cu,Mo and S in subterranean clover

The decreased concentration of Cu in plants on the lateritic podzolic soils and the red-brown earth following application of P may have resulted from increased dry matter production i.e. growth dilution. It is difficult to explain how P decreased the concentration of Cu in subterranean clover on the calcareous sand, despite its negligible effect on the dry matter production. Evidence regarding the mechanism of interaction of P and Cu in soils and plants is not well documented. However, it is possible that application of P to the calcareous sand may have resulted in the formation of a Cu-phosphate of low solubility. Jurinak and Inouye (1962) showed that P reacts with Cu in aqueous systems to form Cu-phosphate which has minimum solubility in the pH range between 7.5 and 8.5.

Figure 3

Effect of $CaCO_3$ application on the pH of the lateritic podzolic soil (Soil 1)



The decreased concentration of Cu and increased concentration of Mo that followed application of $CaCO_3$ to the lateritic podzolic soil (Table 9) could be due to the effects of increased soil pH (Figure 3) on adsorption and precipitation reactions in the soil. Piper and Beckwith (1949) found that application of $CaCO_3$ decreased the concentration of Cu in subterranean clover and increased that of Mo.

Where no Mo was applied to the soil, application of P tended to decrease the concentration of Mo in the plant; this may have resulted from increased plant growth. On the other hand, when Mo was applied to the soil, P increased the Mo concentration in plants. At low Mo supply (0.15 and 0.3 mg per pot) P brought about only small increase in the concentration of Mo in subterranean clover compared with the large increase where 3 mg per pot Mo was applied (Tables 10 and 14).

There are several factors which could have contributed to these results. Firstly, the effects of P in increasing the concentration of Mo in plants may be due to competition of phosphate with molybdate at the adsorption sites in the soil (Gorlach _et al_., 1969; Hingston _et al_., 1971). Phosphate and molybdate are both strongly adsorbed by the lateritic podzolic soils in particular. The bonding energy of these ions decreases with increasing amounts adsorbed. It is likely that the more weakly bound ions formed at higher surface coverage are more readily available to plant roots. Thus, the amount of Mo in weakly bound (readily available) form in the soil is a function of the amounts of Mo and P added. The observed differences in the effects of P on the concentration of Mo in subterranean clover on the lateritic podzolic soil (Soil 1), _viz_ small effects at low Mo supply and marked effects at high Mo supply, reflect the fact that Mo is more strongly adsorbed when present in small amounts than in large amounts. Secondly, P-induced root proliferation of subterranean clover will result in increased exploration of the soil volume for Mo, and thus may increase the concentration of Mo in the plant if top:root ratio is decreased (Barber et al., 1962). Thirdly, Barshad (1951) has speculated that P may enhance the uptake of Mo by the plant, possibly by the formation of a phosphomolybdate complex, which may be more readily absorbed by the plant root than molybdate. In view of the optimum chemical conditions required for the formation of this complex, it seems very unlikely that it would form to an appreciable extent in soils.

In the calcareous sand (pH 8.2) Mo is already so weakly bound by the soil constituents that application of P has no further influence on Mo availability, as evidenced by the lack of effect on the concentration of Mo in subterranean clover on this soil.

In contrast to P, S decreased the concentration of Mo in subterranean clover irrespective of the soil type, and supply of Mo and P. There are two aspects of the P, Mo and S interaction that need to be considered: firstly, the interaction in the soil, and secondly the interaction in the plant. Phosphate, molybdate and sulphate all exist in the soil as anions and are adsorbed by the soil colloids at sites involving surface hydroxyl groups (Hingston et al., 1967 and 1972). Thus, sulphate may compete with molybdate for the adsorption sites in the soil in a manner similar to that already discussed for phosphate. Stout et al. (1948 and 1951) reported that sulphate competes with molybdate at the root absorption sites and/or in the translocation processes in the plant root thereby decreasing the uptake of Mo by the plant. The results of the present investigation showed that the concentration of Mo in subterranean clover was decreased when high amounts of P and S were applied together to the soil (Table 19).

Table 19. Effect of the application of equal amounts of P and S together to different soils on the concentration of Mo in subterranean clover.

Soils	Amounts of P and S applied (mg per pot) P0 + S0	 P45 + S45	 P135 + S135
	Mo concentration (ppm)		
Lateritic podzolic soil (Soil 1)	0.17 f	0.20 f	0.07 g
Lateritic podzolic soil (Soil 2)	0.29 de	0.23 e	0.08 g
Red-brown earth (Soil 3)	0.23 ed	0.28 d	0.07 g
Calcareous sand (Soil 4)	2.87 a	2.62 a	2.12 c

Statistical analyses were performed on log-transformed data. Concentrations of Mo followed by the same letter are not statistically different at P = 0.05. See Appendix 3 for further details.

This suggests that the effect of S in decreasing the absorption of Mo by the plant more than counteracted the effects on Mo uptake of any possible increase in Mo concentration in the soil solution that resulted from exchange with the added sulphate or phosphate. The tendency of phosphate to compete with sulphate for the adsorption sites could increase the availability of sulphate in the soil, which would enhance the inhibition of Mo uptake by the plant. Thus, the effect of P application on the concentration of Mo in subterranean clover depends to a large extent upon the S supply.

The results of these investigations show that it is hard to generalize on the effects of P and S on the concentrations of Cu,Mo and S in subterranean clover, because of the dependence on soil type and supply of Cu,Mo and S. Effects of P on the concentration of Cu in the plant depend upon Cu supply (Table 7) and soil type (Table 12). The effects of P on the concentration of Mo in the plant depended upon soil type, and the supply of Mo and S (Tables 8, 10 and 14) whereas the effects of P on S concentration in the plant depended mainly upon the soil type (Table 15). Application of S increased the concentration of Cu in the plant only on the lateritic podzolic soils (Tables 8 and 13). The effects of S on the concentrations of Mo and S in the plant were independent of the soil type, and the supply of Mo and P (Tables 8, 14 and 15).

5.2 Effects of soil temperature and water content on the dry matter production and concentrations of Cu,Mo and S in subterranean clover

Increasing the soil temperature from 12 to 22°C greatly increased dry matter production and concentrations of Cu and Mo in the tops of subterranean clover. The higher soil temperature (22°C) had only a small effect on the concentration of S in the plant. Root growth was not measured in the present experiments, but Millikan (1957) and Nicolson (1970) reported an increase in root growth of subterranean clover as soil temperature increased from 10 to 20°C. This would provide greater exploration of the soil at the higher temperature. Increasing soil temperature could also result in more rapid decomposition of the soil organic materials with accompanying release of associated nutrients: the association of Cu (McLaren and Crawford, 1974), Mo (Hodgson, 1963; Jaakkola, 1972) and S (Evans and Rost, 1945) with the soil organic matter is well known. Increased production of organic compounds with chelating properties may aid dissolution and movement of trace element cations to the plant root (Elgawhary _et al._, 1970; Stevenson and Ardakani, 1972).The increase in soil temperature from 12 to 22°C resulted in the increased

$CaCl_2$-extractable Cu in the soil (Table 20).

Table 20. Effect of soil temperature and water content on the extractable Cu (0.05 M $CaCl_2$) in post-harvest soil samples

	Soil temperature 12°C			Soil temperature 22°C		
	Soil water content			Soil water content		
	Low	Medium	High	Low	Medium	High
	ppm x 10^{-3}			ppm x 10^{-3}		
Lateritic podzolic soil (Soil 1)	56	62	59	75	81	78
Calcareous sand (Soil 4)	32	27	27	51	44	47
L.S.D. P = 0.05			11			

Increasing the soil temperature from 12 to 22°C had greater effects on the concentration of Cu in plants on the calcareous sand than on the lateritic podzolic soil (Table 13). This may be due to the relatively high organic matter content of the calcareous sand (Appendix 1). Although the increase in extractable Cu with increasing soil temperature was similar on both soils, the $CaCl_2$ extraction does not take into account forms of Cu which are more strongly adsorbed by the soil and still available to plants.

The effect of the higher soil temperature (22°C) in increasing the dry matter production of subterranean clover to a greater extent on the calcareous sand than on the lateritic podzolic soil (Table 17) may be related to the effects of temperature on the trace element nutrition of the plant. Increasing the temperature of the calcareous sand from 12 to 22°C invariably increased not only the concentration of Cu (Table 17) but also that of Zn (Appendix 6) in subterranean clover from concentrations considered to be marginally deficient to those considered adequate (Teakle, 1943; Millikan, 1953). It is possible that Zn deficiency on this soil may have prevented subterranean clover from responding to fertilization with P, S and Cu in Experiment 2.

The results indicate that soil water content had a negligible effect on the concentration of Cu in subterranean clover. This is in agreement with the findings of Adams and Honeysett (1964) and Nambiar (1975).

The experiments of Lavy and Barber (1964) are relevant to the results of the present investigation concerning the effects of soil water content on the concentration of Mo in subterranean clover (Table 17). They showed that movement of Mo to the plant root depended upon water content of the soil and the Mo concentration in the soil solution. The higher uptake of Mo at higher soil temperature (22°C) and high soil water content is consistent with higher mass flow arising from increased transpiration. The overall enhanced uptake of Mo by subterranean clover on the calcareous sand is a reflection of the anticipated higher concentration of Mo in soil solution. The data in Appendix 1 show that the calcareous sand had a much higher concentration of water-soluble Mo than the lateritic podzolic soil (Soil 1). In addition, soil water content through its effects on the mineralization of soil organic matter and thus production of competing ions such as sulphates and phosphates could also influence the concentration of Mo in the plant.

5.3 Concentrations of Cu, Mo and S in pasture plants at different stages of maturity

Concentrations of Cu in the different plants on the lateritic podzolic soils examined in the glasshouse experiment are similar to the values observed in the field sampling study, although additional Cu was applied to the soil in the glasshouse experiment (Tables 16 and 18). The lateritic podzolic soil (Soil 1) had already an adequate supply of Cu for plant growth. These values are also close to those reported by other workers from their field studies; for example, 8 to 14 ppm for subterranean clover (Piper and Beckwith, 1951; Gladstones et al., 1975), 5 to 9 ppm for silvergrass (Dick et al., 1953; Gladstones et al., 1975), 4 to 8 ppm for Wimmera ryegrass (Gladstones et al., 1975) and 10to 18 ppm for capeweed (Beck, 1962; Gladstone et al., 1975).
By contrast, the concentrations of Mo in plants grown in the glasshouse were higher than those in plants from the field sampling study. Although all species in the field sampling study tended to maintain similar, low (0.11 to 0.16 ppm) concentrations of Mo throughout the season (Table 18), the concentrations of Mo in legumes and grasses responded differently to the higher supply of Mo in the glasshouse experiment. When fertilized with Mo, the grasses tended to accumulate more Mo in their shoots than did subterranean clover (Table 16). This corroborates the findings of Dick (1953a). On the other hand, the concentration of Cu in grasses was less than that of subterranean clover in both the glasshouse and field sampling studies (Tables 16 and 18).

The concentrations of Cu and S in all the genotypes declined with advancing season both in the glasshouse and the field sampling studies: there was little change in Mo concentration. Such seasonal changes in the concentration of elements in pasture plants may be due to the changes in environmental factors (soil water, temperature), to depletion of the available nutrients in the soil by plant uptake and to the physiological stage of maturity of the plants which might affect their requirement for nutrients. Cu concentration in the plants decreased despite the fact that the average daily temperatures during the growth period increased from about 10 to 25°C with the advancement in the season. Results in Table 17 indicate that increasing the soil temperature from 12 to 22°C increased the concentration of Cu in subterranean clover. Fluctuations in the daily temperatures in Experiment 3 and the field sampling study could have partly accounted for the differences in the effects of soil temperature on the concentration of Cu in subterranean clover. Although plants in Experiment 3 were of the same age, their physiological stages were different as evidenced from their different dates of flowering (Table 16). Despite the fact that the physiological stage of growth was affected by defoliation by grazing animals, seasonal changes in the concentrations of Cu,Mo and S in plants in the field sampling study followed similar trends to those in the glasshouse experiment. Different plant parts vary widely in their mineral composition and as plants mature, the ratio of these parts to one another can also change and result in seasonal variation in nutrient concentrations in plants (Fleming, 1973).

6.0 GENERAL DISCUSSION

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It is difficult to state precisely the concentrations of Cu, Mo and S in pasture plants necessary for the normal Cu metabolism of grazing ruminants because selective grazing can affect the total intake of elements by the animal: the concentrations in the ingested plant material are not necessarily the same as concentrations in the pasture as a whole (Pritchard et al., 1964; Rossiter, 1966; Wilson and McCarrick, 1967). Moreover, the availability to the animal of the ingested Cu depends upon other factors, such as the interactions with Mo and S.

Nevertheless, it is possible to suggest ranges of the concentrations of these elements in the diet which permit normal Cu metabolism in the animals, viz 4-16 ppm Cu, 0.5 - 2 ppm Mo and 0.1 - 0.4 per cent S (Dick, 1956; Underwood, 1971; Suttle, 1975). If the concentrations in the diet of one or more of these elements fall outside these ranges, Cu toxicity or deficiency in the animal may occur. For example, the occurrence of Cu toxicity in sheep grazing on pastures containing about 10-18 ppm Cu, less than 0.4 ppm Mo and less than 0.2 per cent S has been reported (T.J.I.C., 1947; Bull, 1951; Dick, 1956). On the other hand, Cu deficiency in grazing ruminants was associated with pastures containing less than 4 ppm Cu (Beck, 1941 and 1962), and also when Cu concentrations were greater than 4 ppm but the concentrations of Mo and S were higher than 3 ppm and/or 0.4 per cent respectively (Cunningham, 1951; Suttle, 1975).

Attempts have been made to use Cu:Mo ratios in the plant as an indicator of potential Cu deficiency or toxicity in grazing ruminants, but they have not been very successful (Russell and Duncan, 1956), possibly because S was not taken into account. However, it may be suggested that Cu:Mo ratios between 2 and 32 are safe, provided that S concentrations in the pasture are between 0.1 and 0.4 per cent (Dick, 1956; Underwood, 1971;

Suttle, 1975).

In the present glasshouse and field studies, the concentrations of Cu, Mo and S in pasture plants ranged from those associated with Cu toxicity to those associated with Cu deficiency in grazing ruminants. The results show that on the unfertilized lateritic podzolic soils and the red-brown earth, the concentration of Mo in subterranean clover (Tables 10 and 14) fell within the range considered high enough to induce Cu toxicity in grazing ruminants. However, it was possible to change the concentration of Mo in the plant by the application of Mo fertilizers to provide concentrations of Mo safe for the nutrition of grazing ruminants. But care must be taken to avoid increasing the concentrations of Mo to values that exceed the safe limit for animals. High amounts of Mo, particularly when applied in conjunction with P, can produce forage with concentrations of Mo (Table 10) that are considered sufficient to induce Cu deficiency in grazing ruminants. When high amounts of S were also added, as would be the case if superphosphate was applied, the risk of Mo-induced Cu deficiency would be decreased. On the other hand, application of P and S at low levels of added Mo decreased the concentration of Mo in the plant to levels considered to be potentially Cu toxic to grazing sheep. The results suggest that on soils which fix large amounts of P (such as the lateritic podzolic soils) application of Mo may need to be increased to counteract the effect of S added in superphosphate. Alternatively, triple superphosphate could be used.

As pointed out by McLachlan (1955), Walker et al. (1955) and Anderson (1956), deficiencies of P, S and Mo often occur in plants on acid soils similar to those used in the present investigation (Soils 1, 2 and 3). Application of lime at low Mo could decrease the chances of the occurrence of Cu toxicity in grazing ruminants by increasing the Mo concentration and decreasing that of Cu in the plant (Table 9). On the other hand, in soils where Mo is high or large amounts of Mo are applied, application of lime could lead to Mo-induced Cu deficiency in ruminants. Results of the present investigations show that judicious use of fertilizers containing P, S and Mo is of utmost importance in optimising the yield of subterranean clover without impairing the quality of the forage: fertilizer practice designed to maximise the yield of pasture may prejudice animal health.

On the unfertilized calcareous sand, the Cu:Mo ratio in subterranean clover was about 1, even when Cu was applied, an indication of potential Cu deficiency in grazing ruminants. Although application of Cu to the soil increased the concentration of Cu in the plant, the concentration remained in the deficient range. Under these conditions, foliar application of Cu to the pasture may prove better than soil applications in increasing the concentration of Cu in the plant (Reith *et al.*, 1968). It may also be possible to give the animal Cu therapy and/or access to herbage on other soils. The results of the present investigations suggest that application of superphosphate to the calcareous sand would affect the Cu nutrition of grazing ruminants because S supply would increase the concentration of S in the plant. The increased S concentration would decrease availability of Cu in the animal. The findings of Suttle (1974) are relevant to the present study. He found that an increase in the concentration of S in the diet of sheep from 0.2 to 0.3 per cent at Mo concentrations 2.5 and 4.5 ppm, decreased the Cu availability to the animal: the concentrations of Cu in the blood plasma decreased from 4.3 to 2.4 mg litre^{-1}.

The contrast between the results obtained for the lateritic podzolic soils and the calcareous sand in particular indicate that modification of the

Cu,Mo and S concentrations in pasture by fertilizers is strongly dependent on the soil type.

In addition to the effects of soil type and fertilizer application, the present investigations also indicate that plant species, season and environmental factors have an important bearing on the Cu status of pastures. For example, subterranean clover and capeweed had higher Cu:Mo ratios than grasses and about similar concentrations of S, particularly at the later stages of plant growth (Tables 16 and 18). In potentially Cu deficient areas, it is possible that factors which favour the dominance of subterranean clover and capeweed in the pastures may become important in inducing or accentuating Cu toxicity in grazing ruminants. On the other hand, in potentially Cu-deficient areas, factors that favour the dominance of grasses in the pasture may induce a deficiency of Cu. A number of factors, including fertilizer practice and grazing management, can affect the botanical composition of pastures. Fertilizer practices affecting the botanical composition has been reviewed by Rossiter (1966). Anderson (1942) reported that application of Mo to a lateritic podzolic soil resulted in the production of clover dominant pastures in South Australia. According to Rossiter (1964 and 1966), application of superphosphate generally tends towards clover and capeweed dominance rather than grass dominance in pastures.

Botanical composition of pastures can also be influenced by grazing management. For example, Rossiter and Pack (1956), Jones and Evans (1960) and Rossiter (1966) reported that understocking usually promotes grass dominance in pastures. Mowing for pasture hay, which is practiced in parts of South Australia, often increases the proportion of clover and decreases that of grasses in the pasture (Rossiter, 1966).

Seasonal variation in the botanical composition of pastures can play an important role in the Cu status of the pasture. Heady (1958) reported that the proportion of clover and capeweed in the pasture decreased while that of grasses increased with advancing season (from autumn to spring). The tendency for Cu concentration in pasture plants, more particularly in grasses, to decrease as the season progresses (Tables 16 and 18) would accentuate any Cu deficiency in animals arising from grass dominance in the pastures. In potentially Cu deficient areas, Cu deficiency in grazing animals is more likely to occur at the end of the season (spring) than in the early part of the season (autumn). On the other hand, in potentially Cu toxic areas the problems in animal health are more likely to occur in the autumn.

In addition to the within-year variations in the botanical composition of pastures, between-year variations also occur. A factor of importance here is the opening of the season. Rossiter (1966) reported that early opening of the season usually leads to the dominance of clover and capeweed in the pastures, whereas late openings lead to grass dominance. This may possibly explain the reason for the severe outbreaks of Cu toxicity in sheep in 1947 in the Adelaide Hills which occurred with the early autumn rainfall (T.J.I.C., 1947). Russell and Duncan (1956) also pointed out that 14 outbreaks of Cu poisoning in sheep in Western Australia were possibly associated with the dominance of subterranean clover in pastures.

The results of the present study indicated that in potentially Cu deficient soils, such as the calcareous sand, increasing soil temperature and soil water content can increase the concentration of Mo in plants (Table 17) to levels potentially toxic to animals. It is possible that concentration of Mo in plants on the calcareous sand may increase in the spring season, particularly if it is a wet spring, because of increased temperatures and water content. This may either induce or accentuate the Cu deficiency in grazing ruminants. The effect of temperature in increasing the concentration of Mo in the plant was greater than its effects on Cu concentration. Soil water had no effect on the concentration of Cu in the plant but tended to increase that of Mo. Hence soil temperature and soil water content may have significant effects on Mo-induced Cu deficiency in grazing ruminants, particularly in potentially Cu deficient areas where even small changes could be important.

The more frequent occurrence of Cu deficiency in grazing animals on the calcareous soils of Eyre Peninsula and the Murray Mallee, particularly in the winter, compared to that on the calcareous soils of South-East of South Australia (Hannam and Reuter, 1976) may be partly due to the lower temperatures in the South-East. However, other factors such as the effect of environment on the botanical composition of pastures, and fertilizer management practices could also be responsible for the above observations. For example, Cu fertilizer is less commonly applied to the pastures on the calcareous soils on the Eyre Peninsula and the Murray Mallee than in the South-East of South Australia (Hannam and Reuter, 1976). Cu toxicity or deficiency in grazing animals on the lateritic podzolic soils in the Adelaide Hills have not been reported recently. A good admixture of subterranean clover and grasses in the pastures, as observed at field sampling locations in the present study, may be one of the reasons for this. Moreover, the pastures have been fertilized with Cu, Mo and superphosphate, and as the results of the present study show, fertilizer application can regulate the concentrations of Cu,Mo and S in pasture plants more easily on the lateritic podzolic soils than on the calcareous sand.

In conclusion, results of the present investigations have shown that concentrations of Cu,Mo and S in pasture plants are significantly affected by the soil type, fertilizer application, genotype, season and environmental factors. By proper manipulation of the above factors the concentrations of Cu, Mo and S in pastures can be advantageously regulated. The results indicated that regulation of Cu, Mo and S concentrations in pastures were more easily achieved by fertilizer applications on the lateritic podzolic soils and the red-brown earth than on the calcareous sand, and that fertilizer applications designed to produce maximum dry matter production of plants do not necessarily produce feed with satisfactory chemical composition. In addition to fertilizer practices, grazing management offers another method which would be advantageously used for providing a balanced diet of Cu,Mo and S to grazing ruminants, depending upon the botanical composition of pasture, season and environmental factors.

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APPENDICES

Appendix 1. Physical and chemical properties of the soils used in the pot experiments.

Soil properties	Lateritic podzolic soil Soil 1	Lateritic podzolic soil Soil 2	Red-brown earth Soil 3	Calcareous sand Soil 4
Texture	Loamy sand	Sandy loam	Sandy clay loam	Loamy sand
Particle size analysis (%)				
Coarse sand	66	68	47	60
Fine sand	12	6	14	18
Silt	11	12	13	13
Clay	11	14	26	9
Soil water content (% w/w) at				
0.1 bar suction	25	26	28	27
0.3 " "	15	21	18	19
1.0 " "	9	14	14	13
10.0 " "	5	7	10	10
15.0 " "	5	7	10	10
Soil pH (1:5 soil:water ratio)	5.5	5.6	6.3	8.2
Free $CaCO_3$ (%)	–	–	–	51
Cation exchange capacity (m.e. per 100 g soil)	10	14	21	23
Exchangeable cations (m.e. per 100 g soil) (Ammonium acetate pH 7.0)				
Na	0.05	0.07	0.20	0.30
K	0.3	0.5	1.0	0.7
Ca	1.7	3.7	12.7	21.7
Mg	0.3	0.7	2.0	1.7

Appendix 1. (cont'd)

Soil properties		Lateritic podzolic soil Soil 1	Lateritic podzolic soil Soil 2	Red-brown earth Soil 3	Calcareous sand Soil 4
E.C. (mS)		0.06	0.06	0.08	0.16
Organic carbon (%)		1.4	2.3	1.5	4.0
Total N (%)		0.1	0.2	0.1	0.4
*Extractable P	ppm	5.1	7.2	21.7	6.8
Extractable SO_4	ppm	8.7	9.2	10.4	12.2
Extractable Cu	ppm	2.1	3.2	5.4	0.5
Extractable Mo	ppm	0.06	0.07	0.09	0.6
Water soluble Mo	ppm	<0.01	<0.01	<0.01	0.6
Extractable Fe	%	0.11	0.10	0.13	0.09
Extractable Al	%	0.09	0.08	0.11	0.08

Particle size distribution was done by the method described by Hutton (1955), soil water characteristics were determined by using pressure plate apparatus (Richards, 1965), soil pH by pH meter using glass electrode, cation exchange capacity and exchangeable cations as described by Tucker (1974), organic carbon by Walkley and Black method as described by Allison (1965) and total N according to Piper (1942).

Appendix 1. (cont'd)

* Extractable nutrients in the soil were determined as follows:

Element	Extracting solution	Time of extraction	Reference Extraction	 Determination
P	0.05 M $NaHCO_3$ pH 8.5	30 min.	Olsen *et al.*, (1954)	Watanabe and Olsen (1965)
S	0.10 M $CaCl_2$ pH 7.0	30 min.	Williams & Steinbergs (1959)	Johnson & Ulrich (1959)
Cu	0.10 M EDTA pH 6.0	72 h	Clayton & Tiller (unpublished)	Allan (1961)
Mo	double deionised water	1 h	Gupta & Mackay (1966)	Bingley (1963)
Mo	0.33 M NH_4 oxalate	16 h	Grigg (1953)	"
Fe	sodium dithionite	10 min.	Mitchell et al. (1971)	Jackson (1956)
Al	sodium dithionite	10 min.	Mitchell et al. (1971)	Wang and Wood (1973)

Appendix 2. Basal fertilizer applications

Appendix 2.1 Pot Experiment 1

Nutrients	Element mg per pot	kg ha^{-1}	Salt used
N	45	30	$Ca(NO_3)_2.4H_2O$
K	45	30	K_2SO_4
Ca	64	42	$Ca(NO_3)_2.4H_2O$
Mg	45	30	$MgCl_2.6H_2O$
B	1	0.7	H_3BO_3
Fe	15	10	$FeSO_4.7H_2O$
Mn	15	10	$MnCl_2.4H_2O$
Zn	7.5	5	$ZnSO_4.7H_2O$

15 mg per pot corresponds to 10 kg ha^{-1}. In Experiment 1b, K was added as KCl instead of K_2SO_4 and S was not added in the basal fertilizer.

Appendix 2. Basal fertilizer applications (Continued).

Appendix 2.2 Pot Experiments 2, 3 and 4

Nutrients	Element mg per pot	 kg ha^{-1}	Salt
N	75	50	KNO_3 and $Ca(NO_3)_2.4H_2O$
K	90	60	KNO_3
Ca	67.5	45	$Ca(NO_3)_2.4H_2O$
Mg	45	30	$MgCl_2.6H_2O$
B	1	0.7	H_3BO_3
Fe	15	10	$FeSO_4.7H_2O$
Mn	15	10	$MnCl_2.4H_2O$
Zn	7.5	5	$ZnSO_4.7H_2O$

The weights given are those added to 3 kg soil in Experiments 2 and 3. In Experiment 4 where only 2.5 kg soil was used per pot, 0.8 of the weights of the element shown in the table were added to each pot.

Appendix 3. Statistical Analysis.

In analysis of variance, it is assumed that the variance is homogenous for all treatments. Bartlett's test (Bartlett, 1937) was used to check that the data met this condition. Where the test showed that the variance was heterogeneous analysis of variance was performed on log-transformed data. In presenting the results where transformations were used, the original data are given and letters are used to indicate where the log-transformed data are statistically different at $P = 0.05$.

Appendix 4.

Appendix 4.1 Seasonal changes in Cu concentration in four pasture species on lateritic podzolic soils of the Birdwood Association.

Genotype	Site	1973			1974			
		Oct.	Nov.	Dec.	June	July	Aug.	Sept.
		Concentration of Cu (ppm)						
Subterranean clover	I	8.5	6.6	6.3	14.1	12.1	10.5	8.3
	II	8.4	5.1	6.0	10.5	9.4	7.5	6.4
	III	6.3	5.2	4.3	12.7	10.3	8.3	6.1
	IV	7.7	6.3	5.1	13.3	11.2	9.4	7.7
	V	8.8	7.5	7.5	9.5	8.9	6.4	7.8
	VI	5.4	6.7	4.1	8.6	8.7	5.7	6.3
Silvergrass	I	5.1	3.4	4.0	8.1	8.4	6.8	4.4
	II	4.5	3.7	4.7	6.4	6.0	5.4	3.4
	III	4.4	4.5	5.0	7.5	6.1	6.7	4.7
	IV	3.3	4.1	4.7	5.3	7.5	6.9	3.8
	V	3.8	3.7	4.5	6.1	5.4	5.0	4.0
	VI	5.0	3.4	5.1	5.3	6.1	4.7	4.5
Wimmera ryegrass	I	5.4	4.4	3.5	5.1	7.3	6.3	4.9
	II	4.7	5.7	4.4	6.4	5.1	4.5	5.4
	III	4.5	5.0	3.1	7.0	6.1	5.6	6.7
	IV	5.7	4.7	4.4	6.5	7.3	4.4	5.4
	V	4.4	4.9	3.9	5.4	6.1	5.7	6.7
	VI	5.7	5.0	3.7	5.3	7.0	6.9	4.5
Capeweed	I	10.5	11.1	10.1	18.4	16.7	18.1	15.0
	II	11.4	12.5	9.0	14.5	15.4	12.5	12.1
	III	9.8	10.4	7.5	11.7	12.7	10.5	11.9
	IV	7.8	8.3	6.7	13.1	10.1	8.7	9.7
	V	6.7	7.1	6.1	8.0	9.5	10.9	8.4
	VI	8.1	7.7	8.6	10.2	1.0	9.8	7.1

Appendix 4.

Appendix 4.2 Seasonal changes in Mo concentration in four pasture species on lateritic podzolic soils of Birdwood Association.

Genotype	Site		1973			1974		
		Oct.	Nov.	Dec.	June	July	Aug.	Sept.
		Concentration of Mo (ppm)						
Subterranean clover	I	0.13	0.10	0.12	0.12	0.10	0.18	0.12
	II	0.14	0.13	0.16	0.10	0.14	0.09	0.08
	III	0.11	0.14	0.18	0.14	0.11	0.17	0.15
	IV	0.11	0.10	0.13	0.09	0.11	0.15	0.14
	V	0.14	0.15	0.11	0.09	0.15	0.13	0.13
	VI	0.13	0.16	0.15	0.16	0.14	0.10	0.08
Silvergrass	I	0.12	0.14	0.12	0.13	0.12	0.17	0.10
	II	0.14	0.16	0.14	0.15	0.13	0.13	0.11
	III	0.13	0.11	0.17	0.15	0.10	0.14	0.09
	IV	0.13	0.10	0.14	0.14	0.13	0.18	0.15
	V	0.11	0.14	0.14	0.13	0.11	0.20	0.12
	VI	0.09	0.16	0.15	0.11	0.13	0.15	0.13
Wimmera ryegrass	I	0.12	0.17	0.10	0.10	0.12	0.16	0.17
	II	0.14	0.16	0.17	0.13	0.09	0.13	0.12
	III	0.13	0.17	0.16	0.14	0.17	0.14	0.11
	IV	0.11	0.15	0.10	0.10	0.13	0.10	0.14
	V	0.11	0.14	0.11	0.16	0.10	0.09	0.15
	VI	0.17	0.13	0.09	0.14	0.13	0.14	0.16
Capeweed	I	0.15	0.17	0.11	0.11	0.10	0.17	0.14
	II	0.16	0.17	0.10	0.13	0.12	0.11	0.12
	III	0.14	0.16	0.17	0.14	0.16	0.13	0.14
	IV	0.10	0.14	0.15	0.09	0.13	0.14	0.17
	V	0.09	0.11	0.11	0.14	0.15	0.16	0.11
	VI	0.14	0.12	0.15	0.14	0.16	0.09	0.14

Appendix 4.

Appendix 4.3 Seasonal changes in S concentration in four pasture species on lateritic podzolic soils of Birdwood Association.

Genotype	Site	1973			1974			
		Oct.	Nov.	Dec.	June	July	Aug.	Sept.
		Concentration of S (%)						
Subterranean clover	I	0.203	0.195	0.177	0.239	0.221	0.235	0.210
	II	0.217	0.177	0.180	0.240	0.230	0.224	0.205
	III	0.209	0.181	0.169	0.239	0.232	0.213	0.195
	IV	0.195	0.193	0.173	0.214	0.219	0.231	0.187
	V	0.191	0.175	0.177	0.205	0.226	0.211	0.201
	VI	0.207	0.167	0.159	0.223	0.233	0.241	0.187
Silvergrass	I	0.147	0.153	0.141	0.195	0.184	0.165	0.159
	II	0.144	0.132	0.115	0.203	0.197	0.181	0.168
	III	0.153	0.139	0.119	0.187	0.211	0.175	0.164
	IV	0.157	0.144	0.123	0.193	0.191	0.169	0.147
	V	0.139	0.149	0.120	0.174	0.197	0.175	0.151
	VI	0.150	0.143	0.131	0.181	0.179	0.157	0.173
Wimmera ryegrass	I	0.135	0.128	0.110	0.159	0.144	0.139	0.143
	II	0.199	0.107	0.187	0.140	0.123	0.131	0.111
	III	0.078	0.094	0.081	0.129	0.110	0.198	0.104
	IV	0.117	0.116	0.083	0.124	0.093	0.106	0.096
	V	0.098	0.118	0.092	0.109	0.131	0.123	0.089
	VI	0.120	0.091	0.099	0.117	0.116	0.129	0.137
Capeweed	I	0.152	0.134	0.103	0.176	0.144	0.148	0.141
	II	0.131	0.121	0.097	0.168	0.191	0.164	0.139
	III	0.149	0.089	0.095	0.192	0.179	0.137	0.123
	IV	0.153	0.132	0.089	0.137	0.149	0.117	0.127
	V	0.138	0.121	0.093	0.145	0.137	0.123	0.096
	VI	0.146	0.108	0.102	0.129	0.139	0.118	0.126

Appendix 5. Effects of $CaCO_3$ application to the lateritic podzolic soil (Soil 1) on the concentration of P in the plant and uptake of P by subterranean clover.

$CaCO_3$ applied (g per pot)	$(P,Cu,Mo)_0$		$(P,Cu,Mo)_1$	
	Concentration of P (%)	P uptake (mg per pot)	Concentration of P (%)	P uptake (mg per po
0	0.21	17.8	0.28	41.1
4.5	0.16	18.9	0.23	35.3
13.5	0.10	6.9	0.21	29.4
L.S.D. ($P = 0.05$)	0.02	2.9	0.02	2.9

Appendix 6. Effects of soil temperature and soil water on the concentration of Zn in subterranean clover grown on lateritic podzolic soil (Soil 1) and calcareous sand.[*]

Soils	Soil Temperature 12°C			Soil Temperature 12°C		
	Low	Medium	High[+]	Low	Medium	High[+]
	(Concentration of Zn ppm)					
Lateritic podzolic soil (soil 1)	48	56	65	81	75	79
Calcareous sand (soil 4)	20	23	28	59	51	67
L.S.D. (P = 0.05)			14			

[*] Plants samples were digested with a 15:4:2 mixture of HNO_3, $HCLO_4$ and H_2SO_4 as mentioned in Section 3.3, and zinc was determined in the aqueous extract by atomic absorption spectrophotometry.

[+] Low = -0.1 to -10 bar soil water content
Medium = -0.1 to -1 bar soil water content
High = -0.1 to -0.3 bar soil water content