

IN DETERMINING
THE APPLICATION OF REGIONAL GEOCHEMISTRY [^] THE CAUSES AND
PREDICTED INCIDENCE OF BOVINE HYPOCUPRAEMIA

by

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ABSTRACT

The resurgence of bovine hypocupraemia since 1975 led to a detailed survey of the blood copper status of cattle in England over the period 1977-1980, inclusive. Cattle in 4,088 herds had blood copper values of 0.8mg/l or less; of these, 1,748 were more severely copper deficient with levels of 0.5mg/l or less. Incidence maps compiled from these data showed bovine hypocupraemia to be a widespread problem, occurring in previously unaffected areas of England. In some regions incidence could be related to high levels of molybdenum, and/or low levels of copper in stream sediments. In other areas this was not so, and the involvement of other elements in the aetiology of bovine hypocupraemia was suspected.

In order to identify other possible geochemical factors, ridge regression was carried out for bovine hypocupraemia incidence rates as a function of 23 element stream sediment data. The most statistically reliable subset of predictors (Cu, Mo, Cd, Sr, Al, K, As and Cr) was then used to compile a map of the expected incidence of bovine hypocupraemia over the whole of England and Wales.

Topsoil, subsoil and herbage samples were collected from 79 sites grazed by hypocupraemic stock in six areas of England (Brayford, Glastonbury, Stow-on-the-Wold, Atherstone, Onecote and Masham) and analysed for Cu, Mo, S, Fe, Cd, Zn, Mn, Sr, Al, K, As, Cr and Ca. From these data causal factors of bovine hypocupraemia at the farm level were identified: absolute copper deficiency in Stow-on-the-Wold and Masham; molybdenum induced bovine hypocupraemia in Glastonbury and Onecote; and sulphur and iron-induced bovine hypocupraemia in Atherstone and Brayford areas respectively. Dietary sulphur and iron concentrations clearly played a potentially important, yet previously unrecognised role, in the incidence of bovine hypocupraemia at the farm level. Local extrapolations of the causal factors were made to other areas, but a country-wide prediction was not possible owing to inadequate veterinary data and the very large numbers of soil and herbage samples which would be necessary.

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CHAPTER 1

INTRODUCTION

1.1 BACKGROUND

There is now an increasing awareness of the seriousness of the World's food supply problem, and improvements in agricultural production are therefore necessary to meet the nutritional requirements of the World's population. Better feeding and management of animal production enterprises could increase food output. Moves towards intensifying agricultural systems and increasing agricultural self-sufficiency have been made in recent years, and this has unfortunately placed considerable demands on the adequacy of an animal's diet. Most cattle and sheep in Great Britain are heavily dependent on home-grown feeds, such as grass or grass-based products, and without supplements these are unlikely to meet livestock requirements for many trace elements, particularly copper, cobalt, selenium and iodine. As trace elements fulfill both structural and catalytic roles in livestock acute deficiencies can result in serious nutritional disorders, while less severe imbalances may result in subclinical effects. The former are easily detected by clinical symptoms, whilst the latter often go unnoticed owing to a lack of overt signs. Considerable losses in yield ensue which can be of economic significance.

In cattle, a deficiency of dietary copper lowers the blood copper status thereby causing bovine hypocupraemia. When the deficiency is severe or prolonged, clinical symptoms may appear and the animal is diagnosed as having bovine hypocuprosis. This condition, which has become prevalent in recent years, is of considerable economic significance in the U.K. owing to substantial losses in beef and milk yields.

This research project has been undertaken to investigate possible causes for the resurgence of copper deficiency in cattle. As the animal ultimately depends on the soil for its supply of copper and other minerals, a study of the soil-plant-animal relationship is essential to understanding the aetiology of bovine hypocuprosis.

1.2 THESIS OBJECTIVES

- (a) To compile maps of:
 - (i) The frequency and occurrence of bovine copper deficiency in England and Wales on the basis of recent veterinary data;
 - (ii) The incidence of injectable copper supplementation from current market research data.

- (b) To determine possible geochemical causes of bovine hypocupraemia by applying multiple regression techniques to relate veterinary information to stream sediment geochemical data for England and Wales; and to select the best regression model for mapping the predicted incidence of bovine hypocupraemia as a function of geochemical parameters.

- (c) To determine geochemical causes of bovine copper deficiency at the farm level based on soil and herbage sampling of farms with hypocupraemic stock.

- (d) To use the factors established above to infer the causes of bovine hypocupraemia in other hypocupraemic areas of England and Wales.

- (e) To investigate the possibility of deriving an improved country-wide prediction of the incidence of bovine hypocupraemia using soil and herbage data instead of the original stream sediment predictors.

1.3 THESIS PRESENTATION

A comprehensive literature review of biochemical, pathological, economic and geochemical aspects of bovine copper deficiency is presented in Chapter 2 to provide a background to the multi-disciplinary nature of the project. In Chapter 3, the incidence of bovine hypocupraemia and intensity of copper supplementation in England and Wales is established using data collected from veterinary and market research sources. A new multiple regression technique is described in Chapter 4 for the statistical prediction of bovine low blood copper status from stream sediment geochemical data and the veterinary information of Chapter 3. Chapter 5 outlines the selection of field areas for detailed soil and herbage sampling on farms with hypocupraemic stock. Analytical techniques and methods of quality control are described in Chapter 6.

The results of the soil and herbage analyses are presented, and their significance discussed, in Chapter 7. Summaries, conclusions and suggestions for further work are outlined in Chapter 8. Finally, any data or explanatory material not essential but supportive to the text are presented in the Appendices.

CHAPTER 2

LITERATURE REVIEW OF BIOCHEMICAL, PATHOLOGICAL, ECONOMIC

AND GEOCHEMICAL ASPECTS OF BOVINE HYPOCUPRAEMIA

2.1 INTRODUCTION

Copper deficiency occurs naturally in grazing livestock in many parts of the world under a wide range of soil and climatic conditions. It sometimes occurs as a single deficiency arising from inherently low soil and herbage copper contents. However, the more common form of copper deficiency is conditioned by the presence of dietary factors that interfere with the absorption and utilisation of copper by the animal.

The first suspicion of absolute copper deficiency in cattle in Great Britain was reported by Jamieson and Russel (1946) on land newly reclaimed from heather in Aberdeenshire. Some three years previously, Ferguson, Lewis and Watson (1943) demonstrated the occurrence of a conditioned copper deficiency on the 'teart' pastures of Somerset. They correlated the molybdenum content of the forage with the scouring disease of the cattle there. This scouring was cured by feeding or drenching with copper sulphate. The limiting effect of molybdenum on copper retention was then demonstrated, and, in a classical series of experiments Dick (1952; 1953a; 1953b; 1954) showed that this effect was only exerted in the presence of adequate amounts of inorganic sulphate. These findings indicated a three-way interaction between copper, molybdenum and sulphate; the mechanism of which was the subject of intensive research for many years.

Sulphur compounds were found to be extensively broken down by rumen microflora. It was suggested by Dick (1954), Van Koetsveld (1955) and Spais et al. (1968) that the subsequent formation of insoluble cupric sulphide in the rumen may be responsible for lowering the availability of dietary copper, irrespective of molybdenum content. Suttle (1974a) found that both inorganic sulphate and organic sulphur, as methionine, both had equal effects on reducing plasma copper owing to their common metabolic fates in the rumen (Anderson, 1956).

Experimentation by Suttle (1974b) indicated that dietary supplements of molybdenum alone (4 mg/kg dry matter (DM)) had no effect on

copper availability, whereas sulphur alone (3 g/kg DM) slightly reduced availability. The two elements together produced a 50% reduction in availability which was thought to be due to the formation of a cupric thiomolybdate complex in the rumen. Dick et al., (1975) proposed the following three-step process for the mechanism:

- i) Reduction of sulphate and organic sulphur to sulphide;
- ii) Reaction of sulphide with molybdenum to form thiomolybdate;
- iii) Reaction of thiomolybdate with copper to form cupric thiomolybdate.

The biochemistry of this three-step reaction has been outlined by Mills (1979).

Conditioned copper deficiency may also arise as a result of the isomorphous substitution of the metal component of metallo-enzymes e.g. the metallothionines in the liver, kidney and intestine (Mills, 1979, Davies, 1974). The competitive antagonism between Cu^+ , Cd^{2+} and Zn^{2+} may be explained by their similar electron distributions.

Recent studies at the Rowett Research Institute (RRI) have indicated that iron may also have a conditioning effect on copper metabolism. Bremner et al. (1983) showed that small increases in iron supply within the range of concentrations found in many feedstuffs can significantly reduce the copper status of weaned calves.

2.2 MANIFESTATIONS OF COPPER DEFICIENCY

As the copper available to an animal becomes insufficient for all the many metabolic processes involving the element, certain processes fail, or are diminished in the competition for the inadequate supply. The activity of several copper dependent enzymes including ferroxidase I, amine oxidase, tyrosinase and cytochrome oxidase declines during the progressive development of copper deficiency in ruminants. This manifests itself in a variety of clinical symptoms such as anaemia; skeletal, vascular and cardiovascular disorders; depigmentation of the hair, defective keratinization of the hair; scouring or diarrhoea; delayed maturity and infertility. A full description of the symptomology of bovine copper deficiency is given by Underwood (1977) and the Agricultural Research Council (ARC, 1980).

2.3 METHODS OF THERAPY AND PROPHYLAXIS

The symptoms described above can be prevented and treated by various methods of therapy and prophylaxis. Supplementary copper is available to copper deficient livestock in a variety of preparations. These include oral and parenteral methods.

Traditionally, oral methods involved the addition of copper to feeds, licks and drinking water. More recently, the potential of slowly released copper from the copper oxide needle has been realised (Lassiter and Bell, 1960; Dewey, 1977; Whitelaw et al., 1980; Suttle, 1979; 1981a; 1981b). Although injectable copper preparations are still the most widely used method of copper supplementation in cattle, they are not without their disadvantages. Severe reactions at the site of injection occur when copper is given in chelated forms for slow release (e.g. copper methionate). Readily translocated compounds cause minimal local reaction but are acutely toxic at relatively low dose rates, even to calves (Mylrea and Byrne, 1974). The effectiveness of both oral and parenteral treatments is reduced by small increments in herbage molybdenum concentrations (Suttle, 1983a).

2.4 RECENT OBSERVATIONS ON THE OCCURRENCE OF BOVINE HYPOCUPRAEMIA

With the symptomology and treatment of bovine hypocupraemia well understood, the condition was thought to be under control. This was reflected in the relatively constant numbers of samples received by the Central Veterinary Laboratory for copper analysis over a period of fifteen years or so (Lewis, 1980). It was therefore with some surprise that following 1974 the number of blood and tissue samples received by veterinary investigation centres for the determination of copper, increased dramatically. In 1975 the number nearly doubled and continued to increase until in 1978 nearly 20,000 samples were received (Figure 2.1). This situation was also reflected in the number of diagnoses of hypocupraemia/hypocuprosis recorded by veterinary investigation centres. In 1975, 1,500 diagnoses were made - the number reached to well over 2,000 by 1978, being reported on farms where it had not been seen before (Figure 2.2).

The increased incidence of bovine hypocupraemia in Britain in

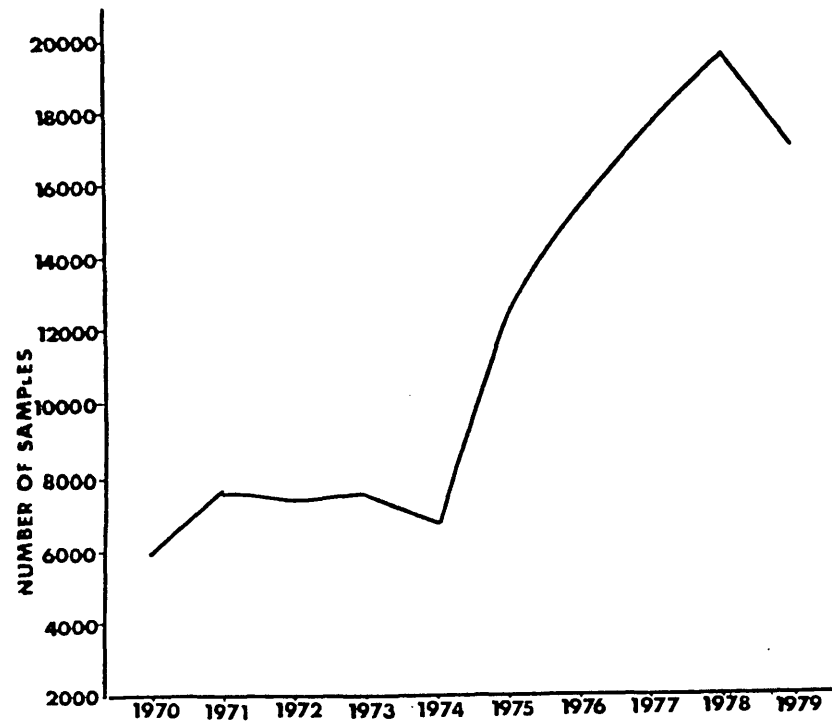


FIGURE 2.1. Number of blood and tissue samples received at the Central Veterinary Laboratory, Weybridge for copper analysis (from, Lewis, 1980)

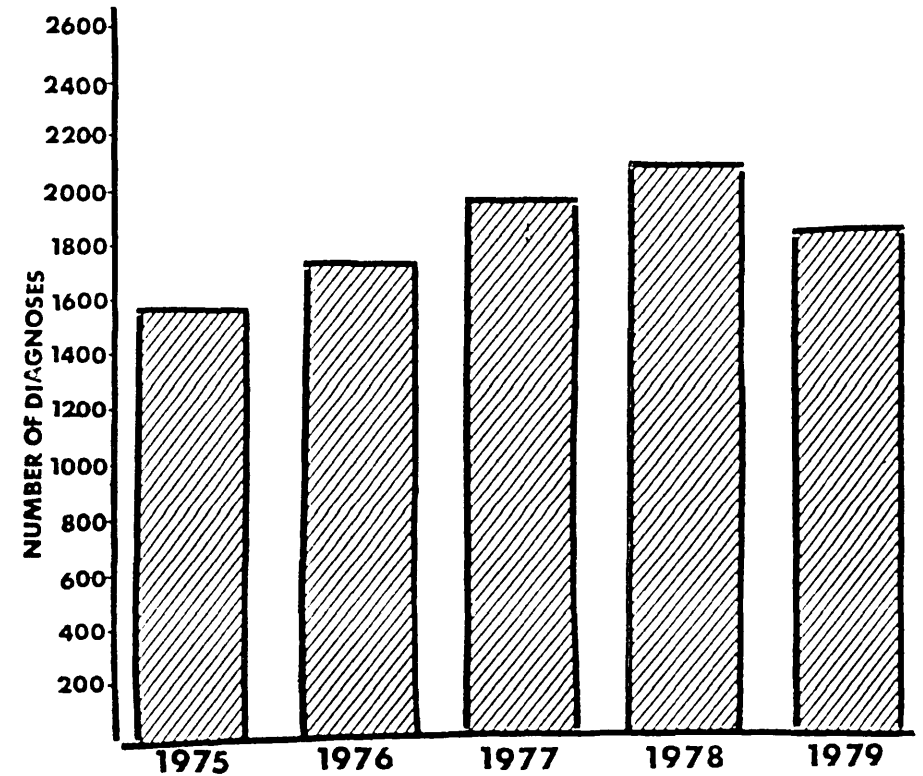


FIGURE 2.2. Diagnoses of hypocupraemia/hypocuprosis recorded by veterinary investigation centres from 1975-1979 (from, Lewis, 1980)

recent years has largely been the result of changes in stock and grassland management in the last 5 - 10 years. These changes are considered below.

2.5 RECENT CHANGES IN STOCK AND GRASSLAND MANAGEMENT

(a) Changes in grassland composition

The desire to increase production from the sward has resulted in a change of the major grass species of the swards. The use of higher levels of fertilisers and increased stocking rates have resulted in an increased proportion of ryegrass (Lolium perenne) in most swards and a reduction in cocksfoot (Dactylis glomerata), timothy (Phleum pratense), fescue (Festuca rubra) and clover (Trifolium repens). Over 80% of the seed now used is perennial and Italian ryegrass which may have resulted in a lowering of the copper content of the pasture sward (MAFF, 1982).

(b) Hay and silage

There has been a considerable increase in silage production since 1970 (MAFF, 1982). This has been accompanied by a greater reliance on fresh herbage and silage in the diet of livestock. The copper in fresh grass and silage however, is less readily absorbed than from hay (Suttle, 1980). Copper intake is therefore reduced.

(c) Higher stocking rates

Trends have been towards higher stocking rates for all grazing livestock (MAFF, 1982). The number of dairy cows has remained stable but herd size has risen by 60%. Beef herds have increased in size, and stocking rates and densities have also risen. Over the same period the total area of grassland has declined from 7,371 million hectares in 1969 to 6,889 million hectares in 1980. Grazing is therefore much more intensive and requirements for copper higher than ever before.

(d) Calving off autumn grass

There are now more cows calving off autumn grass - a time when copper reserves in the dam may be low. This means that the calf frequently starts life with a low copper status, which is exacerbated by

an increase in production of larger, more rapidly growing calves which have a greater demand for copper anyway (Lewis, 1980).

2.6 THE ECONOMIC SIGNIFICANCE OF COPPER DEFICIENCY IN CATTLE

Owing to the increased occurrence of bovine hypocupraemia in recent years, interest has been renewed in ascertaining the financial losses to the farming industry incurred as a result of the condition. There are four main areas where revenue is lost. These are:

- i) Losses arising as a result of reduced milk and beef production and also as a result of reduced fertility. In addition, losses may be incurred due to an increased susceptibility to infection;
- ii) Losses due to the cost of diagnosis;
- iii) Losses due to the cost of treatment;
- iv) Losses due to the cost of prevention.

A working party consisting of Britain's experts on the subject, recently proposed tentative estimates for each of these losses based on the rather limited information available at the present time (MAFF, 1982). Due to the limitations of the data, certain assumptions had to be made concerning the overall incidence of clinical deficiency. It was also not possible to estimate the losses due to preclinical consequences of the deficiency. The calculated minimum cost of copper deficiency in cattle and sheep in Britain is estimated as follows:

	£ Million p.a.
LOSSES DUE TO REDUCED PRODUCTION IN CATTLE	3.6
LOSSES DUE TO REDUCED PRODUCTION IN SHEEP	1.2
LOSSES DUE TO DIAGNOSIS	0.05
LOSSES DUE TO TREATMENT (INJECTIONS)	1.6
LOSSES DUE TO PREVENTION	0.35
TOTAL LOSS	6.80

It is interesting to note that despite the continuing use of copper in feeds and injections, and ongoing diagnostic efforts at a cost of £2 million, the calculations suggest that there is still a minimum loss of £4.8 million per annum. It would therefore seem essential for further investigations to be conducted into the factors limiting copper availability to the ruminant. Preventative measures may then be applied with greater success.

2.7 FACTORS AFFECTING COPPER AVAILABILITY TO THE RUMINANT

Recent studies have revealed a number of factors, some physiological, some genetic and some dietary in origin, which affect the efficiency with which cattle use dietary copper. These are considered below.

(a) Physiological factors

Suttle (1975) has shown that suckling or milk fed animals absorb copper with an efficiency of 60% or more. With the development of a functional rumen, the absorbability of copper from normal diets falls markedly to 10% or less (Suttle, 1975).

(b) Genetic factors

The existence of heritable differences in copper metabolism (Wiener and Field, 1969; Luke and Wienman, 1970) raises the possibility of reducing the incidence of copper deficiency by breeding ruminants which utilise dietary copper more efficiently (Suttle, 1978).

(c) Dietary factors

Studies by Suttle (1981c) of the absorbability of copper from natural foodstuffs using Scottish blackface ewes and a plasma repletion technique have revealed marked variation. Even when molybdenum levels are low (<2 mg/kg DM), grazed herbage provides copper which is poorly absorbed, especially in the autumn. At the other extreme, cereals and leafy brassicas provide copper which is uniformly well absorbed, giving a maximum nine-fold difference between foodstuffs. Hay and swedes are of intermediate absorbability. Silage provides copper which is more absorbable than fresh grass.

Copper absorbability is dependent in the first instance on the concentration of copper and its antagonists in the diet. The levels of copper, molybdenum and sulphur in foodstuffs are initially dependent on the concentration and forms of these elements in the soil. This aspect is considered below.

2.8 COPPER IN ROCKS AND SOILS

Copper occurs in nature both as dispersed forms in sedimentary and igneous rocks, derived sediments and soils, and concentrated in rare mineral deposits. Besides the natural inputs of copper there are also anthropogenic sources. These include industrial emissions and effluents, sewage sludge, pig slurry, composted refuse, fertilisers and pesticides.

Sandstones are usually poor in copper (average 8.5 ppm; Burridge, Reith and Berrow, 1983) because of intensive weathering. Shale contains more copper than sandstone or limestone (4 ppm) because of its higher clay content and it becomes the most copper-rich of the common sedimentary rocks as its organic content increases (shale/clay, 50 ppm; black shale, 70 ppm; Thornton, 1980). Gabbro (41 ppm) has the highest copper concentration of the igneous rocks, and serpentine (15 ppm) and granite (7 ppm) the lowest (Burridge, Reith and Berrow, 1983).

Factors affecting the relationship between the copper concentrations in parent rock and soil include: the degree of weathering; nature and intensity of soil formation; drainage; pH; redox potential; and organic matter content. The average copper concentration in soil in the U.K. is 20 ppm, with a background range of 4 - 60 ppm. This is only exceeded in soils contaminated by old mining and smelting activities where copper levels can be as high as 2000 ppm (Thornton and Webb, 1980).

The actual influence of parent material on the total content and form of copper in the soil is dependent on mobilisation and redistribution of copper (Thornton, 1980). There is often an enrichment of copper in surface horizons because of the accumulation from plant residues and fungi. Movement of copper from highly leached surface horizons has been demonstrated in podzols. Impeded drainage most affects copper mobilisation (Knezek and Ellis, 1980). In gleying conditions many minerals break down and release trace elements, which are then held in the soil in a much more available form.

Copper is the most versatile of all the agronomically important trace elements in its ability to interact with soil-mineral and organic components, as well as to form precipitates with sulphides, carbonates, hydroxides and other anions (MAFF, 1982). Numerous forms of copper are

therefore likely to exist in any given soil environment. Much of the available soil copper is in the organically bound fraction. It is more strongly bound to organic matter than other trace element cations. Copper organic complexes therefore play an important role in regulating copper mobility and availability in the soil (Thornton, 1980).

2.9 MOLYBDENUM IN ROCKS AND SOILS

Metamorphic schists and sedimentary rocks, particularly clays, have the highest molybdenum content (2 ppm). Marine black shales are exceptional in that they contain 1 - 1,000 ppm (Thomson, 1971). Molybdenum content is low in ultrabasic rocks (0.2 - 0.4 ppm), medium in basalts and gabbro (1.4 ppm) and higher in granite (1.9 ppm) - (Aubert and Pinta, 1977).

Molybdenum in British surface soils has been reported to average 1 ppm (Swaine and Mitchell, 1960), although research by the Applied Geochemistry Research Group (AGRG) indicates that soils derived from marine black shales of Cambrian, Ordovician, Silurian, Jurassic, Carboniferous, Cretaceous and Holocene age in England, Wales and Ireland may contain from 1 - 100 ppm Mo; while the majority of soils derived from other rock types contain less than 2 ppm (Thornton, 1977).

Information on molybdenum distribution in the soil profile is limited. In general there are higher concentrations in the subsoil than surface horizons in freely and imperfectly drained soils, but not in poorly drained profiles. Molybdenum is present in soils as molybdate in solution, adsorbed molybdate and firmly held molybdate (Barrow, 1975). Adsorption occurs as a result of a specific reaction between molybdate and atoms of aluminium or iron on the surface of the soil particles, and reaches a maximum at pH values of less than 4. Hence availability to plants increases at pH greater than 4, and the addition of lime to soil therefore increases the uptake of Mo by plants.

2.10 SULPHUR IN SOILS

The total sulphur content of mineral soils usually lies in the range 0.01 - 0.06% dry weight, whilst in organic soils total sulphur may exceed 0.5% dry weight (Halstead and Rennie, 1977). Sulphur is present

in soils in oxidation states +6 (SO_4^{2-}) to -2 (S^{2-}). Total sulphur is made up of organically-bound sulphur and a range of plant-available and -unavailable inorganic forms.

Most sulphur in well-drained temperate agricultural soils is combined with organic compounds; ester organic sulphur and carbon bonded sulphur. Inorganic forms are usually much less abundant and typically account for less than 25% of total soil sulphur (Halstead and Rennie, 1977). Halstead and Rennie (1977) list five categories of inorganic sulphur; easily soluble sulphate, adsorbed sulphate, insoluble sulphate, insoluble sulphate co-precipitated with CaCO_3 and reduced inorganic forms. The predominant form of inorganic sulphur in well-drained soils is sulphate, the most soluble form of which, is readily available to plants (usually 1 - 10% of the total, (Brown, 1982)).

2.11 COPPER, MOLYBDENUM AND SULPHUR CONTENTS OF PASTURE HERBAGE

The uptake of copper, molybdenum and sulphur by plants is dependent on a number of factors, including:

- i) The amount and forms of the elements in the soil (Section 2.8-2.10);
- ii) Botanical composition of the sward;
- iii) Maturity of the plant;
- iv) Season of the year;
- v) Liming and fertiliser application;
- vi) Drainage/water regime of the soil.

Botanical composition of the sward

Numerous studies have shown that legumes and herbs are richer sources of minerals such as copper, zinc and iron than grasses (Fleming, 1963; Whitehead, 1966). On soils of low copper status differences in copper content between grass and legumes may be small. Molybdenum content is also greater in legumes than common grass species, with the exception of Yorkshire fog (Holcus lanatus). Ferguson et al. (1943), working on the 'teart' pastures of Somerset suggested that it was the presence of clovers which caused 'improved' pastures to become more teart at that time. The use of basic slag increased their growth. In contrast, the Hill Farming Research Organisation (HFRO) has shown that the sulphur content of ryegrass was double that of white clover (MAFF, 1982).

Maturity of the plant

As a crop matures the concentration of trace elements in its dry matter usually decreases. Copper levels have been shown to decline with advancing maturity (Fleming, 1963; MAFF, 1982). Marked reductions in sulphur levels also occur in grasses such as ryegrass and cocksfoot when they are left to mature (Whitehead, 1966).

Season of the year

There is no seasonal trend for copper levels in pasture herbage. Molybdenum in contrast, has long been recognised in the temperate areas to peak during the spring and autumn flush of grass - the highest values being found in the autumn (Ferguson et al., 1943). Where soil molybdenum levels are lower, less marked seasonal trends occur, although minima are still found during July (Whitehead, 1966). Analysis of monthly ryegrass growth indicated that herbage sulphur contents were generally higher in July, August and September, than earlier in the season (Whitehead, 1966).

Fertiliser and lime application

The evidence for the effect that nitrogen fertilisers have on herbage copper contents is conflicting. A higher nitrogen regime has resulted in an increased proportion of ryegrass in most swards and a reduction in timothy, cocksfoot, fescue and clover. The overall copper content is therefore likely to be lower as the proportion of legumes in the sward is reduced. There is little evidence that nitrogen application has a direct effect on the molybdenum content of herbage. A reduction might be expected though, through a reduction in the amount of clover. Experimentation by Whitehead, Jones and Barnes (1977) to determine the influence of fertiliser nitrogen on the nitrogen, sulphur and mineral content of perennial ryegrass showed that even at the highest rate of application, sulphur supply was more than adequate for crop growth, and sufficient for ruminants.

Evidence for the feasibility of raising herbage copper levels by application of copper fertilisers is also conflicting. Several workers (Mitchell, 1957 ; MAFF, 1982 ; Burridge, Reith and Berrow, 1983) have had little long term success in increasing herbage copper contents by

treating either the soil or herbage with copper. Jarvis (1978) studying copper uptake and accumulation by perennial ryegrass grown in soil and solution culture, found that increased addition of copper only increased the copper content of the roots. The shoots showed small ranges of copper content over large changes of concentration in the soil or solution culture. This was because the roots of ryegrass restrict the movement of copper along the pathway from soil to plant.

Although the use of lime has been reduced in recent years through the removal of the lime subsidy, it can still have devastating effects on the molybdenum content of herbage. Limited results available from the Macauley Institute indicate that increases in herbage molybdenum content in the order of 2 - 5 fold occur between pH 5.5 and 6.5. The increases in uptake between pH 4.5 and pH 5.5, and between pH 6.5 and pH 7.5 are smaller and of the order of 2 fold (MAFF, 1982).

Soil moisture status

Results of several workers have shown that soil moisture status markedly effects the uptake of many elements. Both copper and molybdenum contents of herbage increase under poorly drained conditions (Davey, 1957; MAFF, 1982)

2.12 CRITICAL LEVELS OF COPPER, MOLYBDENUM AND SULPHUR IN PASTURE HERBAGE

The quantitative interrelationships between copper, molybdenum and sulphur are complex. This is because of the effects that these elements exert both singly and together on ruminant nutrition. When molybdenum and sulphur levels are low then pasture copper levels of 8 mg/kg DM or less are usually associated with an uncomplicated bovine hypocupraemia. Critical levels of molybdenum and sulphur are not so easy to define. When the conditioning effect of molybdenum was first discovered it was the absolute concentration that was considered crucial. Levels of 5 mg/kg DM molybdenum and greater, in the diet were thought to significantly influence copper metabolism in the ruminant, even when the copper content was quite high. More recently, Suttle (1974b) has shown that molybdenum influences copper requirements at much lower dietary concentrations than was originally believed. Where molybdenum levels are 2 - 5 mg/kg DM the minimum copper requirement of sheep is approximately 10 mg/kg DM.

Considerable experimentation has shown clearly that the toxicity of any particular level of molybdenum is affected by the ratio of dietary copper to dietary molybdenum. According to a Canadian study (Multimore and Mason, 1971) a Cu : Mo ratio of less than 2 : 1 can result in conditioned copper deficiency in cattle. The results of an English study (Alloway, 1973) suggest that a ratio closer to 4 : 1 is necessary to avoid hypocuprosis in sheep. As cattle are known to be the more susceptible ruminants to molybdenum toxicity, the critical ratio for cattle might be expected to be higher than that for sheep.

It has sometimes been difficult to relate the occurrence of bovine hypocupraemia with either low copper, high molybdenum or a low Cu : Mo herbage ratio. Some of these anomalies may be explained by high levels of organic and inorganic sulphur in the diet. Until recently, the scarcity of data on total sulphur intakes has precluded even approximate description of the individual and combined effects of molybdenum and sulphur on the copper status of ruminants. Suttle and McLauchlan (1976) sought a closer relationship by relating availability of dietary copper to the sulphur and molybdenum concentrations in a large number of experimental diets for sheep. The availability of copper was predicted from responses in plasma copper using a repletion technique (Suttle, 1974c). The concentrations of sulphur and molybdenum in the basal semi-purified diets varied within the normal range for herbage (i.e. 1,000 - 4,000 mg/kg DM sulphur and 0.5 - 4.5 mg/kg DM molybdenum). Of the relationships examined the best fit was obtained from the following equation (Equation 1)

$$\text{Log } A_{\text{Cu}} = -1.153 - (0.076S) - (0.013S.Mo) \quad (r = 0.93)$$

This equation implied that sulphur exerted a predominant and independent effect on copper availability (A_{Cu}), whereas molybdenum had a lesser and sulphur-dependent role. This supported earlier suggestions of sulphur's independent effect on copper availability to sheep and cattle (Wynne and McClymont, 1956; Allcroft and Lewis, 1956; Goodrich and Tillman, 1966; Hartmans and Van der Grift, 1964). From Equation 1 the responses to dietary sulphur and molybdenum are seen to be exponential rather than linear, indicating that increments at the lower end of the normal ranges of concentration, have relatively large depressing effects on copper availability.

More recently, copper availability has been estimated in natural foodstuffs (Suttle, 1980). No correlation between observed A_{Cu} and that predicted from the equation for semi-purified diets was found. It seemed that specific equations were required for each type of forage examined. This is possibly because of differences in the rate or extent to which molybdenum and sulphur are solubilised or degraded from a particular foodstuff during digestion in the rumen. Copper in brassicas, hay and dried grass was invariably more absorbable than predicted, whereas copper in fresh grass and silage was in general less absorbable. The presence of non-degradable forms of sulphur, such as methyl cysteine sulfoxide and thioglycosides in brassicas could result in the underestimation of copper absorbability from the semi-purified diet equation. Conversely, the rapid degradation of silage and fresh grass might result in overestimations (Suttle, 1981c).

Suttle (1983) has derived a separate equation (Equation 2) for predicting absorbability of copper from summer herbage;

$$A_{Cu} = 5.71 - (1.279S) - (2.785 \log_e Mo) + (0.227Mo.S) \quad (r = 0.90)$$

This differs substantially from Equation 1. Suttle (1983b) recommends that Equation 2 be used to replace Equation 1 for predicting the absorption of copper from pasture. The data from which this equation is derived do not contain sulphur and molybdenum concentrations greater than 4,000 and 6 mg/kg DM respectively. The equation should not be used to extrapolate to higher concentrations.

Accepting that some revision of Equation 2 may prove necessary for cattle, this approach is still more practical than the presentation of arbitrary statements about critical levels of molybdenum and sulphur, and is preferable to the use of Cu : Mo ratios in herbage.

2.13 EFFECTS OF OTHER DIETARY ANTAGONISTS ON COPPER AVAILABILITY

(a) Iron

Iron has been shown to reduce copper availability in cattle and sheep (Campbell and Mills, 1974; Abdellatif, 1968). More recently, studies at the Rowett Research Institute have indicated that diets

containing more than 350 mg/kg DM can strongly inhibit copper utilisation by cattle (Scottish Agricultural Colleges, 1982). Such concentrations of iron are sometimes exceeded in diets based on silage and winter herbage contaminated with soil. Quantitative aspects of this antagonistic effect of iron have not yet been fully described, but it is already evident that approximately 700 mg/kg DM iron in the diet can have an inhibitory effect upon copper utilisation similar to that caused by 5 mg/kg DM molybdenum. Experimentation by Bremner et al. (1983) has shown that relatively small increases in iron supply, within the ranges found in many feedstuffs and mineral supplements, can significantly reduce plasma and liver copper status of weaned calves. However, this has not been accompanied by the appearance of clinical symptoms that are usually associated with a low copper status. A threshold of approximately 500 mg/kg DM iron in the diet has been suggested by the Rowett as the level above which adverse effects on copper utilisation should be expected (Scottish Agricultural Colleges, 1982).

(b) Cadmium and zinc

The possible isomorphic substitution of Cd^{2+} and Zn^{2+} for Cu^{+} has already been mentioned. Mills and Dalgarno (1972) found that 7 mg/kg DM cadmium added to the diet of a pregnant ewe reduced the copper status of her offspring. More recently, levels as low as 3 mg/kg DM cadmium have been found to adversely affect copper retention and growth of lambs (Campbell, cited in ARC (1980)). Increasing the dietary concentration of zinc from 18 to 100 mg/kg DM has been shown to reduce the accumulation of copper in the liver of lambs on a semi-purified diet (Mills, 1974). It is difficult to set critical levels of cadmium and zinc until more is known of the form and biological effectiveness of the contaminant. In the 'Nutrient Requirements of Ruminant Livestock' (ARC, 1980), the Agricultural Research Council have suggested that A_{Cu} is likely to be 3% or less when levels of cadmium and zinc in the diet exceed 3 and 200 mg/kg DM respectively.

(c) Soil ingestion

Grazing animals involuntarily ingest soil along with grass. Previous work in Britain and New Zealand has shown that cattle may ingest from 1 - 10% of their dry matter intake in the form of soil (Field and

Purves, 1964; Healy, 1967; Healy, 1968; Healy, 1970; Suttle, Alloway and Thornton, 1975). The extent of soil ingestion is inversely related to the amount of herbage available. It therefore reaches a maximum in autumn and winter.

In copper repletion trials conducted at the Moredun Institute, diets containing 100 g/kg soil, irrespective of their molybdenum content, reduced the availability of copper fed as copper sulphate by more than 50% (Suttle, Alloway and Thornton, 1975). The mechanisms for the effect of soil ingestion on copper metabolism are probably complex. The physico-chemical properties of soils could result in the occlusion of copper from the gastrointestinal tract by adsorption on the organo-mineral cation exchange complex; by sorption on hydrous oxides of iron, manganese and aluminium; and by the formation of stable complexes with soil organic matter. It is also probable that elements well known for their antagonistic effect on copper metabolism, are released from the ingested soil and so reduce copper availability (Suttle, Alloway and Thornton, 1975).

2.14 THE COPPER REQUIREMENTS OF CATTLE

The Agricultural Research Council (1980) have proposed estimates for the net and gross requirements of cattle and sheep for copper. The estimated requirements are based on a simple mathematical approach which integrates the components of net and gross requirements. Suttle (1983b) considers these requirements to be substantially overestimated. He has recalculated new factorial requirements which are 50% below those suggested by the ARC (1980). Suttle (1983b) suggests gross dietary copper requirements ranging from 6.2 - 7.5 mg/kg DM for growing bullocks according to their liveweight and growth rate. These replace the ARC (1980) recommended copper requirements of 11 - 13 mg/kg DM. The suggested requirements must be met by the diet, with supplements if necessary, to prevent the onset of bovine hypocupraemia.

2.15 THE IMPORTANCE OF REGIONAL GEOCHEMISTRY IN DELINEATING THE INCIDENCE OF BOVINE HYPOCUPRAEMIA

Geochemical reconnaissance techniques, initially developed for mineral exploration found application to British agriculture in the late 1960's (Webb and Atkinson, 1965; Webb, Thornton and Fletcher, 1968;

Thornton, Webb and Moon, 1969). The method generally used is stream sediment sampling at a density of about 1 sample/ 2km². By virtue of its origin, the stream sediment approximates to a naturally derived composite of the soil and rock upstream from the sampling point. It has been shown that the trace element content of this active sediment broadly reflects that of the soils and rocks as a whole. Reconnaissance maps based on this method have successfully delineated areas of recognised trace element disorders in livestock as well as focussing attention on areas where unrecognised subclinical deficiency may be limiting agricultural production. Examples of previous work relating to bovine hypocupraemia in the U.K. include:

(a) Co. Limerick

Webb and Atkinson (1965) identified anomalous molybdenum and selenium levels related to an outcrop of marine black shales in an area from which possible molybdenum-induced copper deficiency had previously been recorded. Detailed studies showed the sediment anomaly to be related to molybdeniferous soils and herbage. Later investigations (Thornton, Atkinson, Webb and Poole, 1966) demonstrated a highly significant correlation between the levels of molybdenum in the stream sediment and blood copper levels in the grazing cattle.

(b) North Wales

Molybdeniferous stream sediments overlying black shale of Ordovician age in the Crafnant and Llyn peninsula of Caernarvonshire, and on coastal alluvium near Harlech in North Wales, correlate with a high incidence of swayback and bovine hypocupraemia in the area (Thornton and Webb, 1967).

(c) Southern Pennines

Geochemical reconnaissance indicated molybdenum anomalies extending over 156 km² (Webb, Thornton and Fletcher, 1968). Clinical bovine hypocuprosis was previously only recognised over some 39 km² of the anomaly. Analysis of blood from 350 cattle in 26 herds from the molybdeniferous area around Ashbourne and Onecote and a non-molybdeniferous control area nearby, indicated a consistently higher

incidence of copper deficiency in animals of all age groups in the molybdeniferous area. 77% of the total stock not fed minerals were deficient in the molybdeniferous area compared to 37% in the control. (Thornton, Kershaw and Davies, 1972a). Supplementation trials using injectable copper were carried out over a two year period in the molybdeniferous area. Increases in liveweight of 10 - 70% were recorded for those animals receiving injections when compared to those not receiving supplementary copper (Thornton, Kershaw and Davies, 1972b).

(d) Lower Lias

Preliminary studies of the Lower Lias in areas of Somerset and Warwickshire, lying outside the teart area, indicated anomalous patterns of molybdenum (>3 ppm) in the stream sediments and soils over part of the outcrop, compared with normal levels elsewhere (Thornton, 1968). Subsequent geochemical reconnaissance over the entire formation showed a total area of over 907 km² with molybdenum levels greater than 3 ppm. Further research by Thomson (1971) delineated additional molybdeniferous areas derived from marine black shales ranging in age from Ordovician to Cretaceous. The Cu : Mo ratio of these pastures was within the range associated with molybdenum-induced copper deficiency in cattle. Widespread bovine hypocupraemia was therefore suspected over the extensive black shale formations.

CHAPTER 3

THE INCIDENCE OF BOVINE HYPOCUPRAEMIA IN ENGLAND

3.1 INTRODUCTION

The resurgence of bovine hypocupraemia in England in the 1975 - 1979 period has led to a countrywide survey to establish the incidence of the condition in recent years. Such a survey may be used to discover more information about the nature of the condition. This may then lead to 'in depth' geochemical studies in certain key areas.

3.2 THE CHOICE OF DIAGNOSTIC CRITERIA

Much controversy surrounds the choice of diagnostic criteria for determining the existence of bovine hypocuprosis in any survey. Overt symptoms such as a reduction in weight gain, weight losses and a decline in milk yield are all important, but non-specific features of the copper deficiency syndrome in cattle. Similarly, changes in hair pigmentation and the loss of coat lustre are not caused solely through a lack of copper. It is therefore necessary to employ supporting diagnostic techniques followed by an appraisal of the effects of administering copper to substantiate the existence of copper deficiency. The supporting diagnostic techniques that are available are considered below.

(a) Decline in blood and hepatic copper reserves

Clinical signs of bovine hypocuprosis rarely arise if blood and liver copper exceed 0.5 mg/l and 30 mg/kg DM , respectively (MAFF, 1982). However, data from blood and liver analyses cannot be used to discriminate those animals in which clinical deficiency is imminent from those likely to remain in ostensibly normal health, despite a low copper status. Nevertheless blood and hepatic copper levels are commonly accepted criteria owing to their accessibility for sampling. It is assumed, without direct evidence, that blood copper is in equilibrium with tissue copper at functional sites, which when depleted, induce metabolic effects that have economic or clinical significance (MAFF, 1982).

Decisions as to the appropriate threshold value for the copper content of blood or liver are arbitrary, but are of value for identifying groups of animals in which the risk of development of copper deficiency is high. The generally accepted thresholds (Scottish Agricultural Colleges, 1982) are 5 mg/l for blood and 30 mg/kg DM for liver (Table 3.1). Dutch advisory workers (Committee on Mineral Nutrition, T.N.O., 1973) recognised some of the problems of the interpretation of blood and liver analyses, and proposed criteria which take greater account of the stage of development of the animal at the time of sampling (Table 3.2). These workers tend to raise the 0.5 mg/l threshold to 0.75 mg/l to take greater account both of the stage of development of the animals and of the seasonal differences in the availability of copper from herbage and feedingstuffs. Workers such as Rogers and Poole (1976), would dispute that even the 0.5 mg/l threshold is too high. They have shown that only 20% of an extensive population of cattle with blood copper less than 0.5 mg/l grew faster after copper treatment. Field and experimental studies have shown that no deleterious effects may be seen in some animals with plasma copper maintained at less than 0.5 mg/l for up to a year (e.g. Smith and Coup, 1973). However, recent chemical and pathological studies with copper deficient cattle have suggested that a wide variety of lesions in cardiac muscle, and vascular and connective tissue may well become established before overt signs of copper deficiency develop (Mills, 1975). It would therefore seem sensible to maintain the blood copper threshold at 0.5 mg/l, if not at 0.75 mg/l.

(b) Decline in blood copper enzyme activity

Several copper-containing enzymes are synthesised intracellularly and are either secreted into blood plasma or packaged into erythrocytes. A loss of activity when copper supply is inadequate to maintain the rate of synthesis of such enzymes, offers an alternative approach to the assessment of copper status of the tissue within which they are synthesised.

- i) Plasma caeruloplasmin activity - this is closely related to plasma copper (Todd, 1970), but is often undetectable when blood copper is less than 0.2 mg/l. It is thought to be of no more diagnostic value than plasma copper.
- ii) Erythrocyte superoxide dismutase (SOD) - its activity is thought to reflect copper status at the time of erythrogenesis

Blood copper (mg/ℓ)	
> 0.5	Normal
0.5 - 0.3	Clinical symptoms of copper deficiency possible
< 0.3	Clinical symptoms likely

Liver copper (mg/kg DM)	
>30	Copper deficiency unlikely
30 - 10	Inadequate hepatic copper reserves
<10	Clinical copper deficiency likely

TABLE 3.1. Thresholds for blood and liver copper levels
(After Scottish Agricultural Colleges, 1982)

Blood copper (mg/ℓ)			
Calf	Yearling	Adult	
0.7 - 1.0	0.65 - 1.0	0.75 - 1.2	Adequate copper
	0.50 - 0.65	0.60 - 0.75	Marginal copper status
<0.5	<0.3	<0.4	Clinical copper deficiency likely

Liver copper (mg/kg DM)			
Calf	Yearling	Adult	
200 - 400	200	150	Normal
	50	25	Marginal - acceptable only at the end of the grazing season
<50	<20	<10	Clinical copper deficiency very likely

TABLE 3.2. Thresholds for blood and liver copper levels set by Dutch advisory workers (After Committee on Mineral Nutrition, TNO, 1973)

Caeruloplasmin	10 units/ℓ
Superoxide dismutase	0.5 mg/g Hb - in young stock
	0.3 mg/g Hb - in adult stock

TABLE 3.3. Thresholds for caeruloplasmin and superoxide dismutase
(After Scottish Agricultural Colleges, 1982)

and this provides a retrospective index of chronic low copper intake. McMurray (1981) suggests that SOD concentrations of less than 0.5 mg/g Hb in cattle is indicative of chronic copper depletion. However, low SOD concentrations are not directly related to the appearance of clinical signs of copper deficiency. A low activity may indicate circumstances in which the risk of development of clinical symptoms are appreciable.

- iii) Leucocyte cytochrome oxidase - its activity declines during copper depletion but unidentified variables cause marked fluctuations in activity (Boyne, 1978). Until the nature of these is known, this relatively simple assay cannot be used as a routine diagnostic aid.

Thresholds for caeruloplasmin and superoxide dismutase activities are given in Table 3.3.

(c) Defective neutrophil function

A decline in microbicidal activity of blood neutrophils has been detected at a very early stage of copper depletion in cattle (Boyne and Arthur, 1981). However, this is not specific, also occurring in selenium depleted cattle when blood selenium is less than 0.02 mg/l.

3.3 PREVIOUS RESEARCH ON THE INCIDENCE OF BOVINE HYPOCUPROSIS / HYPOCUPRAEMIA

Three previous studies have led to the publication of maps showing the incidence of bovine hypocuprosis / hypocupraemia in Great Britain. The first by Allcroft (1952) was produced in response to the early discoveries of 'conditioned' copper deficiency in various parts of the country. This worker was quick to realise the possible economic importance of a subclinical copper status in cattle and prompted a blood copper survey throughout the country. In 1952 well over 100 herds had been investigated of which one third were found to have a low copper status associated with a variety of clinical symptoms. A map of the distribution of these herds as recorded in 1952 is shown in Figure 3.1.

This map was updated some seven years later by Allcroft and

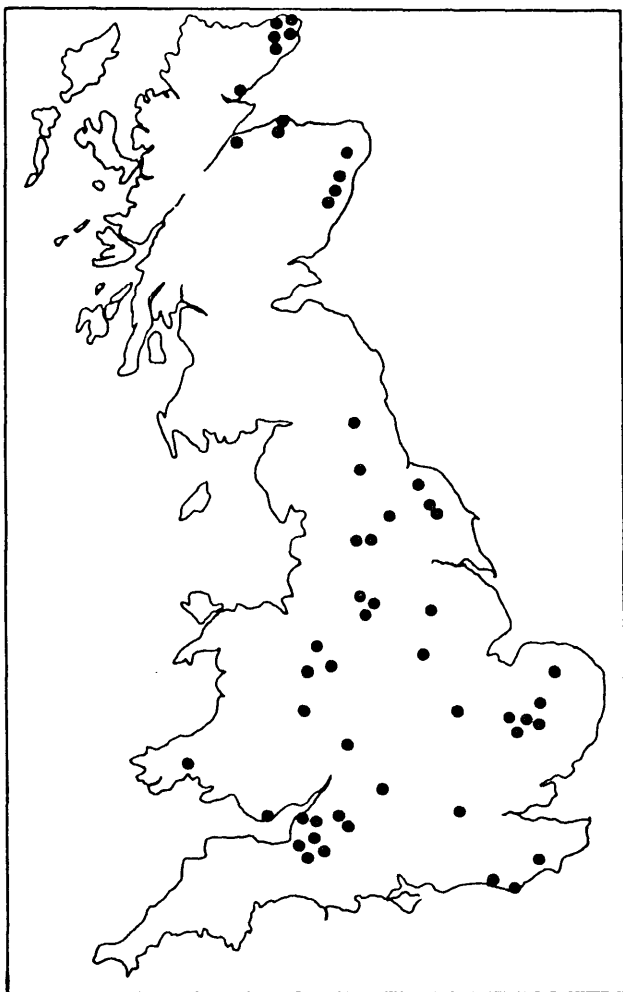


FIGURE 3.1. Map showing the distribution of bovine hypocuprosis in Britain in 1952 (redrawn from Allcroft, 1952)

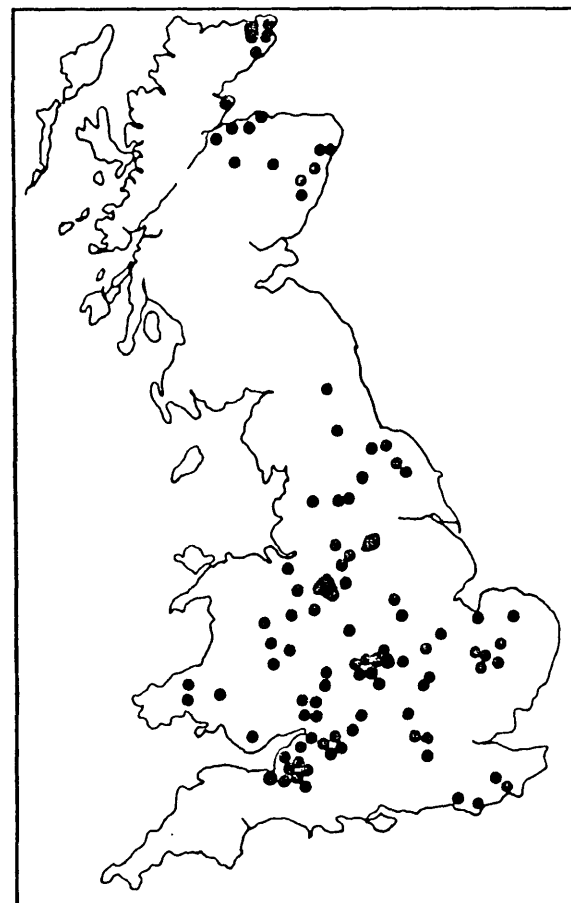


FIGURE 3.2. Map showing the distribution of bovine hypocuprosis in Britain in 1959 (redrawn from Allcroft and Uvarov, 1959)

Uvarov (1959). These workers showed from the data collected between 1952 and 1959 that the incidence of bovine copper deficiency was even more widespread (Figure 3.2).

It was then not until 1972 that Thomson, Thornton and Webb (1972) plotted a further map to show the incidence of bovine hypocuprosis with particular reference to the outcrop of the principal marine black shales in England and Wales (Figure 3.3). The data were made available by the Veterinary Investigation Service and local veterinary practices and are complete to January 1970. Thomson (1971) states that these data are far from comprehensive - bovine hypocupraemia is often treated on the farm and not systematically recorded; a bias is therefore imparted by veterinary practitioners who record this information. Nevertheless, Thomson found that 65% of the herds with hypocuprosis occurred at localities underlain by marine black shales, or in some coastal areas, recent black muds. From Figure 3.3 a high frequency of hypocuprosis is seen to occur on the Lower Lias in the teart pastures of Somerset and also in Glamorgan and Warwickshire, with further groupings associated with the lower / middle Carboniferous black shales in the southern Pennines and north Lancashire. In all these areas molybdenum-induced bovine hypocuprosis was confirmed. Diffuse patterns of hypocuprosis are also found over Oxford and Kimmeridge clay formations in Wiltshire, Berkshire, Oxfordshire and Buckinghamshire.

3.4 THE INCIDENCE OF BOVINE HYPOCUPRAEMIA 1977 - 1980

After very careful consideration of the facts given in 3.2, it was decided to use the copper content of whole blood as the diagnostic aid in determining cattle of low copper status. The advantage of this indicator at the present time, is an abundance of fairly standardised data which unfortunately none of the other diagnostic criteria listed in 3.2 have. Using blood copper levels in preference to confirmed cases of hypocuprosis has the further advantage of including cattle with possible subclinical deficiency. All the disadvantages of using blood copper were appreciated and any extrapolations based on ensuing clinical effects and their economic significance, were to be considered with caution.

The blood copper data were collected from the Central Veterinary

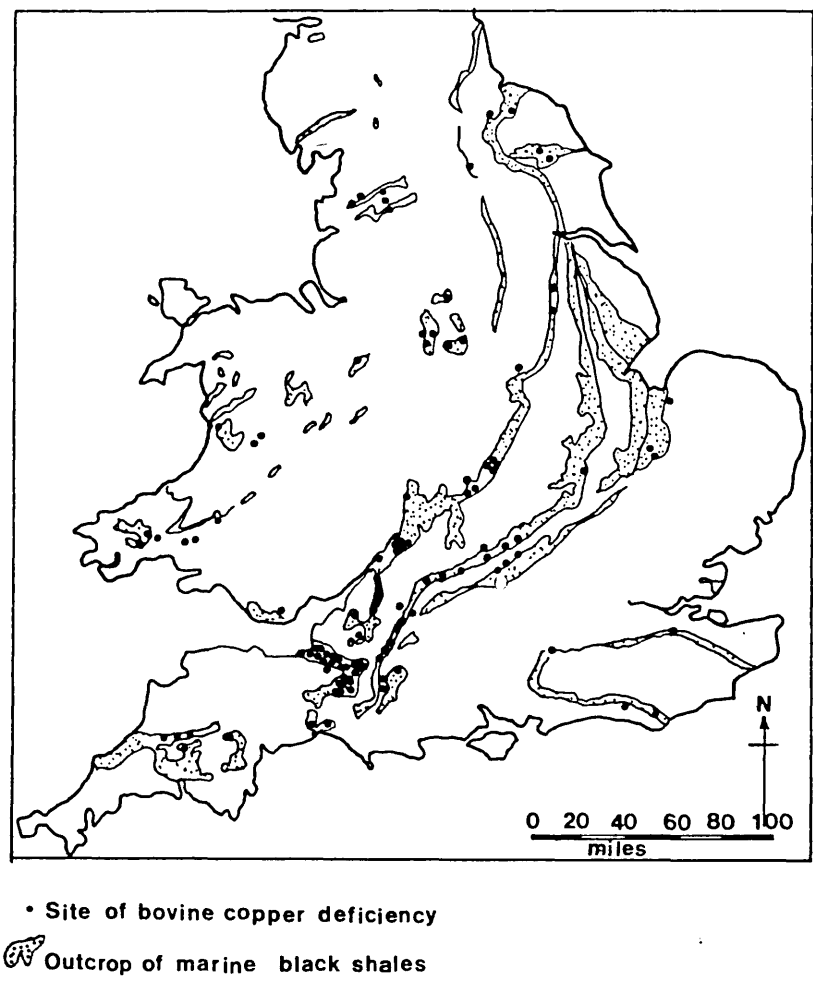


FIGURE 3.3. The distribution of bovine copper deficiency in localities underlain by marine black shale (redrawn from Thomson et al., 1972)

Laboratory, Weybridge and the Veterinary Investigation Centres at Shrewsbury and Sutton Bonington. The data collected, covered the years 1977 - 1980 inclusive, and so reflected the resurgence of the condition in the post 1975 period. As data from Wales were not of a strictly comparable form (i.e. they were based on serum copper rather than on plasma copper) they have been omitted. The equation (McMurray and Blanchflower, 1983):-

$$\text{serum copper (mg/l)} = 11.7 + 0.66 \times \text{plasma copper (mg/l)}$$

could have been used for the conversion of serum to plasma copper levels but was unavailable at the time this survey was conducted.

In the compilation of maps from these data the lowest blood copper values were recorded if they fulfilled the following two criteria:

- i) There was more than one low value for the herd;
- ii) It was less than or equal to 0.8 mg/l.

The high threshold was applied so as to allow for the stage of development of the animal at the time of sampling, as suggested by Dutch advisory workers (Committee on Mineral Nutrition, T.N.O., 1973). It also made allowance for an increase in blood copper due to the stress imposed on the animal due to blood sampling. Cattle with blood copper levels 0.6 - 0.8 mg/l were classified as marginally deficient. Those with levels less than or equal to the widely accepted threshold of 0.5 mg/l were considered as deficient. No record was made of the time of sampling or age of the animal, as it was not practical to do so.

Owing to the confidentiality of the data the exact locations of the farms with hypocupraemic cattle could not be used. Instead the two-letter, four-figure British National Grid References of the approximate centres of the enclosing parishes were recorded (Ordnance Survey, 1975). These two-letter, four-figure grid references were converted to eight-figure British National Grid Co-ordinates approximating to the centre of each parish, using a simple computer program. These data, along with the parish name and blood copper value were stored as a direct access computer file. This data file was the input information to a mapping routine (Howarth, unpublished). This enabled the following two Kingmatic plotted maps to be produced

from the veterinary data:

- i) The distribution of bovine hypocupraemia over the period 1977 - 1980 (blood copper ≤ 0.8 mg/l) (Figure 3.4);
- ii) The distribution of bovine hypocupraemia over the period 1977 - 1980 (blood copper ≤ 0.5 mg/l) (Figure 3.5).

It is interesting to note that over the period 1977 - 1980 there were cattle in 4,088 herds that had blood copper values less than or equal to 0.8 mg/l (i.e. ≤ 0.08 mg/100ml). Of these, 1748 had blood copper values of 0.5 mg/l (0.05 mg/100ml) or less.

3.5 THE INFLUENCE OF COPPER SUPPLEMENTATION ON THE REPORTED INCIDENCE OF BOVINE HYPOCUPRAEMIA

Whilst the maps plotted from the veterinary information show bovine hypocupraemia to be a widespread problem, it was thought that these would generally be underestimates of the extent of copper deficiency in England. This is because numerous effective treatments and preventative measures against bovine copper deficiency have been available for many years, and might have been used to such an extent so as to have masked the true size of the problem. There might have also been a bias, as reported by Thomson (1971), imparted by the non-systematic way in which bovine hypocupraemia is recorded.

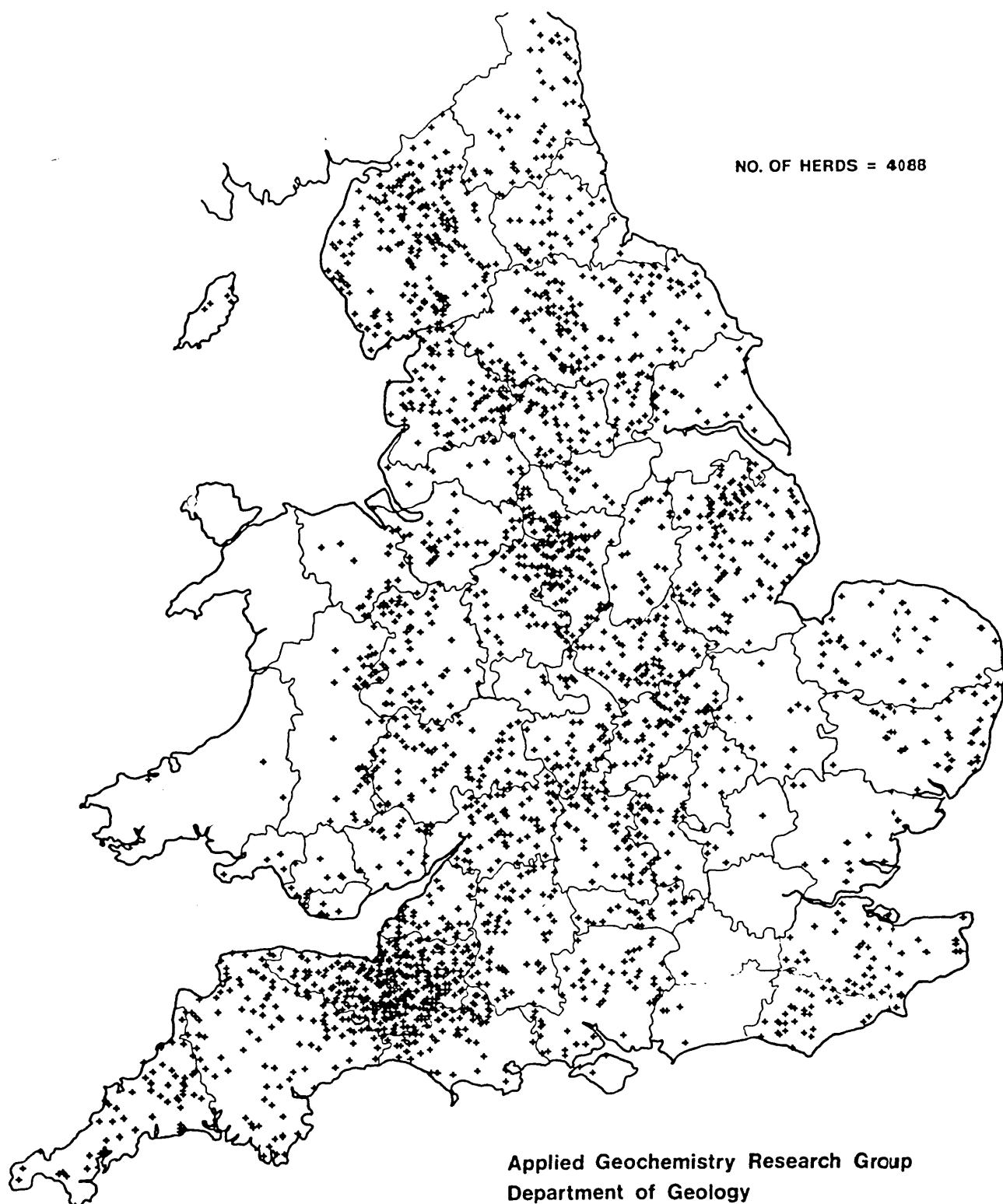
In order to overcome some of these problems a measure of intensity of copper supplementation was sought. The copper product manufacturers Glaxovet (for Coprin), C-Vet (for Copavet), Rycovet (for Bovicoppa) and Tasman Vaccine Laboratory (for Cujec) were consulted, but none could be of specific assistance. Consequently, Agridata, an agricultural market research company was contacted. This commercial organisation collects information concerning the usage of animal health products by farmers. Their 'Animal Health and Nutrition Index' involves the collection of data in spring and autumn to cover the previous six months from panels of 950 cattle, 750 sheep and 500 pig farmers. Panels are supposed to be statistically representative of the farming enterprise covered.

Limited information on copper supplementation to cattle was available. Nevertheless, data obtained from 1,372 questionnaires gave

FIGURE 3.4

THE DISTRIBUTION OF BOVINE HYPOCUPRAEMIA
OVER THE PERIOD 1977-1980

(BLOOD COPPER $\leq$ 0.08 mg / 100 ml WHOLE BLOOD)

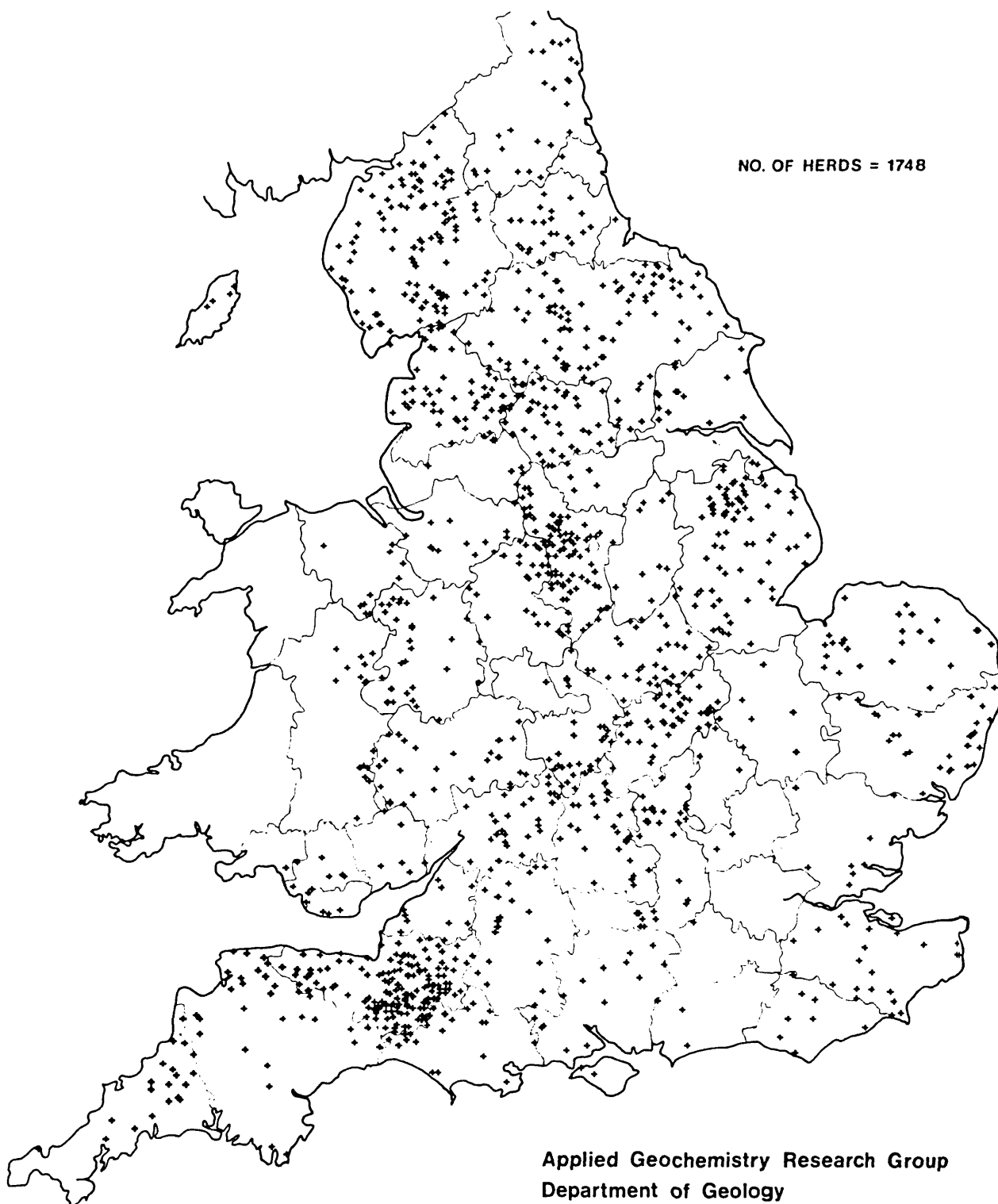


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Imperial College

FIGURE 3.5

THE DISTRIBUTION OF BOVINE HYPOCUPRAEMIA
OVER THE PERIOD 1977-1980

(BLOOD COPPER ≤ 0.05 mg / 100 ml WHOLE BLOOD)



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Department of Geology
Imperial College

an indication of the location and number of farms on which the parenteral treatment of copper deficiency by injectable copper supplementation was being administered to cattle over the period April 1980 - March 1981. Again, owing to the confidential nature of the data, the exact location of the farms could not be used. The two-letter, four-figure grid references for the enclosing parishes were recorded. The eight-figure British National Grid Co-ordinates were calculated as before, and these plus parish names and numbers of copper injections administered, were stored as an indirect access computer file. This information was used to plot 'The location of injectable copper supplementation to cattle in England and Wales 1980 - 1981' (Figure 3.6). Of the 1,372 questionnaires analysed, 89 herds were found to be undergoing supplementation (6.5%), totalling 3920 injections.

Agridata weight their data according to the Ministry of Agriculture's June 1977 census which provides information on the distribution of holdings by herd size in terms of numbers of animals. The usage of copper injections, as indicated by the quota sampling, can then be expanded to represent the national usage. In this way, Agridata have calculated that for the six month period, October 1978 - April 1979 a total of 281,000 injections were administered to cattle in England, Wales and Scotland. Five percent of all cattle in Great Britain therefore underwent copper supplementation in the winter period. A similar, if not slightly higher value might have been expected for the summer.

In addition to parenteral copper supplementation, copper is also routinely added to compound feedingstuffs for dairy cattle. Some 200 different brands of mineral supplement are marketed containing from 200 - 8,000 mg/kg Cu. No information on areas of heaviest usage was available.

It would seem that whilst the occurrence of bovine hypocupraemia is widespread in England, so too is the usage of copper supplements. Taken together, the cost of low blood copper status in cattle through diagnoses, treatment, prevention and subclinical losses in yield, must be significant.

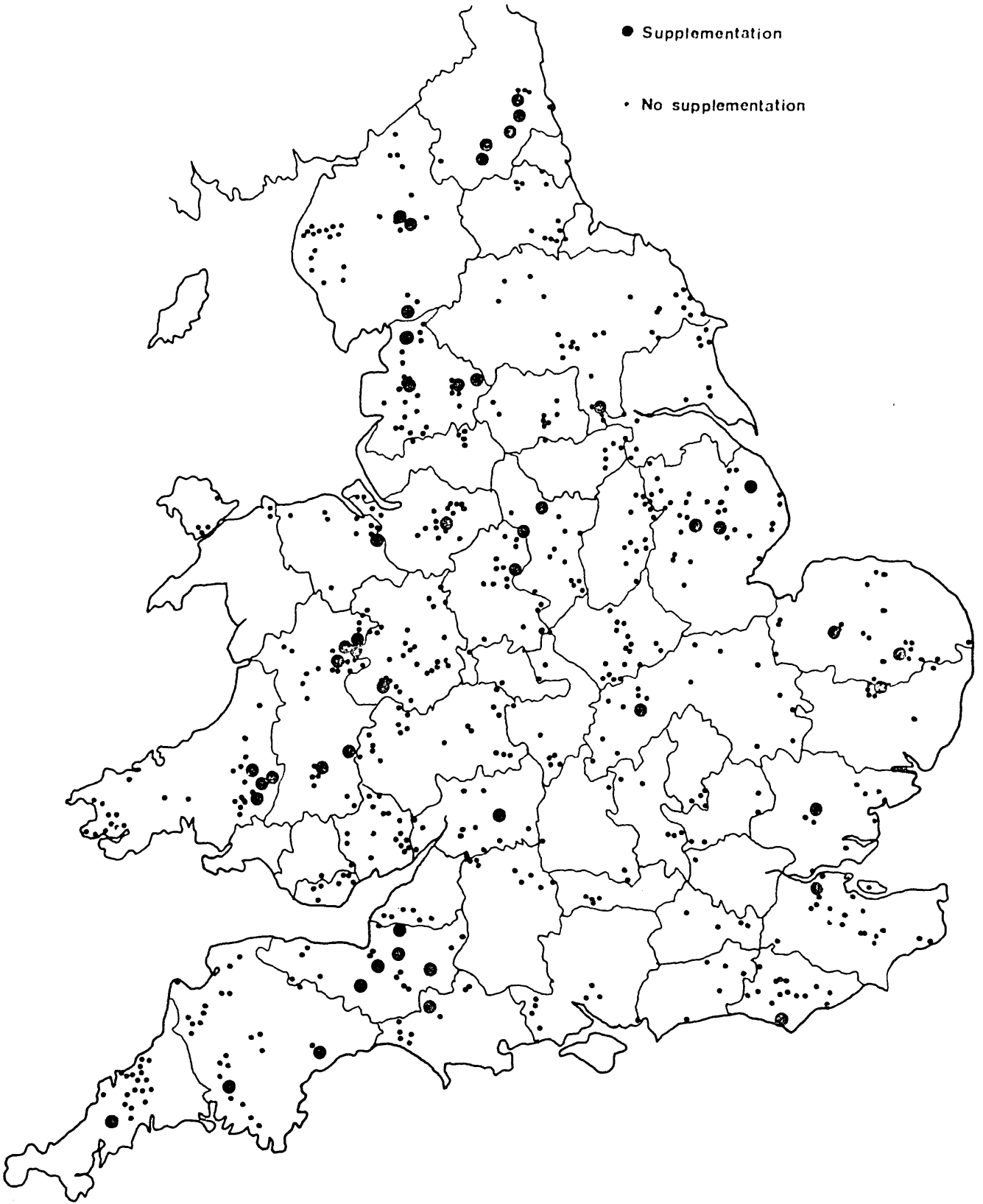


FIGURE 3.6. The location of injectable copper supplementation to cattle in England and Wales 1980-1981 (Agridata)

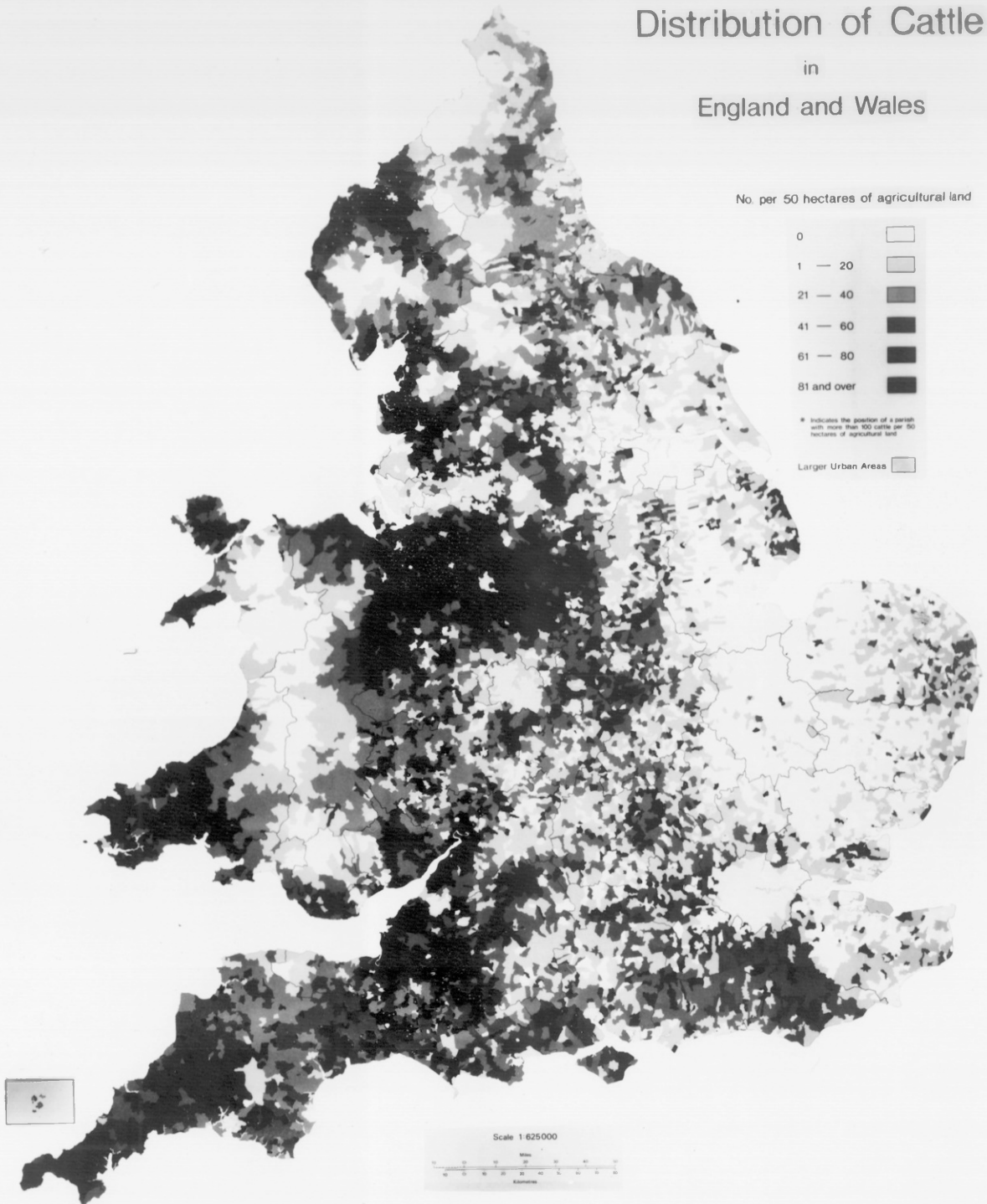
3.6 INTERPRETATION OF THE MAPS PRESENTED

The maps plotted from the veterinary information and supported by the map of injectable copper supplementation confirm that bovine hypocupraemia does still pose a considerable problem to agriculture. Reference to the 'Distribution of cattle' map (MAFF, 1978) in Figure 3.7 shows that most of the cattle populated regions of England exhibit some herds of low copper status - the number is far higher, though somewhat more controversial, for marginal deficiencies.

Attention is immediately drawn to Somerset where there is an abundance of points on all maps. Not only does it have the greatest frequency of herds exhibiting low blood copper status, but also the highest number of copper injections administered. This high frequency of hypocupraemia has been recognised for many years and attributed to excess molybdenum in soils and pastures (Ferguson et al., 1943). As discussed in section 2.12 it is not so much the absolute level of molybdenum or copper that is important, but the ratio of Cu : Mo in soils and herbage. The geochemical map for Mo - Cu (Figure 3.8) taken from 'The Wolfson Geochemical Atlas of England and Wales' (Webb et al., 1978), helps to delineate areas where Cu : Mo ratios in stream sediments are low and hence might be expected, given the right pH and drainage conditions, to support herbage with such characteristics. Such areas are seen in Somerset, Bristol, Warwickshire, Staffordshire and Derbyshire. These areas, underlain by molybdeniferous rocks, are associated with a high incidence of bovine hypocupraemia in the current study and also support earlier work on the subject (Webb and Atkinson, 1965; Thornton, Atkinson, Webb and Poole, 1966; Thornton and Webb, 1967; Webb, Thornton and Fletcher, 1968; Thornton, Kershaw and Davies, 1972a and b; Thornton, Webb and Moon, 1968; Thomson, 1971; Thomson, Thornton and Webb, 1972).

It is interesting to note that several areas of bovine hypocupraemia fall outside the highly molybdeniferous regions. Areas of north Devon, Warwickshire, Yorkshire, Lancashire and Cumbria are all examples of this, and serve to illustrate that bovine hypocupraemia/hypocuprosis in England and Wales is not only caused by excessive molybdenum, or low Cu : Mo ratios in the diet. The involvement of elements other than molybdenum has been considered by other workers

FIGURE 3.7
Distribution of Cattle
in
England and Wales



(MAFF, 1978)

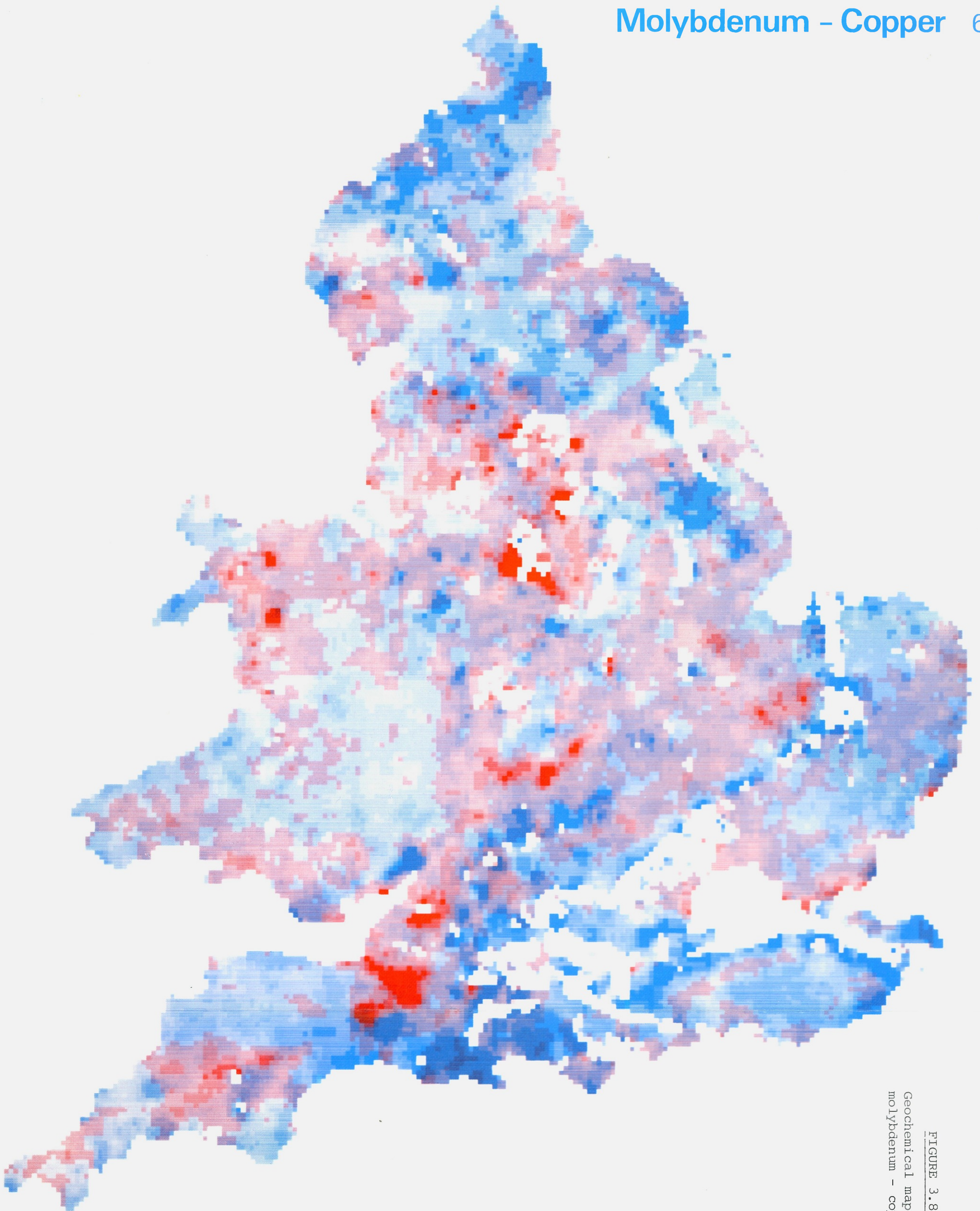


FIGURE 3.8
Geochemical map for
molybdenum - copper

Combined element map for empirical **Molybdenum** (red), increasing colour contribution with increasing element concentration; and percentile **Copper** (blue), increasing colour contribution with *decreasing* element concentration.

and a review of their work has already been given in section 2.13. Whilst particular attention will be paid to levels of copper and molybdenum in soils and herbage, a wide range of other elements such as sulphur, iron, cadmium and zinc will also be surveyed in an attempt to explain the occurrence of bovine hypocupraemia in non-molybdeniferous areas.

3.7 THE IMPORTANCE OF VETERINARY SURVEYS

The need for a good veterinary data bank has become apparent from the current survey. The main source of information available at present, to indicate the extent of copper deficiency in cattle is the Veterinary Investigation Diagnosis Analysis II (VIDA II) system. This computer-based system has been recording diagnostic data from 24 veterinary investigation centres in England and Wales and 9 centres in Scotland since 1974. VIDA data are based on a referral system that is dependent on a veterinary practitioner referring a case to a veterinary investigation laboratory for confirmation of a provisional diagnosis of copper deficiency. If this provisional diagnosis is confirmed then the incident is entered in the VIDA compilation. As many diagnoses are made without reference to a V.I. laboratory, they are not recorded by VIDA. The specimens received by V.I. centres therefore represent a biased sample of the field problem of animal disease. Caution must therefore be shown when interpreting and using diagnostic statistics as they do not bear any simple or direct relationship to the number of cases of disease in national animal populations (ADAS, 1980). Clearly there is still some room for improving veterinary data collection and collation. Increased use of computer facilities is one way of achieving this.

CHAPTER 4

THE STATISTICAL PREDICTION OF LOW BLOOD COPPER STATUS IN CATTLE FROM GEOCHEMICAL DATA

4.1 INTRODUCTION

It was established in Chapter 3 that bovine hypocupraemia posed a considerable problem to agricultural production in England. It was thought that the causal factor in several cases was not attributable to excessive molybdenum in the diet. It was therefore decided to investigate the geochemistry of the hypocupraemic areas further, to see whether any other geochemical parameters could be related to the occurrence of low blood copper status in cattle in England.

4.2 GEOCHEMISTRY OF THE HYPOCUPRAEMIC AREAS

Several of the most severely hypocupraemic regions in England and the Welsh borders were defined using the following criteria:

- i) Each area had a high frequency of herds with blood copper levels of 0.5 mg/l or less;
- ii) Each area was underlain by only one major geological formation;
- iii) There were no main towns in the area.

These hypocupraemic areas were best defined by the irregular shapes (polygons) illustrated in Figure 4.1. In all, twenty one hypocupraemic polygons were defined. It was also hoped to delineate some non-hypocupraemic polygons but this was much more difficult, as an absence of low blood copper data did not automatically indicate an unaffected area. This was mainly because of the bias, referred to in Section 3.3, which was imparted by the non-systematic recording of the occurrences of bovine hypocupraemia. Consequently, only one 'normal' polygon was defined with any degree of certainty at all (polygon 16). The polygon coding is given in Table 4.1.

The source of the geochemical data used in the current study was The Wolfson Geochemical Atlas of England and Wales (Webb et al., 1978). This atlas was based on sampling some 60,000 stream sediments at tributary-road intersections wherever the upstream catchment area did not exceed 25 km². At each site, care was taken to avoid locally collapsed

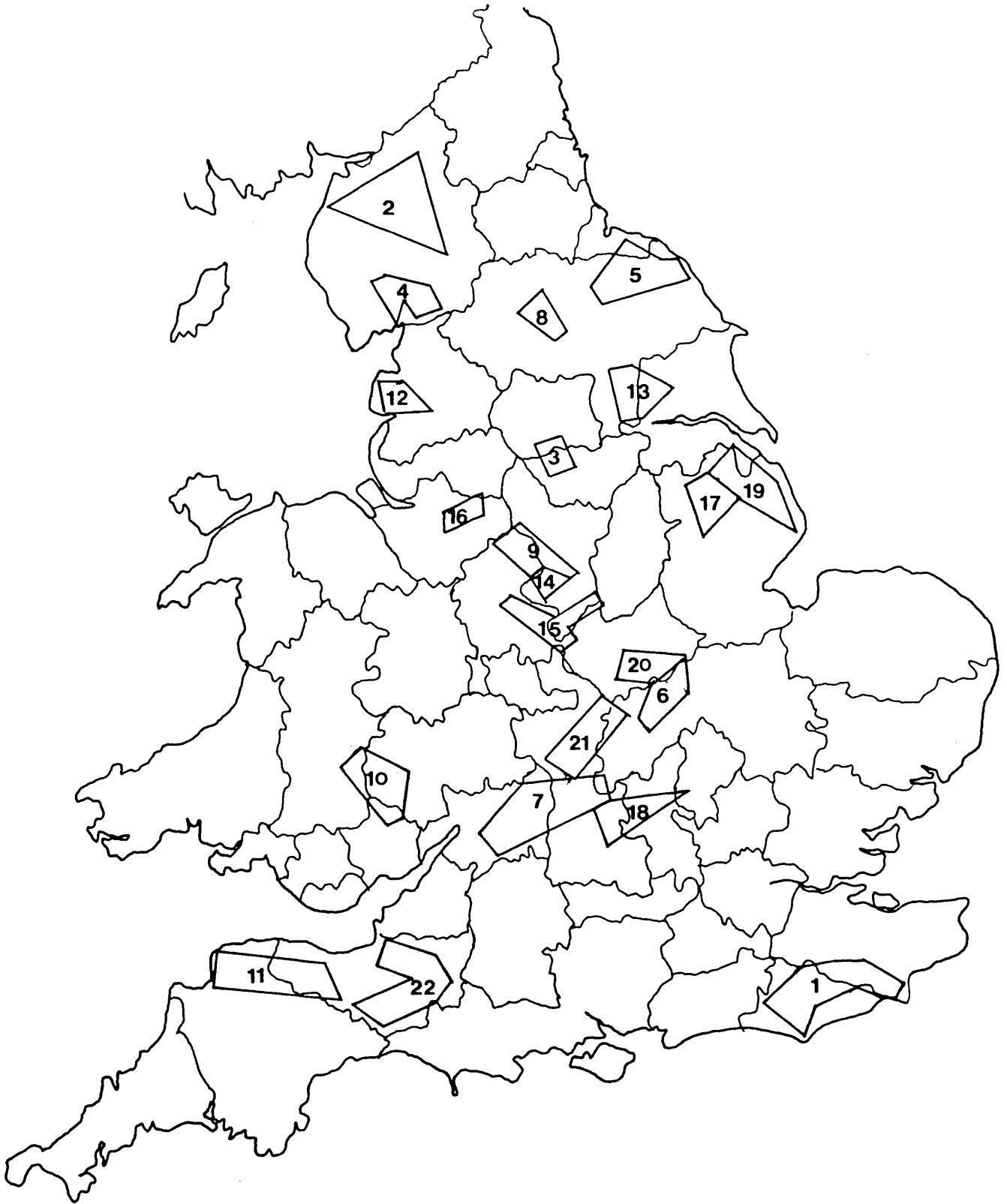


FIGURE 4.1. The location of hypocupraemic polygons
(polygon coding is given in Table 4.1)

- 1 Hastings Beds: East Sussex and Kent
- 2 Carboniferous Limestone and Bunter Sandstone: Cumbria
- 3 Coal Measures: West and South Yorkshire
- 4 Silurian: Cumbria
- 5 Upper and Lower Oolite: North Yorkshire
- 6 Upper and Lower Oolite: Northamptonshire
- 7 Upper and Lower Oolite: Gloucestershire and Oxfordshire
- 8 Millstone Grit and Culm Measures: North Yorkshire
- 9 Millstone Grit and Culm Measures: Staffordshire and Derbyshire
- 10 Upper and Middle Old Red Sandstone: Herefordshire and Worcestershire
- 11 Lower Old Red Sandstone: North Devon and Somerset
- 12 Bunter Sandstone/Keuper Marl and Sandstone: Lancashire
- 13 Bunter Sandstone/Keuper Marl and Sandstone: North Yorkshire and Humberside
- 14 Keuper Marl and Sandstone: Derbyshire
- 15 Keuper Marl and Sandstone: Staffordshire, Warwickshire and Lancashire
- 16 Keuper Marl and Sandstone: Cheshire
- 17 Purbeck and Portland Beds, Kimmeridge and Oxford Clays: Lincolnshire
- 18 Purbeck and Portland Beds, Kimmeridge and Oxford Clays: Oxfordshire and Buckinghamshire
- 19 Chalk: Lincolnshire
- 20 Liassic and Rhaetic: Leicestershire
- 21 Liassic and Rhaetic: Warwickshire
- 22 Liassic and Rhaetic: Somerset

TABLE 4.1. Polygon coding based on bedrock geology

bank material and highly organic sediment. Sampling was restricted over ground underlain by massive calcareous rocks where there was little surface drainage; and conurbations were not sampled.

The samples were analysed for As, Mo, Cd, Zn, Al, Ba, Co, Cr, Cu, Fe, Ga, Li, Mg, Mn, Ni, Pb, Sc, Si, Ti and V, and moving-average smoothed 1:1 M scale maps prepared for publication. The smoothed maps were based on a grid of 254 rows and 209 columns, each map cell being approximately 6.5 km² in area. Averaging was based on a 3-by-3 cell window. Full details are given in Webb et al (1978). The smoothed, rather than original data, were used here in order to have maximum compatibility with the veterinary data at a regional scale.

Before geochemical data could be obtained for each of the polygons, these had to be converted into equivalent map cells, identical to those used for the Wolfson Geochemical Atlas. A computer program was written to carry out the following operations:

- i) The conversion of polygon areas to map cells;
- ii) The calculation of the number of map cells / polygon;
- iii) The calculation of the number of low (<0.5 mg/ℓ) blood copper values in each polygon;
- iv) The standardisation of blood copper values to the number of deficiencies / 100 map cells, for easy comparisons between polygons.

Having defined the polygons in terms of row and column numbers, it was possible to merge the relevant geochemical data using computer software. The final output data file contained the concentration of 23 elements in each map cell, and within each polygon, together with the standardised blood copper data for each polygon. Mean element values were calculated for each polygon using the Minitab statistical package (Ryan et al., 1981). These are presented in Table 4.2. This information forms the basis to the subsequent statistical prediction of low blood copper status from geochemical parameters.

4.3 REGRESSION ANALYSIS

The method most commonly employed to describe the relation of one variable to a number of other independent variables is multiple linear regression. It is assumed that the model is:

Polygon	Al	Ca	Fe	K	Mg	Si	Ga	Sc	Cu	Pb	V	Va	Co	Cr	Ni	Mn	Sn	Sr	Li	Mo	As	Zn	Cd	bcr
1	3.6	1.8	2.6	0.7	2	30	5	6	15	25	35	139	10	40	19	658	0	49	21	0.9	1.2	84	0.1	6
2	4.2	0.6	2.2	0.9	3	37	5	6	9	23	21	297	7	30	17	594	1	45	44	0.7	0.7	61	0.1	22
3	6.7	1.2	4.3	0.9	3	35	9	10	42	78	39	396	20	42	35	1763	4	51	52	2.3	1.7	153	0.7	11
4	7.0	0.7	4.2	1.6	6	31	13	9	25	65	74	271	17	70	41	1633	1	35	46	1.2	2.2	202	1.6	19
5	4.8	0.8	3.0	0.6	2	39	5	6	10	19	31	143	10	62	18	268	2	30	36	1.2	0.8	76	0.1	20
6	5.1	2.8	5.4	1.0	2	35	9	6	19	38	73	178	10	63	27	462	2	56	43	1.1	1.6	127	0.1	25
7	3.9	11.1	3.3	1.1	3	26	6	2	13	28	61	175	4	60	9	507	0	109	12	1.4	1.7	111	0.2	16
8	3.7	2.9	2.4	0.4	3	38	4	4	12	78	13	288	3	23	7	439	1	62	28	0.6	0.4	183	0.9	27
9	6.4	0.8	4.0	0.9	3	36	12	5	26	44	52	308	16	59	34	738	1	21	45	3.1	1.0	167	2.0	49
10	5.8	1.1	3.0	0.8	4	33	9	6	24	21	53	205	10	94	30	816	1	38	37	0.5	0.4	71	0.1	17
11	8.2	0.5	4.0	1.3	4	34	11	8	22	22	66	208	14	58	38	625	2	39	56	0.9	1.6	120	0.9	24
12	4.9	1.4	3.1	0.9	4	37	4	5	22	37	19	218	8	31	122	439	2	42	40	1.3	0.7	116	0.2	12
13	4.2	1.9	2.4	1.0	3	35	4	5	13	26	20	203	5	27	12	371	1	42	35	1.0	0.4	73	0	7
14	4.6	1.9	2.9	1.0	2	38	9	5	38	158	42	1433	10	39	22	539	1	46	40	3.3	1.2	230	2.3	38
15	4.3	1.4	2.7	1.4	3	35	9	5	17	45	36	350	11	36	20	489	1	30	41	1.3	0.9	138	0.5	11
16	3.9	0.9	2.5	1.2	2	37	6	5	29	50	37	293	7	46	21	306	3	45	40	1.0	0.8	100	0.2	0
17	3.5	4.9	2.4	1.0	3	33	4	4	5	18	21	169	2	16	2	309	0	82	11	1.0	0.9	67	0.1	21
18	4.1	9.4	3.7	1.1	2	26	7	1	18	31	55	181	5	57	12	594	0	89	16	1.4	1.6	118	0	23
19	4.5	5.8	3.4	1.2	4	27	6	3	17	18	50	211	4	47	11	477	0	109	22	1.2	1.5	98	0	7
20	4.7	2.8	4.5	1.3	3	34	10	5	22	41	68	221	13	61	28	566	2	49	44	1.3	1.6	108	0.2	13
21	5.4	3.3	4.3	1.4	4	30	10	5	28	38	75	215	11	62	26	542	1	61	50	2.1	1.5	130	0.1	13
22	4.6	6.3	2.6	1.0	3	30	6	3	18	38	44	260	4	57	13	481	0	142	14	3.1	0.8	120	0.4	65

TABLE 4.2. Mean element values and standardised blood copper incidence rates (bcr) for the polygon areas

Note: Al, Ca, Fe, K, Mg and Si are in %; Ga, Sc, Cu, Pb, V, Ba, Co, Cr, Ni, Mn, Sn, Sr, Li, Mo, As, Zn and Cd are in ppm

$$Y = b_0 + b_1.X_1 + b_2.X_2 + . . . b_p.X_p + e$$

where,

Y is the dependent variable; $X_1, X_2 . . . X_p$ are the predictor variables; $b_0, b_1, b_2 . . . b_p$ are the regression coefficients and e is the error of the X variables, assumed to be Normally distributed.

In the classical method of least squares, the regression estimators are chosen such that the sum of the squared deviations of the sample data points from the estimated regression equation, is minimised (Bradley and Anderson, 1977). It is in this sense that the least squares estimates provide a 'best fit' to the sample data. If the data are ill-conditioned (i.e. there is substantial inter-correlation between the independent variables) the least squares solution yields coefficients whose absolute values are too large and whose signs may actually reverse with negligible changes in the data (Marquardt and Snee, 1975). There may also be a tendency for the more important predictors to be masked. In achieving optimum fit to the estimation data, least squares often destroys good prediction of new data.

Ridge regression is a technique for reducing the effects of ill-conditioned data. It stresses minimum mean square deviation between true and estimated coefficients, rather than stressing least squares. This achieves a reduction in the mean square error at the expense of a very small bias in the estimator. The residual sum of squares is larger with the ridge solution, but a much more stable set of estimators is achieved as a result. A small constant k is added to the diagonal elements of the correlation matrix to form a new matrix, which is much better conditioned than the original, and should therefore give much better estimates of the regression coefficients. k is called the ridge constant. (Hoerl and Kennard, 1970).

The mean square error of the ridge regression estimators is the sum of two terms: one represents a measure of bias, the second, a measure of variance. Increasing k, increases the former and decreases the latter. There is a value of k therefore, for which the expected mean square error is at a minimum. Variance inflation factors (VIF) are the sum of the inverse eigenvalues for each solution, i.e. for a given k, and may be regarded as indicators of the degree of multicollinearity in the data. For an orthogonal (uncorrelated) solution the eigenvalues will all equal

one. Very large VIFs therefore indicate an ill-conditioned solution. When VIFs are between one and ten it is generally agreed to be a satisfactory solution and the most important variables in the regression will have the largest standardised coefficients. Optimum k is determined from inspection of the estimated regression coefficients graphed as a function of k (the ridge trace). The stable solution occurs where the estimates of the coefficients (α) do not change rapidly with k . The classical least squares solution corresponds to the result when $k = 0$. In the presence of correlated predictors the coefficients are collectively unstable, and with small increases in k , many coefficients change in both sign and magnitude.

4.4 THE APPLICATION OF RIDGE REGRESSION TO THE PREDICTION OF LOW BLOOD COPPER STATUS FROM GEOCHEMICAL PARAMETERS

Ridge regression analysis was performed on the geochemical data and low blood copper incidence rates given in Table 4.2, using a computer program developed by Dr R J Howarth of the Applied Geochemistry Research Group. This program was run several times to eliminate variables with the least prediction power. Ultimately, a subset of the strongest predictor variables remained. These comprised the most statistically sound regression equations. The procedure that was followed is outlined below, which for clarity, is divided into individual runs of the computer program.

RUN 1

The first run of the ridge regression program utilised data for all twenty two polygons and all twenty three elements of the Wolfson geochemical data bank. This meant that there were 22 observations on 24 variables (1 dependent variable and 23 independent ones). As the number of variables exceeded the number of observations, the program could not execute to completion. Nevertheless, sufficient information about the relative importance of each element as a predictor of low blood copper status, could be gained. The table of ridge constants (Table 4.3) shows the classical least squares solution to occur where $k = 0$. Had this method of multiple linear regression been employed Si, Sr, Al and Pb would have been the strongest positive predictors, and Sn and Ba the strongest negative ones. With a small increase in k however, several of

STANDARDISED ESTIMATORS

	0	.05	.10	.20	.30	.40	.50	.60	.70	.80	.90	1.00
AL	.7500	.3658	.3110	.2552	.2211	.1962	.1766	.1607	.1474	.1362	.1265	.1180
CA	.1250	-.0263	-.0207	-.0091	-.0012	.0041	.0079	.0106	.0127	.0142	.0154	.0163
FE	-.2500	.0212	.0361	.0350	.0296	.0247	.0208	.0176	.0150	.0130	.0112	.0098
K	-.2500	-.1270	-.1388	-.1493	-.1485	-.1436	-.1374	-.1308	-.1243	-.1182	-.1125	-.1072
MG	-.5938	-.3067	-.1909	-.1031	-.0687	-.0512	-.0411	-.0346	-.0301	-.0270	-.0246	-.0228
SI	1.4375	.3900	.3114	.2309	.1851	.1547	.1328	.1162	.1032	.0927	.0840	.0767
GA	.6875	.3470	.2463	.1727	.1408	.1218	.1087	.0990	.0912	.0849	.0796	.0751
SC	-.3750	-.0185	-.0153	-.0257	-.0334	-.0381	-.0409	-.0425	-.0434	-.0437	-.0438	-.0436
CU	0	-.4197	-.3472	-.2602	-.2085	-.1736	-.1481	-.1287	-.1133	-.1009	-.0906	-.0819
PB	.7500	.0125	-.0188	-.0264	-.0224	-.0169	-.0116	-.0068	-.0027	.0009	.0039	.0065
V	-.5000	.0911	.0636	.0449	.0366	.0316	.0282	.0257	.0238	.0223	.0210	.0199
BA	-.7500	-.0576	-.0359	-.0191	-.0087	-.0009	.0053	.0101	.0141	.0173	.0199	.0220
CO	-.2500	-.1487	-.1074	-.0713	-.0539	-.0435	-.0367	-.0318	-.0282	-.0255	-.0233	-.0215
CR	.5000	.0911	.1119	.1130	.1072	.1009	.0950	.0897	.0848	.0804	.0763	.0726
NI	.3047	.1215	.0608	.0164	-.0003	-.0083	-.0127	-.0153	-.0169	-.0178	-.0184	-.0188
MN	.3750	.2060	.1032	.0293	.0025	-.0097	-.0159	-.0191	-.0207	-.0214	-.0216	-.0215
SN	-1.2500	-.2661	-.2131	-.1746	-.1565	-.1446	-.1353	-.1277	-.1212	-.1154	-.1103	-.1056
SR	1.2500	.5958	.4705	.3508	.2877	.2470	.2180	.1961	.1788	.1647	.1531	.1432
LI	.1250	.0368	-.0119	-.0384	-.0452	-.0473	-.0477	-.0473	-.0466	-.0457	-.0447	-.0437
MO	.3125	.4410	.4385	.4069	.3744	.3464	.3226	.3021	.2845	.2691	.2555	.2433
AS	0	-.3067	-.2505	-.1963	-.1675	-.1481	-.1335	-.1218	-.1122	-.1039	-.0969	-.0907
ZN	-.2500	.0580	.0851	.0943	.0944	.0927	.0906	.0885	.0863	.0842	.0822	.0803
CD	0	.2185	.2352	.2340	.2240	.2128	.2021	.1923	.1834	.1754	.1680	.1613

TABLE 4.3

Standardised estimator values and ridge constants for the prediction of low blood copper status from 23 stream sediment predictor variables

VARIANCE INFLATION FACTORS

(ORTHOGONAL SOLUTION WHEN ALL = 1.0)

	0	.05	.10	.20	.30	.40	.50	.60	.70	.80	.90	1.00
AL *	.596E+13	6.0062	4.0121	2.5720	1.9390	1.5698	1.3244	1.1482	1.0150	.9104	.8260	.7564
CA *	.326E+14	7.5068	4.8416	2.9434	2.1443	1.6960	1.4071	1.2047	1.0548	.9390	.8468	.7717
FE *	.383E+14	5.8619	3.9286	2.4989	1.8727	1.5114	1.2735	1.1038	.9760	.8761	.7956	.7293
K *	.547E+14	3.8120	2.5802	1.7432	1.3752	1.1552	1.0047	.8933	.8067	.7369	.6793	.6306
MG *	.330E+13	4.1223	2.9585	1.9746	1.5196	1.2509	1.0709	.9407	.8415	.7631	.6992	.6460
SI *	.339E+14	9.6284	5.3956	3.0365	2.1621	1.6939	1.3989	1.1948	1.0446	.9293	.8377	.7632
GA *	.274E+14	11.2108	6.4019	3.5375	2.4697	1.9061	1.5563	1.3174	1.1436	1.0113	.9071	.8228
SC *	.526E+14	8.3427	5.0555	2.9548	2.1291	1.6786	1.3921	1.1926	1.0452	.9316	.8411	.7673
CU *	.262E+14	6.7971	4.1966	2.5669	1.9053	1.5327	1.2896	1.1170	.9874	.8861	.8045	.7373
PB *	.145E+15	11.6264	6.2220	3.3092	2.2880	1.7620	1.4393	1.2203	1.0614	.9406	.8456	.7688
V *	.339E+15	11.5100	6.3414	3.4312	2.3807	1.8334	1.4959	1.2663	1.0997	.9731	.8734	.7929
BA *	.146E+15	6.6412	4.0902	2.4997	1.8547	1.4910	1.2537	1.0853	.9589	.8602	.7809	.7156
CO *	.221E+14	10.5245	6.0627	3.4014	2.3994	1.8653	1.5309	1.3008	1.1325	1.0036	.9018	.8192
CR *	.105E+15	4.4925	2.8998	1.8856	1.4613	1.2143	1.0480	.9265	.8330	.7582	.6968	.6453
NI *	.601E+12	2.0824	1.6612	1.2846	1.0838	.9497	.8505	.7730	.7100	.6575	.6129	.5744
MN *	.502E+14	5.2918	3.5566	2.2736	1.7161	1.3952	1.1835	1.0319	.9173	.8273	.7545	.6942
SN *	.117E+15	6.8501	4.0490	2.4225	1.7909	1.4410	1.2145	1.0543	.9341	.8402	.7646	.7023
SR *	.202E+14	5.7929	3.8531	2.4373	1.8279	1.4782	1.2479	1.0835	.9595	.8624	.7840	.7193
LI *	.440E+14	7.6244	5.0248	3.0689	2.2327	1.7629	1.4603	1.2485	1.0917	.9707	.8745	.7961
MO *	.127E+14	3.6800	2.5977	1.7947	1.4222	1.1944	1.0367	.9194	.8203	.7549	.6942	.6432
AS *	.146E+14	6.9370	4.3229	2.6109	1.9183	1.5329	1.2841	1.1090	.9782	.8766	.7951	.7281
ZN *	.476E+14	7.0351	4.4357	2.6940	1.9787	1.5784	1.3195	1.1371	1.0011	.8955	.8110	.7416
CD *	.766E+14	8.0215	4.8753	2.8621	2.0663	1.6310	1.3539	1.1610	1.0184	.9085	.8209	.7495

TABLE 4.4

Variance inflation factors for the prediction of low blood copper status from 23 stream sediment predictor variables

these estimators show a rapid decrease in predictive importance. It is clear from the table of variance inflation factors (Table 4.4) that at $k = 0$, most of the variables show high multiple correlation. It is therefore apparent that the least squares solution produced predictors that are unreliable and may well be meaningless from an interpretational point of view. An optimum value of k was selected after careful consideration of the facts presented in Section 4.3. It was felt that a stable solution occurred where $k = 0.5$. At this point certain variables could be eliminated from the regression, either because of their instability or because of their low predictive importance (i.e. those with the smallest values of α). Elimination left the following predictor variables in decreasing order of predictive importance:

Mo > Sr > Cd > Al > Cu > K > As > Si > Ga > Cr > Zn

RUN 2

With the data set now reduced to 22 observations on 12 variables, the ridge regression program could execute to completion. The ridge trace (Figure 4.2) and table of VIFs (Table 4.5) indicate the optimum ridge constant to be at $k = 0.45$. At this value, Mo, Sr and Cd are the best positive predictors and Cu the best negative one. The equation produced from the predictor variables may be tested for its statistical significance. For this the multiple correlation coefficient (R^2) and F-ratio must be considered. R^2 is the maximum correlation between the dependent variable and all the independent variables i.e.

$$R^2 \propto \frac{\text{explained variation}}{\text{total variation}}$$

It is a good indicator of the 'goodness of fit' of the regression line to the observed points. Thus, if the line is a good estimator of the data, the ratio approaches one. In this particular case (Table 4.6), $R^2 = 0.7455$, which indicates a reasonable fit. The F-ratio is designed to test the null hypothesis that the variance about the regression line is no different from the variance of the observations. The test statistic follows an F distribution, with in this case, degrees of freedom $V_1 = 11$ and $V_2 = 10$. The critical region therefore consists of values exceeding $F_{.05} = 2.98$. The computed test value (Table 4.6) falls outside the critical region because it equals 2.66. The null hypothesis cannot therefore be rejected. Consequently, a better regression equation is

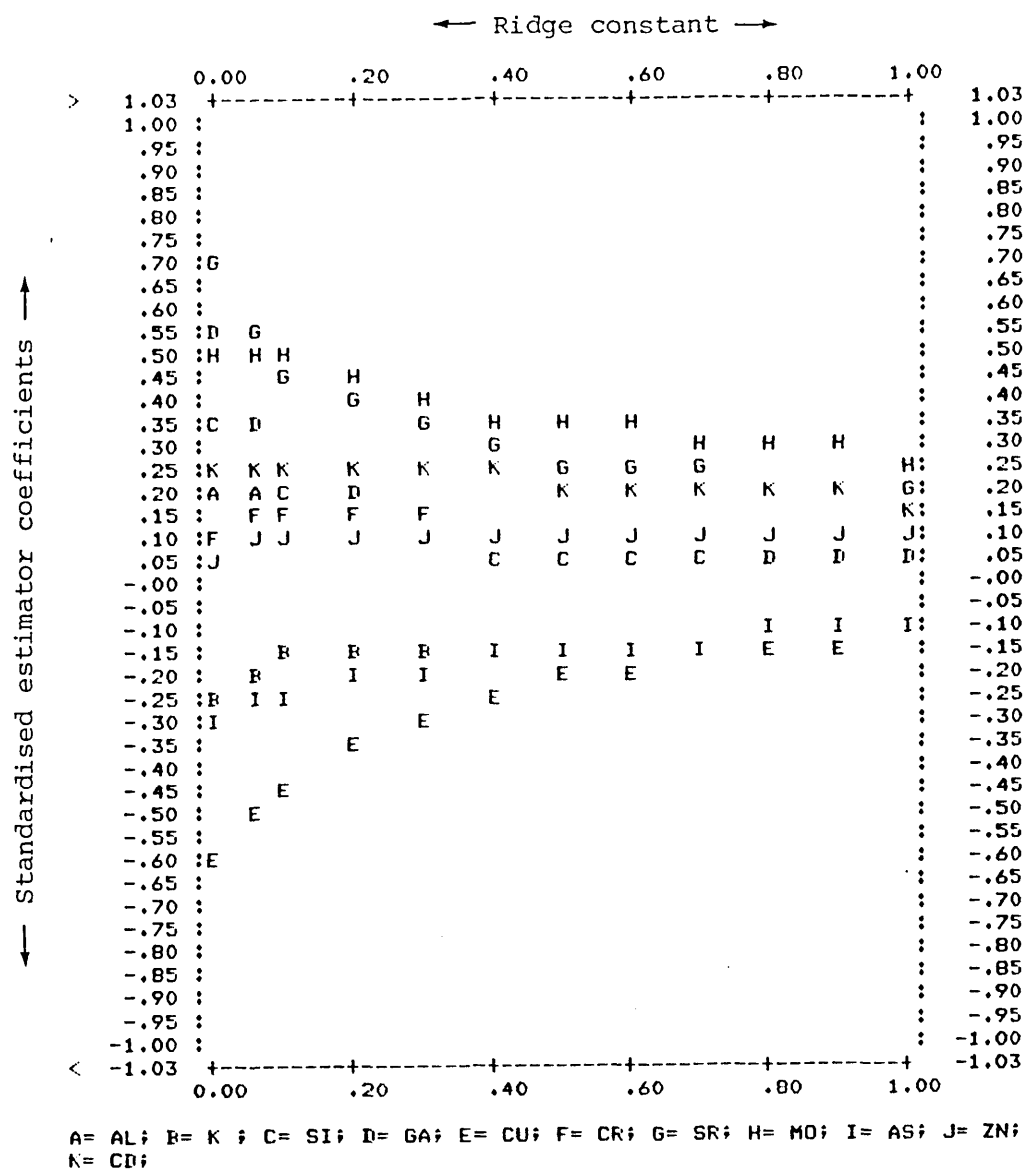


FIGURE 4.2. Ridge trace - Run 2

VARIANCE INFLATION FACTORS		(ORTHOGONAL SOLUTION WHEN ALL = 1.0)											
		RIDGE CONSTANT											
		0	.05	.10	.20	.30	.40	.50	.60	.70	.80	.90	1.00
AL		3.2766	2.4636	2.0668	1.6128	1.3400	1.1523	1.0135	.9063	.8206	.7503	.6917	.6419
K		2.9325	2.1090	1.7521	1.3754	1.1583	1.0097	.8991	.8127	.7428	.6848	.6359	.5938
SI		4.1611	3.1134	2.5199	1.8483	1.4727	1.2307	1.0612	.9353	.8379	.7601	.6965	.6434
GA	13.1262	6.8098	4.6426	2.8706	2.0943	1.6561	1.3737	1.1760	1.0297	.9169	.8272	.7540	
CU	2.9818	2.2944	1.9446	1.5380	1.2901	1.1174	.9883	.8875	.8063	.7393	.6830	.6350	
CR	3.4778	2.3427	1.8799	1.4241	1.1788	1.0178	.9013	.8118	.7404	.6816	.6323	.5902	
SR	4.7856	3.1112	2.4119	1.7290	1.3757	1.1540	.9999	.8857	.7972	.7263	.6680	.6192	
MO	3.3133	2.3830	1.9559	1.4990	1.2397	1.0664	.9403	.8436	.7666	.7035	.6508	.6060	
AS	3.1424	2.4807	2.0952	1.6296	1.3472	1.1541	1.0125	.9037	.8171	.7466	.6878	.6380	
ZN	6.3510	3.9415	2.9572	2.0414	1.5893	1.3132	1.1248	.9869	.8812	.7973	.7288	.6719	
CD	7.5246	4.5886	3.3671	2.2507	1.7166	1.3986	1.1859	1.0327	.9166	.8253	.7516	.6906	

TABLE 4.5 Variance inflation factors for the prediction of low blood copper status from 12 stream sediment predictor variables

ANALYSIS OF VARIANCE : RIDGE

SOURCE OF VARIATION	SUM OF SQUARES	DEGREES OF FREEDOM	MEAN SQUARES	F-TEST
REGRESSION	3411.51	11	310.14	2.6625
RESIDUAL	1164.85	10	116.48	
TOTAL VARIATION	4576.36	21		

MULT. R-SQUARED = .7455

TABLE 4.6 Analysis of variance for the second ridge solution

required. As Zn and Si had least predicting power at the optimum ridge constant, they were eliminated from the data set.

RUN 3

The ridge regression program was run with the reduced data set of 22 observations on 10 variables. The optimum ridge constant was deemed to be 0.4 where Mo, Cd and Sr were the best positive predictors and Cu the best negative one. The computed R^2 value equals 0.7505, which is a slight improvement on Run 2 and is indicative of a reasonable fitting regression equation. The critical value of $F_{.05}$ in this case is 2.8. As the computed value is 4.01, the null hypothesis can be rejected in favour of the regression.

Subsequent runs of the ridge regression program were aimed at improving on the result obtained in Run 3. A summary of these results is given in Table 4.7.

From a variety of possible alternatives, all of which had significant F-ratios, the preferred solution in Table 4.7 was Run 6, as it had the largest R^2 value. This had Mo, Cd and Sr as the best positive predictors and Cu as the best negative one (Figure 4.3).

Other information to be considered is: residual sum of squares; sum of inverse eigenvalues; total variance of the estimators; and the residual probability plot. There seems to be no quantitative rule concerning permitted increases in the residual sum of squares, when ridge regression is employed. Turner (1980) obtained percentage increases of up to 28.64%, which he considered satisfactory. In the case of Run 6, a percentage increase of 69% was observed. The sum of inverse eigenvalues in Run 6 equals 9.01, with eight independent variables. This indicates a good approximation to the uncorrelated, orthogonal system aimed at. The total variance of the estimators in Run 6 is 0.5744 for the least squares solution and 0.4569 for the ridge solution. The residual probability plot (Figure 4.4) is approximately linear, indicating that the residuals are relatively normally distributed, with no obvious outliers. The derived regression equation in Run 6:

$$\text{LOW BLOOD COPPER INCIDENCE RATE} = 3.7329 + (2.025\text{Al}) - (7.32\text{K}) - (0.4343\text{Cu}) \\ + (0.1219\text{Cr}) + (0.1348\text{Sr}) + (7.713\text{Mo}) - (0.5018\text{As}) + (7.49\text{Cu})$$

SOLUTION	PREDICTING ELEMENTS	R ²	F-RATIO
1	Al, Ca, Fe, K, Mg, Si, Ga, Sc, Cu, Pb, V, Ba, Co, Cr, Ni, Mn, Sn, Sr, Li Mo, As, Zn, Cd	Unavailable	Unavailable
2	Al, K, Si, Ga, Cu, Cr, Sr, Mo, As, Zn, Cd	.7455	2.6625 ^{NS}
3	Al, K, Ga, Cu, Cr, Sr, Mo, As, Cd	.7505	4.0107 ^X
4	Al, K, Ga, Cu, Sr, Mo, As, Cd	.7390	4.6008 ^{XX}
5	K, Ga, Cu, Cr, Sr, Mo, As, Cd	.7323	4.4451 ^{XX}
6	Al, K, Cu, Cr, Sr, Mo, As, Cd	.7704	5.4528 ^{XX}
7	Al, Cu, Cr, Sr, Mo, As, Cd	.7556	6.1819 ^{XX}
8	Al, K, Cu, Sr, Mo, As, Cd	.7385	5.6485 ^{XX}
9	K, Cu, Cr, Sr, Mo, As, Cd	.7515	6.0555 ^{XX}
10	Al, K, Cu, Cr, Sr, Mo, Cd	.7497	5.9898 ^{XX}
11	Cu, Mo, Cd	.5858	8.4874 ^{XX}
12	Cu, Sr, Mo, Cd	.6247	7.0740 ^{XX}
13	Cu, Sr, Mo, As, Cd	.6679	6.4367 ^{XX}

TABLE 4.7. R² and F-ratio statistics of the 13 ridge regression solutions derived for the prediction of bovine low blood copper status from geochemical parameters

Note: NS Not significant
X Significant at F._{.05}
XX Significant at F._{.01}

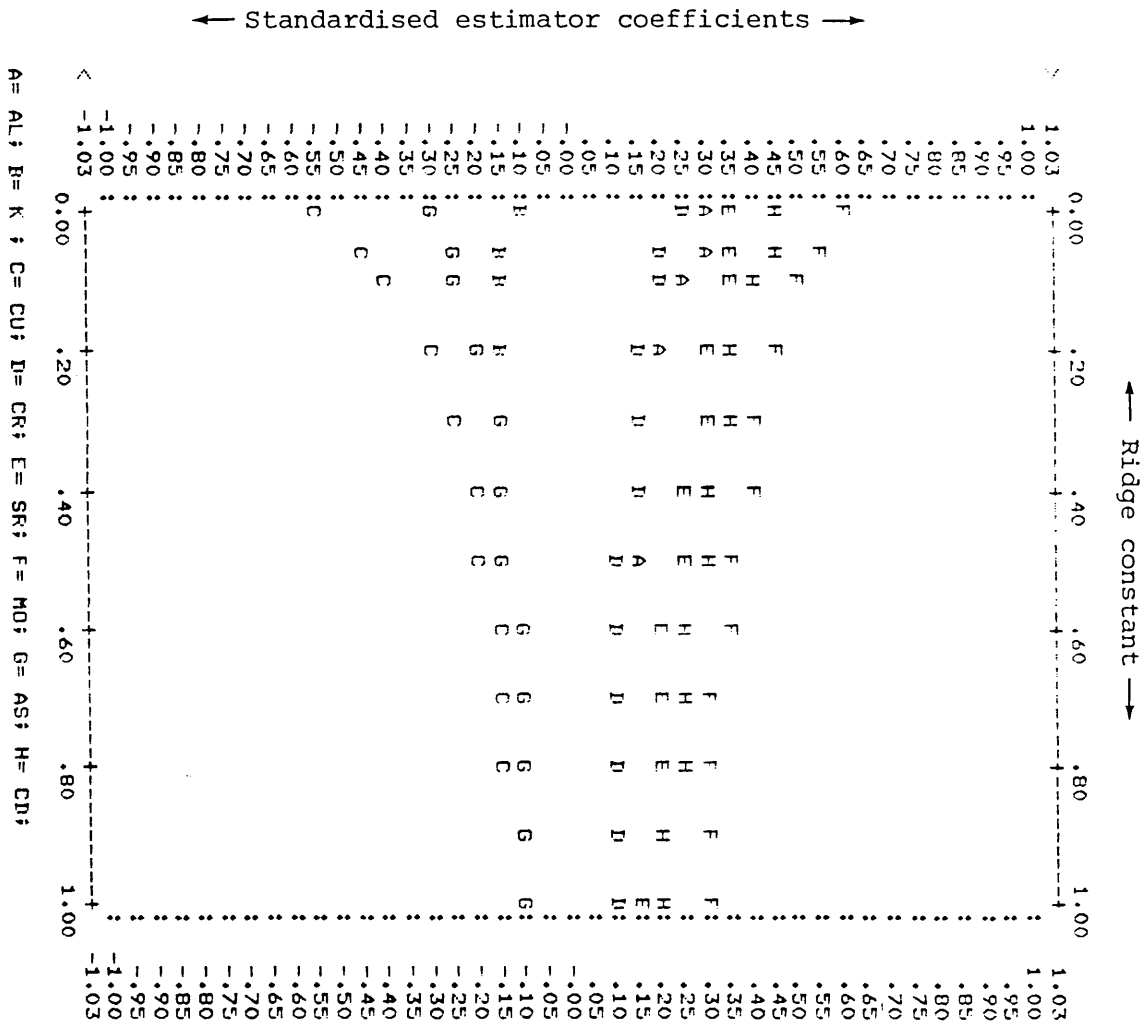


FIGURE 4.3 Ridge trace - Run 6

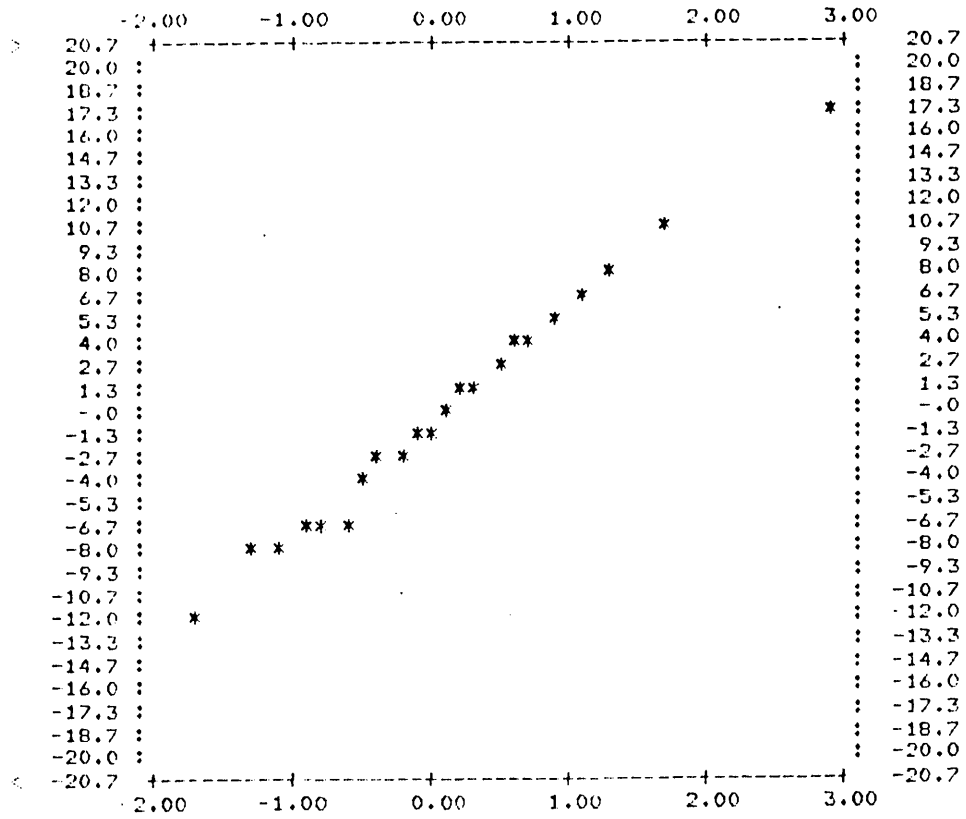


FIGURE 4.4. Residual probability plot - Run 6

is therefore considered to be significant. The concentrations of Cu, Cr, Sr, Mo, As and Cd are in ppm and the concentrations of Al and K are in %.

The above equation may now be used to predict incidence rates of bovine low blood copper status throughout England and Wales. The smoothed Wolfson Geochemical Atlas data were substituted into the regression equation to produce a map of the predicted low blood copper incidence rate for each map cell (Figure 4.5). It was necessary to assume a uniform cattle distribution owing to the lack of suitable information on cattle density throughout England and Wales. It is therefore satisfactory to note that whilst the prediction map is based solely on stream sediment data (and hence only approximates to the chemical content of rocks, soils, plants and animals) the results it indicates are very sensible. Despite the omission of cattle density data, many of the areas predicted to be severely affected by bovine hypocupraemia (e.g. Lower Lias of Somerset, Bristol and the Vales of Evesham and Gloucester, in addition to the Carboniferous limestone and Namurian shales of Derbyshire) have already been recognised as such by the veterinary profession. Other areas predicted to have low bovine blood copper status (yet are not noted for bovine hypocupraemia) occur in large regions of North Yorkshire, Devon, Cambridgeshire, central and north Wales. Subclinical copper deficiency as yet undetected by farmers and veterinary surgeons could still be limiting milk and beef production. Although further investigations are required, the potential of ridge regression analysis as a technique for predicting trace element disorders in livestock is quite evident.

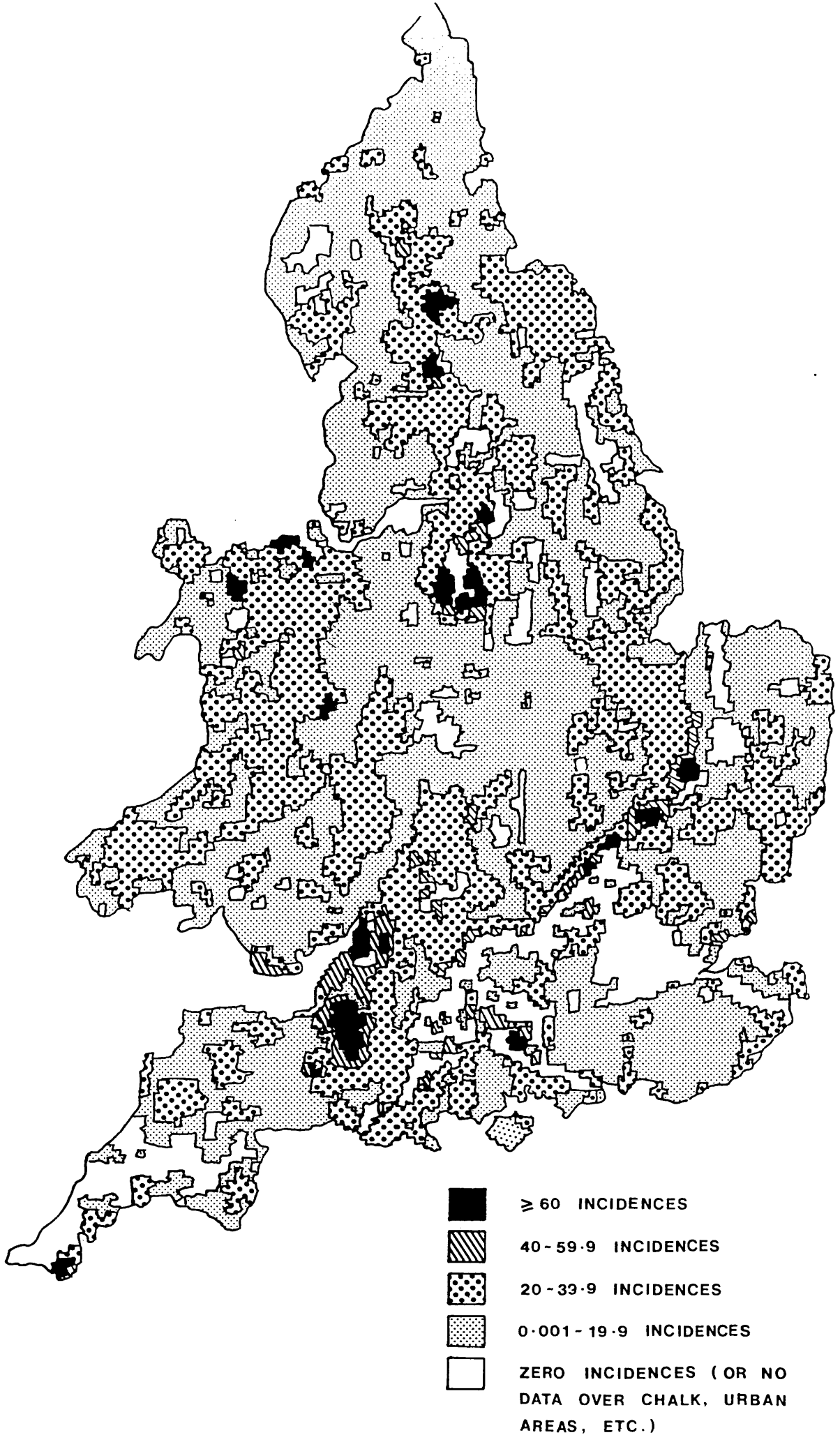


FIGURE 4.5 Map showing the predicted incidence of bovine low blood copper status in England and Wales

CHAPTER 5

A DESCRIPTION OF THE AREAS SELECTED FOR SOIL AND PASTURE STUDIES

5.1 INTRODUCTION

In order to determine some of the underlying geochemical causes of low blood copper status in cattle, soil and herbage sampling was considered necessary in areas of bovine hypocupraemia. Twenty two areas exhibiting various degrees of bovine hypocupraemia and encompassing a wide range of geological situations were originally selected for the initial model-making stage of the investigation, based on stream sediment geochemistry. As it was not practical to pursue follow-up soil and herbage sampling in all twenty two areas, they had to be grouped on the basis of their similar geochemistries, so that one typical area could be sampled from each group.

5.2 THE SELECTION OF FIELD AREAS

Various methods of classification were available to group the hypocupraemic areas on the basis of their similar geochemistry. The two chosen here were a hierarchical cluster analysis and a non-linear mapping algorithm.

(a) Hierarchical cluster analysis

Hierarchical cluster analysis was carried out using a computer program, written by Dr R J Howarth of The Applied Geochemistry Research Group, with the geochemical data for the eight most important predictors of bovine hypocupraemia (Section 4.4) as the criteria for classifying the twenty two polygons. These data were standardised and the Euclidean distance computed between each pair of polygons according to their similarities in Cu, Mo, Cd, Cr, Sr, K, Al and As concentrations. The shorter the Euclidean distance the greater was their similarity. The most similar pair of polygons which formed the initial group were 6 and 20. All other distances were then compared. If any polygon was more similar to the polygons already grouped, then it was assigned to that group, otherwise a new group was formed from the next most similar pair of polygons. This proceeded until all the polygons were arranged into a hierarchy. The level of similarity between polygons was recorded and

represented in dendrogram form (Figure 5.1). The endpoints of the branches represent polygons and the height at which branches join corresponds to the similarity level for admission to a particular polygon group.

(b) Non linear mapping

This algorithm developed by Sammon (1969) is based on the non-linear point mapping of high dimensional data in two dimensions, such that the inherent structure of the data is approximately preserved. An initial two-dimensional configuration for the vectors is found by projecting the L-dimensional data orthogonally onto the two-dimensional space spanned by the two original co-ordinates with the largest variances. Next, all the two-dimensional space interpoint distances are computed, and are used to define the mapping error E. E represents how well the present configuration of points fits the N points in the original L-dimensional space. The two-dimensional configuration is adjusted so as to decrease this error (Howarth, 1973). The non-linear mapping analysis was carried out using a computer program written by Dr R J Howarth. The eight element geochemical data for the twenty two polygons were again the input information used in the classification procedure. After fifty iterations the mapping error was 0.057, which was acceptable. The final two-space configuration may be seen in the scatter plot in Figure 5.2. The polygons closest to each other are the most similar.

If the arbitrary threshold of 0.75 is chosen to divide the dendrogram into a reasonable number of groups, four polygon clusters can be identified:-

- i) 6, 20, 21, 7, 18 19
- ii) 2, 13, 12, 17, 1, 5, 15, 16, 8
- iii) 4, 11
- iv) 9, 14

leaving three individuals ungrouped (22, 10 and 3). Similar groupings can be identified from the two-dimensional scatter plot. However, this non-linear mapping technique splits the first two dendrogram clusters into tighter groups:-

- i) 7, 19, 18
- ii) 6, 20, 21
- iii) 13, 1, 2, 8
- iv) 15, 12, 16
- v) 4, 11
- vi) 9, 14

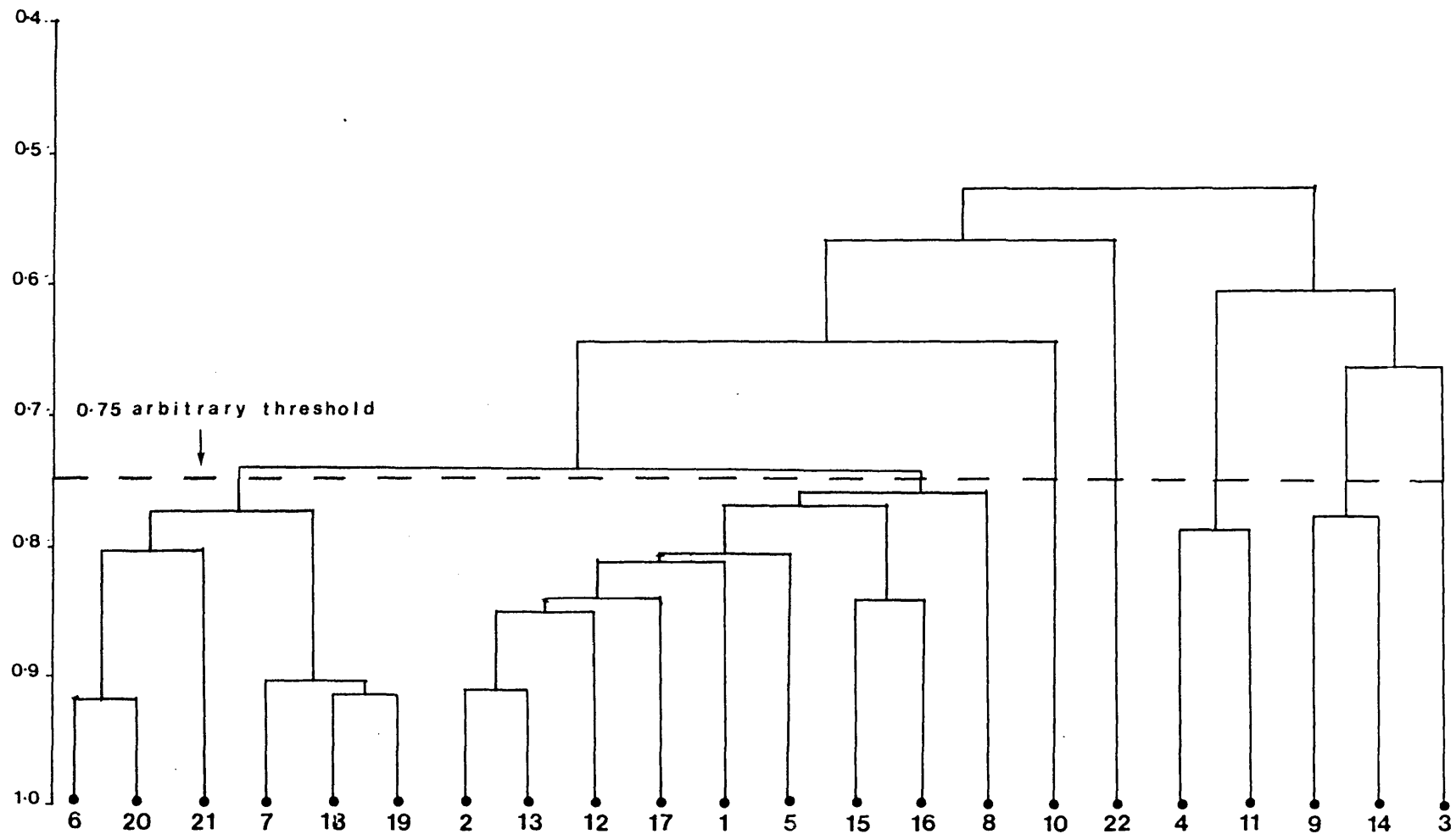


FIGURE 5.1. A dendrogram representing the polygon groupings produced by hierarchical cluster analysis (polygon coding is given in Table 4.1)

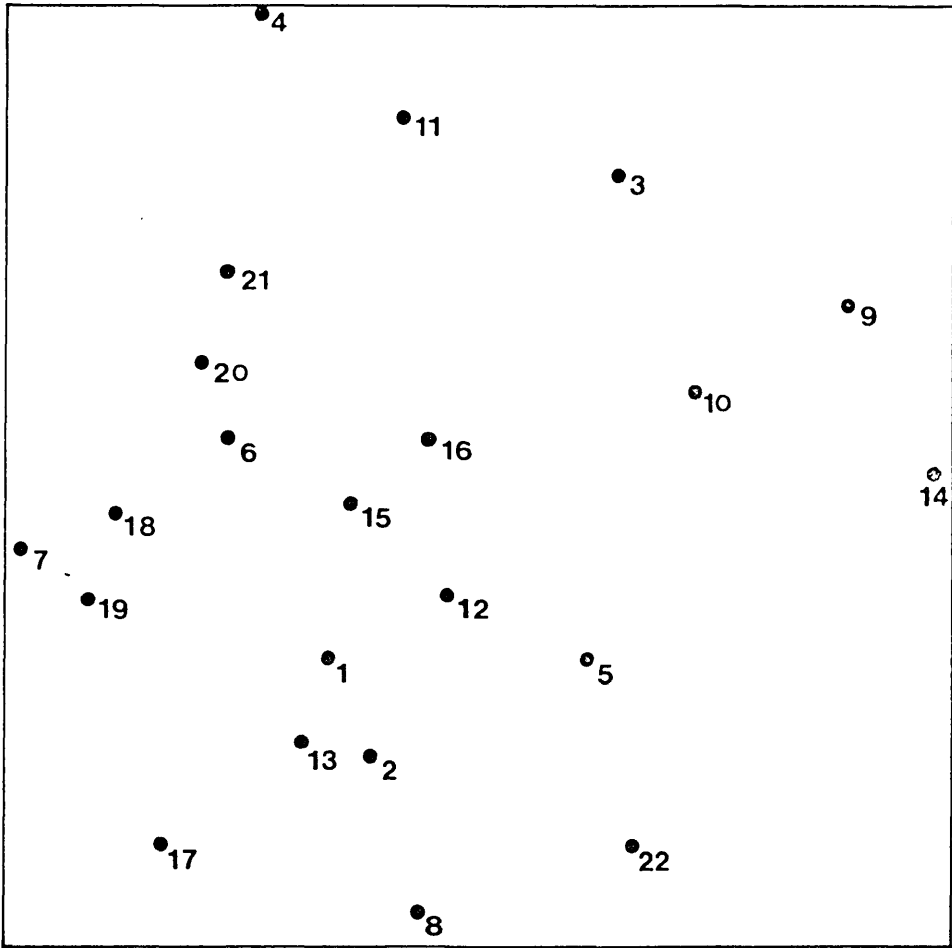


FIGURE 5.2. A two dimensional scatter plot representing the polygon clusters produced by non-linear mapping (polygon coding is given in Table 4.1)

leaving five individuals ungrouped (22, 10, 3, 17 and 5).

The polygon groupings derived from the non-linear mapping analysis are likely to be more reliable than the ones from the hierarchical classification for the reasons outlined by Sammon (1969):

- i) The two-dimensional scatter plot is easily appreciated by eye and is suited to the visual detection of clusters based on point density. The evaluation of a cluster configuration in a dendrogram is more difficult;
- ii) The non-linear mapping algorithm does not depend on any control parameters like the subjectively chosen threshold for defining dendrogram groups;
- iii) The non-linear mapping algorithm is more efficient at identifying complex data structures;
- iv) The dendrogram may have considerable distortion inherent in the two-dimensional tree representation. If two clusters are close, samples intermediate between them tend to form a bridge and cause spurious mergers in the dendrogram.

Castillo-Munoz (1973) also carried out an extensive comparison of dendrogram and non-linear mapping analysis on a set of regional geochemical data from the United Kingdom. This worker found that non-linear mapping generally gave more separable clusters than the hierarchical technique but was computationally more expensive for large data sets.

From the evidence given by Sammon (1969) and Castillo-Munoz (1973) it was decided to use the tighter polygon groupings produced by non-linear mapping for the selection of field areas.

As a preliminary step in determining possible geochemical causes of bovine hypocupraemia, the stream sediment concentrations of Cu, Mo, Cd, Sr, Cr, Al, As and K were superimposed on the two dimensional scatter plot for each polygon (using data from Table 4.2). Examples for copper and molybdenum are given in Figure 5.3 and 5.4. From these plots, the following hypotheses about underlying causes of low blood copper status in cattle were proposed:

- i) An absolute copper deficiency in areas with low stream sediment copper levels (i.e. < 20 ppm). This applies to the polygons numbered 13, 1, 2, 8, 7, 19, 18, 17 and 5.

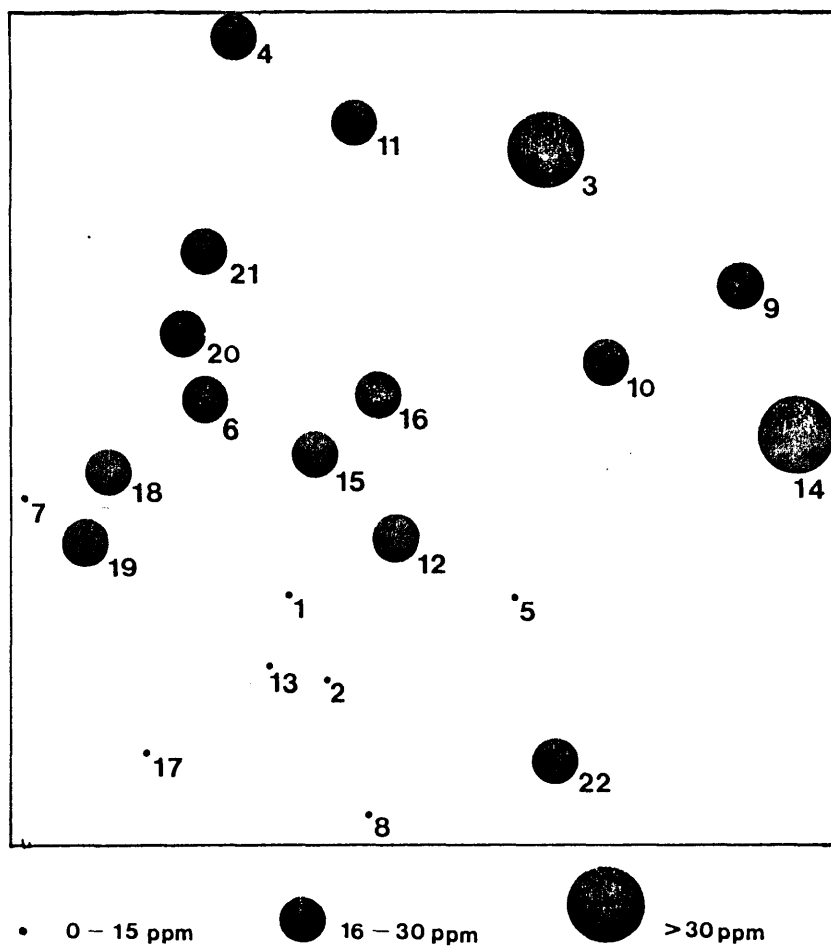


FIGURE 5.3. Scatter plot showing stream sediment copper concentrations in hypocupraemic polygons

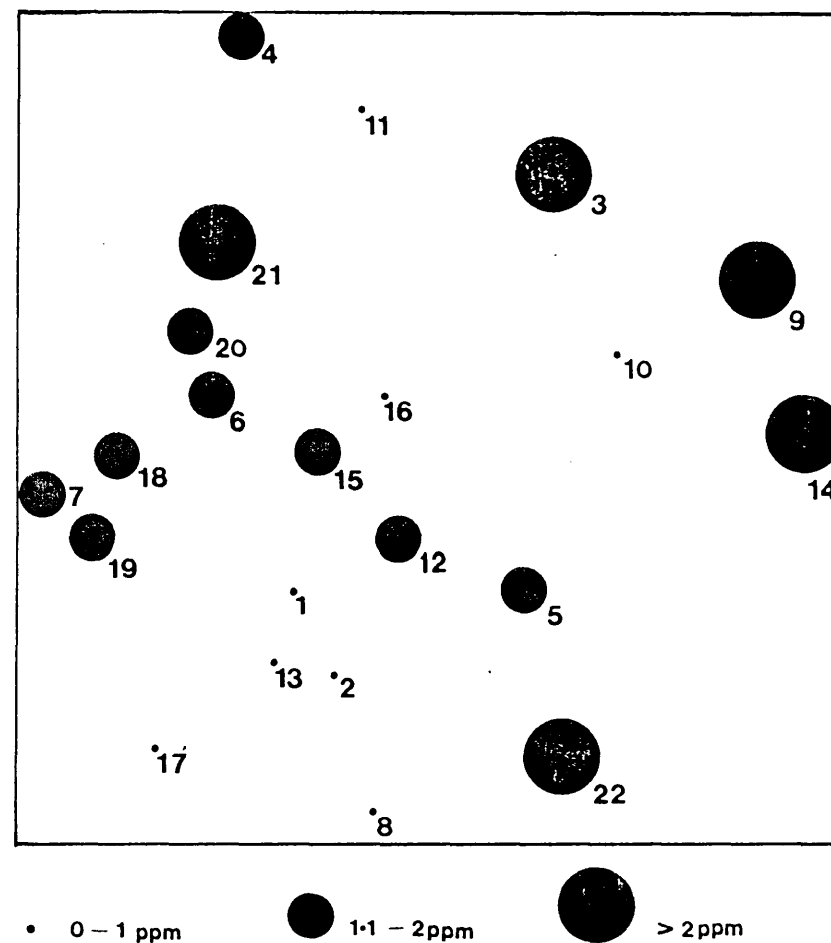


FIGURE 5.4. Scatter plot showing stream sediment molybdenum concentrations in hypocupraemic polygons

- ii) A molybdenum induced copper deficiency in areas with medium to high levels of stream sediment molybdenum (i.e. > 2 ppm). This applies to polygons numbered 6, 20, 21, 22 and 3. A molybdenum plus cadmium antagonism is possible in polygons 9 and 14 as both molybdenum and cadmium levels are above background concentrations.
- iii) Unknown cause/causes of copper deficiency where stream sediment levels of copper are adequate and levels of molybdenum and cadmium are low. This applies to polygons numbered 15, 12, 16, 4 and 11.

It was hoped that soil and pasture samples taken from various polygon areas would enable:

- i) The above hypotheses to be either accepted or rejected and
- ii) More information about other possible causes of bovine hypocupraemia to be gained.

The polygon with the highest incidence of bovine hypocupraemia was selected from each of the six polygon groups for follow-up fieldwork, using the incidence data presented in Table 4.2. Sampling locations within the chosen polygons were restricted to areas mapped by the Soil Survey of England and Wales. The six field areas were therefore located as follows:

1	Brayford, North Devon	(polygon 11) 24 cases/100 map cells
2	Glastonbury, Somerset	(polygon 22) 65 cases/100 map cells
3	Stow-on-the-Wold, Gloucestershire	(polygon 7) 25 cases/100 map cells
4	Atherstone, Warwickshire	(polygon 15) 12 cases/100 map cells
5	Onecote, Staffordshire	(polygon 9) 49 cases/100 map cells
6	Masham, North Yorkshire	(polygon 8) 27 cases/100 map cells

5.3 FIELDWORK PROCEDURES AND SAMPLE COLLECTION

Local veterinary practitioners were visited in each area to obtain information on the location of farms with hypocupraemic stock. Permission was then sought from the relevant farmers to carry out a sampling programme over the 1982 grazing season.

Each farmer was first interviewed for background information about the severity of bovine hypocupraemia on his farm and about the amount of copper supplementation administered to his stock. This data is presented in Appendix 1. A field was then selected for sampling,

provided that it fulfilled the following two criteria:

- i) The soil type in the field chosen was typical of the soils in other fields on that farm. Where two major soil series were present, two fields were sampled - one from each series.
- ii) The field chosen was grazed by hypocupraemic stock.

With these two criteria satisfied, soil and herbage sampling was undertaken. Soils were sampled on one occasion only as there is little seasonal variation in the trace element content of the soil. Herbage was collected on three occasions during the grazing season - in May, in July and again in September, owing to considerable seasonal variation in the trace element content of pasture.

Herbage sampling

Herbage samples were collected before soil samples so as to reduce the chance of trampling the pasture under-foot and so contaminating it with soil. Pasture herbage was clipped with sharp stainless steel shears one inch above the soil surface, care being taken to avoid extraneous soil and faecal material. Samples were collected at regular intervals along a 'W' transect of the field to make up 25 sub-samples, which were bulked in a labelled collecting envelope. Samples were taken back to the laboratory for analysis.

Soil sampling

Soil was sampled using a 2.5 cm diameter hand auger with a stainless steel screw. 25 samples of 0 - 15 cm depth were again collected at regular intervals along a 'W' transect of the field. These were bulked in a labelled collecting envelope. In addition to the topsoil samples, a bulked subsoil sample was also obtained. This consisted of 5 - 10 sub-samples taken at 30 - 45 cm depth. Under stony or heavy clay conditions it was sometimes physically impossible to obtain these.

5.4 FIELD AREA DESCRIPTION

(a) Brayford, North Devon

The Brayford district, covered by the Ordnance Survey 1:25,000 map sheet SS 63, is in North Devon between Exmoor to the north and east, and

Barnstaple to the west. It lies across the upper parts of the stratigraphic succession of the Devonian and includes a small area of lower Carboniferous rocks. The geology has been described in detail by De La Beche (1839), Sedgewick and Murchison (1840), Evans (1922) and Edmonds et al. (1975). It is summarised here in Figure 5.5.

The location of the fourteen farms sampled in the Brayford district plus data on underlying geologies and soil types are summarised in Table 5.1. Fourteen soil map units have been identified in the area (Hogan, 1981). Free-draining loamy brown earths and brown podzolics are by far the most widespread around Brayford. These are formed in stony drift (Head) derived from the underlying slates, mudstones, siltstones and sandstone in which slaty cleavage is developed to varying degrees. Of the fourteen map units present only three types were sampled in this study, in connection with the incidence of bovine hypocupraemia. These were the Highweek, Dartington and Loxhore series which comprise 54%, 8% and 16% of the map area respectively. A detailed account of the soils of the Brayford district is given in the Soil Survey Record No. 71 (Hogan, 1981).

(b) Glastonbury, Somerset

The Glastonbury area forms an extensive plain in the centre of Somerset. It is covered by the Ordnance Survey 1:63,630 map sheet ST 296. The district is flanked in the north by the Mendip Hills and embraces wide alluvial flats known as moors or levels. Underlying rocks are mainly of Triassic and Jurassic origin. The geology of the Glastonbury area is presented in Figure 5.6.

The location of the fifteen farms sampled in the Glastonbury district plus data on underlying geological units and soil types are summarised in Table 5.2. Four major soil groups have been recognised in the area - brown earths, calcareous soils, gleys and organic soils. These groups are broad and may be further divided to make up a total of 31 different soil map units. The most extensive are the brown earths with gleying, and the calcareous soils with gleying. Of the 31 soil series, only eight were sampled in this study. These were the Worcester, Somerton, Evesham, Charlton Bank, Long Load, Midelney, Sedgemoor and Turbary Moor series and complexes, which comprise 5%, 13%, 17%, 5%, 3%, 9%, 10% and 3% of the map area respectively. A detailed account of the Glastonbury soils

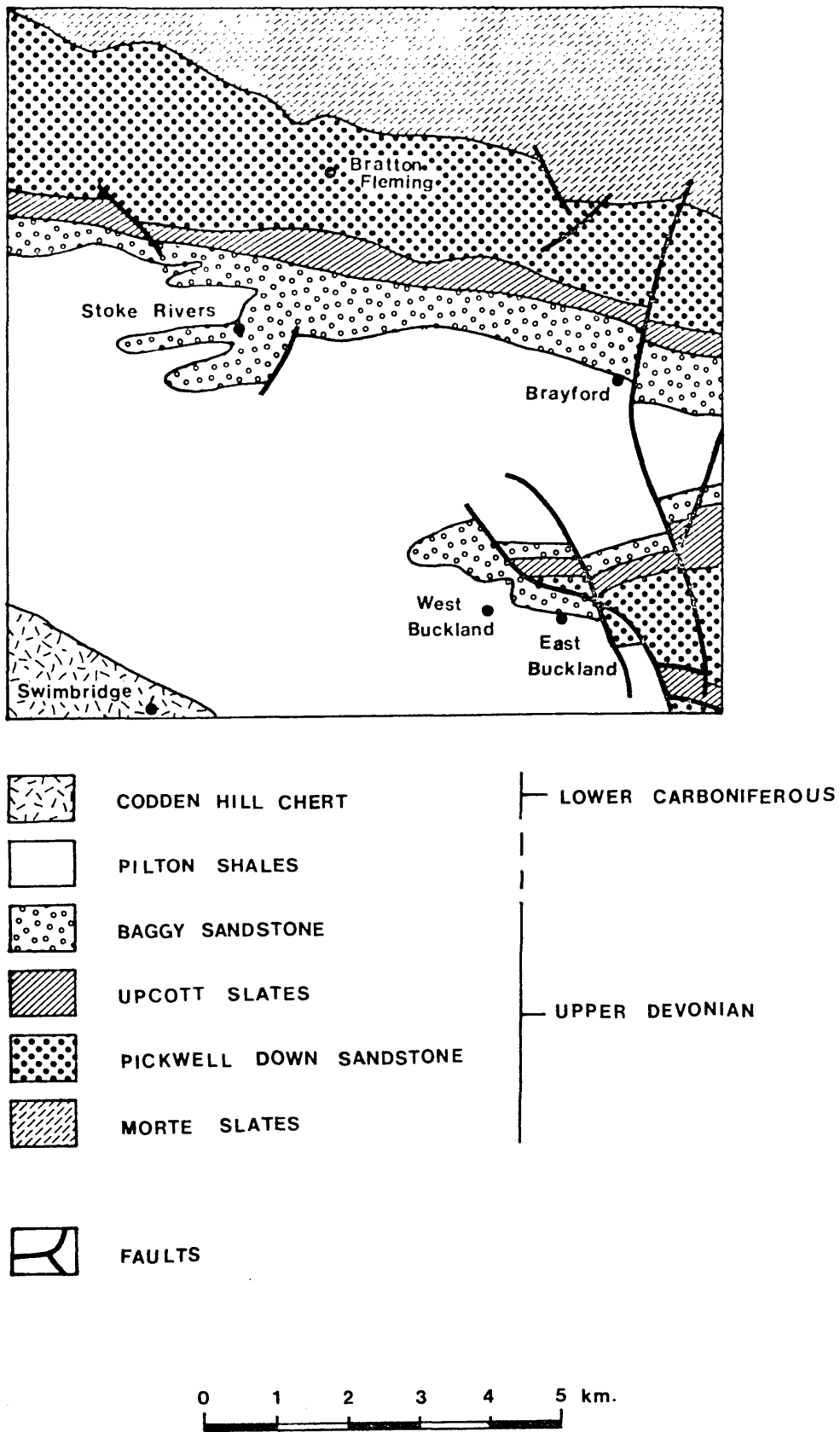


FIGURE 5.5 Geology of the Brayford district of north Devon (redrawn from Hogan, 1981)

SAMPLE NUMBER	SAMPLING LOCATION	PARENT MATERIAL	SOIL SERIES	SOIL GROUP	DRAINAGE
1	26521331	Head-slate, silt, mud & sandstone	Highweek	Brown earth	Free
2	26921366	Head-sandstone	Loxhore	Brown podzolic	Free
3	27051349	Head-slate, silt, mud & sandstone	Highweek	Brown earth	Free
4	27081364	Pickwell Down sandstone	?		
5	26771352	Head-slate, silt, mud & sandstone	Highweek	Brown earth	Free
6	26951350	Head-slate, silt, mud & sandstone	Highweek	Brown earth	Free
7	26661347	Head-slate, silt, mud & sandstone	Highweek	Brown earth	Free
8	26801337	Head-slate, silt, mud & sandstone	Highweek	Brown earth	Free
9	26941403	Head-slate, silt, mud & sandstone	Dartington	Brown podzolic	Free
10	26821374	Head-slate, silt, mud & sandstone	Highweek	Brown earth	Free
11	26511347	Head-slate, silt, mud & sandstone	Highweek	Brown earth	Free
12	27101375	Head-slate, silt, mud & sandstone	Highweek	Brown earth	Free
13	26351351	Head-slate, silt, mud & sandstone	Highweek	Brown earth	Free
14	26851356	Head-slate, silt, mud & sandstone	Highweek	Brown earth	Free

TABLE 5.1. Sampling locations with details of underlying geologies and soil types in Brayford, North Devon

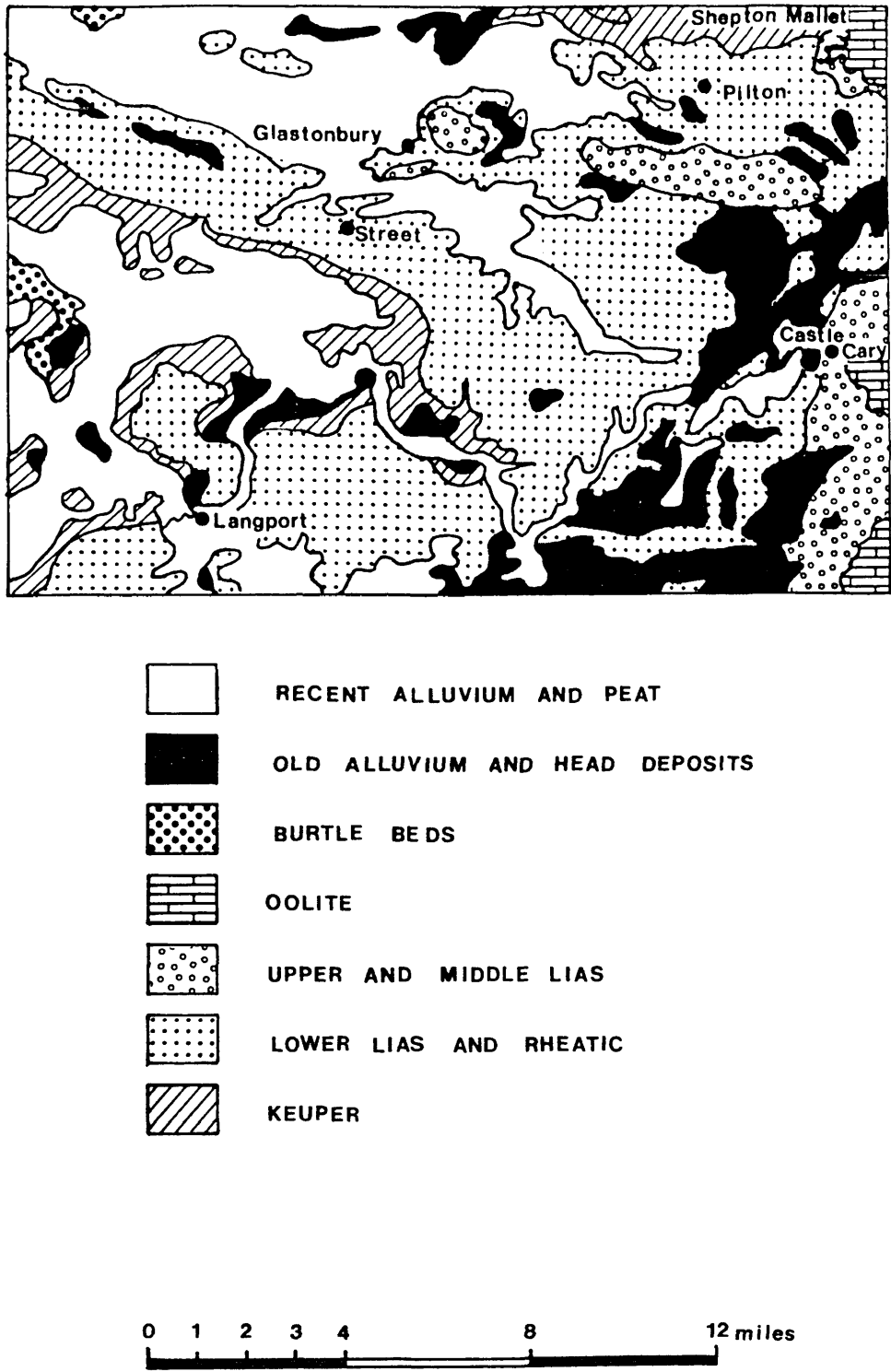


FIGURE 5.6 Geology of the Glastonbury district of Somerset
(redrawn from Avery, 1955)

SAMPLE NUMBER	SAMPLING LOCATION	PARENT MATERIAL	SOIL SERIES	SOIL GROUP	DRAINAGE
15	35321321	Lower Lias clays	Evesham	Gleyed calcareous	Imperfect
16	35181343	Lower Lias clays	Evesham	Gleyed calcareous	Imperfect
17	34911361	Lower Lias clays	Evesham	Gleyed calcareous	Imperfect
18	34581347	Fen peat	Sedgemoor	Organic	Poor
19	34691347	Riverine clay over peat	Midelsey	Ground water gley	Poor
20	35291395	Lower Lias clay	Charlton Bank	Surface water gley	Poor
21	34281425	Raised moss	Turbary Moor	Organic	Imperfect
22	34271468	Lower Lias clay	Charlton Bank	Surface water gley	Poor
23	34071394	Mid/Upper Lias siltstone	Long Load	Surface water gley	Poor
24	34051407	Fen peat	Sedgemoor	Organic	Poor
25	33681404	Lower Lias limestone & shale	Somerton	Brown calcareous	Free
26	34421354	Keuper marl	Worcester	Gleyed brown earth	Imperfect
27	34401350	Fen peat	Sedgemoor	Organic	Poor
28	34751270	Lower Lias limestone & shale	Somerton	Brown calcareous	Free
29	31611279	Lower Lias clay	Evesham	Gleyed calcareous	Imperfect

TABLE 5.2. Sampling locations with details of underlying geologies and soil types in Glastonbury, Somerset

is given by Avery (1955).

(c) Stow-on-the-Wold, Gloucestershire

The Stow-on-the-Wold district is located in the northern Cotswolds and is covered by the Ordnance Survey 1:25,000 map sheet SP 12. This area may be divided into three physiographic units - the limestone upland, the scarps and the clay vales. Stow-on-the-Wold geology is dominated by Jurassic rocks, particular Inferior Oolite and Chipping Norton limestone. The geology is covered by the Geological Survey sheet 217 Moreton-in-Marsh and is described by Richardson (1929). Arkell (1947) summarised later work. The geology is presented here in Figure 5.7.

The location of the nine farms sampled in the Stow district plus information on underlying geological units and soil types are summarised in Table 5.3. Ten soil map units have been differentiated in the Stow-on-the-Wold area of which only three were sampled in this current study. These were the Sherborne, Denchworth and Waltham series, comprising 50%, 7% and 3% of the map area respectively. A detailed account of the Stow soils is given in the Soil Survey Record No. 52 (Courtney and Findlay, 1978).

(d) Atherstone, Warwickshire

The Atherstone district is in north Warwickshire and is covered by the Ordnance Survey 1:25,000 map sheet SP 29. The area comprises an uplifted block of Carboniferous and Cambrian sediments which is bounded by a gently rolling Keuper landscape. The geology has been described by the Institute of Geological Sciences in a memoir accompanying the 1:63,630 map sheet numbered 155 (Fox-Strangways) and was remapped in 1975 by Horton (priv. comm. to Soil Survey). The geology of the Atherstone area is presented here in Figure 5.8.

The location of the fourteen farms sampled in the Atherstone area plus data on the underlying geological units and soils are summarised in Table 5.4. Of the 38 soil map units delineated, only seven were sampled in this study. These were the Shifnal, Dodmoor, Whimple, Gatacre, Merevale, Baxterley and Bardsey series and reclaimed land, comprising 8%, 8%, 11%, 1%, 1%, 4%, 4%, and 3% of the area respectively. A detailed account of the Atherstone soils is given in the Soil Survey Record No. 66

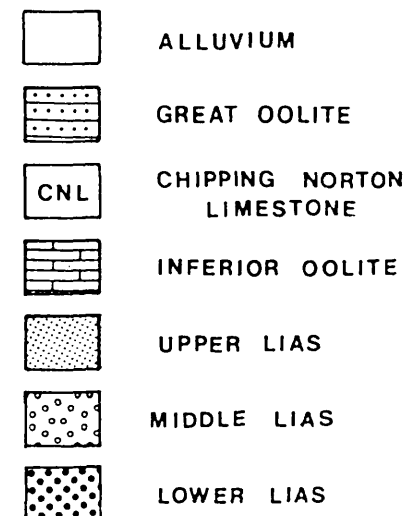
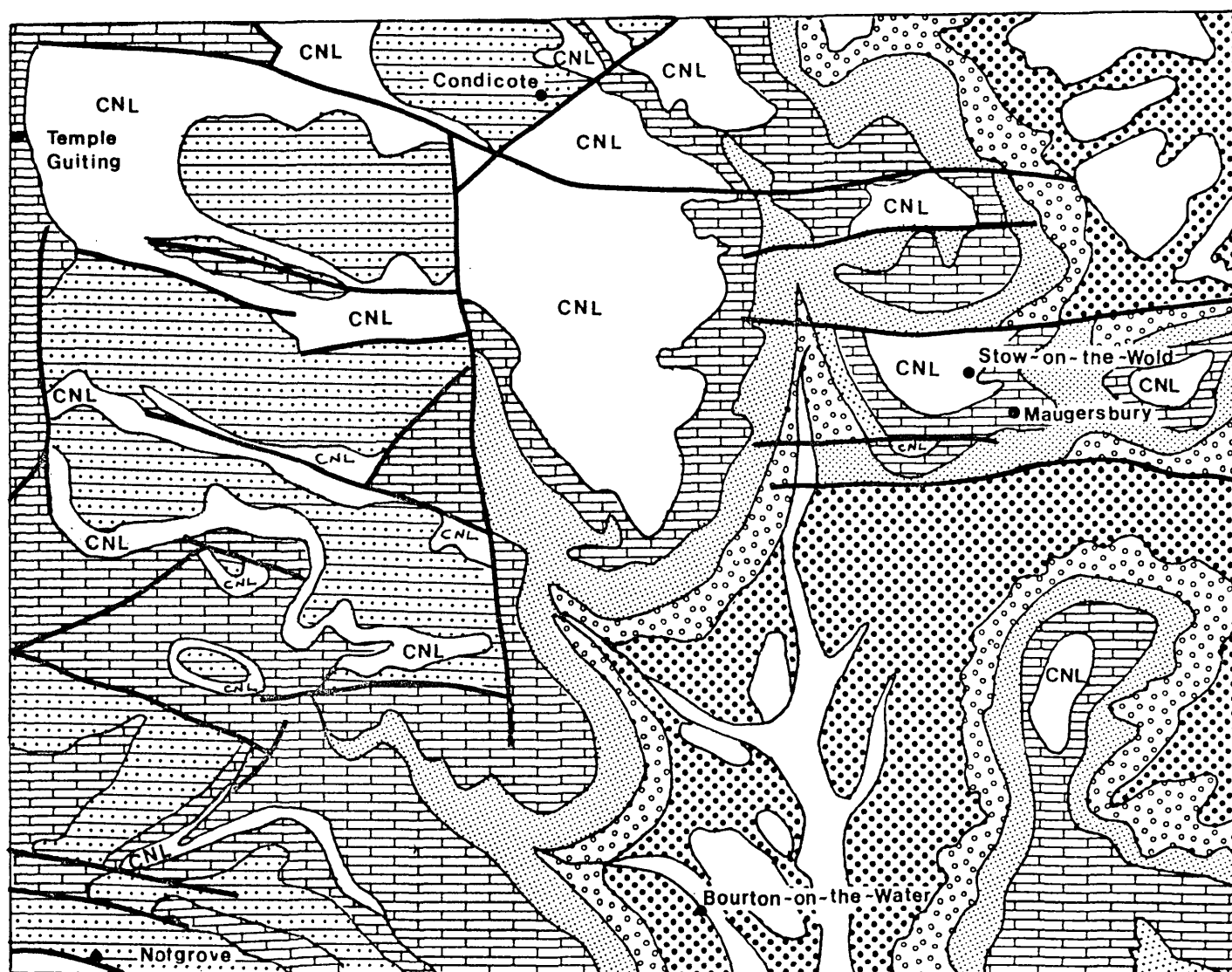


FIGURE 5.7 Geology of the
Stow-on-the-Wold district
Gloucestershire



SAMPLE NUMBER	SAMPLING LOCATION	PARENT MATERIAL	SOIL SERIES	SOIL GROUP	DRAINAGE
30	41682238	Jurassic limestone	Sherbourne	Brown rendzina	Free
31	41742238	Jurassic clay shale/mudstone	Denchworth	Pelo-stagnogley	Poor
32	42042254	Jurassic clay shale/mudstone	Denchworth	Pelo-stagnogley	Poor
33	41052274	Jurassic limestone	Sherbourne	Brown rendzina	Free
34	41122269	Jurassic sandy limestone	Waltham	Brown earth	Free
35	41402196	Jurassic limestone	?		
36	41472318	Jurassic limestone	Sherbourne	Brown rendzina	Free
37	41552184	Jurassic limestone	Sherbourne	Brown rendzina	Free
38	41592183	Jurassic limestone	?		

TABLE 5.3. Sampling locations with details of underlying geologies and soil types in Stow-on-the-Wold, Gloucestershire

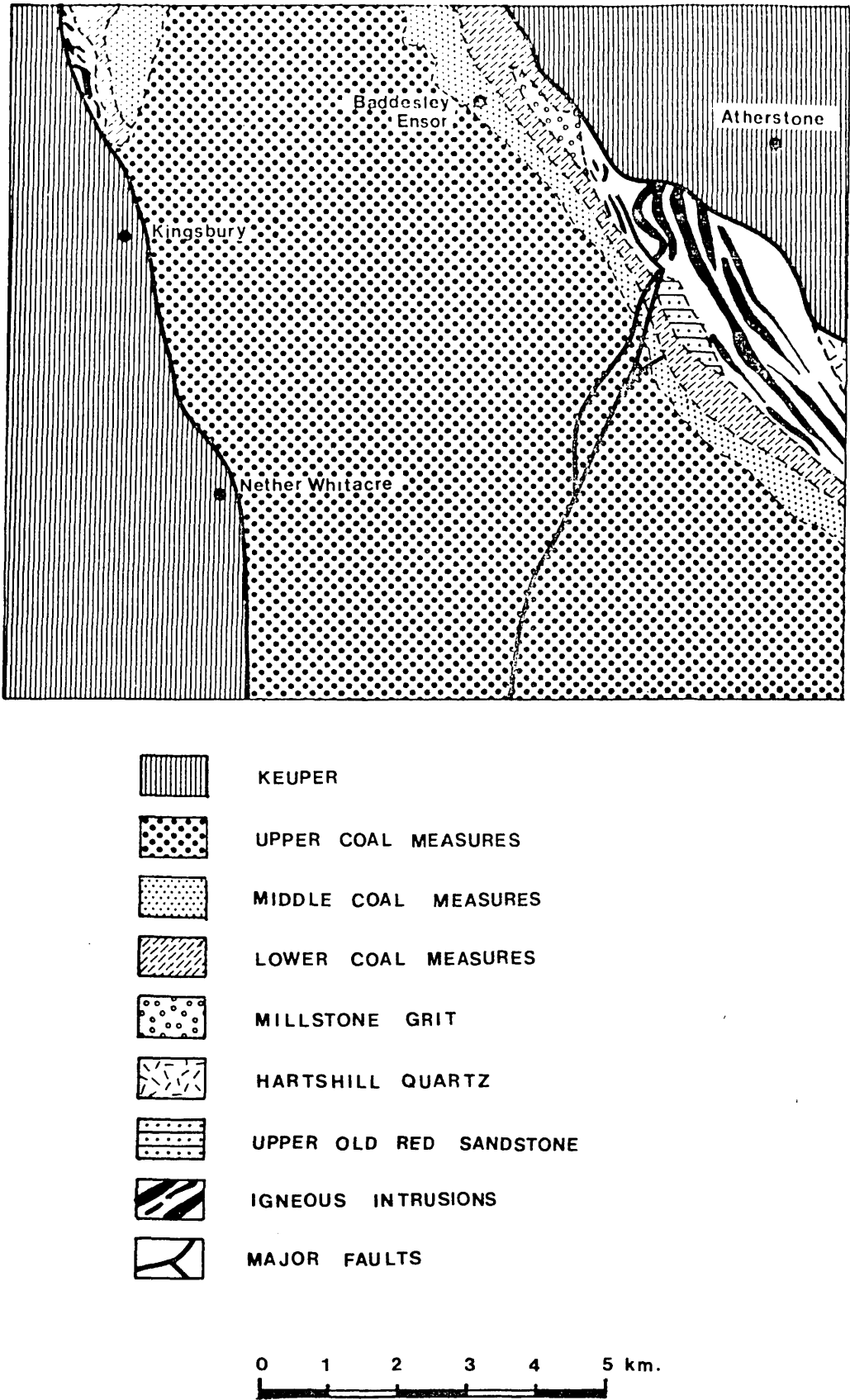


FIGURE 5.8 Geology of the Atherstone district of Warwickshire (redrawn from Whitfield and Beard, 1980)

SAMPLE NUMBER	SAMPLING LOCATION	PARENT MATERIAL	SOIL SERIES	SOIL GROUP	DRAINAGE
39	42892968	Carboniferous greyshale/sandstone	Reclaimed	Not applicable	Poor
40	42922972	Drift-L. Paleozoic mudstone/clay shale	Merevale	Argillic brown earth	Imperfect
41	42782942	Red Carboniferous sandstone	Shifnal	Brown earth	Free
42	42552964	Red Carboniferous sandstone	Shifnal	Brown earth	Free
43	42542957	Red Carboniferous sandstone	Shifnal	Brown earth	Free
44	42552959	Drift-red Carboniferous mudstone/clay shale	Gatacre	Argillic brown earth	Imperfect
45	42712974	Drift-red Carboniferous mudstone/clay shale	Baxterley	Stagnogley	Poor
46	42682975	Drift-Carboniferous clay shale/silty shale	Bardsey	Cambic stagnogley	Poor
47	42742969	Drift-red Carboniferous mudstone/clay shale	Baxterley	Stagnogley	Poor
48	42432949	Drift-red Carboniferous mudstone/clay shale	Dodmoor	Argillic brown earth	Imperfect
49	42652930	Red Carboniferous sandstone	Shifnal	Brown earth	Free
50	42662928	Drift-red Carboniferous mudstone/clay shale	Dodmoor	Argillic brown earth	Imperfect
51	42382902	Drift-red Triassic mudstone/clay shale	Whimble	Argillic brown earth	Imperfect
52	42862976	Drift-Carboniferous clay shale/silty shale	Bardsey	Cambic stagnogley	Poor

TABLE 5.4. Sampling locations with details of underlying geologies and soil types in Atherstone, Warwickshire

(Whitfield and Beard, 1980).

(e) Onecote, Staffordshire

The Onecote district is situated to the east of Leek in north Staffordshire and is covered by the Ordnance Survey 1:25,000 map sheet SK 05. Carboniferous rocks underlie the whole area but their succession and tectonics are complex. The geology is presented here in Figure 5.9.

The location of the fifteen farms sampled in the Onecote area plus details of underlying geological units and soils are summarised in Table 5.5. Only nine of the twenty seven soil series delineated in the area were sampled in connection with the incidence of bovine hypocupraemia. These were the Malham, Heapy, Bodden, Dale, Ticknall, Wilcocks, Onecote, Brickfield and Brickfield/Dale/Bardsey soil series comprising 3%, 2%, 1%, 15%, 6%, 17%, 5%, 9% and 7% of the area respectively. A detailed account of the Onecote soils is given in the Soil Survey Record No. 29 (Hollis, 1975).

(f) Masham, North Yorkshire

The Masham area extends from Leyburn in the north to Pately Bridge in the south. Its geology is dominated by Millstone Grit bordered by Magnesian limestone to the east, and Carboniferous limestone to the west. The geology is presented in Figure 5.10.

The Masham district was the only field area not covered by a Soil Survey memoir. The neighbouring Boroughbridge district has been surveyed, but could not be selected for fieldwork because of it being a predominantly arable region. The relatively few cattle that do graze here do not exhibit bovine hypocupraemia (Hannan, personal communication). In the absence of a soil memoir, information concerning soil series in the area was obtained by a personal communication from the northern division of the Soil Survey of England and Wales. The location of the eleven farms sampled plus details of underlying geology and soil types are summarised in Table 5.6.

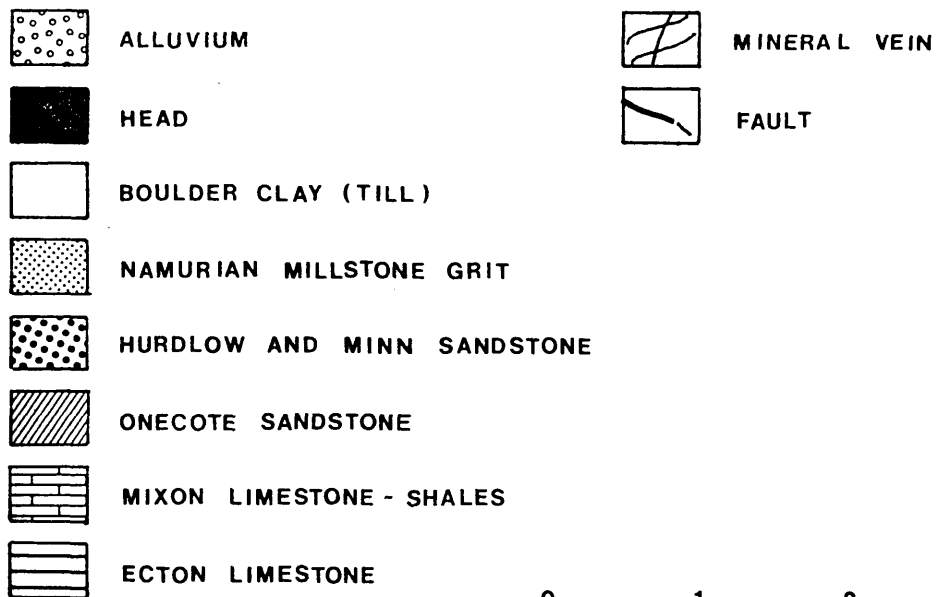


FIGURE 5.9 Geology of the Onecote district of Staffordshire

SAMPLE NUMBER	SAMPLING LOCATION	PARENT MATERIAL	SOIL SERIES	SOIL GROUP	DRAINAGE
53	40373554	Carboniferous shale/sandstone	B/D/B	Cambic stagnogley	Poor
54	40353558	Carboniferous clay/silt shale	Onecote	Cambic stagnohumic gley	Poor
55	40353564	Carboniferous drift	Wilcox	Cambic stagnohumic gley	Poor
56	40933514	Carboniferous limestone	Malham	Brown earth	Free
57	40863507	Carboniferous drift	Bodden	Stagnogleyic argillic brown earth	Imperfect
58	40743520	Carboniferous shale/silt	Ticknall	Cambic stagnogley	Poor
59	40723533	Carboniferous clay shale	Dale	Pelo-stagnogley	Poor
60	40313574	Carboniferous clay shale	Dale	Pelo-stagnogley	Poor
61	40083569	Carboniferous drift	Brickfield	Cambic stagnogley	Poor
62	40143565	Carboniferous shale/sandstone	B/D/B	Cambic stagnogley	Poor
63	40193558	Carboniferous shale/sandstone	B/D/B	Cambic stagnogley	Poor
64	40773559	Carboniferous clay shale	Dale	Pelo-stagnogley	Poor
65	40853575	Carboniferous clay shale	Dale	Pelo-stagnogley	Poor
66	40723579	Carboniferous drift	Brickfield	Pelocambic-stagnogley	Poor
67	40723576	Carboniferous shale/silt	Heapy	Stagnogleyic brown earth	Poor

TABLE 5.5. Sampling locations with details of underlying geologies and soil types in Onecote, Staffordshire

Note: B/D/B is Brickfield, Dale, Bardsey

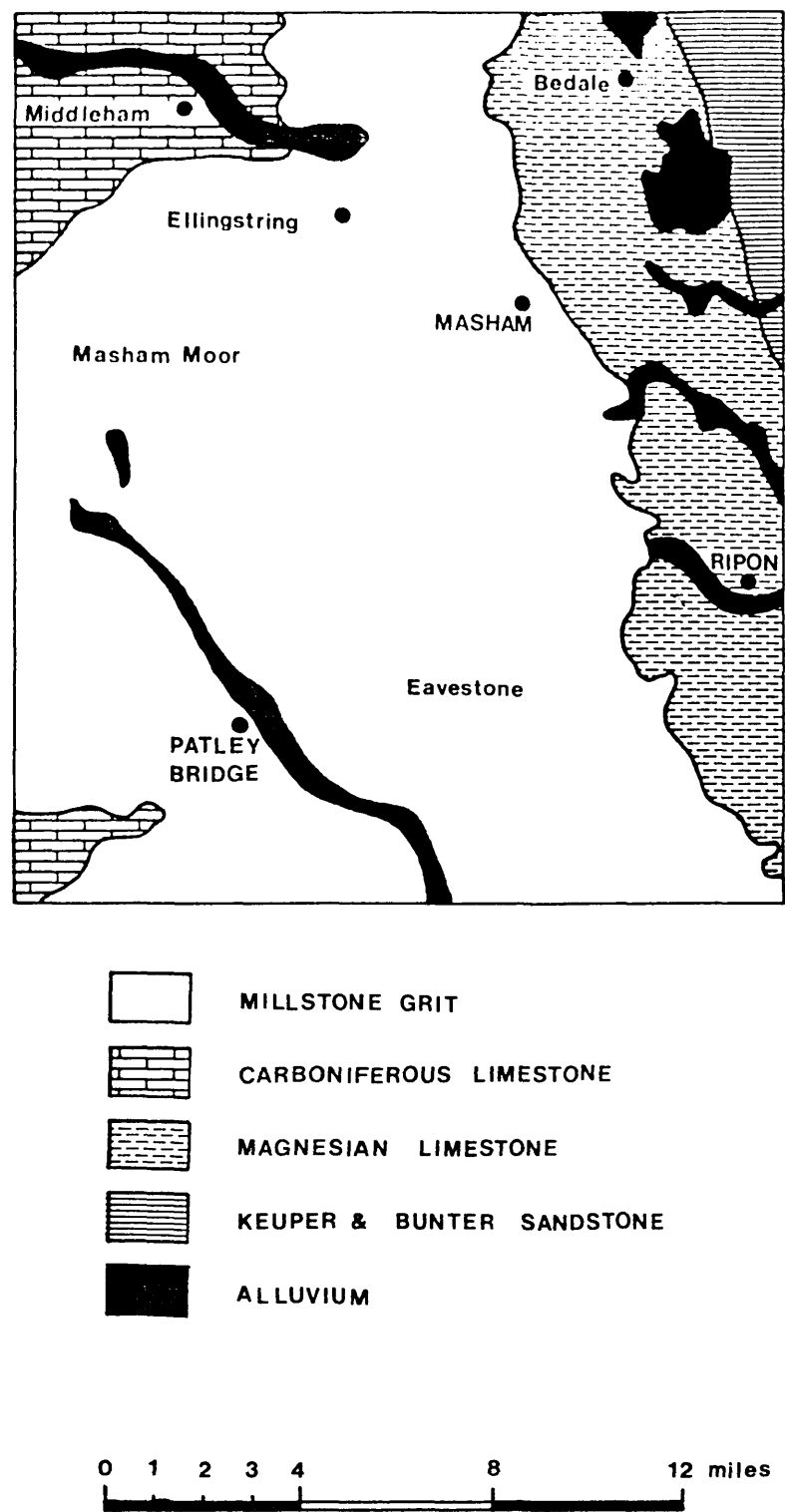


FIGURE 5.10 Geology of the Masham district of North Yorkshire

SAMPLE NUMBER	SAMPLING LOCATION	PARENT MATERIAL	SOIL SERIES	SOIL GROUP	DRAINAGE
68	41574828	Carboniferous-clay, silt, shale	Wilcocks	Cambic stagnohumic gley	Poor
69	41704818	Millstone Grit	?		
70	42154795	Glaciofluvial river terrace	Wick	Brown earth	Free
71	42184831	Drift with silicious stones	East Keswick	Brown earth	Free
72	42134833	Drift with silicious stones	East Keswick	Brown earth	Free
73	42214685	Carboniferous shale	Dunkeswick	Stagnogley	Poor
74	41434670	Carboniferous shale	Dunkeswick	Stagnogley	Poor
75	42014693	Carboniferous-clay, silt, shale	Wilcocks	Cambic stagnohumic gley	Poor
76	41964715	Millstone Grit	?		
77	42334793	Glaciofluvial river terrace	Wick	Brown earth	Free
78	42284787	Millstone Grit	?		
79	41544785	Carboniferous shale	Dunkeswick	Stagnogley	Poor

TABLE 5.6. Sampling locations with details of underlying geologies and soil types in Masham, North Yorkshire

CHAPTER 6

ANALYTICAL PROCEDURES AND QUALITY CONTROL

6.1 SAMPLE PREPARATION

SOILS

On return to the laboratory the soils were spread out thinly on clean paper and loosely covered with paper to protect them from contamination. They were allowed to dry at room temperature for three days. Once fully dry, the soil samples were disaggregated using a porcelain pestle and mortar and sieved through a 2 mm (10 mesh) nylon sieve. Each sample was then split into two - one subsample to be used for pH measurement, the other to be analysed for its trace element content. As homogeneity is essential for trace element determinations the latter subsample was ground finely in an agate mill to less than 2 μ m (80 mesh). This finely divided sample was then split into two - one subsample to be dried at 105°C (for general elements), the other to be dried at 70°C (for arsenic).

HERBAGE

On return to the laboratory the herbage samples, still in their sample bags, were dried in an air circulation oven at 50°C for five days. In the context of this work it was thought that the trace element content of unwashed herbage would be more relevant to the actual trace element intake of grazing livestock, than the content of washed herbage, which is more useful for plant uptake studies. Consequently, all analyses were performed on unwashed herbage. Once fully dry, the herbage was finely milled using a Christy and Norris mill especially designed to minimise trace element contamination i.e. the beater cross was made from cast iron. The milling equipment was dusted thoroughly between samples.

6.2 PREPARATION OF THE ANALYTICAL EQUIPMENT

As the work involved the determination of low concentrations of elements in soils and herbage, all equipment was kept scrupulously

clean. Before each analysis, glassware were washed in hot water using the detergent Teepol. Individual items were rinsed in water and soaked in nitric acid (10%) overnight. This was followed by three rinses in de-ionised water (DIW) and drying in a low temperature oven. Equipment was covered in Kimwipe tissue when not in use to prevent contamination. Contamination was also minimised in sample analysis by the use of AnalaR grade chemicals.

6.3 INSTRUMENTATION

The three types of instrumentation used in the current study to determine elemental concentrations in soil and plant material were the inductively coupled plasma (ICP) system, atomic absorption spectrophotometry (AAS) and colorimetry.

(a) ICP

This instrument measures the great majority of elements in the periodic table with low detection limits, good precisions and linear calibrations over several orders of magnitude. As it is a solution method, matrix effects and interference effects can be minimised. Most important, all the elements can be measured simultaneously in a matter of seconds, making it the most rapid of all analytical methods.

The elements to be analysed are presented in aqueous solution. The solution is pumped by a peristaltic pump into the spray chamber where the flow of pure argon gas converts a proportion of the solution to an aerosol. This is sprayed into the centre of the argon plasma. The inductively coupled plasma is a stream of argon atoms which are heated by inductive heating using a radio frequency coil which is run from a generator. Inductive heating of argon as it flows through the radio frequency field effectively strips electrons away from the argon atoms and produces a plasma of argon ions with an operating temperature at its centre of 6,000 - 10,000^oK. The plasma is ignited by a high frequency spark.

The solution to be analysed is thus sprayed into a very high temperature flame which is stable and of sufficient temperature to dissociate the chemical bonds and excite a large number of spectral

lines. The light emitted is then focussed into a conventional air path 1.5 m spectrometer in which the spectral lines are detected by fixed photomultipliers mounted along the Rowland circle of the spectrophotometer. The signal from the photomultiplier may be output directly onto the teletype to give intensity measurements. Alternatively, calibration lines can be prepared from the intensity readings obtained from solutions containing known concentrations of the elements, and these calibration lines may be stored on the computer linked to the system.

(b) AAS

Before the introduction of the ICP, atomic absorption spectrophotometry was the best technique for determining concentrations of major and trace elements. The spectrophotometer is a relatively cheap instrument to buy and run. It is easy to calibrate and readily automated.

AAS involves the absorption of light-type energy by atoms. For this to be achieved the sample generally has to be brought into solution. The solution containing the element to be analysed is aspirated by means of a flame, fuelled by a mixture of air and acetylene. This has a temperature sufficiently high (200°C) to reduce most of the droplets into an atomic form. The result is that most of the elements in the vapour exist as neutral, unbound atoms in their ground state. The vapour containing the element to be analysed is illuminated by a light source, usually a hollow cathode lamp of the same element as that being determined. It is an inherent characteristic of the atoms in their ground state, in the vapour, that they will absorb at discrete frequencies, the incident energy radiating from the hollow cathode lamp. Radiation from the lamp is then isolated by means of a monochromator, and its energy now reduced because of absorption in the flame, measured on a detector. The amount of this absorption is proportional to the concentration of the element in the vapour. By measuring the decrease in energy being received by the detector, a quantitative determination of the amount of the element in the vapour can be made. One reason for the great sensitivity of the AAS method is that about 95% of the atoms are in their atomic state in the vapour and are thus available for absorbing radiation from the hollow cathode tube. Poor detection limits for some elements, as well as major element

interferences and non-linear calibration limits are the three main disadvantages of the AAS technique.

(c) Colorimetry

The formation of coloured compounds in solution by reaction of an element with a specific chemical reagent is the basis of colorimetry. When white light passes through this coloured solution some wavelengths are selectively absorbed; non-absorbed radiation is transmitted by the solution and causes its colour. Solutions of coloured substances can therefore be characterised by their transmission and absorbance curves for visible light. Providing the solution of the coloured substance is sufficiently dilute, the relationship between its concentration and absorption is given by Beer's law:-

$$\text{ABSORBANCE (A)} = \log\left(\frac{I_o}{I_t}\right) = abc$$

where ' I_o ' and ' I_t ' are the intensities of incident and transmitted light, ' a ' is a constant (absorbivity), ' b ' is the length of the light path and ' c ' is the concentration. The concentration of the coloured substance can be estimated either visually or instrumentally by measuring the absorption of the complementary colour with a spectrophotometer. Colorimetry has the advantage of simplicity, low cost and portability but unfortunately also has poor sensitivity and applicability to one-element analysis at any one time.

6.4 DETERMINING THE CHEMICAL CONTENT OF SOIL AND HERBAGE MATERIAL

(a) Nitric acid - perchloric acid digestion of soils to determine their total Cu, S, Fe, Zn, Cd, Al, K, Ca and Mn content using ICP instrumentation

A description of the method is given in Appendix 2a. A mixture of nitric and perchloric acids is commonly used for sample digestion as it provides a rigorous attack on minerals owing to the higher temperatures that can be attained during the reaction (180°C). It is the perchloric that is the effective reagent. The nitric is added to react with readily oxidisable material which otherwise might cause a violent

reaction with the perchloric alone. The nitric-perchloric mixture yields about 95% extraction of the metals in sulphides and weathered products which is almost total extraction. It cannot be used for the extraction of As, Sb and Se owing to their partial volatilisation during the attack. Alternative methods have to be employed (Section 6.4 (d)).

(b) Nitric acid - perchloric acid digestion of herbage samples to determine their total Cu, Mo, Fe, Zn, Cd, Al, Ca and Mn content using ICP instrumentation

A description of the method is given in Appendix 2b. This is similar to the method for soils but because larger volumes of plant material are required, the attack has to be conducted on a larger scale and therefore necessitates smaller batches. Again, owing to the partial volatilisation of As, and in this case S, alternative procedures were followed (Section 6.4 (e)).

(c) The determination of total Mo in soils using colorimetry

A description of the method is given in Appendix 2c. This procedure is based on the nitric acid - perchloric acid digestion of soils given in 6.4 (a). However, owing to considerable interference from Al when Mo was determined on the ICP, alternative instrumentation had to be employed. This involved a colorimetric method using the Gilford 300N microsample spectrophotometer. Through a series of reactions based on Stanton and Hardwick's (1967) method, any molybdenum present was made to form a green complex with dithiol which was then extracted in toluene. The colour intensity is proportional to the molybdenum concentration. To prevent interference effects the following precautions were taken:-

- i) Any Fe(III) was converted to Fe(II) by the reducing solution;
- ii) Any W was masked by the addition of the citric acid (in the reducing solution);
- iii) Cu was suppressed by the addition of potassium iodide.

(d) The determination of total As in soils using magnesium nitrate ashing followed by hydride generation and AAS

A description of the method is given in Appendix 2d(from

Pahlavanpour, Pullen and Thompson, 1980). Magnesium nitrate is successfully used as an ashing aid by preventing losses of As by volatilisation. The As residue is readily soluble in dilute hydrochloric acid where it is subsequently reduced to hydride by sodium tetrahydroborate(III) solution in a continuous flow system. This may be directly injected into the Perkin Elmer AA403 for determination of As alone, or into the ICP for simultaneous determination of As, Sb and Te. Even with ashing aids, losses of As have been reported and attributed to :-

- i) Inadequate mixing before ashing;
- ii) Insufficient ashing aid and
- iii) Heating too rapidly.

Particular attention was therefore paid to these sources of error to minimise losses.

(e) The simultaneous determination of As and S in herbage

The difficulty in determining As because of its volatility has already been discussed. Problems also arise in the determination of S in organic materials because S may be present as, or easily converted to, volatile compounds such as hydrogen sulphide, , thiols etc. It was therefore hoped to use the magnesium nitrate attack proposed by Pahlavanpour, Thompson and Thorne (1981) for the simultaneous determination of As and S in herbage. This procedure which is based on Pahlavanpour, Pullen and Thompson's (1980) soil method was found to be unsatisfactory. During the ashing phase, a considerable amount of plant material was disseminated in the muffle furnace, causing cross contamination and losses. A Ministry of Agriculture, Fisheries and Food method (1981) for the determination of total S in plant material was subsequently attempted. A larger volume of more dilute magnesium nitrate solution was used as the ashing aid. The herbage - magnesium nitrate mixture was maintained on a hot plate raised to 280°C before completing the ashing in a muffle furnace at 450°C. Cross contamination occurred once more. To try to prevent this, the following precautions were taken:-

- i) Herbage samples were more finely ground in order to increase the surface area on which the ashing aid could act;
- ii) Wetting agents were considered but only acetone could be used owing to a considerable S input from detergents. It was hoped

that the wetting agent would enhance the effect of the ashing aid;

- iii) A larger volume of more dilute magnesium nitrate solution was used, again to ensure efficient mixing of herbage with ashing agent.

Although the amount of herbage movement in the furnace was reduced, it was not eliminated. An alternative strategy was therefore pursued. This involved a pre-digestion of the herbage with fuming nitric acid prior to magnesium nitrate ashing. This proved to be very effective and was successfully employed for all the samples (Appendix 2e).

6.5 THE DETERMINATION OF SOIL pH

pH may be determined either in the field or under laboratory conditions. The latter was chosen as it had the advantage of allowing a standard procedure to be adopted. 10g of air-dried, 2 mm soil was weighed into a clean 100 ml beaker. 25 ml of DIW was added and mixed to a slurry with a glass rod. The slurry was left for 15 minutes before making the pH readings. These were obtained using a Pye Unicam pH meter model 293, that had been calibrated to pH 4.0, pH 7.0 and pH 9.2 using buffer solutions. Duplicate analyses were included to check the repeatability of the readings. The glass electrode was always washed with DIW between readings, and the calibration checked every ten readings.

6.6 THE DETERMINATION OF SOIL ORGANIC MATTER BY LOSS OF WEIGHT ON IGNITION

Several methods are available for the estimation of organic matter or organic carbon content of the soil (Hesse, 1971). For the purpose of this work estimation by loss of weight on ignition was considered to provide a convenient and rapid technique for dealing with the number of samples involved. The technique adopted is that of Avery and Bascombe (1974), where the loss of weight of an oven dry (105°C) sample is estimated after ignition at 375°C for 16 hours. This was chosen in preference to ignition at 850°C as errors due to the loss of structural waters from clays were minimised (Ball, 1964). It was appreciated that the technique employed destroys elemental C as well as organic forms. Weight loss due to the decomposition of carbonates

in calcareous soils was also realised as another possible source of error. Smith (1983) calibrated the loss on ignition method against one for the determination of organic carbon and found a good linear correlation between the two determinations ($r = 0.999$).

6.7 ANALYTICAL QUALITY CONTROL PROCEDURES

Analytical quality control is an integral part of geochemical analysis. The two parameters used to assess analytical quality are accuracy and precision. The term accuracy is used to denote the extent to which the mean approaches the true concentration of the analyte. As the true value cannot be ascertained, a consensus of estimates, made by a variety of analytical methods, is used. This is known as the preferred or accepted value (Thompson, 1983). Accuracy is therefore referred to as the extent to which the mean approaches the preferred value. Random errors are assumed to follow a normal Gaussian distribution about the mean concentration. Precision refers to the spread of results around the mean. It is a function of the coefficient of variation and is usually defined in geochemistry as:

$$P = (2s \div \bar{x}) \cdot 100\%$$

i.e. indicating, that relative to the concentration of the analyte the range in which approximately 95% of the observations fall (Thompson, 1983). Accuracy and precision may be used to monitor:-

- i) Within batch variations;
- ii) Between batch variations and
- iii) Overall accuracy

by the following methods of analytical quality control:-

(a) Use of machine calibrators to monitor machine performance. They are prepared using BDH standard solutions and are made up in a matrix similar to that of the samples. The machine is calibrated with them initially and this calibration checked every twenty samples. If the calibration deviates by 10% or more, the machine is recalibrated before analysis is continued. In this way systematic errors due to drift can be detected and corrected.

(b) Use of duplicates to monitor within batch precision. In each batch, 10% of the samples were analysed in duplicate and included randomly in the batch - each set of subsamples being taken through the

whole analytical procedure independently.

Precision can be rapidly tested against an empirical standard of precision using the special control chart presented in Figure 6.1. This chart, which is based on the methodology of Thompson and Howarth (1973; 1976; 1978) and Howarth and Thompson (1976) necessitates the calculation and subsequent plotting of $|x_1 - x_2|$ and $(x_1 + x_2 / 2)$, the absolute difference between pairs of duplicate analyses and the mean value, respectively. Figure 6.1 illustrates acceptance of 5% precision for the determination of copper in soils. The results are distributed on the chart such that $> 95\%$ of the points fall below the 5% line. A similar chart for the determination of copper in herbage material, given in Figure 6.2, illustrates 10% precision.

Precision control charts were plotted for all elements. Table 6.1 summarises the precision of each element for both soil and herbage analyses. Precisions of all elements are acceptable although those for soils are generally better than those for herbage. This could be due to :

- i) Soil samples being more homogeneous than the plant material and,
- ii) A better developed method for the chemical determination of soils through rigorous temperature control.

Difficulties in the use of duplicates to estimate precision arise when differences between them do not follow a Gaussian distribution. This happens if:

- i) The sample is heterogeneous and sampling errors are skewed;
- ii) The concentrations are close to the resolution of the analytical method and results are reported as discrete values and therefore give a discontinuous distribution;
- iii) The concentrations are close to their detection limit. Values less than the detection limit are either set to zero or to the detection limit;
- iv) Systematic bias arises within a batch.

(c) Use of departmental and international reference materials to monitor accuracy. These were included at random to make up 10% of the batch. If reference materials are all put at the beginning or end of a batch then estimates of precision will be optimistically biased, and any

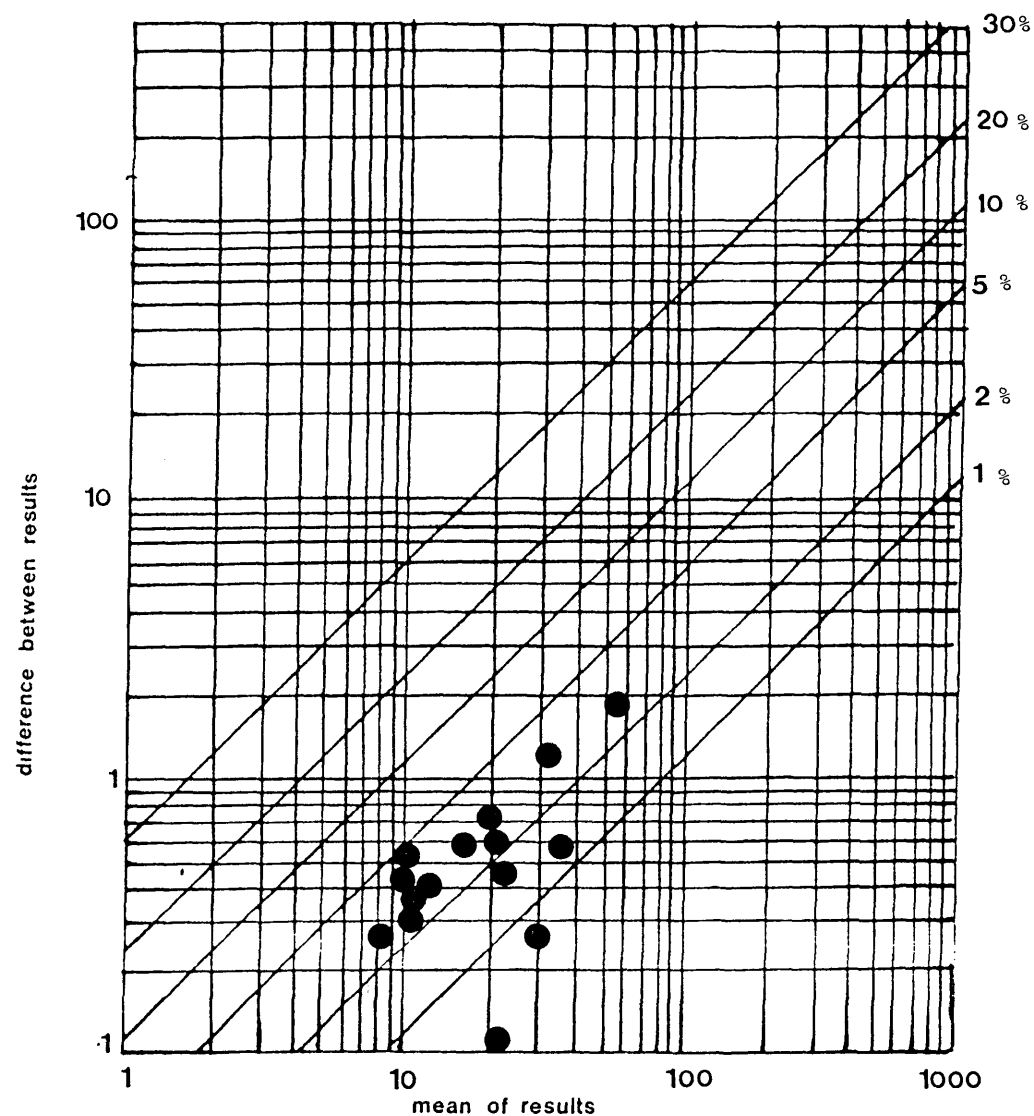


FIGURE 6.1. Precision control chart for duplicate soil copper results (5% precision)

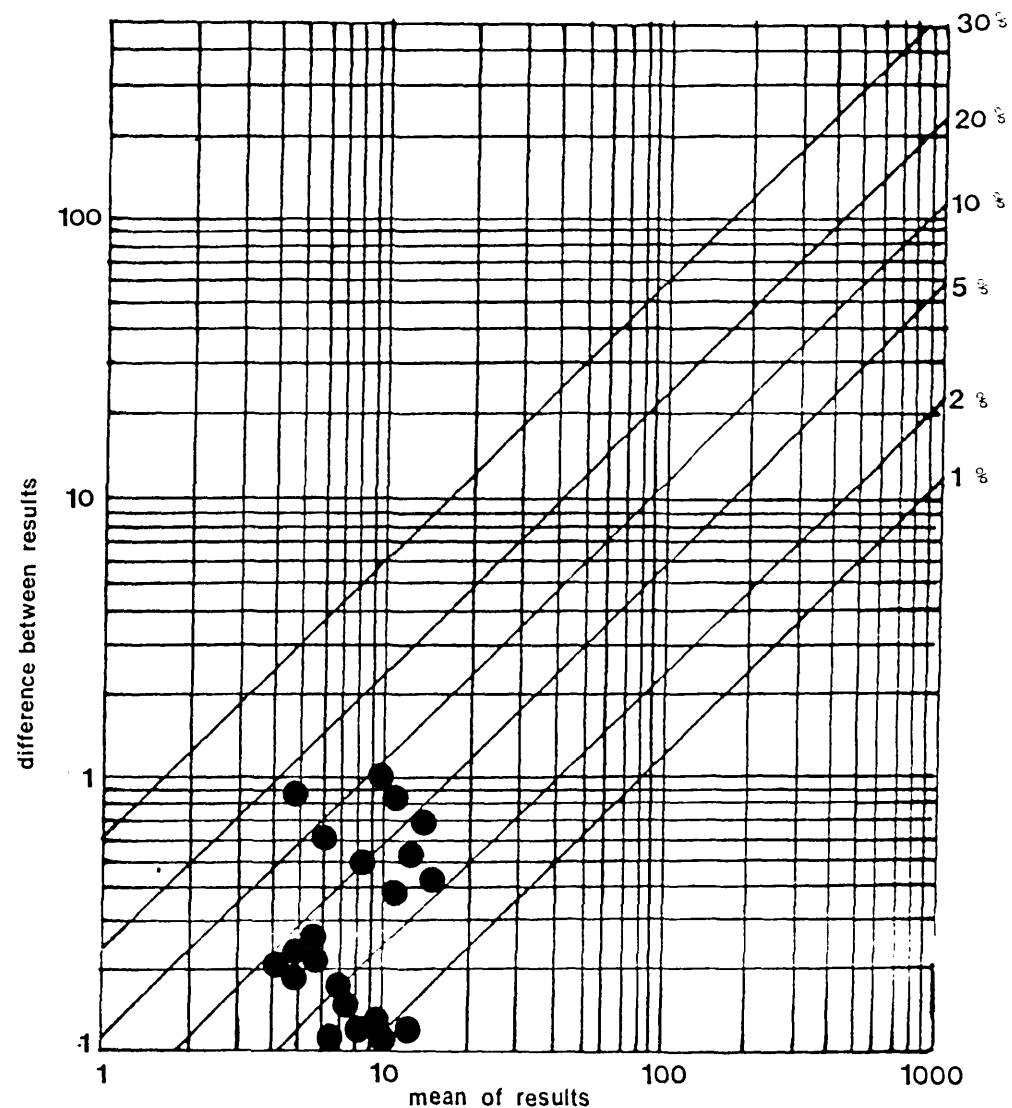


FIGURE 6.2. Precision control chart for duplicate herbage copper results (10% precision)

ELEMENT	SOIL	HERBAGE
Cu	5% (1)	10% (2)
Mo	25% (3)	15% (2)
S	5% (1)	10% (5)
Fe	5% (1)	20% (2)
Zn	10% (1)	15% (2)
Al	5% (1)	25% (2)
Sr	10% (1)	10% (2)
Cr	30% (1)	25% (2)
K	5% (1)	-
As	10% (4)	20% (5)
Mn	15% (1)	15% (2)
Ca	10% (1)	20% (2)

TABLE 6.1. Estimates of precision based on duplicate analyses

Note:

- (1) Nitric acid - perchloric acid attack for soils
- (2) Nitric acid - perchloric acid attack for herbage
- (3) Colorimetric molybdenum determination in soils
- (4) Magnesium nitrate ashing for arsenic determination in soils
- (5) Magnesium nitrate ashing for arsenic and sulphur determinations in herbage

systematic effects will be missed. If the reference materials are evenly spaced throughout the batch then variance may be underestimated if the frequency of the standards corresponds with the periodic variation. It is also important that the reference materials are of a similar compositional and mineralogical make-up to the sample, and that the particle sizes are similar.

Where available, two reference materials were selected with the concentration of the analyte at background and threshold levels. As there were no departmental reference herbage materials with preferred S and As values, the international standards 'Citrus leaf' and 'Pepperbush' were used instead.

The overall means, standard deviations and accuracies were calculated for each reference material. These results are presented in Tables 6.2 and 6.3. All accuracies were acceptable, although better values were obtained when the analyte concentrations were at their threshold rather than background levels. This is to be expected however.

(d) Use of reagent blanks to assess background concentrations and possible contamination errors. Contamination can occur when anomalous and background samples are analysed together, either through sample carry-over at the preparation stage or as memory caused through instrumental carry-over. Contamination may also arise as a result of the addition of extraneous material to the sample. Tables 6.4 and 6.5 illustrate the mean element concentrations of the reagent blanks for each batch. These were generally below the detection limit. Where blanks were above the detection limit their concentrations were generally less than 10% of the concentration of the lowest sample. Two exceptions were:

- i) The mean zinc concentration in batch 1 of the soil analyses was 4.1 ppm. This was 20% of the concentration recorded for the lowest sample.
- ii) The mean chromium concentration in batch 1 of the herbage analyses was 1.1 ppm. This was as high as some of the sample readings.

The use of reagent blanks in the soil and herbage analyses of this study have shown there to be few errors due to contamination of the samples. As overall precisions and accuracies were also good, the results of the soil and herbage analyses can be accepted with a degree of confidence.

REFERENCE MATERIAL	RESULT	Cu	Mo	Fe	Zn	As	Al	Sr	Cr	Mn	Ca	K
HRM 1	ESTABLISHED	3.0	1.5	8200	22	49	4500	6	13	85	450	590
	FOUND	2.34	0.83	7946	19.9	4.3	4251	6.36	10.9	89.3	420	553
	S.D.	0.5	0.23	237	2	0.2	206	0.7	2.8	10.8	53	34
	n	8	6	8	8	7	8	8	8	8	8	8
	% ACCURACY	22	45	3.1	9.5	12	5.5	6	16	5	6.6	6.3
HRM 2	ESTABLISHED	590	3.8	46000	400	150	32000	1200	450	1300	5000	5500
	FOUND	579	3.5	47704	394	168	35654	1268	415.8	1278	5279	6416
	S.D.	12.1	0.6	1390	11	13	707	25	31	51	142	202
	n	8	8	8	8	8	8	8	8	8	8	8
	% ACCURACY	1.9	7.9	3.7	1.5	12	11.4	7.1	7.7	1.7	5.6	16.7

TABLE 6.2. Overall accuracy of the soil analyses

Note : (1) All concentrations are in ppm

(2) S not included in table above as there was no established value

REFERENCE MATERIAL	RESULT	Cu	Mo	S	Fe	Zn	Al	Sr	Cr	Mn	Ca
HRM 11	ESTABLISHED	8.11	1.03	3300	263.2	45.07	121.4	11.59	1.29	95.13	3819
	FOUND	7.65	0.88	3488	251.7	43.57	114.15	11.35	1.17	91.59	3678
	S.D.	0.29	0.05	173	17.1	1.5	8.5	0.6	0.2	4.5	193
	n	4	4	4	4	4	4	4	4	4	4
	% ACCURACY	5.7	14.6	5.6	4.6	3.3	6	2.1	9.3	3.7	3.7
HRM 14	ESTABLISHED	6.28	1.57	-	1585	35.11	784.3	48.84	19.65	72.56	5621
	FOUND	5.87	1.3	-	1544	33.4	776.5	48.87	19.03	66.8	54.98
	S.D.	0.29	0.09	-	163	2.2	9.5	2	3.5	7.1	302
	n	4	4	-	4	4	4	4	4	4	4
	% ACCURACY	6.5	17	-	2.6	4.9	1	0	3	7.9	2.2

TABLE.6.3 Overall accuracy of the herbage analyses

Note : (1) All concentrations are in mg/kg DM

(2) As not included in table above. The use of 2 additional reference materials, citrus leaf and pepperbush, indicated an accuracy of 10.2% (n = 8) and 1% (n = 7) respectively.

(3) The accuracy of S determination for pepperbush was 19% (n = 8).

ELEMENT	BATCH 1	BATCH 2
Cu	BDL	BDL
Mo	BDL	BDL
S	5.6	0.3
Fe	9.4	35.5
Zn	4.1	0.7
Al	187	15.9
Sr	0.2	0.1
Cr	BDL	1.1
K	BDL	BDL
As	.04	.04
Mn	BDL	BDL
Ca	BDL	4.8

TABLE 6.4. Element concentrations in 'blanks'
incorporated randomly within batches of soil samples

Note:

All concentrations are in ppm.

BDL - below detection limits i.e. 0.2 ppm for Cu,
0.5 ppm for Mo, 0.4 ppm for Cr, 4 ppm for K, 0.6 ppm
for Mn and 2 ppm for Ca.

ELEMENT	BATCH 1	BATCH 2	BATCH 3	BATCH 4
Cu	BDL	BDL	BDL	BDL
Mo	BDL	BDL	BDL	BDL
S	20.8	10.6	BDL	-
Fe	5.0	6.0	2.9	2.4
Zn	0.4	0.3	0.4	0.4
Al	9.8	5.7	4.1	3.8
Sr	0.03	0.1	0.08	0.06
Cr	1.1	0.17	0.07	BDL
As	0.02	0.025	-	-
Mn	BDL	BDL	BDL	BDL
Ca	2.1	3.8	4.1	2.4

TABLE 6.5. Element concentrations in 'blanks' incorporated randomly within batches of herbage samples

Note:

All concentrations are in ppm.

BDL - below detection limits i.e. 0.03 mg/kg DM for Cu, 0.05 mg/kg DM for Mo, 0.05 mg/kg DM for Cr and 0.08 mg/kg DM for Mn.

CHAPTER 7

INTERPRETATION OF SOIL AND PASTURE HERBAGE DATA, AND THE SUBSEQUENT DETERMINATION OF GEOCHEMICAL CAUSES OF BOVINE HYPOCUPRAEMIA

7.1 INTRODUCTION

Topsoil, subsoil and herbage samples were collected from six areas of England exhibiting varying degrees of bovine hypocupraemia. These underwent chemical analyses for their Cu, Mo, S, Fe, Zn, Cd, As, Al, K, Ca, Sr and Cr contents. Subsequent analytical quality control showed these results to have acceptable precision and accuracy with negligible contamination errors. The data could be interpreted therefore with some degree of confidence. It is the aim of this chapter to analyse the soil and herbage results with a view to determining geochemical causes of bovine low blood copper status.

7.2 TOPSOIL DATA

(a) Data presentation

Topsoil data for the elements listed above are presented for each field area in Appendix 3 (a-e). In order to aid interpretation of these data, boxplots (Wainer, 1981) were computer-plotted for each element. These are redrawn here in Figure 7.1 (a-n). Boxplots display the main features of the data set and permit simple comparisons between sites. The box encloses the central 50% of the data for each area. The overall median is marked with a '+'. The right hand end of the box is at the upper hinge (median of the top 50%, approximately the 75th percentile). The left hand end of the box is at the lower hinge (median of the bottom 50%, approximately the 25th percentile). Lines or whiskers extend from the hinges to the extremes of the data lying within ± 1.5 of the concentration interval between the hinges outwards from the hinges. The position of unusual values are indicated on either side of the box with special symbols: possible outliers are indicated by '•' and probable outliers by 'o'. Outliers were only subsequently removed from the data set if their presence caused scaling of the display, such that the boxes for all the groups were condensed at one end of the plot, so as to obscure

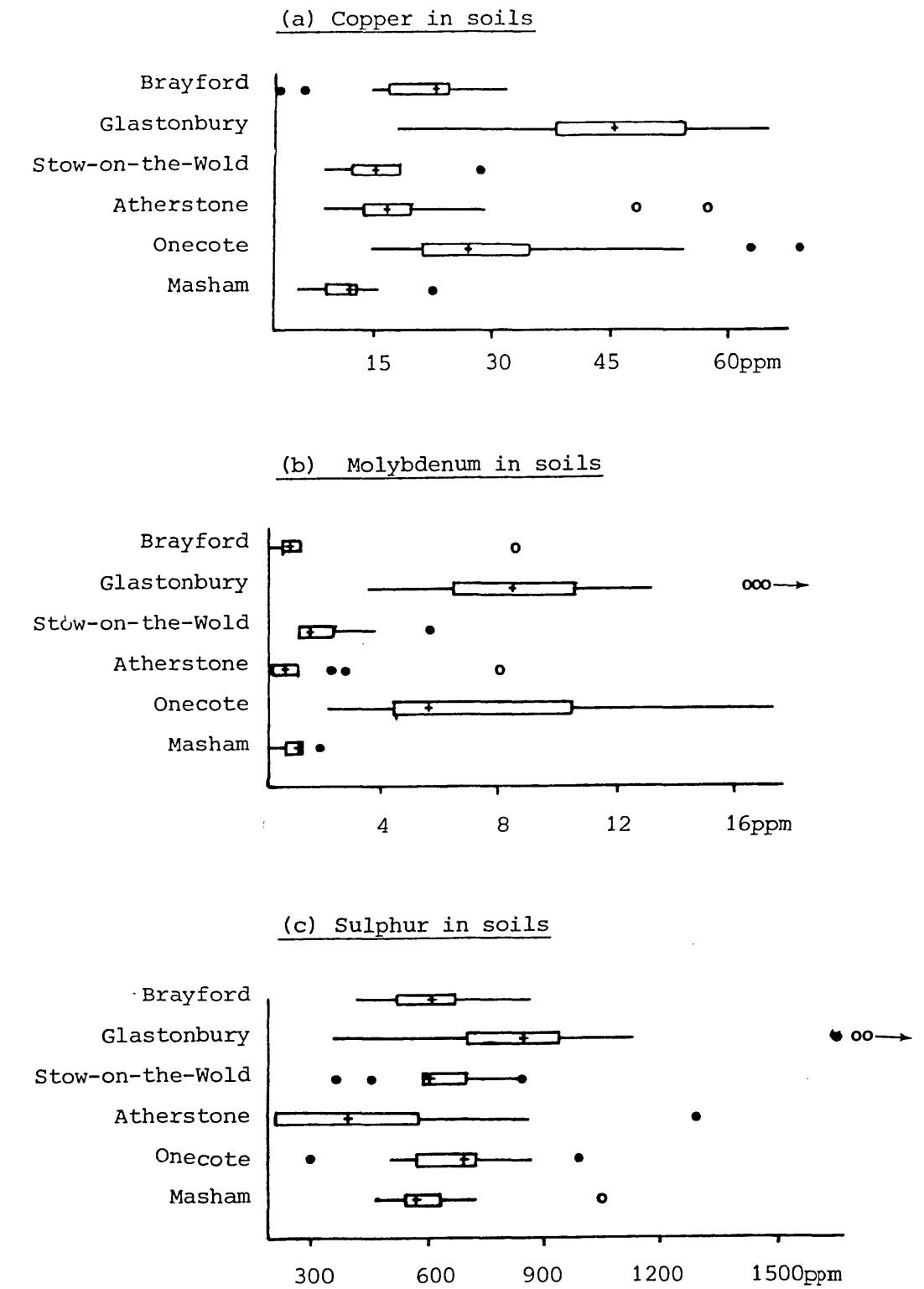
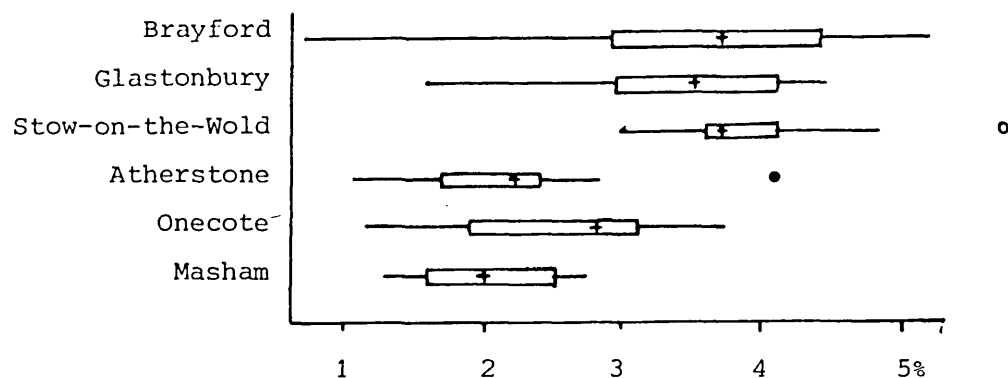
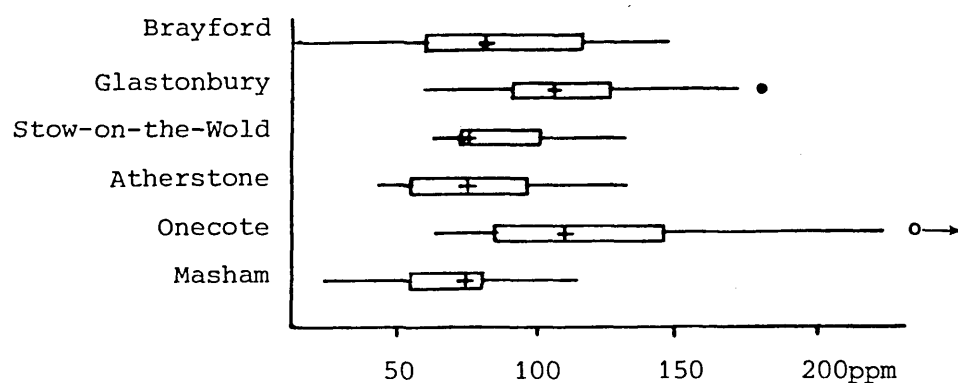


FIGURE 7.1. Boxplots of soil data - copper, molybdenum and sulphur

(d) Iron in soils



(e) Zinc in soils



(f) Strontium in soils

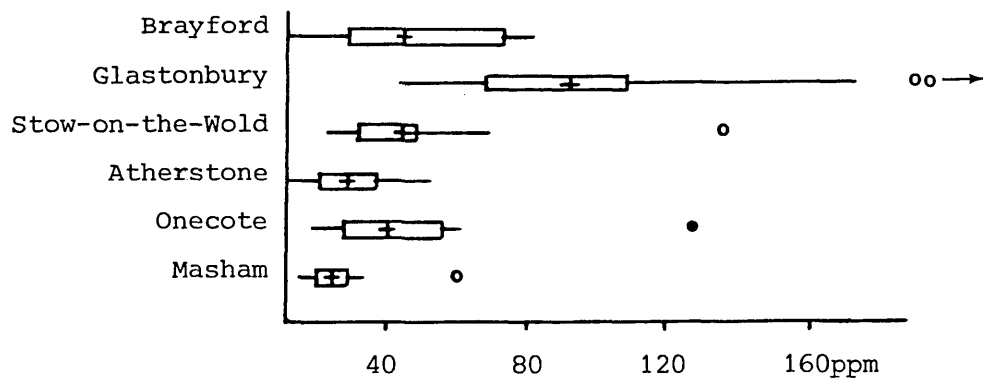


FIGURE 7.1. Boxplots of soil data - iron, zinc and strontium

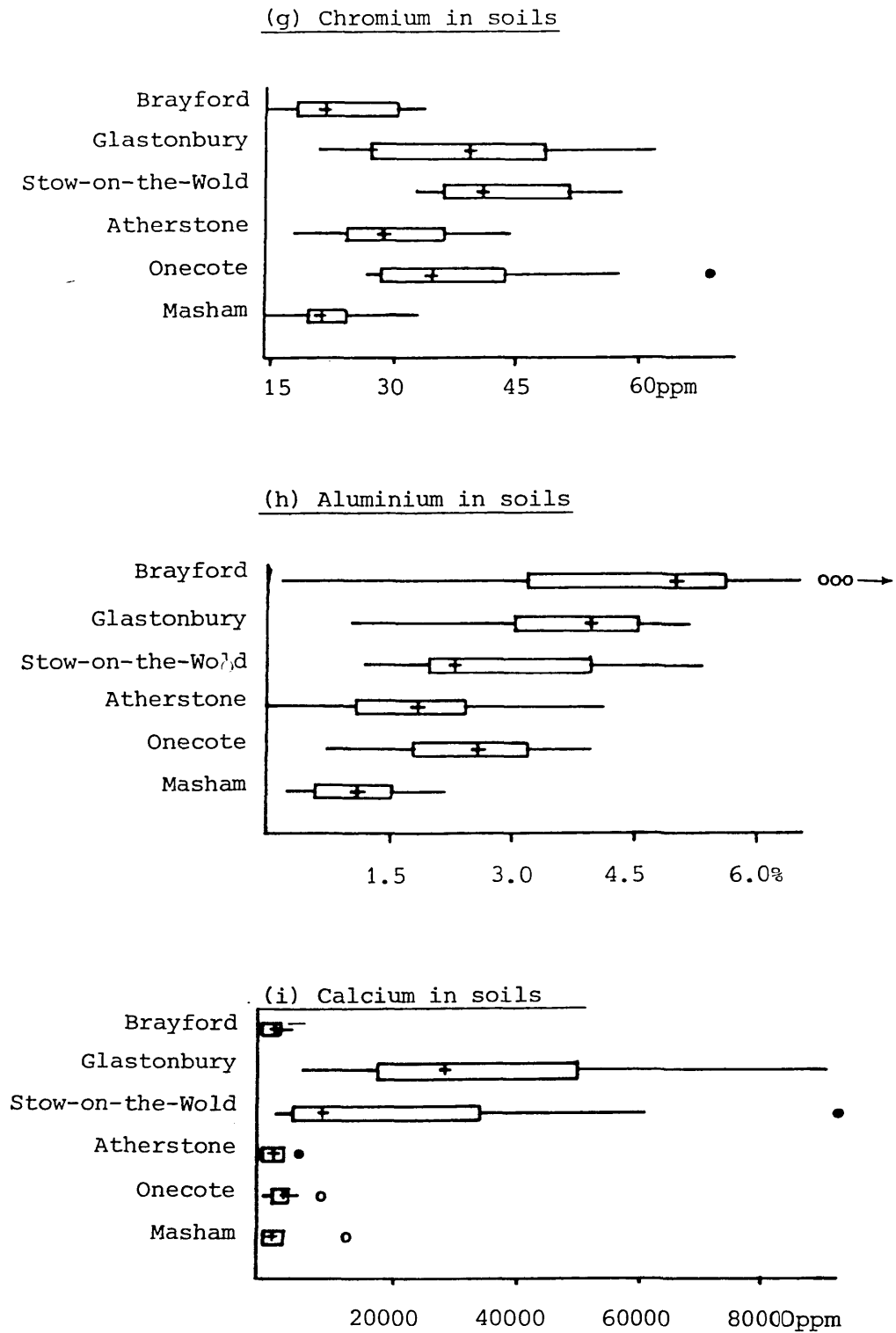


FIGURE 7.1. Boxplots of soil data - chromium, aluminium and calcium

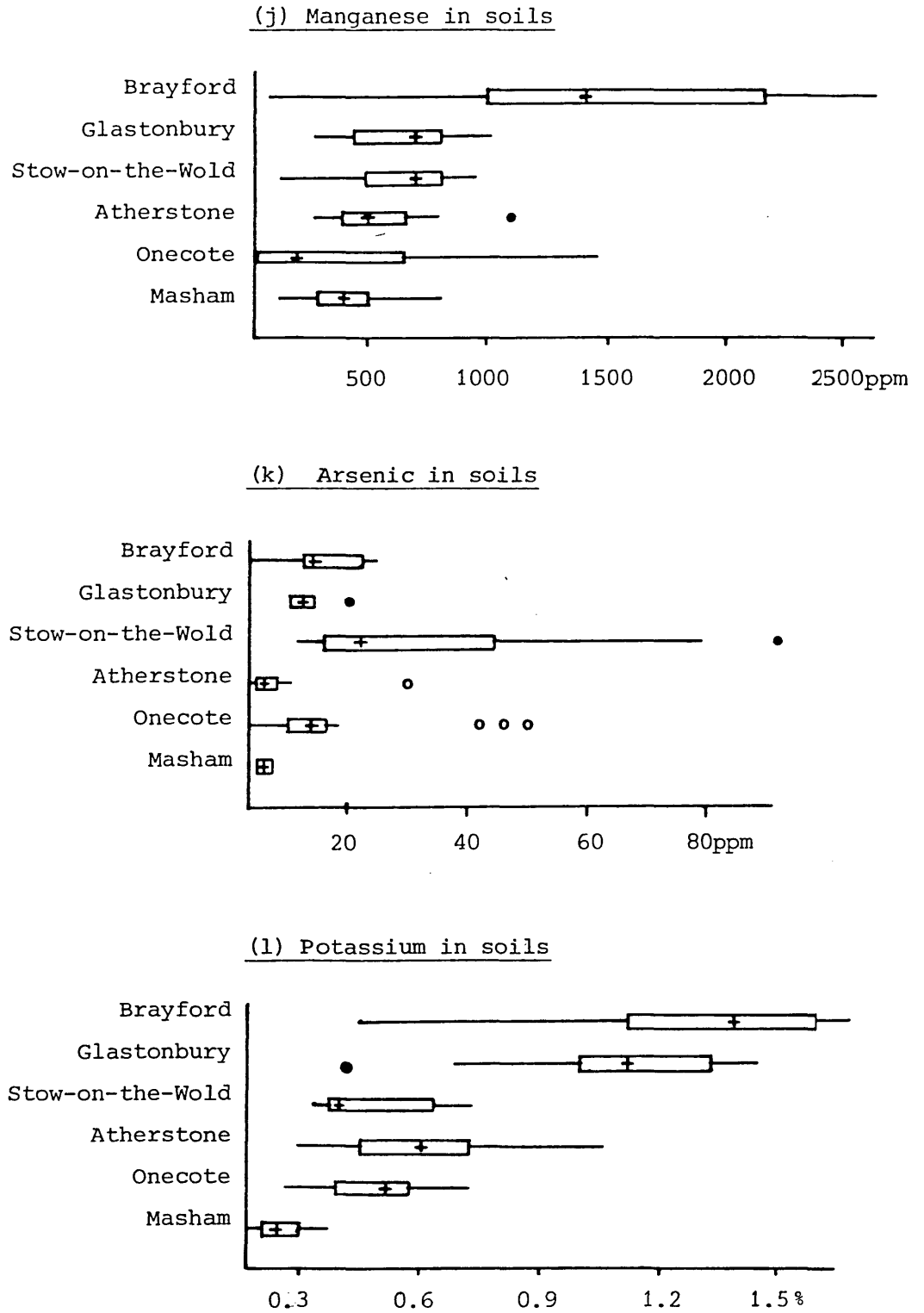


FIGURE 7.1. Boxplots of soil data - manganese, arsenic and potassium

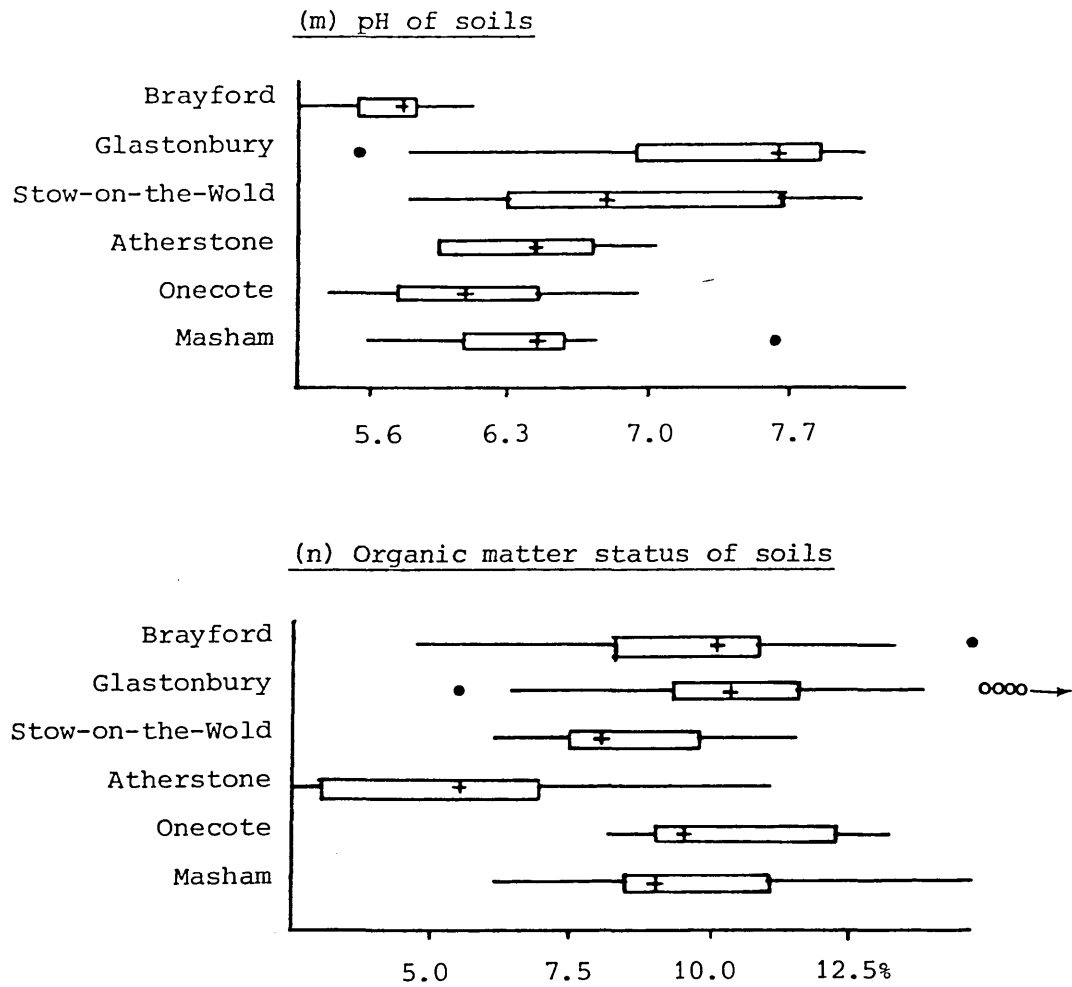


FIGURE 7.1. Boxplots of soil data - pH and organic matter status

details of interest.

(b) Interpretation of topsoil data

Summary topsoil data for each area are given in Table 7.1. These may be compared to published data on the ranges of element concentrations in British surface soils, given in Table 7.2. Each element is considered individually below.

Copper

The mean content of copper in British surface soils is generally taken as 20 ppm. Soils from three of the six areas have contents less than this, which is attributable to their coarse-grained parent material i.e. Millstone Grit in North Yorkshire, Carboniferous sandstones and mudstones in Warwickshire and Oolitic limestone in Gloucestershire. Highest copper concentrations are to be found in the Liassic shales of Somerset, Namurian and Visean shales of Staffordshire and the Old Red Sandstone of north Devon, where copper ores may be developed locally.

The majority of the soils sampled in the Brayford district were from the Highweek series. These fine loamy brown earths, derived from Devonian slate, mudstone, siltstone and sandstone, have a mean copper content of 23.7 ppm, which is slightly above the British average. The two podzols sampled in this area belonging to the Loxhore and Dartington series are both depleted in topsoil copper with a mean of 11 ppm. This is due to the eluviation of copper to lower horizons.

Soil series in the Glastonbury district are all enriched in copper. The Evesham, Middelney and Long Load series have the highest copper contents of all (>50 ppm), although the Somerton and Charlton Bank series are also substantially enriched (38-58 ppm Cu). The high clay content of these soils no doubt plays a vital role in maintaining high topsoil copper concentrations.

The soil series sampled in Stow-on-the-Wold have, with one exception, topsoil copper contents less than the British average (10-19 ppm). This is a reflection of the Oolitic limestone bedrock which is itself

		Cu	Mo	S	Fe	Zn	Sr	Cr	Al	Ca	Mn	As	K	pH	OM
BRAYFORD (n=14)	Mean	20.4	1.8	631	3.7	83.3	51.3	23.2	6.2	0.3	1525	15.5	1.3	5.8	10.0
	S.D.	8.1	1.9	106	1.1	36.5	22.5	6.1	1.6	0.07	741	6.0	0.3	0.2	2.4
	Min.	3.0	0.9	450	0.8	15.0	15.0	16.0	1.9	0.1	134	5.0	0.5	5.3	7.9
	Max.	32.0	8.7	860	5.2	145.0	86.0	34.0	8.1	0.3	2600	24.0	1.7	6.2	14.8
GLASTONBURY (n=15)	Mean	44.0	14.7	2288	3.5	112.6	132.6	3.9	5.3	3.8	708	13.1	1.1	7.3	18.5
	S.D.	14.1	11.9	3463	0.8	32.7	97.6	1.2	1.1	2.3	218	2.7	0.3	0.8	15.3
	Min.	19.0	4.1	380	1.8	61.0	47.0	2.2	2.7	0.8	350	11.0	0.4	5.6	5.5
	Max.	65.0	41.3	11600	4.5	181.0	420.0	6.2	6.8	9.2	1060	21.0	1.5	8.1	58.4
STOW-ON-THE WOLD (n=9)	Mean	16.2	2.7	658	4.1	90.1	57.2	4.4	4.6	2.9	607	37.8	5.1	7.0	8.4
	S.D.	5.3	1.4	148	0.8	19.5	31.8	0.8	1.4	2.9	296	28.0	1.4	0.8	1.6
	Min.	10.0	1.7	870	3.1	65.0	28.0	3.3	2.9	0.3	220	12.0	3.9	5.9	6.4
	Max.	29.0	6.2	380	5.8	133.0	140.0	5.8	7.0	9.4	1050	93.0	7.4	8.1	11.6
ATHERSTONE (n=14)	Mean	21.9	2.0	500	2.3	82.7	33.6	3.0	3.5	0.3	609	8.8	6.1	6.4	5.7
	S.D.	13.6	1.9	294	0.7	27.4	11.5	0.8	1.2	0.1	208	6.1	2.0	0.4	2.7
	Min.	10.0	0.7	230	1.3	47.0	17.0	1.9	1.6	0.1	330	4.0	3.1	6.0	2.5
	Max.	58.0	8.4	1310	4.2	130.0	55.0	4.4	5.7	0.6	1160	30.0	10.8	7.1	11.0
ONECOTE (n=15)	Mean	32.4	7.5	694	2.6	138.8	49.5	3.9	4.2	0.5	403	19.5	5.1	6.2	10.6
	S.D.	16.2	4.1	161	0.7	70.8	26.0	1.2	0.9	0.28	380	14.0	1.2	0.5	1.8
	Min.	16.0	0.7	320	1.3	66.0	23.0	2.8	2.4	0.3	77	4.0	2.9	5.5	8.5
	Max.	69.0	17.6	1030	3.6	330.0	132.0	7.0	5.6	1.1	1500	50.0	7.3	7.0	13.4
MASHAM (n=12)	Mean	12.3	1.5	643	2.1	71.3	30.9	2.4	2.8	0.4	480	6.3	2.8	6.5	9.9
	S.D.	4.1	0.4	150	0.4	21.5	10.5	0.5	0.5	0.3	191	0.7	0.5	0.5	2.5
	Min.	7.0	0.8	500	1.5	29.0	21.0	1.5	2.0	0.1	220	5.0	1.9	5.7	6.3
	Max	23.0	2.4	1090	2.8	110.0	35.0	3.3	3.8	1.3	840	8.0	3.8	7.7	14.9

TABLE 7.1. Summary Data : Means, standard deviation, minima and maxima of element concentrations in topsoils

Note: Concentrations of Fe, Al, Ca, K and OM (organic matter) are in % ; concentrations of Cu, Mo, S, Zn, Sr, Cr, Mn and As are in ppm.

ELEMENT	MEDIAN CONC. (mg/kg)	CONC. RANGE (mg/kg)
Cu	20	2 - 100
Mo	2	1 - 5
S	-	100 - 800
Fe	-	3000 - 50000
Zn	50	10 - 300
Sr	300	50 - 1000
Cr	100	5 - 3000
Al	-	50000 - 150000
Ca	-	5000 - 200000
Mn	800	500 - 1000
As	6	5 - 100
Cd	0.5	0.01 - 7
K	-	3000 - 25000

TABLE 7.2. Normal range of trace and major elements in naturally occurring British soils of interest in the aetiology of bovine hypocupraemia (from Aubert and Pinta, 1977; Mitchell, 1971; Swaine, 1955; Thornton and Webb, 1980)

inherently depleted in copper. The samples from the Denchworth series might have been expected to have higher copper contents than those recorded (10 and 18 ppm), owing to their derivation from Liassic clay shales.

Soil series in the Atherstone field area, like those above, have topsoils with less than 20 ppm copper. Again, this is a reflection of the coarse-grained Carboniferous sandstone/mudstone parent material which is inherently low in copper. The coarse nature and low organic matter status compound a copper-depleted situation. The only sites enriched in copper are those in close proximity to old coal workings i.e. samples 39, 40 and 52 which have copper levels of 29, 48 and 58 ppm, respectively. The remaining soils of the Shifnal, Dodmoor, Whimble and Baxterley series are copper-depleted (i.e. < 20 ppm).

Onecote soil samples generally have copper contents greater than the national average. The Dale series derived from Carboniferous clay shales, is particularly enriched in copper (mean of 56.3 ppm) reflecting to some extent the extensive copper mining activity last century. The other soil series sampled are not derived solely from the Carboniferous clay shales, like the Dale series, and as such have much less copper enrichment.

The copper status of Masham soil samples is, with one exception, considerably lower than the British average. Dunkeswick, Wick and East Keswick series do not exceed 13 ppm copper in their topsoils. This low copper status is accompanied by low potassium and aluminium levels, indicative of low silt and clay content and perhaps indicative generally of the poor trace element status of the parent material/transported overburden in the area.

Molybdenum

The mean molybdenum content of British soils has been reported as 1 ppm (Swaine and Mitchell, 1960). Somerset and Staffordshire field areas have considerable enrichment of molybdenum attributable to the underlying marine black shales of Jurassic and Carboniferous ages, respectively. These may contain from 1-100 ppm Mo, whilst the majority of soils derived from other shales, clays, limestones, sand, sandstones and

granite contain less than 2 ppm (Thornton, 1977). The trace element content of black shales is strongly influenced by the quality and composition of the organic matter present in the rock and depositional environment. Molybdenum, vanadium, nickel and copper are identified as the suite of elements frequently associated with the organic matter in marine black shales (Thomson, 1971). This explains the copper enrichment noted in both Somerset and Staffordshire soil series.

In all but one sample, the Highweek, Loxhore and Dartington series from Brayford, have background levels of molybdenum. The enrichment in sample No. 12 is possibly due to small pockets of molybdeniferous soil on the farm.

Glastonbury soil series, unlike those from Brayford, exhibit topsoil molybdenum concentrations far in excess of the national average. The highest levels are found in the Evesham and Middelney clays (mean of 36.5 ppm) and the lowest levels in the Worcester, Charlton Bank and Somerton series (mean of 6.7 ppm). Differences in molybdenum content between the soil series merely reflect differences in parent material. Where soils are derived directly from the Lower Lias calcareous claystone, molybdenum concentrations are considerably higher than soils derived from mixed origin.

Three of the four samples from the Sherborne series in Gloucestershire have slightly elevated levels of molybdenum (mean of 3.8 ppm). The reason for this enrichment is not known. Samples from the other soil series of Stow-on-the-Wold have background levels of molybdenum.

The Shifnal, Dodmoor, Gatacre, Whimble and Baxterley series in Warwickshire have background levels of molybdenum. Three anomalous samples from reclaimed land, Merevale and Bardsey series (Nos. 39, 40 and 52) perhaps reflect molybdenum-rich clay shale parent material or an anthropogenic source of molybdenum.

All the soil series sampled in Staffordshire have elevated levels of molybdenum. The Dale series has the highest concentrations of all (mean of 13.5 ppm). The variation in molybdenum content reflects the variation in underlying parent material. Those soils developed solely on Carboniferous clay shales have inherently higher molybdenum contents

than those developed from a mixture of shales, limestones and sandstones.

The Dunkeswick, Wick, East Keswick and Wilcocks soil series in North Yorkshire have background levels of molybdenum (less than 2 ppm) as they are derived from non-molybdeniferous parent material.

Sulphur

No great variation in sulphur content between areas is detectable. Atherstone soils have slightly lower sulphur levels than elsewhere, but even these fall within the normal range for British soils. Outlying data points with sulphur concentrations exceeding 1000 ppm typify organic peat and peaty clay soils. These are found in Somerset on the Sedgemoor soil complex.

Owing to the small variation in total sulphur between areas, attention is drawn to overall trends. There is for instance, a slight tendency for gleyed topsoils to have a generally higher sulphur content than their freely drained counterparts. Light textured, freely drained soils of the Shifnal, Sherborne, Highweek, Dartington and Loxhore series generally have topsoil sulphur concentrations of less than 600 ppm, whilst those heavily gleyed soils of the Dale, Wilcox, Onecote and Charlton Bank series generally have sulphur concentrations greater than 700 ppm. This observation is probably explained in terms of higher sulphur accumulation in the organic fraction of gleyed soils, together with a reduction in leaching of soluble sulphate from poorly drained soils. Lighter textured soils would be considerably more prone to rapidly leaching of sulphate. The higher levels of total sulphur in the calcareous soils of Glastonbury are due to the retention of insoluble calcium sulphate in these topsoils e.g. Somerton series.

Iron

Topsoil iron concentrations are greatest in Brayford, Glastonbury and Stow-on-the-Wold soil series. This is attributable to the underlying parent material which, in Devon was locally mined for iron. Small amounts of iron occur as lattice components of the clay aluminosilicates, however, most iron is present either as hydrous ferric

sesquioxide, in particulate form, or as a coating on other mineral particles.

Chromium

With the exception of samples from Brayford, topsoil chromium levels parallel those of iron. All sites contain less than the British mean of 100 ppm, which is probably due to inevitable losses of chromium during the nitric acid-perchloric acid digestion of soils. Lowest chromium concentrations occur in soils developed from Old Red Sandstone in north Devon and Millstone Grit in North Yorkshire.

Aluminium

Aluminium forms a part of the structural lattice of clay minerals, and under acid conditions, enters the soil exchange complex. In most agricultural soils of neutral pH, aluminium is a good indicator of clay content. On this basis most of the soils sampled had moderate clay content. Notable exceptions are the coarse grained soils of the Sherborne and Somerton series, as well as the Dunkeswick, Wilcocks, Wick and East Keswick series.

Potassium

High levels of topsoil potassium tend to reflect the finer grained soils. Soil potassium is usually derived from alumino-silicate minerals such as feldspars and micas, and consequently finer textured soils, with higher clay and silt content, tend to have higher potassium contents. Levels of potassium closely follow levels of aluminium in the soil series sampled. Hence the coarse grained soils of the Sherborne, Somerton, Wick, and Wilcocks and East Keswick series have the lowest levels of potassium. Chemically, potassium is a mobile element; its simple compounds are very soluble in water and, were it not for its incorporation into the fairly stable lattice structure of alumino-silicates it would be rapidly lost by leaching. Black (1968) cites a mean figure of 99.6% of total potassium to be in the form of a non-exchangeable component of the soil matrix.

Calcium

The calcium content of soils is widely variable. Rendzinas derived from calcium-rich parent material may have calcium contents of 16-20%, while acid sandy soils have less than 0.5%. In non-carbonate soils the calcium originates from the alumino-silicate and calcium-magnesium silicate minerals with the consequence that calcium content and clay content are often correlated.

Soils developed from the calcareous Lias of Somerset and Oolitic limestone of Gloucestershire exhibit significantly higher calcium levels in their topsoils than soils from other non-calcareous rocks in Devon, Warwickshire, Staffordshire and North Yorkshire. Highest levels of topsoil calcium are found in the Evesham (mean of 7%) and Sherborne (mean of 5.3%) series whilst lowest levels occur in soils of the Shifnal (mean of 0.18%) and Highweek (mean of 0.16%) series.

Zinc

The mean concentration of zinc in British soils is quoted as 100 ppm (Mitchell, 1971). The median values for the six field areas are all in the range of 70-110 ppm Zn. The soils sampled from the Dale series contain most zinc (mean of 240 ppm) and those from the Dunkeswick series the least (mean of 57 ppm). As zinc is held on the exchange complex, and in combination with organic matter, soils low in silt, clay and organic matter status are likely to be zinc depleted i.e. North Yorkshire soils.

Strontium

Strontium, like calcium, varies appreciably with parent material. A normal range of 50-1,000 ppm Sr might be expected in British soils (Swaine, 1955). Areas underlain by calcareous parent material appear to be those with elevated levels of strontium; the highest concentrations being recorded in Somerset for the organic Sedgemoor complex (mean of 268 ppm) and the Evesham series (mean of 104 ppm).

Manganese

In soil, manganese behaves similarly to iron, and consequently it shows similar distribution patterns in profiles. The total manganese

content may vary from a few ppm to 1%. The only area showing substantial manganese enrichment is in Brayford where soil samples from the Highweek series contain a mean manganese concentration of 1,600 ppm. This enrichment may possibly be attributed to past mining activities at several locations in the vicinity.

Arsenic

The mean range for arsenic in British soils is 5 - 100 ppm (Thornton and Webb, 1980). Samples from the soil series in Devon, Somerset, Warwickshire, Staffordshire and North Yorkshire have arsenic contents at the lower end of the range. Soils from Gloucestershire however, have slight arsenic enrichment in the Sherborne series (mean of 64 ppm).

Cadmium

Soil cadmium levels were generally below the detection limit (0.2 ppm). The few samples that exceeded this had values at the lower end of the normal range expected for British soils. Consequently cadmium data has been excluded from further discussion.

pH status

The six field areas may be ranked in order of decreasing pH units according to topsoil pH:

Glastonbury > Stow-on-the-Wold > Atherstone > Masham > Onecote > Brayford

alkalinity —————> neutrality —————> acidity

This trend follows the trend for total calcium content of topsoils. The highest pH values recorded are for the Evesham (mean of 7.9), Somerton (mean of 7.9) and Sherborne (mean of 7.8) series. The lowest pH values recorded are for the Highweek, Dartington and Loxhore series (mean of 5.8).

Organic matter status

Little variation in organic matter status is observed between the areas or soil series. Most samples have loss on ignition values of 7-13%. The main exception to this is the Sedgemoor organic complex in Somerset which has up to 58% organic matter content. The soils of Warwickshire exhibit a slight tendency to have generally lower organic contents than

elsewhere, probably due to the coarse grained parent material.

7.3 THE VERTICAL DISTRIBUTION OF ELEMENTS IN SOILS

In the present study it has not been possible to examine the distribution of metals within the soil profile in detail. Nevertheless, samples taken at two depths (0-15 cm and 30-45 cm) provide data illustrating the generalised relationships between the metal content of topsoils and subsoils. The ratios of topsoil:subsoil for each element, in each field area are presented in Tables 7.3-7.8. Ratios greater than one indicate topsoil enrichment, those less than one, topsoil depletion.

Pedological factors involved in profile differentiation include: surface enrichment due to organic matter accumulation; leaching of mobilised constituents; gleying; podzolisation; and microbiological activity on mobilisation. Certain trends can be identified from the tables of topsoil:subsoil ratios:

(a) An increase of sulphur in the topsoil of all soils except two organic peats from the Sedgemoor soil complex in Somerset. A mean ratio of two for sulphur is attributable to organic matter degradation of plant remains near the soil surface.

(b) An increase of calcium in the topsoil from all regions except Somerset. Enrichment is due to liming of agricultural land to maintain an optimum pH of about 6. Subsoils are inherently acidic when not derived from calcareous parent material. Soils in the Glastonbury area exhibit an apparent topsoil depletion of calcium, but this is an artefact caused by the calcareous bedrock. In absolute terms, the calcium content of Somerset topsoils is in the order of ten times greater than the other non-calcareous areas. If subsoil samples had been taken from the Sherborne series of Gloucestershire then these too would be expected to exhibit calcium enrichment for similar reasons.

(c) An increase of zinc and manganese in topsoils attributable to the zone of plant enrichment. Here zinc and manganese combine with organic matter or with secondary iron and manganese oxides and become immobilised.

(d) A decrease of topsoil molybdenum in soils of the Glastonbury district.

SAMPLE	SOIL SERIES	Cu	Mo	S	Fe	Zn	Sr	Cr	Al	Ca	Mn	As	K
1	Highweek	1.2	2.0	2.1	0.9	1.3	1.0	1.2	0.9	1.9	0.8	0.7	1.0
2	Loxhore	1.2	1.8	2.2	0.9	1.8	1.1	1.6	1.1	1.9	1.0	1.5	1.1
3	Highweek	0.6	1.2	2.1	0.9	1.0	1.0	1.0	1.0	1.4	1.2	0.9	1.0
5	Highweek	1.0	1.1	1.5	1.1	1.1	1.0	2.7	1.0	1.2	1.5	1.0	1.0
6	Highweek	1.0	0.7	2.1	1.0	1.1	1.1	2.1	1.0	2.0	1.1	1.1	0.9
7	Highweek	0.9	0.6	2.5	0.8	1.0	0.8	1.4	0.8	2.8	0.9	1.2	0.8
10	Highweek	0.7	0.9	2.1	0.7	1.1	0.9	1.4	0.7	1.5	0.8	0.9	0.7
11	Highweek	0.5	0.8	2.1	0.7	0.4	1.3	0.9	1.0	3.2	0.7	0.9	1.4
12	Highweek	1.1	0.6	2.2	0.9	1.1	0.9	4.0	1.0	2.1	1.3	0.8	0.9
13	Highweek	1.1	5.4	2.5	0.9	1.2	1.2	2.4	1.1	2.4	1.5	2.3	1.1

TABLE 7.3. Topsoil: Subsoil Ratios from Brayford, North Devon

SAMPLE	SOIL SERIES	Cu	Mo	S	Fe	Zn	Sr	Cr	Al	Ca	Mn	As	K
15	Evesham	1.0	0.8	3.3	1.0	1.1	0.6	2.0	0.9	0.7	1.3	1.1	0.9
16	Evesham	1.1	0.9	2.6	1.2	1.4	0.7	2.0	1.1	0.6	0.9	1.2	1.0
17	Butleigh	1.2	0.9	2.7	1.0	1.3	1.1	1.5	0.9	1.2	1.0	1.3	0.9
18	Sedgemoor	2.2	1.2	0.6	2.4	2.4	0.6	1.7	2.3	0.4	0.8	2.1	2.3
19	Midelney	1.0	0.8	1.8	1.0	1.2	1.0	1.2	0.9	1.2	1.9	1.0	1.0
20	Charlton Bank	0.8	1.0	2.9	0.9	0.9	0.9	1.4	0.8	1.4	0.5	1.0	0.7
22	Charlton Bank	0.8	1.1	2.9	1.1	1.3	0.3	1.1	0.9	0.3	1.2	1.3	0.9
24	Sedgemoor	1.1	1.0	0.9	1.0	1.4	0.8	1.4	1.1	0.7	1.5	1.4	1.0
25	Somerton	1.1	1.2	1.0	1.1	1.2	0.6	1.3	1.1	0.6	1.1	1.3	1.1
26	Worcester	1.2	0.9	1.7	1.0	1.2	1.1	1.5	1.0	1.2	1.0	1.5	1.3
29	Evesham	1.1	1.0	2.1	1.2	1.4	0.8	1.5	1.1	0.8	1.1	1.3	1.1

TABLE 7.4 Topsoil : subsoil ratios from Glastonbury, Somerset

SAMPLE	SOIL SERIES	Cu	Mo	S	Fe	Zn	Sr	Cr	Al	Ca	Mn	As	K
30	Denchworth	1.7	1.6	2.0	0.9	1.3	1.3	0.8	1.3	1.8	1.2	0.9	1.3
32	Denchworth	1.6	1.6	1.9	0.9	1.3	1.2	0.8	1.1	1.5	0.5	0.9	1.3
34	Waltham	1.0	1.0	1.4	1.0	1.1	1.0	1.0	0.9	1.1	0.9	1.4	0.9
35	?	0.9	1.2	1.9	1.1	0.6	1.7	1.2	1.1	3.0	1.0	0.9	1.0
38	?	1.1	1.8	2.0	1.0	1.3	0.9	1.4	0.9	1.4	1.3	1.7	0.9

TABLE 7.5 Topsoil : subsoil ratios from Stow-on-the-Wold, Gloucestershire

SAMPLE	SOIL SERIES	Cu	Mo	S	Fe	Zn	Sr	Cr	Al	Ca	Mn	As	K
39	Reclaimed	0.9	1.3	1.2	1.0	1.1	0.9	3.3	0.7	4.0	2.2	1.2	0.6
40	Merevale	0.9	1.1	1.9	0.9	1.5	0.8	3.6	0.7	1.8	1.7	1.0	0.6
41	Shifnal	1.1	2.0	1.8	1.0	1.2	2.3	2.0	1.0	1.0	1.2	1.3	1.0
43	Shifnal	1.0	0.3	1.2	1.0	1.1	0.8	1.0	1.0	0.9	0.8	1.0	0.9
44	Gatacre	1.2	0.8	2.2	1.2	1.7	1.3	2.1	1.3	1.5	1.1	1.4	1.2
45	Baxterley	1.7	1.4	2.3	0.9	2.0	0.9	1.6	0.9	1.5	0.6	1.1	0.9
46	Bardsey	1.3	0.4	1.7	1.1	1.4	1.0	1.1	0.9	1.1	1.4	1.5	0.9
47	Baxterley	0.8	0.7	2.1	0.6	1.1	1.1	2.6	0.6	1.0	0.4	0.8	0.9
48	Dodmoor	0.6	0.7	0.9	0.9	0.9	1.0	1.7	0.9	1.3	0.8	0.9	0.8
49	Shifnal	0.8	1.1	1.5	0.7	0.8	0.6	1.8	0.6	1.1	1.0	0.8	0.5
52	Bardsey	2.0	1.3	3.5	1.0	2.0	1.2	3.2	0.9	2.1	1.1	1.5	0.9

TABLE 7. 6 Topsoil : subsoil ratios from Atherstone, Warwickshire

SAMPLE	SOIL SERIES	Cu	Mo	S	Fe	Zn	Sr	Cr	Al	Ca	Mn	As	K
53	B/D/B	1.3	1.2	3.1	0.9	1.6	1.0	0.8	1.0	1.5	1.6	1.3	0.9
54	Onecote	1.0	0.5	3.2	0.5	1.2	0.8	0.6	0.7	1.1	1.5	0.6	0.5
55	Wilcox	1.0	0.4	2.7	0.5	1.1	0.8	0.8	0.7	1.1	0.9	1.0	0.6
56	Malham	0.9	1.0	1.6	0.8	1.2	0.9	0.8	0.8	1.3	0.8	1.2	0.8
57	Bodden	1.1	0.9	2.2	0.9	1.6	1.0	1.2	0.9	1.6	1.2	1.2	0.9
58	Ticknall	0.8	0.6	2.8	0.6	1.8	0.9	1.1	0.8	2.0	1.5	0.7	0.6
59	Dale	0.7	0.4	3.1	0.7	1.1	0.7	0.9	0.7	0.9	0.8	0.8	0.5
60	Dale	0.9	0.7	1.7	0.7	1.0	0.2	0.9	0.8	1.4	1.5	0.8	0.8
61	B/D/B	1.9	1.1	4.0	1.0	2.9	1.1	0.9	1.0	2.4	2.2	1.6	0.9
63	Brickfield	1.2	1.3	2.0	0.8	1.6	0.9	1.0	0.9	1.7	0.8	1.2	0.8
64	Dale	1.0	-	2.6	0.8	1.2	0.8	1.1	0.8	1.8	1.0	0.9	0.6
65	Dale	1.0	0.9	1.4	0.8	1.3	0.9	0.7	0.9	1.3	0.7	1.1	0.9
66	Brickfield	1.1	0.9	1.9	0.7	1.0	1.0	0.6	0.6	1.1	1.2	-	0.9

TABLE 7.7 Topsoil : subsoil ratios from Onecote, Staffordshire

(B/D/B is Brickfield, Dale, Bardsey series)

SAMPLE	SOIL SERIES	Cu	Mo	S	Fe	Zn	Sr	Cr	Al	Ca	Mn	As	K
68	Dunkeswick	2.1	0.6	2.9	0.7	1.2	1.0	1.1	0.8	6.8	0.4	2.6	0.9
73	Wilcocks	1.0	1.1	1.9	0.9	1.2	1.1	1.1	1.0	2.1	1.1	1.4	0.9
74	?	2.6	0.8	1.9	1.0	1.7	1.1	1.0	1.1	2.0	1.0	1.7	1.0
75	Wick	1.3	1.4	2.4	1.0	2.0	0.9	0.8	0.8	1.8	0.8	2.3	0.8
76	?	1.6	1.1	2.5	1.2	1.7	0.9	1.3	1.0	2.0	1.3	2.6	0.8
77	E. Keswick	1.3	1.2	0.8	1.0	1.3	1.4	1.1	1.0	4.2	1.0	1.8	1.1
78	E. Keswick	1.2	1.2	1.1	0.8	1.1	0.9	1.0	0.8	1.0	0.9	1.5	0.8
79	Wick	1.1	0.9	2.4	0.7	1.3	1.0	0.9	0.9	1.8	0.5	1.3	0.8

TABLE 7. 8 Topsoil : subsoil ratios from Masham, North Yotkshire

Most of the soils in this area are imperfectly drained and of high pH. Lewis (1943) noted that the molybdenum content of these soils increased with depth, over the Lower Lias. The same trend is apparent here. Molybdenum (present in Somerset soils as the mobile MoO_4^{2-} ion) is susceptible to leaching down the profile. The prevailing high pH conditions in the topsoil also favour plant uptake of molybdenum, which further adds to topsoil depletion of this element. Where there is low topsoil pH (e.g. sample 24) or a very high organic matter content (e.g. sample 18) molybdenum is retained by sorption onto clay minerals, ferric oxides or in combination with organic matter. Consequently ratios greater than one were obtained for these two samples.

(e) Topsoil depletion of molybdenum, iron, aluminium, potassium, strontium and chromium in the soils in the Onecote district of Staffordshire. This may be attributed to mobilisation of these elements during gleying of the poorly drained profiles, typical of the area.

No other consistent trends in topsoil:subsoil ratios are detectable. Variations are small and probably of no major significance. Owing to no subsoil data for the two podzols in Devon, the vertical distribution of elements in a podzolised profile cannot be discussed.

7.4 HERBAGE DATA

(a) Data presentation

Mean levels of major and trace elements in herbage samples are presented in Appendix 3f - 3k. These represent element means over the grazing season. Variations in herbage content between areas and seasons are summarised in boxplots (Figure 7.2 (a)-(k)). These are based on May, July and September herbage data.

(b) Interpretation of herbage data

Copper

There is a clear increase in herbage copper content over the grazing season. Lowest levels of copper are consistently found in herbage samples from

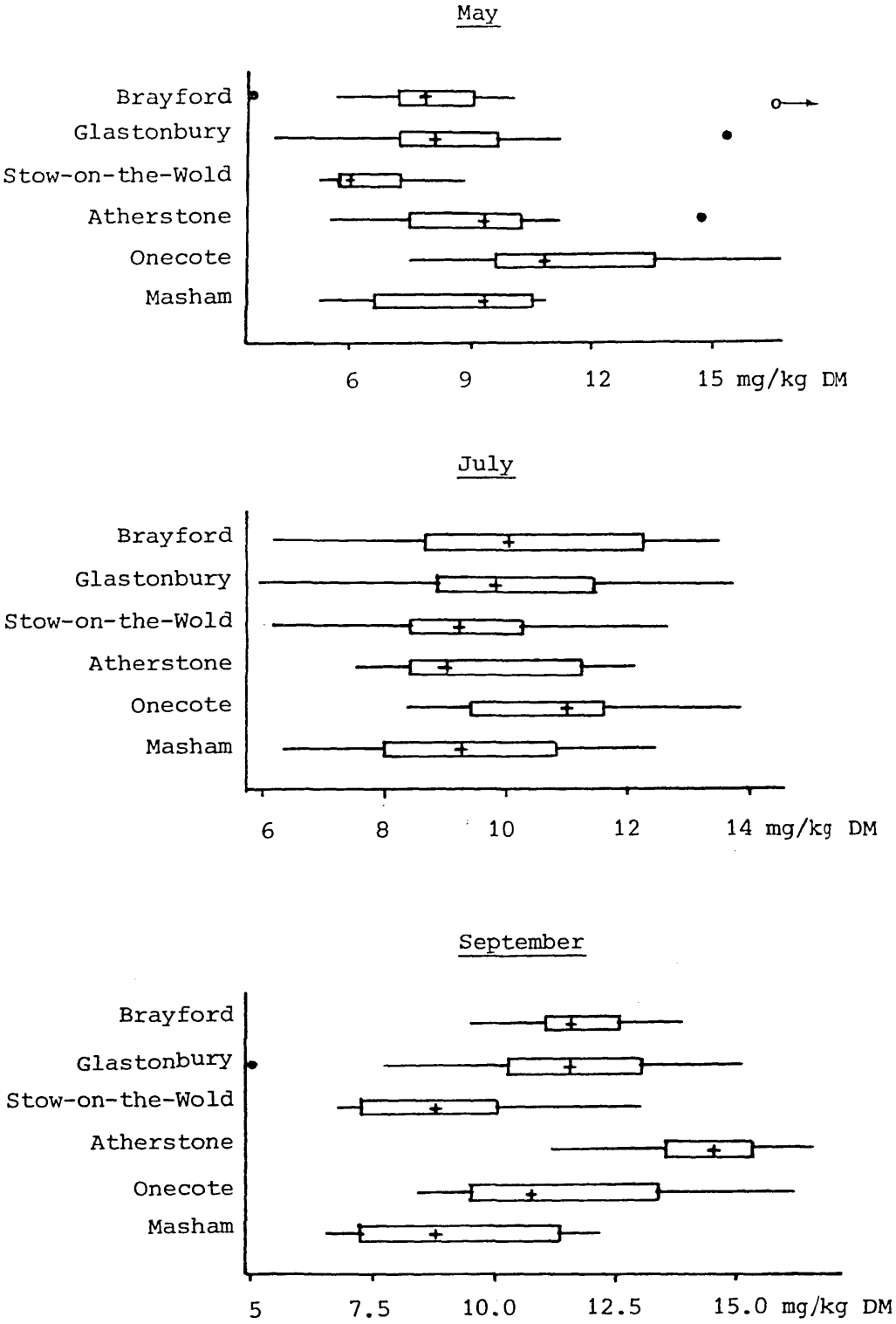


FIGURE 7.2. Boxplots of herbage data - (a) Copper in herbage

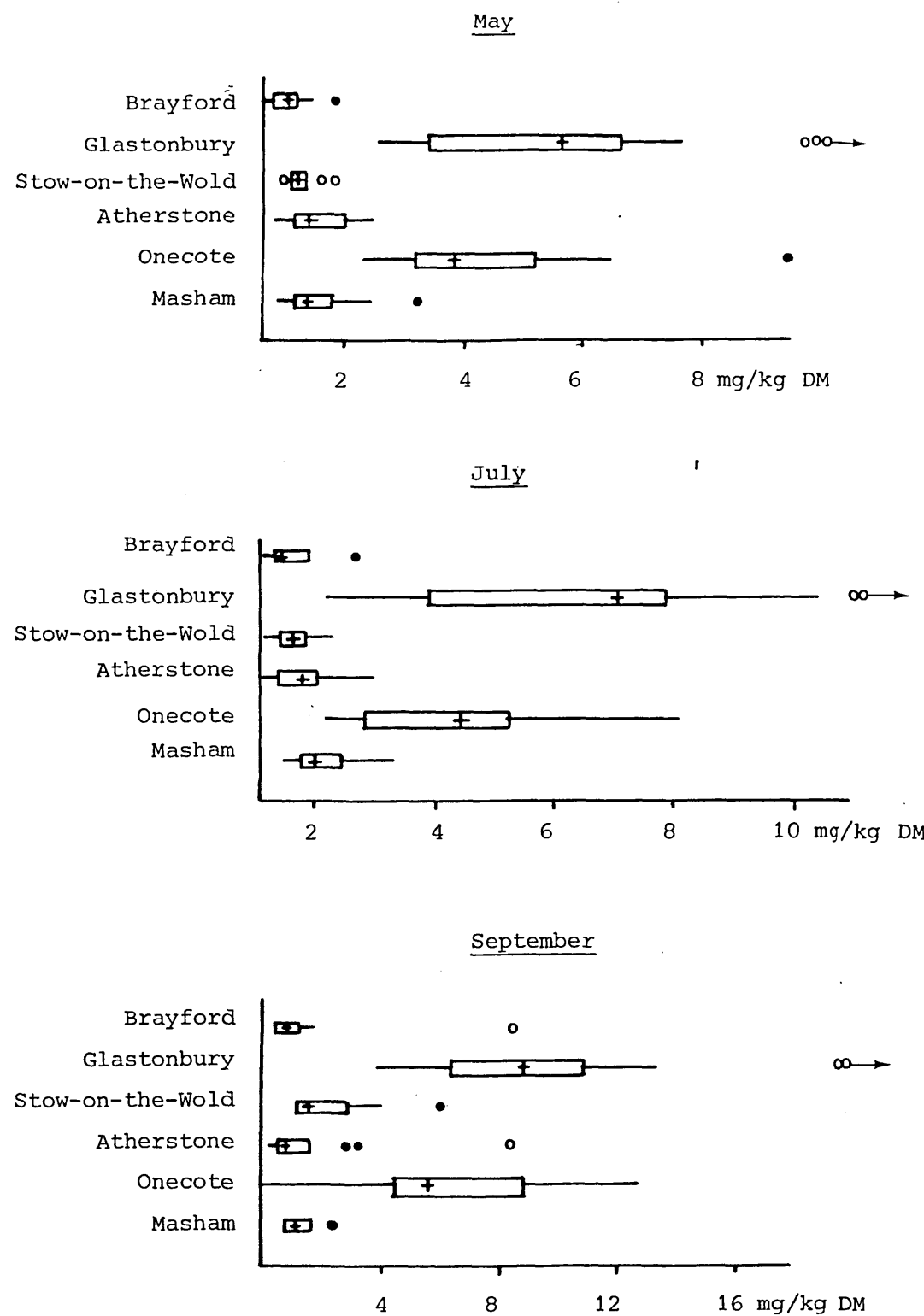


FIGURE 7.2. Boxplots of herbage data - (b) Molybdenum in herbage

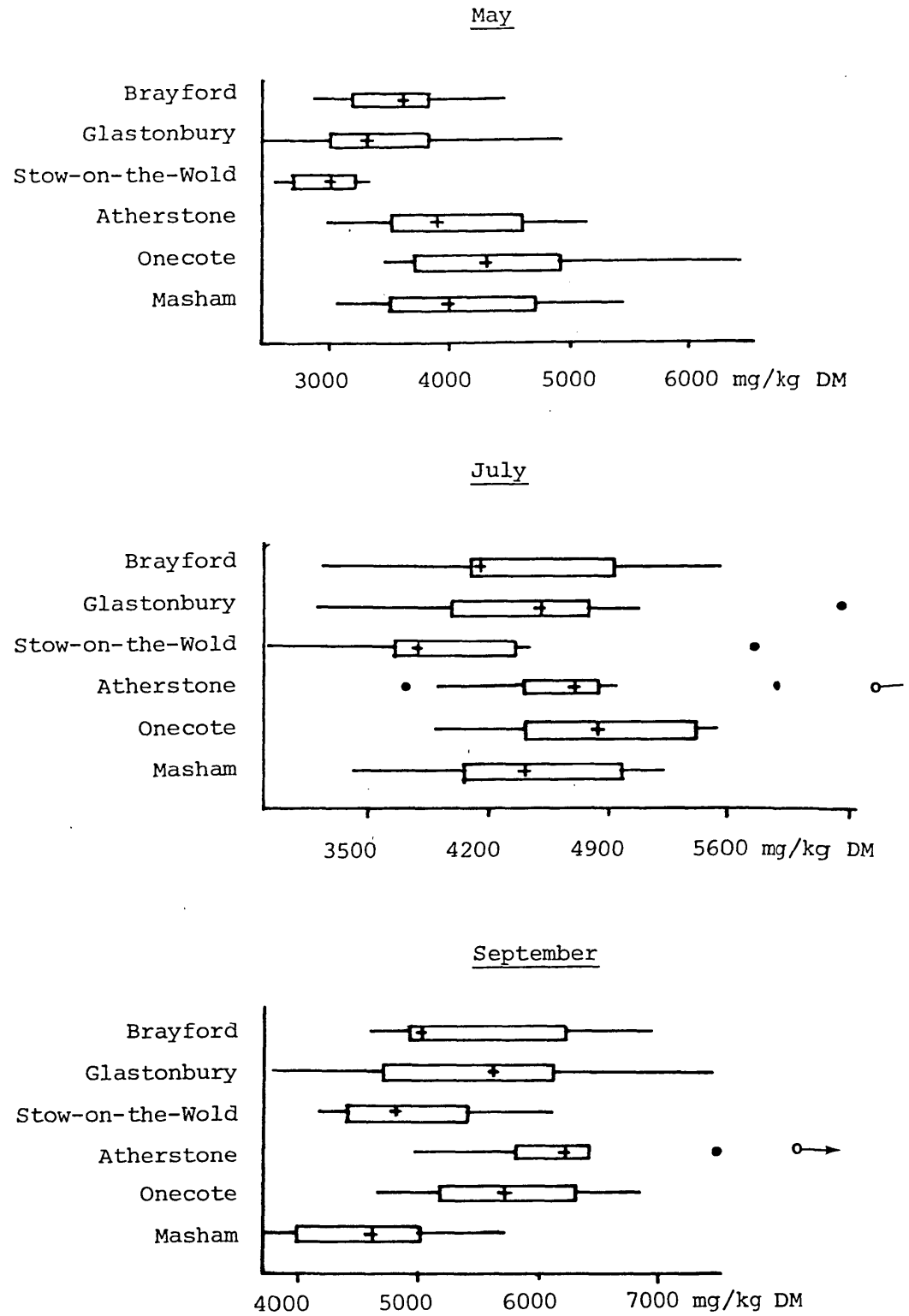


FIGURE 7.2. Boxplots of herbage data - (c) Sulphur in herbage

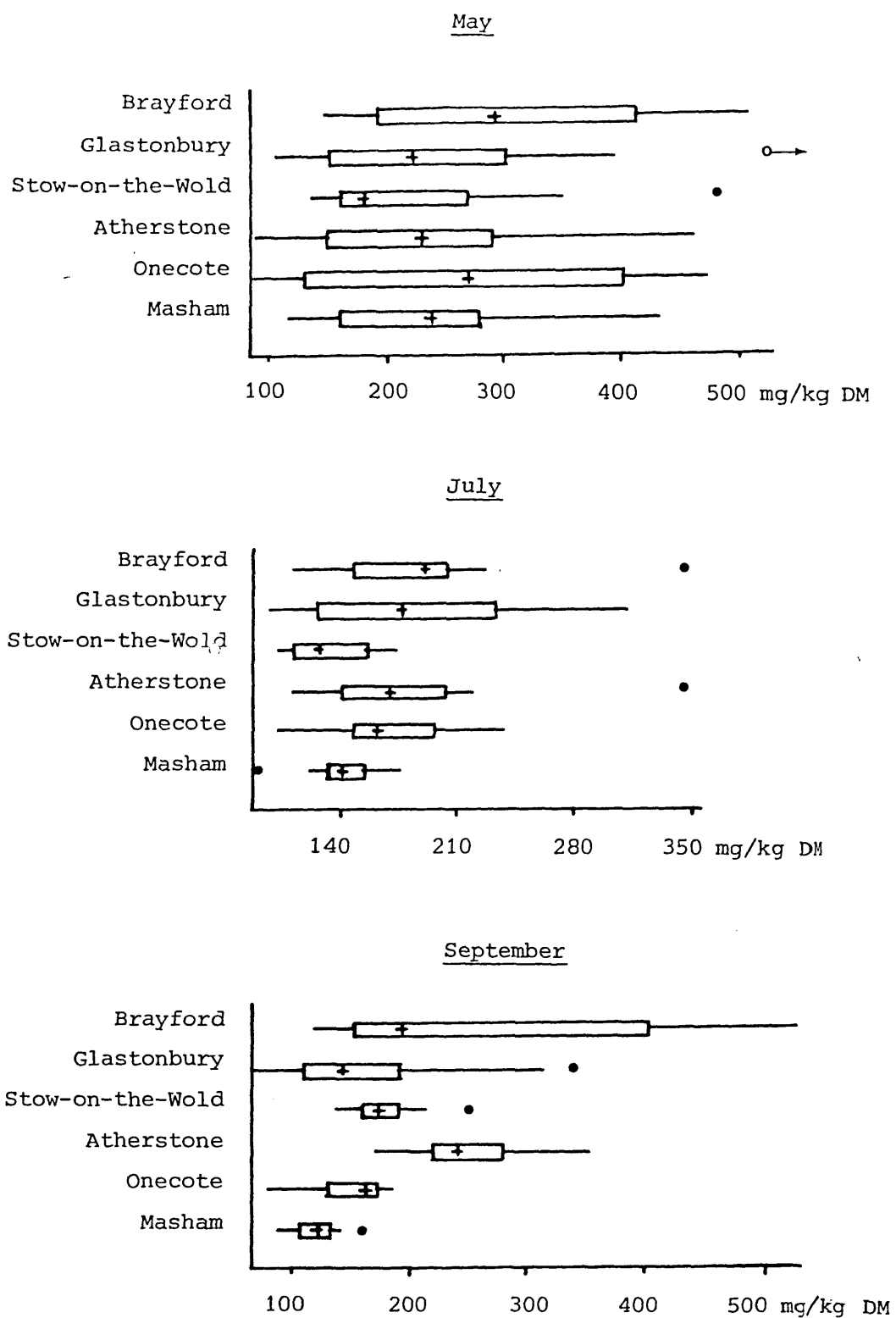


FIGURE 7.2. Boxplots of herbage data - (d) Iron in herbage

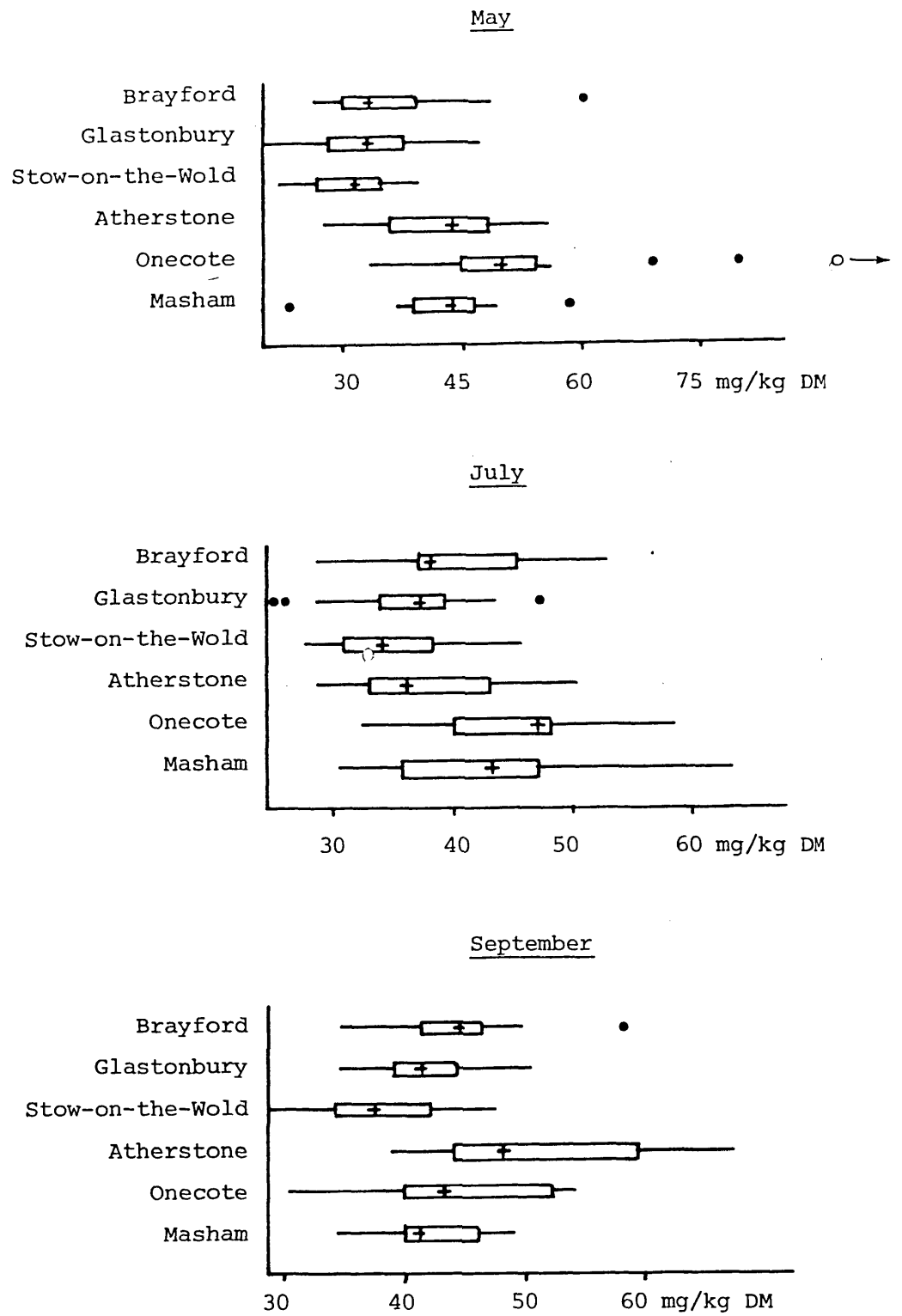


FIGURE 7.2. Boxplots of herbage data - (e) Zinc in herbage

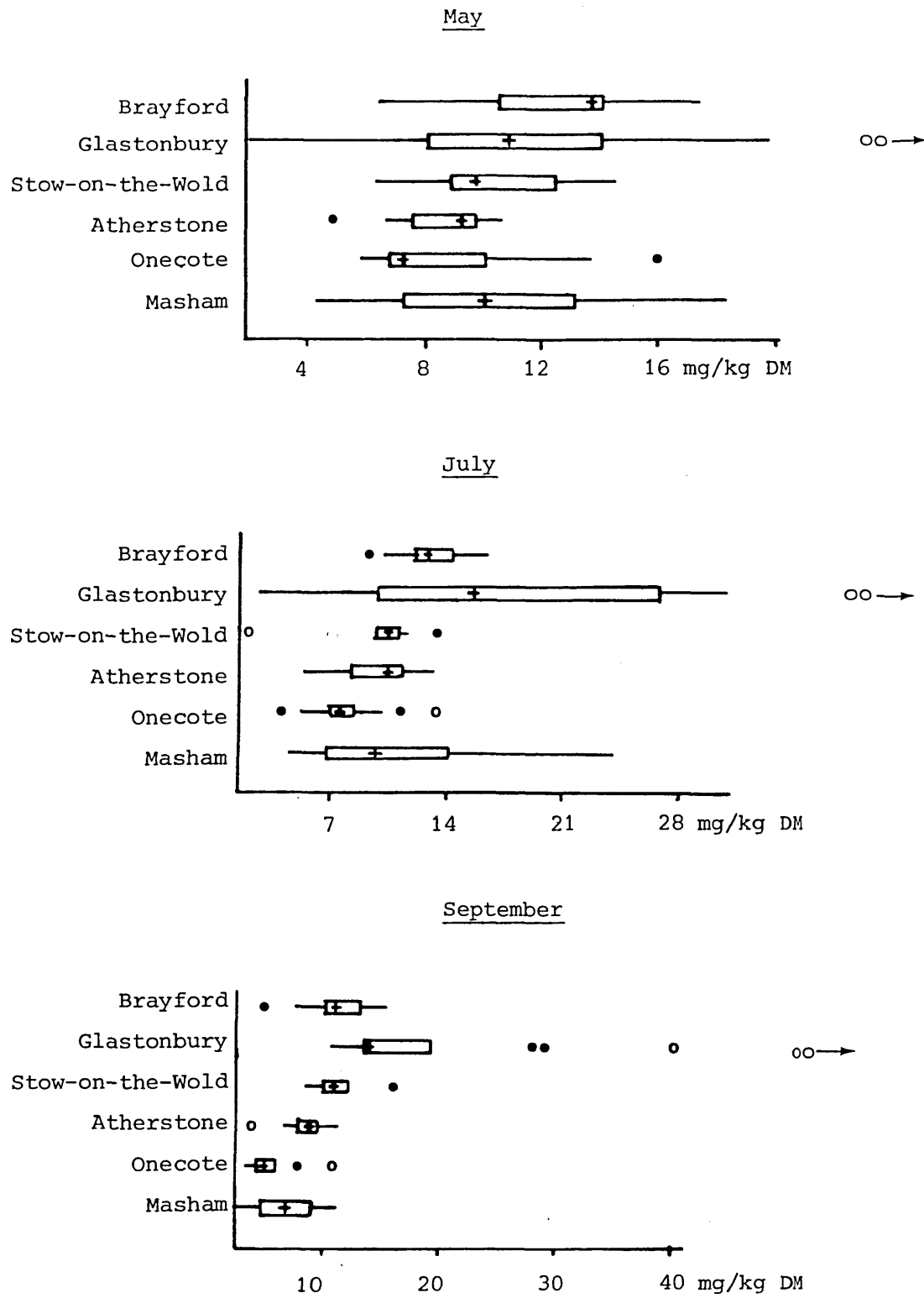


FIGURE 7.2. Boxplots of herbage data - (f) Strontium in herbage

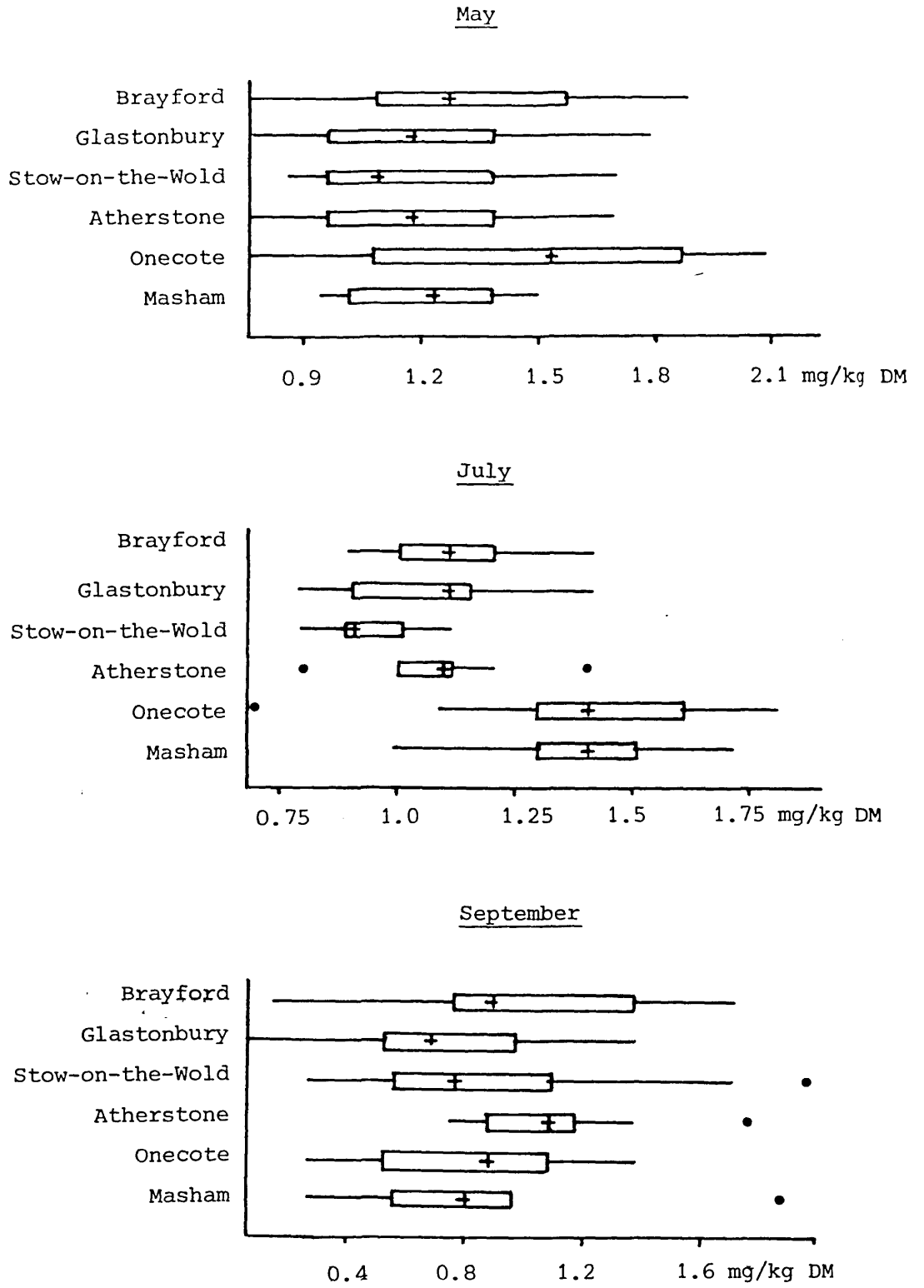


FIGURE 7.2. Boxplots of herbage data - (g) Chromium in herbage

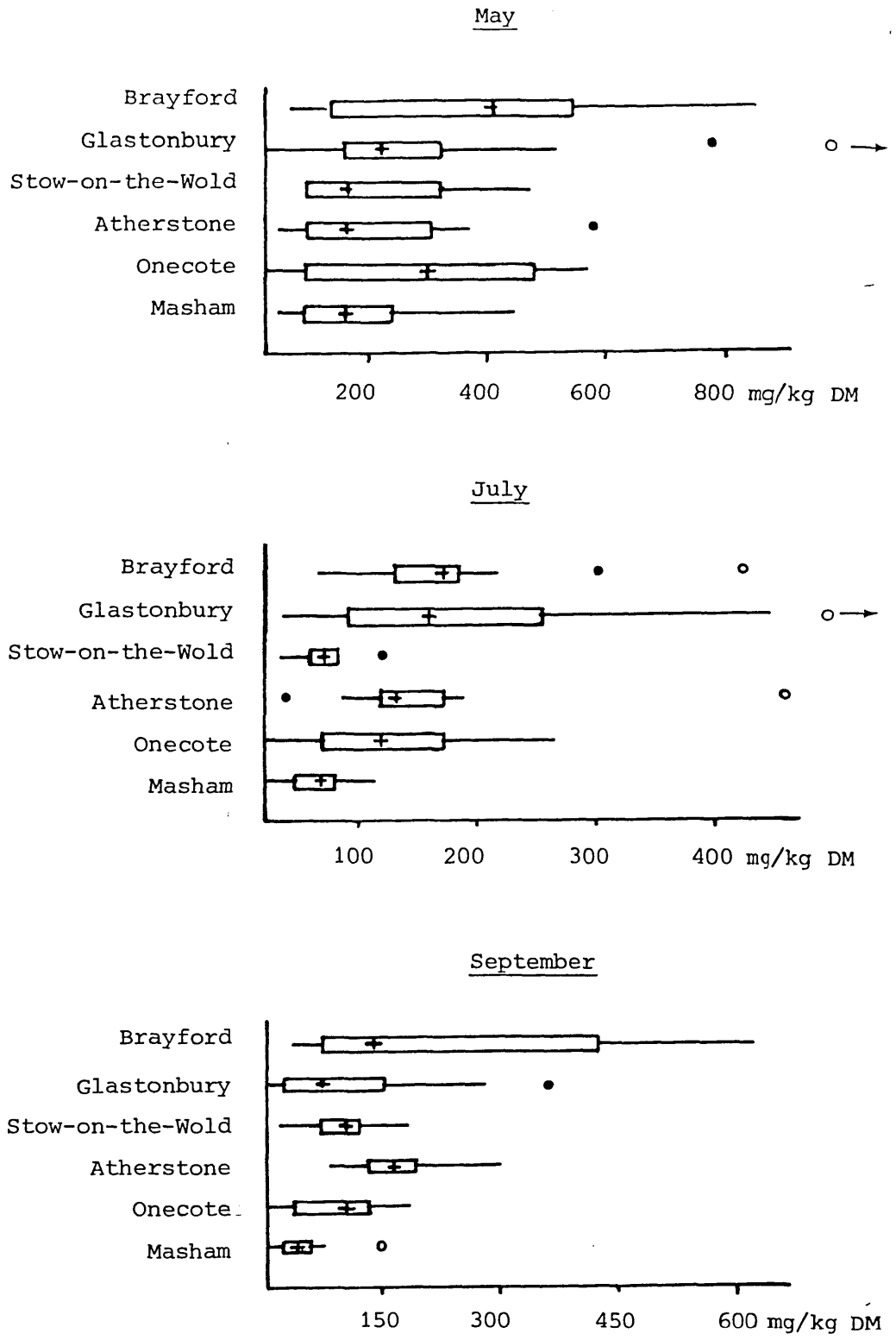


FIGURE 7.2. Boxplots of herbage data - (h) Aluminium in herbage

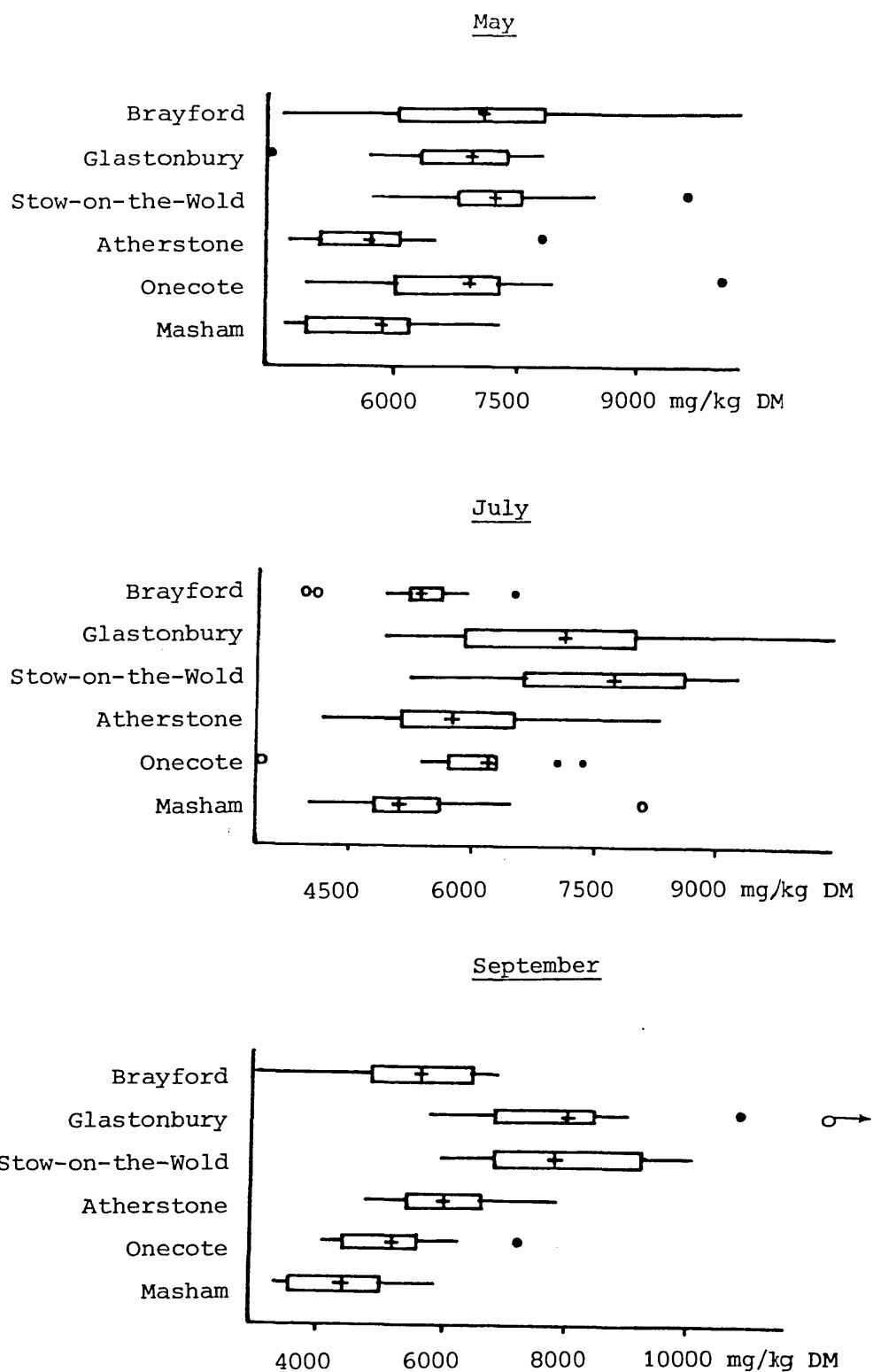


FIGURE 7.2. Boxplots of herbage data - (i) Calcium in herbage

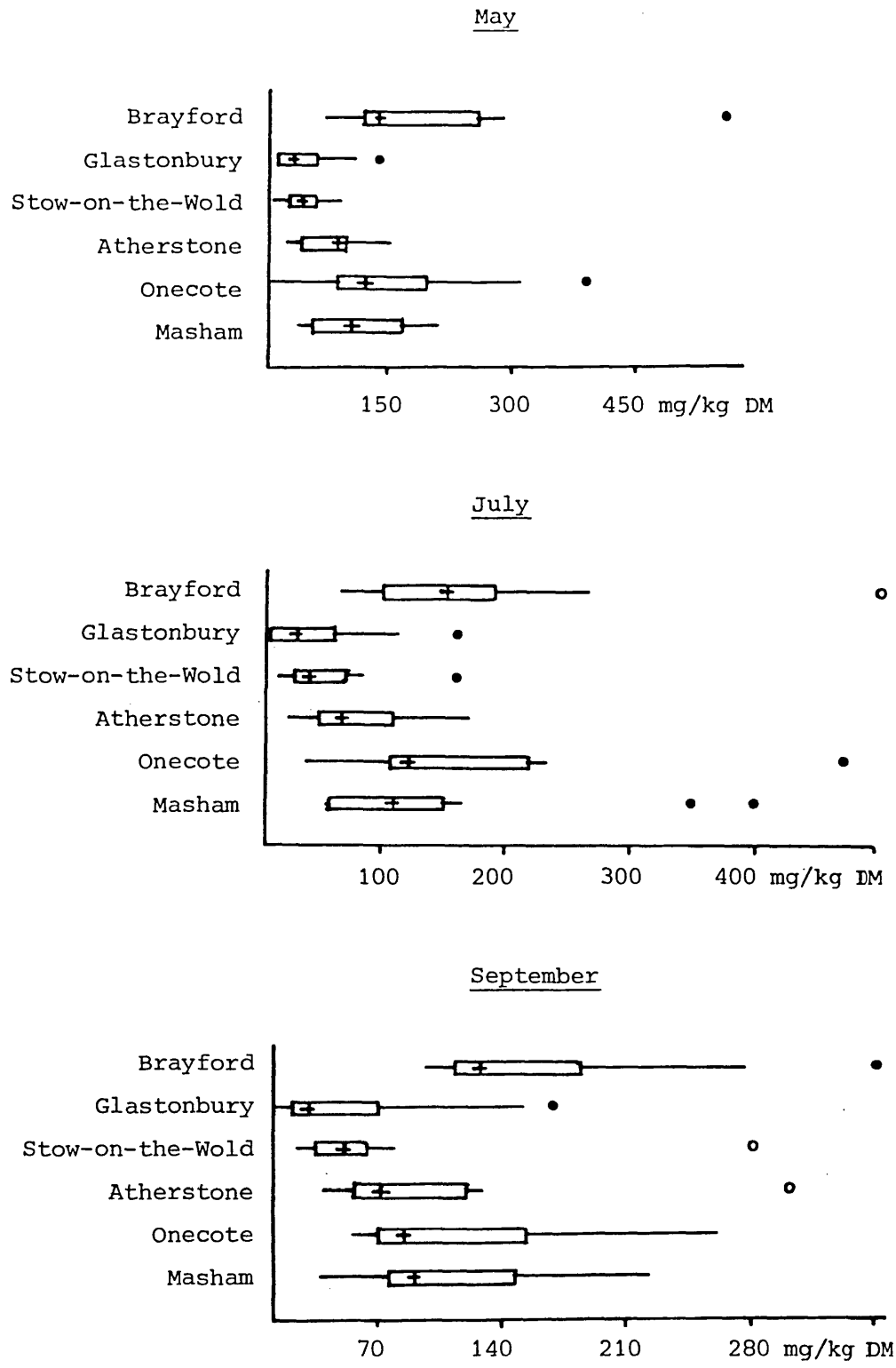


FIGURE 7.2. Boxplots of herbage data - (j) Manganese in herbage

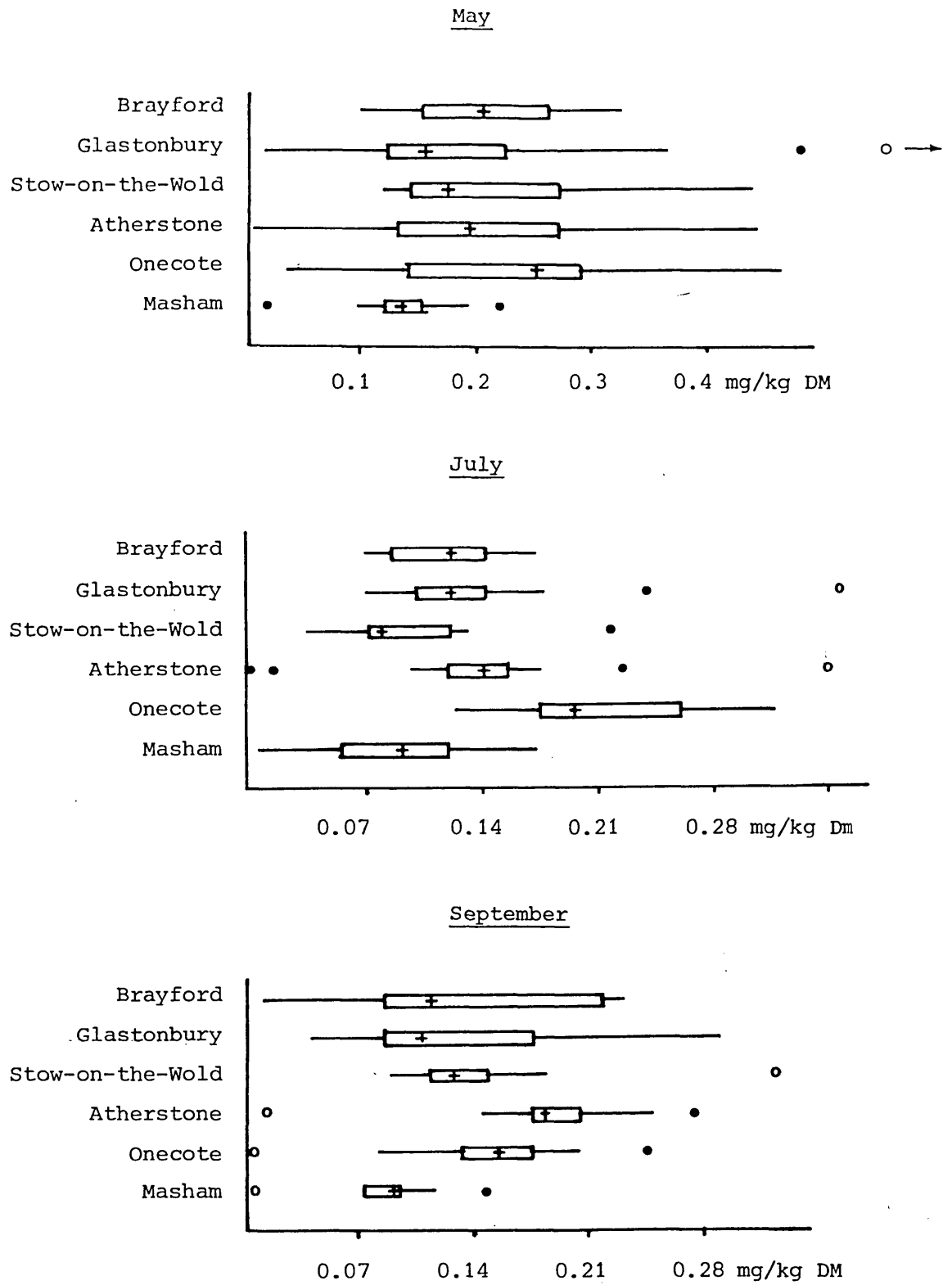


FIGURE 7.2. Boxplots of herbage data - (k) Arsenic in herbage

Gloucestershire and North Yorkshire farms which may be attributed to their low soil copper levels (both < 20 ppm). As high soil pH results in low copper availability, herbage samples from the Sherborne series in Gloucestershire have the lowest copper contents of all. (< 10 mg/kg DM). The lower pH status of North Yorkshire soil series maintains their limited copper supply in an available form for plant uptake. Consequently, herbage copper levels are higher than in Gloucestershire. It is interesting to note that soils rich in copper (Evesham, Middelney, Charlton Bank and Somerton series) do not support herbage proportionally rich in copper. This is partly due to their high pH reducing copper availability and partly due to exclusion mechanisms of the plant itself.

Molybdenum

A seasonal increase in herbage molybdenum content is detectable, particularly in Somerset. Levels are greatest in Glastonbury and Onecote, where concentrations far exceed the normal range for British forages (0.1–3.0 mg/kg DM). This is attributable to their molybdeniferous soils – Evesham and Middelney series contain 41 ppm in the soil and up to 38 mg/kg DM in associated herbage. The prevailing high pH status of these soils facilitates molybdenum uptake. In Staffordshire, where soil pH is considerably lower, molybdenum uptake is partially restricted which results in lower levels of herbage molybdenum.

Sulphur

Herbage sulphur shows the same seasonal increases as herbage copper and molybdenum. The range in concentration (from 3,000 mg/kg DM in May, to 6,000 mg/kg DM in September) is consistent with that recorded by Whitehead (1966) and Whitehead, Jones and Barnes (1978) for ryegrass. Surveys of grasslands in Pembrokeshire (Williams, 1975) and southern Scotland (McLaren, 1975) however, have indicated herbage sulphur levels lower than these, possibly because permanent grasslands contain less sulphur.

Herbage sulphur levels far exceed soil levels. The boxplots (Figure 7.1 (c) and 7.2 (c)) and raw data tables (Appendix 3) indicate no clear relationship between soil sulphur content and the corresponding herbage concentration. Soils in Atherstone for example, have the lowest sulphur contents of all yet they support herbage with the highest sulphur levels.

The Shifnal series in the area has a mean soil sulphur concentration of 248 ppm and a mean herbage sulphur level of 5,100 mg/kg DM. In contrast, Sherborne series in the Stow-on-the-Wold district has a mean sulphur content of 675 ppm and only 3,580 mg S/ kg DM in associated herbage. Clearly total soil sulphur is not a good index of plant-available sulphur. The relationship is probably complicated by the atmospheric input of sulphur to plants. As unwashed herbage was analysed, dry deposition and uptake of SO_2 by the plant could have obscured any soil-plant sulphur relationship. The significance of atmospheric inputs of sulphur to pasture, and hence the bovine, is discussed in Section 7.9.

Iron

Uncontaminated herbage contains less than 200 mg/kg DM iron. Whitehead (1966) has even recorded levels <100 mg/kg DM for washed ryegrass. As the relative accumulation ratio (plant content/soil content) is low for iron, herbage is particularly susceptible to soil-iron contamination. The boxplots (Figure 7.2 (d)) show herbage iron levels to be at a maximum in May, when they frequently exceed the 200 mg/kg DM typical of uncontaminated herbage. Soil contamination is likely to have arisen in May through soil splash and ruminant poaching, and aggravated by the short height of the pasture at this time of the year. Highest levels of herbage iron are recorded for Brayford and Onecote samples.

Zinc

The range in herbage zinc concentration (30-60 mg/kg DM) is consistent with that recorded by Whitehead (1966). The lack of any detectable seasonal variation in zinc herbage levels is also compatible with his findings. The higher levels of soil zinc in Staffordshire and Somerset are only expressed to a limited extent in herbage zinc contents. The high soil pH status impedes zinc uptake by herbage in Somerset. Consequently, it is only the herbage associated with the Dale soil series in Staffordshire that reflects soil zinc enrichment to any extent.

Other elements

Chromium, aluminium and arsenic, like iron, are good indicators of soil contamination in herbage. Consequently, herbage contents of these

elements are greatest in May and lowest in July. Where soil contamination is low, the levels of these elements fall in the normal range expected for forages. Little can be inferred about seasonal uptake of aluminium, chromium and arsenic from the unwashed herbage data, owing to the effects of soil contamination.

Total manganese content of soils gives no idea of its availability to plants as it may occur in the di-, tri-, or tetra-valent state, of which only the first is particularly soluble. Nevertheless, soil enrichment in north Devon is reflected to a certain degree in raised herbage manganese levels. This is due to the prevailing low pH status of these soils maintaining manganese availability to the plant. In Somerset soils, where the pH regime is very high, the tetra-valent manganese ion predominates making manganese unavailable to plants.

Herbage cadmium levels, like soil concentrations, were either below the detection limit (0.06 mg/kg DM) or at the lower end of the normal range for forages (i.e. were ≤ 0.5 mg/kg DM). Consequently, they have been excluded from further discussions. Cadmium is unlikely to be a causal factor of bovine hypocupraemia on any of the farms sampled.

7.5 ROBUST CORRELATIONS BETWEEN SOIL AND HERBAGE DATA FOR MAY, JULY AND SEPTEMBER

Robust estimates

It has long been recognised that the presence of samples of unusual composition in a data set will affect estimation statistics such as mean, standard deviation, correlation coefficient, regression equations etc. The root of the trouble is that such outliers have an unusually large influence on the least squares estimators, essentially biasing the least squares 'fit' towards them (Howarth, 1984). Inferences based on classical means and covariances may be misleading therefore. Accordingly, robust (resistant) techniques have been developed which ensure that a few extraordinary values do not distort the results of the analysis. This is achieved by down-weighting the influence of outliers far from the multi-variate mean.

A computer program (kindly made available by Dr N. A. Campbell, CSIRO, Perth) to calculate robust Pearson (linear) correlations was used.

Full weight (1.0) was given to observations assumed to come from the main body of the data. Atypical data points were given an iteratively derived weighting ($0 \leq 1$) to reduce their influence. The actual weight given to each observation, in the calculation of weighted estimates, was an inverse function of the Mahalanobis distance of the data point from the overall mean composition*. The influence of the few observations with unduly large Mahalanobis distances was therefore greatly reduced.

Robust correlation matrices for May, July and September soil and herbage data are presented in Tables 7.9 - 7.11. Correlations significant at the 0.001 level are underlined.

7.6 INTERPRETATION OF ROBUST CORRELATION MATRICES

Copper

Soil copper levels are significantly correlated with many factors. Correlation with potassium and aluminium reflect association of copper with clay minerals on the exchange complex. Similarly, correlation with iron is also probably related to clay content through combination with iron oxides. Correlation of soil copper with soil molybdenum reflects the copper-nickel-molybdenum-vanadium association in the shales of Somerset and Staffordshire. Finally, the positive correlation between zinc and copper in soils is well recognised (Vinogradov, 1959) - with zinc concentrations approximately twice those of copper.

Herbage copper levels are most consistently correlated with herbage zinc concentrations for all three seasons. This might be anticipated from their similar chemical and biochemical properties. As stated above, copper and zinc are correlated in soils, and as similar factors control plant uptake of these two elements, copper and zinc are consequently correlated in herbage.

There was no significant correlation between soil copper and herbage copper levels. This might be expected since total soil copper rarely reflects that which is available for plant uptake. As the September

* The Mahalanobis distance is defined as $(X - \bar{X})^t S^{-1} (X - \bar{X})$, where S is the covariance matrix, $\bar{X}$ the mean vector and X the sample composition.

	CuH	MoH	SH	FeH	ZnH	SrH	CrH	AlH	CaH	MnH	AsH	pH	OM	CuS	MoS	SS	FeS	ZnS	SrS	CrS	AlS	CaS	MnS	AsS	KS
CuH	1	.27	<u>.58</u>	.31	<u>.53</u>	-.17	.26	.22	-.13	.10	.22	-.10	-.06	.16	.14	-.07	-.16	.25	-.15	.06	-.08	-.15	.06	-.22	-.07
MoH		1	-.17	.04	-.10	.11	-.01	.05	.07	-.26	-.05	<u>.45</u>	.18	<u>.63</u>	<u>.93</u>	.17	.15	<u>.40</u>	<u>.39</u>	.36	.18	<u>.49</u>	-.13	-.04	.20
SH			1	.14	<u>.68</u>	-.25	.24	.05	-.20	.24	.15	-.33	-.02	-.23	.26	-.05	-.50	-.12	<u>-.38</u>	-.23	-.33	<u>-.39</u>	-.11	-.33	-.26
FeH				1	.32	.30	<u>.77</u>	<u>.86</u>	.16	.28	<u>.59</u>	-.20	.01	.19	-.01	.02	.25	.23	.02	.07	.23	-.13	.36	.01	.24
ZnH					1	-.27	<u>.39</u>	.19	-.33	.32	.36	<u>-.41</u>	-.08	-.19	-.21	-.12	<u>-.47</u>	-.09	<u>-.42</u>	-.16	-.36	<u>-.42</u>	-.20	-.28	-.33
SrH						1	.22	.37	.29	.24	.13	-.08	<u>.40</u>	.24	.13	<u>.56</u>	.31	.19	<u>.49</u>	-.11	<u>.40</u>	.07	<u>.42</u>	-.08	<u>.47</u>
CrH							1	<u>.74</u>	.14	.29	<u>.57</u>	-.23	.11	.07	-.05	.04	.01	.11	-.06	.03	.06	-.18	.15	-.05	.08
AlH								1	.19	.37	<u>.48</u>	-.29	.07	.24	.03	.06	.33	.20	.09	.01	<u>.43</u>	-.16	<u>.50</u>	-.02	<u>.43</u>
CaH									1	.07	.12	.03	.15	.01	.06	.04	.21	.10	.20	.09	.24	.15	.18	.10	.19
MnH										1	.35	<u>-.68</u>	.08	-.33	-.27	-.06	-.10	-.15	-.24	-.25	.03	<u>-.42</u>	.22	-.11	.00
AsH											1	-.22	.04	.01	-.09	.09	.04	.10	.04	.14	.03	-.07	.06	.12	-.01
pH												1	-.12	<u>.40</u>	<u>.44</u>	.06	.26	.19	<u>.50</u>	<u>.49</u>	-.06	<u>.77</u>	-.23	.32	.01
OM													1	.22	.23	<u>.74</u>	.04	.21	.29	.06	.19	.02	-.06	.00	.10
CuS														1	<u>.69</u>	.26	<u>.46</u>	<u>.63</u>	<u>.63</u>	.36	<u>.54</u>	<u>.45</u>	.21	.04	<u>.54</u>
MoS															1	.22	.29	<u>.50</u>	<u>.43</u>	<u>.44</u>	.28	<u>.46</u>	-.08	.09	.24
SS																1	.09	.19	<u>.43</u>	.19	.13	.13	-.01	.06	.10
FeS																	1	<u>.55</u>	<u>.53</u>	<u>.40</u>	<u>.79</u>	.37	<u>.63</u>	<u>.63</u>	<u>.68</u>
ZnS																		1	.35	<u>.45</u>	<u>.50</u>	.21	.13	.24	.41
SrS																			1	.22	<u>.52</u>	<u>.73</u>	.24	.31	<u>.54</u>
CrS																				1	.07	<u>.44</u>	-.13	<u>.58</u>	-.06
AlS																					1	.14	<u>.71</u>	.24	<u>.91</u>
CaS																						1	-.07	<u>.47</u>	.17
MnS																							1	.11	<u>.74</u>
AsS																								1	.08
KS																									1

TABLE 7.9 Robust correlation matrix for May data

Note: $n = 79$, $n' = n - \sum(1 - \text{non unit weight}) = 70$, $r_{.001} = \pm 0.38$, H = herbage, S = soil

	CuH	MoH	Sh	FeH	ZnH	SrH	CrH	AlH	CaH	MnH	AsH	pH	OM	CuS	MoS	SS	FeS	ZnS	SrS	CrS	AlS	CaS	MnS	AsS	KS
CuH	1	.14	.26	.17	<u>.51</u>	-.01	.02	.06	.22	.22	.04	-.20	-.15	.20	.09	-.06	.21	.20	.15	.07	.18	-.01	.23	.12	.11
MoH		1	.04	.05	.04	-.15	.24	.06	.33	.05	.24	.23	.20	<u>.39</u>	<u>.68</u>	.16	-.03	.28	.38	.13	.02	<u>.46</u>	-.30	.11	-.04
SH			1	-.11	.02	-.04	.03	-.06	.37	-.07	.17	.16	-.22	.07	-.02	-.07	.00	.04	.03	.06	.09	.05	.06	-.08	.09
FeH				1	.29	.15	.25	<u>.93</u>	-.12	.16	.29	-.25	.16	<u>.48</u>	.08	.17	.16	.34	.31	-.01	.33	.01	.12	.07	.30
ZnH					1	.02	<u>.64</u>	.15	-.14	<u>.66</u>	.22	<u>-.57</u>	.15	-.10	.00	.05	-.16	.09	-.10	-.04	-.09	-.37	-.16	.06	-.24
SrH						1	.00	.22	.08	.08	-.22	-.10	.23	.03	-.04	.37	.33	.22	.35	.09	.31	.06	.26	.13	.28
CrH							1	.15	.05	<u>.51</u>	.22	-.32	.27	-.04	.11	.07	-.26	.05	-.03	-.17	-.15	-.26	-.25	-.13	-.32
AlH								1	-.12	.17	.30	-.26	.17	<u>.56</u>	.13	.19	.27	.34	<u>.42</u>	.02	<u>.49</u>	.04	.22	.10	<u>.46</u>
CaH									1	-.30	-.00	<u>.39</u>	-.04	.11	.24	.03	.10	.14	.30	.25	.07	.37	-.15	.12	-.02
MnH										1	.29	<u>-.61</u>	.26	-.12	.04	.09	-.11	-.07	-.01	-.09	.07	.33	-.04	.08	-.04
AsH											1	-.18	-.01	.27	.35	.05	-.15	.19	.03	.14	.04	-.13	-.21	.25	-.10
pH												1	-.20	.17	.19	-.05	-.03	.04	.13	.28	-.24	<u>.71</u>	-.22	-.13	-.13
OM													1	.17	.28	<u>.75</u>	.08	.17	<u>.41</u>	.06	.19	.21	-.07	.27	.06
CuS														1	<u>.58</u>	.23	<u>.51</u>	<u>.64</u>	<u>.56</u>	.36	<u>.53</u>	<u>.51</u>	.25	<u>.45</u>	<u>.43</u>
MoS															1	.26	.26	<u>.57</u>	<u>.42</u>	<u>.43</u>	.22	<u>.45</u>	-.18	<u>.49</u>	.07
SS																1	.07	.17	<u>.54</u>	.16	.12	.26	-.01	.15	.05
FeS																	1	<u>.52</u>	<u>.55</u>	.22	<u>.85</u>	.25	<u>.69</u>	<u>.64</u>	<u>.75</u>
ZnS																		1	.38	<u>.49</u>	<u>.43</u>	.26	.09	<u>.61</u>	.24
SrS																			1	.04	<u>.64</u>	<u>.54</u>	.35	.34	<u>.59</u>
CrS																				1	.03	.33	-.13	<u>.46</u>	-.14
AlS																					1	.11	<u>.69</u>	<u>.51</u>	<u>.90</u>
CaS																						1	.07	.21	.18
MnS																							1	.24	<u>.75</u>
AsS																								1	.29
KS																									1

TABLE 7.10 Robust correlation matrix for July data

Note: $n = 79$, $n' = n - \sum(1 - \text{non unit weight}) = 67$, $r_{.001} = \pm 0.39$, H = herbage, S = soil

	CuH	MoH	SH	FeH	ZnH	SrH	CrH	AlH	CaH	MnH	AsH	pH	OM	CuS	MoS	SS	FeS	ZnS	SrS	CrS	AlS	CaS	MnS	AsS	KS
CuH	1	.09	<u>.46</u>	<u>.48</u>	<u>.56</u>	.11	.24	.39	.26	-.00	.35	-.07	<u>-.42</u>	.34	.10	-.14	.11	.07	.13	-.01	.18	.01	.10	.05	.27
MoH		1	.15	-.22	-.04	-.01	-.18	-.18	.13	-.12	.03	.23	.28	<u>.44</u>	<u>.63</u>	.19	-.17	.33	.36	.17	-.07	<u>.42</u>	<u>-.41</u>	.06	-.11
SH			1	.23	.34	-.05	.04	.17	.20	-.05	<u>.47</u>	-.06	-.16	.20	.12	-.05	-.14	-.02	-.00	.02	.05	-.15	-.15	.04	.09
FeH				1	.35	.22	<u>.62</u>	<u>.97</u>	.17	.06	<u>.45</u>	-.18	-.28	.18	-.20	-.09	.30	.09	.08	-.11	.36	-.09	.37	.08	<u>.43</u>
ZnH					1	-.13	.32	.22	-.12	.20	.11	-.34	-.09	-.04	-.04	-.07	-.24	-.13	-.16	-.10	-.18	-.17	-.20	-.09	-.12
SrH						1	.01	.23	<u>.50</u>	-.01	.08	.14	.29	.32	.11	<u>.64</u>	<u>.49</u>	.20	<u>.74</u>	.03	<u>.49</u>	<u>.44</u>	.38	.23	<u>.52</u>
CrH							1	<u>.60</u>	.23	.05	.24	-.08	-.26	.05	-.18	-.12	.18	.02	-.13	.02	.08	-.14	.17	.01	.11
AlH								1	.15	.12	<u>.41</u>	-.23	-.19	.22	-.15	-.05	.37	.17	.12	-.10	<u>.45</u>	-.10	<u>.42</u>	.11	<u>.48</u>
CaH									1	<u>-.42</u>	.13	<u>.52</u>	-.10	<u>.41</u>	.21	.07	<u>.41</u>	.27	.36	<u>.41</u>	.31	.55	.08	.27	.30
MnH										1	-.11	<u>-.70</u>	.11	-.21	-.09	.01	.08	-.02	-.00	-.17	.16	<u>-.41</u>	.17	.13	.08
AsH											1	-.12	-.20	.26	.06	.03	.08	.24	-.06	.08	.10	-.13	.10	.20	.11
pH												1	-.20	.24	.21	-.05	.02	.01	.16	.23	-.19	<u>.71</u>	-.20	-.13	-.07
OM													1	.12	.27	<u>.74</u>	.01	.15	<u>.40</u>	.04	.14	.20	-.11	.21	.03
CuS														1	<u>.60</u>	.21	<u>.51</u>	<u>.62</u>	<u>.57</u>	.36	<u>.49</u>	<u>.54</u>	.28	<u>.47</u>	<u>.44</u>
MoS															1	.27	.27	<u>.62</u>	<u>.43</u>	<u>.47</u>	.19	.39	-.17	<u>.52</u>	.07
SS																1	.05	.16	<u>.53</u>	.13	.09	.26	-.02	.16	.04
FeS																	1	<u>.48</u>	<u>.52</u>	.22	<u>.87</u>	.24	<u>.73</u>	<u>.65</u>	<u>.76</u>
ZnS																		1	.33	<u>.46</u>	<u>.40</u>	.21	.10	<u>.64</u>	.22
SrS																			1	-.00	<u>.61</u>	<u>.59</u>	.35	.31	<u>.59</u>
CrS																				1	.02	.30	-.11	<u>.52</u>	<u>-.15</u>
AlS																					1	.15	<u>.74</u>	<u>.54</u>	<u>.89</u>
CaS																						1	-.07	.14	.22
MnS																							1	.27	<u>.77</u>
AsS																								1	.32
KS																									1

TABLE 7.11 Robust correlation matrix for September data

Note: $n = 79$, $n' = n - \Sigma(1 - \text{non unit weight}) = 66$, $r_{.001} = \pm 0.40$, H = herbage, S = soil

data show, factors such as soil organic matter content are important in determining availability. A negative correlation between September herbage copper and organic matter status ($r = -0.42$) is evident owing to copper fixation by the organic components of the soil.

Molybdenum

Soil molybdenum levels are significantly correlated with those elements also enriched in the Liassic and Namurian shales i.e. with copper, zinc, strontium, chromium and calcium.

Herbage molybdenum is very significantly correlated with total soil molybdenum concentrations. Correlation coefficients of 0.93, 0.68 and 0.63 were obtained for May, July and September data, respectively. Decreasing levels of correlation with season may reflect small, unrecorded changes in soil molybdenum concentrations over the grazing season. Herbage molybdenum is also positively correlated with pH and calcium status of the soil. The underlying cause for this association is the increasing availability/mobility of the MoO_4^{2-} ion with increasing pH and calcium status. At low pH and calcium concentrations, molybdenum becomes strongly bound by iron and aluminium hydrates, phosphates and soil colloids, thus rendering it unavailable for plant uptake.

Sulphur

Soil sulphur levels are only correlated significantly with soil organic matter and strontium status. The association of sulphur and organic matter status is to be expected, as most of the sulphur in well-drained, temperate agricultural soils is in combination with organic compounds. Correlation of sulphur with strontium may reflect an inorganic component of sulphur in soil. Sulphur, as insoluble strontium sulphate, is probably an important but unavailable source of sulphur in areas enriched in strontium e.g. in Somerset.

Sulphur herbage levels show little overall correlation with any of the variables measured. Literature (Brown, 1982) indicates soil sulphate-sulphur to be the best predictor of herbage sulphur, but there was insufficient time for this determination to be made here. The lack of correlation between total soil sulphur and plant sulphur levels support the

findings of other workers (Freney, Barrow and Spencer, 1962; Scott, 1966) and confirm that total sulphur determinations show little promise as an index of available sulphur. Much of the total soil sulphur is in an organic form which is largely unavailable to plants. Negative correlations of herbage sulphur with soil iron, strontium and calcium levels are evident from May data. This is a result of soil sulphur becoming locked up in inorganic forms e.g. CaSO_4 , SrSO_4 and FeS , which are insoluble and unavailable for plant uptake. These observations are again consistent with earlier studies which showed the importance of pH and iron oxides on the availability of sulphur (Jones, Cowling and Lockyer, 1972; Chao, Harward and Fang, 1962; and Kamprath, Nelson and Fitts, 1956). Other significant correlations include those of herbage sulphur with herbage copper and zinc levels, which might be a reflection of the biochemical importance of all these elements in plant enzyme systems and structural components of the cell.

Iron

Soil iron levels show significant positive correlations with many soil variables. Correlations are greatest for soil potassium and aluminium levels which reflect the association of these elements in clay minerals and sesquioxides.

Any correlations with herbage iron in May and September are inevitably distorted by soil contamination at these times of the year. Elements with low relative accumulation ratios consequently appear to have significant positive correlations with herbage iron levels i.e. aluminium, chromium and arsenic. The significant positive correlation ($r = 0.93$) between herbage iron and aluminium in July is not due to soil contamination of pasture at this time of the year, but perhaps a reflection of similar mechanisms of plant uptake and translocation. This is peculiar though, as there is as yet, no conclusive evidence that aluminium (unlike iron) performs any essential function in plants, animals or micro-organisms. (Underwood, 1977).

Zinc

Soil zinc levels, like those for copper, are correlated with many soil factors. Of these, correlations with copper, iron and aluminium levels

are the most consistent. This again reflects the incorporation of zinc, as a divalent cation on the adsorption complexes of clays, particularly aluminosilicates and iron oxides.

Zinc herbage levels are negatively correlated with the calcium, strontium and pH status of soils. This is a reflection of a plant's inability to assimilate zinc from alkaline soils of high calcium, pH and strontium status, owing to fixation by precipitation reactions. The negative correlation between herbage zinc and soil iron levels may be attributable to the fixation of soil zinc by iron oxides.

Other significant correlations

Predictably, soil pH has a significant positive correlation with soil calcium status. It also has a significant negative correlation with herbage manganese content owing to the effect of high pH on manganese availability to plants. In calcareous soils for example, manganese (as Mn^{4+}) is fixed by sesquioxides in an unavailable form. Acid soils however, predominantly contain Mn^{2+} ions which are held at exchange sites ready for plant uptake.

Correlations of soil aluminium and potassium with soil calcium, copper, iron, zinc and strontium levels are detectable and attributable to cation exchange of these elements at the exchange sites of clay minerals.

Finally, the lack of significant correlations of herbage chromium and arsenic levels with any of the soil variables measured is noteworthy. Chromium, present in soil as Cr^{3+} , is very immobile and therefore unavailable to plants. Arsenic is strongly fixed by soil iron, aluminium, and to a lesser extent calcium, such that availability to plants is severely restricted.

7.7 QUANTITATIVE RELATIONSHIPS BETWEEN SOIL AND HERBAGE PARAMETERS

The total element composition of soils gives no more than a general indication of plant composition. Ideally, extractants to assess the soil availability of each element would be used in preference to total extraction, to gain more information about plant composition. However, as much controversy still exists over which extractants to use and how to interpret

the results, only total extractions were carried out here.

Graphs showing the relationship between the total soil and herbage contents for copper, molybdenum and sulphur in May, are presented in Figure 7.3 (a)-(c). Of these, only the plot for molybdenum shows any strong degree of linearity. To investigate the linearity of soil-plant data for all the elements in May, July and September, simple linear regression was performed on each set of soil and herbage data. The Minitab statistical package (Ryan et al., 1981) was employed to carry out the plots and regression analysis. R^2 values for each regression equation are given in Table 7.12. From these statistics it is apparent that there are few reliable linear relationships between soil and plant contents. Herbage molybdenum levels are probably the most reliably predicted from total soil concentrations, although herbage strontium is also quite reliably predicted during May and September. Soil contamination of herbage affects the regression equations for iron, aluminium, chromium and arsenic, making them unrepresentative of the true soil-plant relationship.

Pasture herbage levels can also be related to soil concentrations by their relative accumulation ratios (i.e. plant content/soil content). If relative accumulation ratios are low, plant uptake only accounts for a small proportion of the total soil concentration of that element (e.g. iron, arsenic, aluminium and chromium). Where ratios are higher (i.e. approaching 1) differences between total soil and plant concentrations are small (e.g. copper, zinc and manganese). Relative accumulation ratios have been plotted against soil concentrations for copper, molybdenum and sulphur in May (Figure 7.4 (a)-(c)). The plots for copper and sulphur have distinct hyperbolic curves. Log-transformation of the data co-ordinates transforms these hyperbolic curves to linearity resulting in statistically significant inverse trends (Figure 7.5 (a)-(c)). The R^2 statistics for the regression equations predicting relative accumulation ratios from soil data, after log-transformation, are presented in Table 7.13. These indicate that log-transformed relative accumulation ratios and soil data yield better linear prediction models overall, than soil and herbage data alone.

The trace element content of herbage is not only controlled by soil variables, but also by the plant itself, in mechanisms of selective uptake and translocation. The hyperbolic curves for copper and sulphur indicate

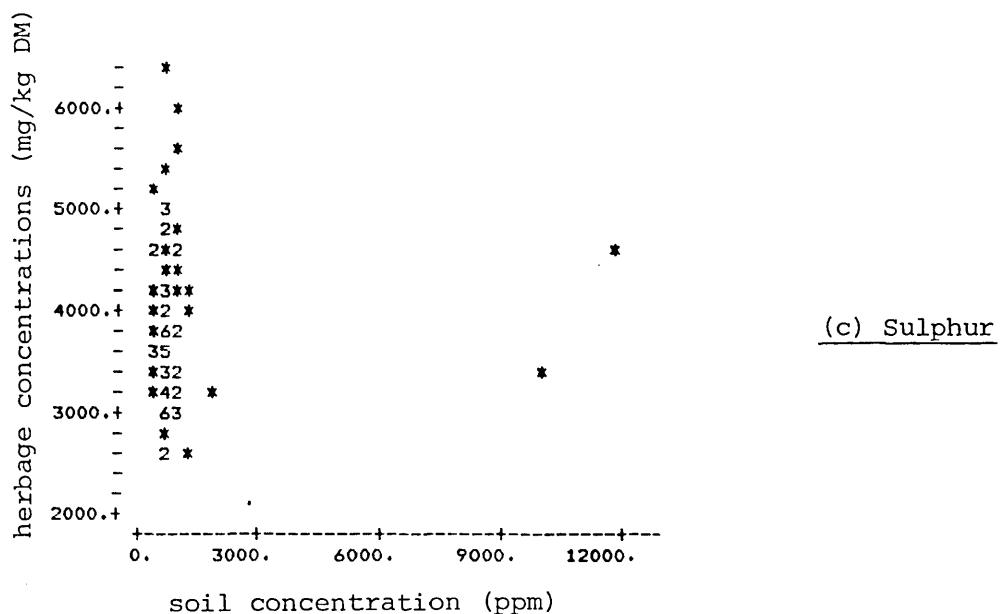
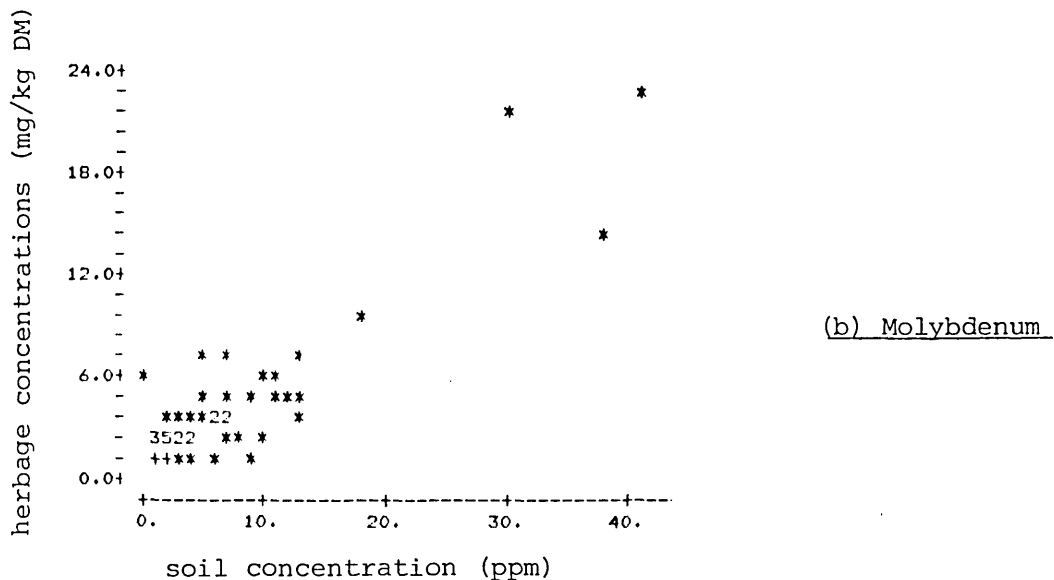
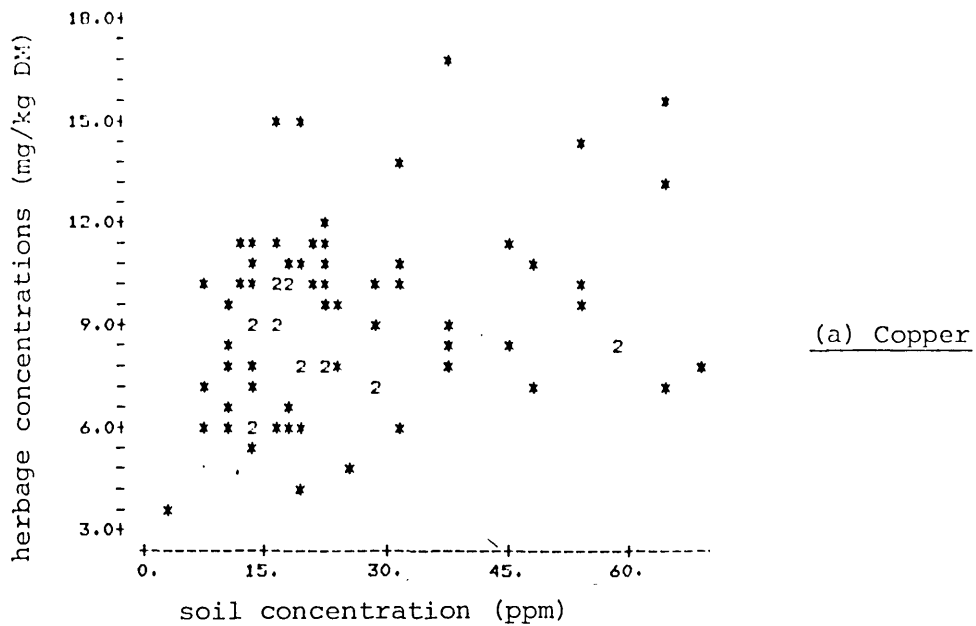


FIGURE 7. 3 (a)-(c) The relationship between total soil and herbage contents for copper, molybdenum and sulphur in May (*=1 data point, 2- 9=2- 9 coincident points, + = ≥ 10 coincident points)

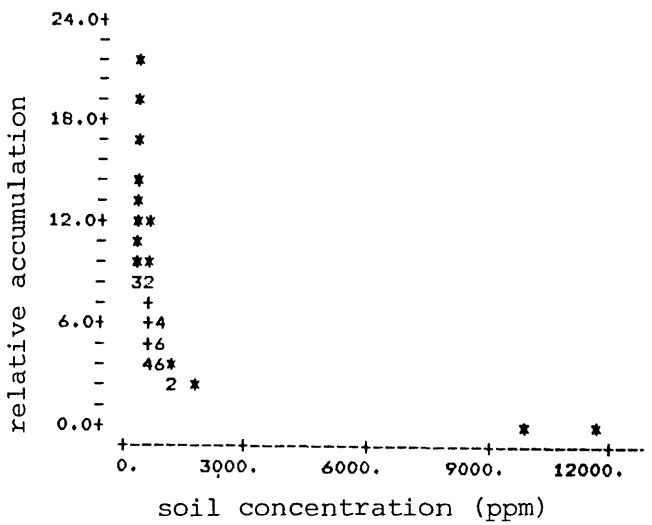
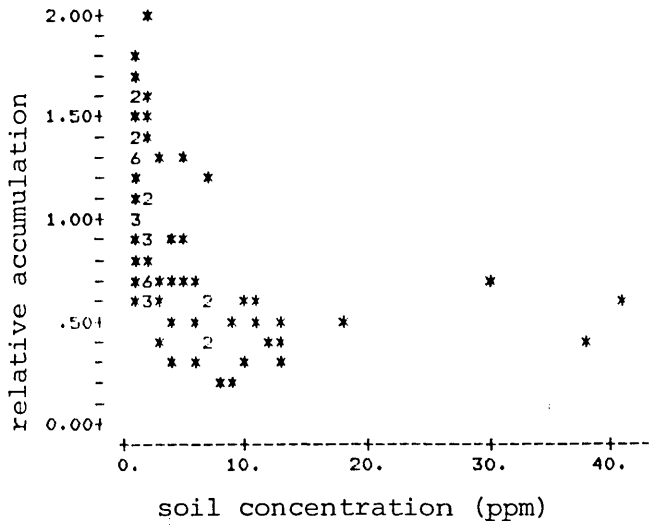
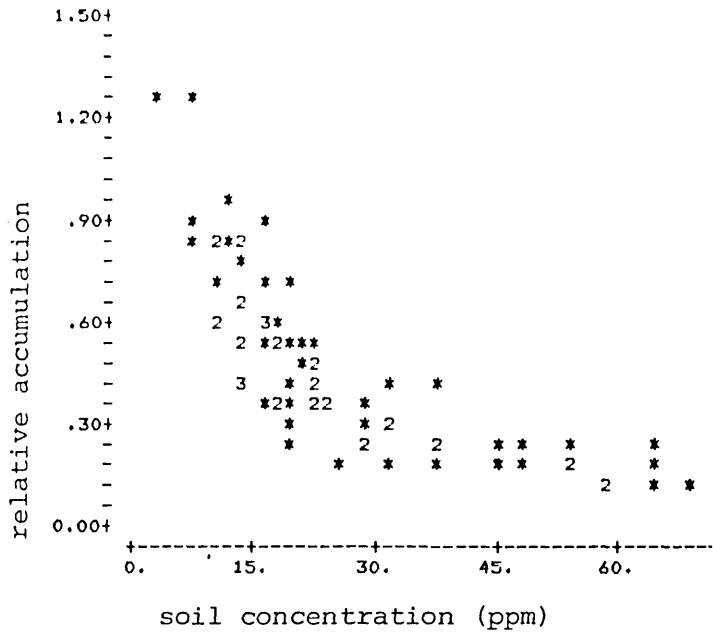


FIGURE 7. 4 (a)-(c) The relationship between total soil content and relative accumulation for copper, molybdenum and sulphur in May

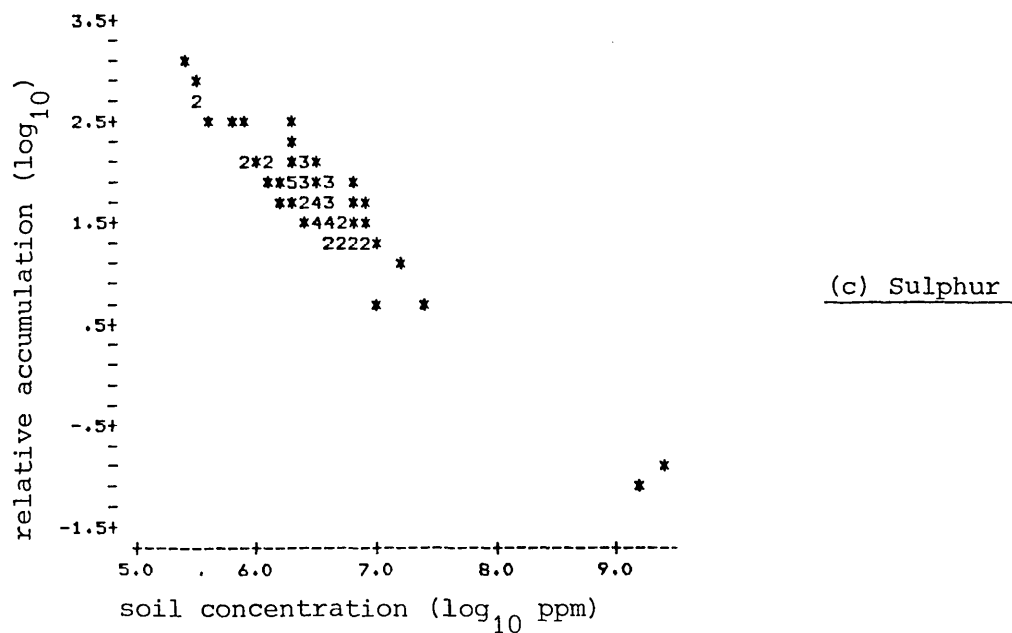
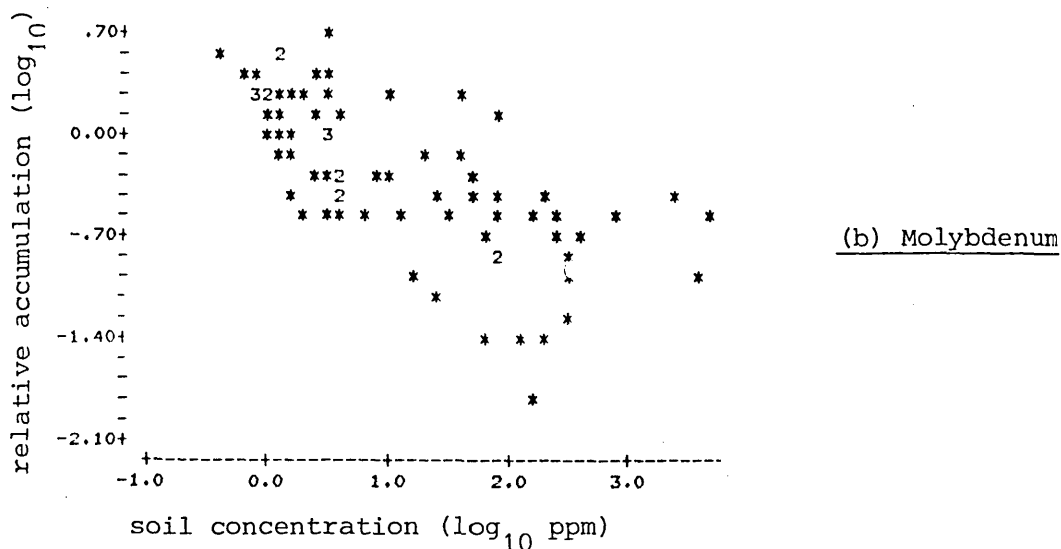
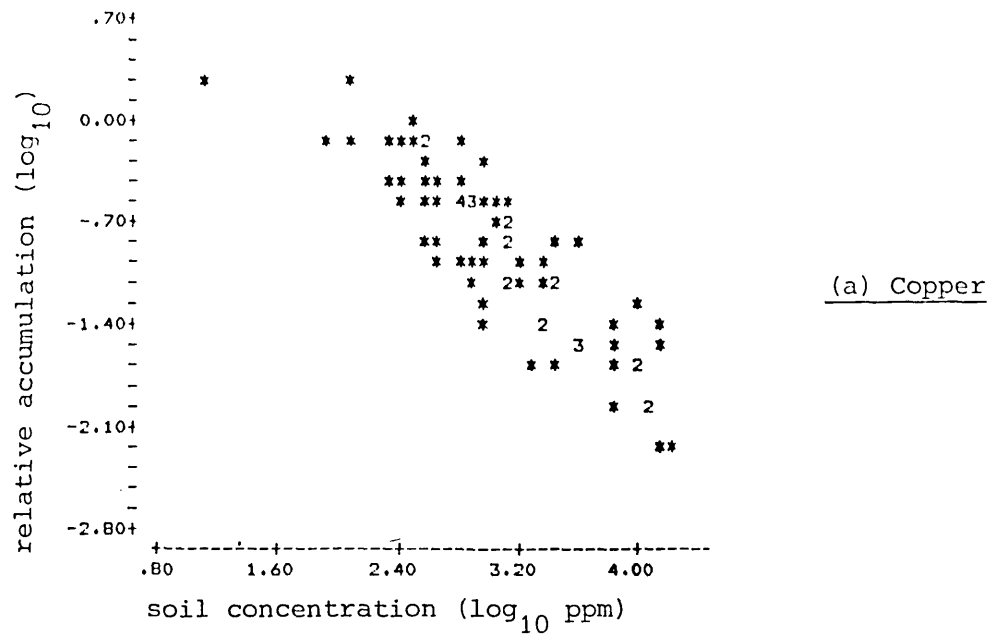


FIGURE 7.5 (a)-(c) The relationship between log transformed soil and relative accumulation data for copper, molybdenum and sulphur in May

ELEMENT	MAY	JULY	SEPTEMBER
Cu	6.8	7.7	10.8
Mo	83.4	54.8	46.2
S	0.2	0.6	0.3
Fe	7.1	0.9	6.3
Zn	9.6	3.6	0.0
Sr	72.4	24.0	83.7
Cr	2.3	35.2	46.7
Al	17.8	17.4	16.6
Ca	2.3	35.2	46.7
Mn	3.8	0.0	3.2
As	0.7	5.8	5.4

TABLE 7.12 R^2 values for the linear regression of herbage data on soil data

ELEMENT	MAY	JULY	SEPTEMBER
Cu	77.5	89.3	84.5
Mo	49.0	58.2	47.5
S	89.9	93.9	92.3
Fe	27.2	63.1	38.5
Zn	68.4	85.5	86.4
Sr	50.5	43.6	26.6
Cr	34.9	77.4	27.4
Al	5.6	9.0	6.1
Ca	97.4	97.1	95.9
Mn	57.9	58.4	57.1
As	42.6	39.0	32.0

TABLE 7.13 R^2 values for the linear regression of the log relative accumulation ratios on the log soil data

that the plant actively excludes these elements at high concentrations. Timperley et al., (1980) noted similar exclusion mechanisms for copper and zinc in Northern Ireland herbage. They concluded that when soil concentrations of copper and zinc were below a physiologically required level, plants would readily accumulate both elements until the required concentration was reached. At soil concentrations greater than this partial exclusion would occur. This seems consistent with the results for all the essential elements determined here. Aluminium, a non-essential element, does not follow the hyperbolic relationship observed for essential elements. However, unpublished work in the Applied Geochemistry Research Group has established a hyperbolic curve for the non-essential element lead. So the connection between hyperbolic curves and essentiality must therefore be questioned. It is possible that the hyperbolic relationships observed here have no connection with mechanisms of selective plant uptake but are artefacts caused by plotting total, rather than available, soil concentrations. (There will also be some induced correlation inherent in the plots of y/x versus x and $\log(y/x)$ versus $\log(x)$).

7.8 MULTIPLE REGRESSION TECHNIQUES TO PREDICT COPPER, MOLYBDENUM AND SULPHUR LEVELS IN PASTURE HERBAGE

It is apparent from the results of the linear regression analyses above, that simple relationships between soil and herbage composition do not generally exist; factors effecting plant uptake are complex. Drainage, organic matter status, pH, concentrations of other elements and microbial activity, all affect plant uptake to varying degrees. For this reason, regression of herbage composition against more than one variable gives potential for the development of models which may account for a greater proportion of the variation. The method most commonly employed for this is multiple linear regression. As geochemical predictor variables are frequently correlated, classical least squares regression is inappropriate. More reliable estimates of the regression coefficients are obtained from ridge regression analysis - a technique fully described in Section 4.3 and 4.4.

Copper, molybdenum and sulphur are the three most important and well-established factors affecting bovine copper metabolism. Consequently any information regarding the quantitative nature of their uptake from soil to plant could be of significance in the control of bovine hypocupraemia.

Ridge regression analysis was applied to the May, July and September data to determine the best predictors of herbage copper, molybdenum and sulphur levels over the grazing season. The most statistically reliable solutions, as determined by R^2 and F-ratio statistics, are presented in Tables 7.14 - 7.16.

Interpretation of ridge regression solutions

The equations presented in Tables 7.14 - 7.16 show that the prediction of herbage copper and molybdenum levels is successfully achieved by ridge regression analysis. Both sets of equations are statistically reliable and account for more than 75% of the overall variation. The ridge solutions for predicting herbage sulphur are generally not statistically reliable, although they account for more of the overall variation than the bivariate models.

The prediction of herbage copper levels

All three equations derived to predict herbage copper levels over the grazing season show herbage zinc to be the most important positive predictor. This is consistent with the significant robust correlation between herbage zinc and copper levels. Soil copper content is a much less important positive predictor as anticipated from its low level of correlation with herbage copper levels and the non-significant bivariate model. The incorporation of additional factors determining plant-available copper in the ridge model has therefore accounted for more of the variation than soil copper levels alone. The appearance of soil calcium as a negative predictor is to be expected from its effect on increasing soil pH, resulting in impeded plant uptake of copper. Similarly, the occurrence of soil sulphur as a negative predictor may be related to the organic matter status of the soil, which complexes copper, rendering it unavailable for plant uptake. The presence of soil iron as a positive predictor of copper in September herbage may relate to the availability of copper on free iron oxides at that time. Finally, the reason for the incorporation of herbage calcium and sulphur in the prediction equations is not known, but may be of potential biochemical significance.

Season	Prediction Equations	R ²	F-ratio
May	$\text{Log CuH} = 0.0273 + 0.6556\log \text{ZnH} + 0.1965\log \text{CuS} - 0.1436\log \text{SS}$	76.5	68.5*
July	$\text{Log CuH} = -1.5904 + 0.6982\log \text{ZnH} + 0.0934\log \text{CuS} + 0.0878\log \text{MnS} + 0.3362\log \text{CaH}$	73.0	31.6*
September	$\text{Log CuH} = -1.7586 + 0.6984\log \text{ZnH} + 0.1022\log \text{CuS} - 0.1147\log \text{AsS} + 0.2109\log \text{FeS} + 0.3585\log \text{SH}$	78.3	43.3*

TABLE 7.14 Prediction equations for herbage copper from ridge regression analysis

Season	Prediction Equations	R ²	F-ratio
May	$\text{MoH} = 1.2967 + 0.3667\text{MoS} + 0.0001\text{CaS} - 3.035\text{AsS}$	87.9	161.8*
July	$\text{MoH} = 1.567 + 0.422\text{MoS} + 0.00018\text{CaS} - 3.826\text{AsS}$	88.3	145.5*
September	$\text{MoH} = 1.341 + 0.1282\text{MoS} + 0.0656\text{OM} + 0.04061\text{CuS} - 0.000944\text{MnS}$	74.6	42.8*

TABLE 7.15 Prediction equations for herbage molybdenum from ridge regression analysis

Season	Prediction Equations	R ²	F-ratio
May	$\text{Log SH} = 3.206 + 0.244\log \text{ZnH} + 0.166\log \text{CuH} + 0.0461\log \text{SS} - 0.19\log \text{FeS} - 0.29\log \text{pH}$	73.8	33.8*
July	$\text{SH} = 4,054.3 + 114.6\text{CuH} + 3706.6\text{AsH} + 7.77\text{KS} - 226.33\text{FeS} - 0.506\text{MnS}$	48.3	11.0*
September	$\text{Log SH} = 1.947 + 0.244\log \text{ZnH} + 0.129\log \text{CuH} + 0.244\log \text{AlS} + 0.202\log \text{CaH} - 0.342\log \text{FeS}$	57.5	15.9*

TABLE 7.16 Prediction equations for herbage sulphur from ridge regression analysis

(note H = Herbage; S = Soil; * = F-ratio significant at $\alpha = 0.001$; all herbage concentrations are in mg/kg DM; soil Cu, S, Mn and As are in ppm; soil Ca, Fe, Al, K and OM are in %)

The prediction of herbage molybdenum levels

The prediction of herbage molybdenum levels by ridge regression analysis makes only a small improvement over the classical bivariate linear regression model, which is itself very statistically reliable. Soil molybdenum content is consistently the most important positive predictor. This is supported by highly significant correlations between soil and herbage molybdenum levels throughout the grazing season, as well as statistically reliable bivariate models. The only other consistent positive predictor (of much less importance) is soil calcium content, which controls pH status and hence molybdenum availability. Where pH is high (6) molybdenum, as the molybdate ion, becomes mobile and available for plant uptake. The presence of herbage arsenic as a negative predictor of herbage molybdenum cannot be explained.

The prediction of herbage sulphur levels

Comparatively poor regression models are obtained for herbage sulphur. Herbage zinc and copper levels are consistent positive predictors, which may be of biochemical significance due to the importance of copper and zinc in sulphur-containing enzymes and structural components of cells. In terms of soil predictors, soil sulphur levels are relatively poor predictors, present in the May equation only. This is to be expected however, as total soil sulphur is a poor index of plant content owing to the high percentage locked-up in organic matter, and unavailable to plants. Soil iron is the strongest negative predictor of herbage sulphur due to the importance of iron in the formation of insoluble and unavailable iron sulphide. The significance of the other predictors is not understood.

It has previously been shown that levels of soil sulphate-sulphur and the input of atmospheric SO_2 are important in the determination of herbage sulphur levels (Cowling and Jones, 1970; Probert, 1970; and Fowler, 1976). This being so, it is of no surprise that the regression equations, omitting these parameters, are of low statistical significance.

7.9 CAUSAL FACTORS OF BOVINE HYPOCUPRAEMIA IN THE SIX FIELD AREAS

The soil-plant relationships discussed in the previous sections make a useful basis for determining geochemical causes of bovine hypocupraemia at the farm level. Three important criteria, described in Section 2.12, are used to establish possible causes. These are;

- (i) Critical herbage levels of Cu, Mo, S, Fe, Zn and Cd;
- (ii) Critical Cu:Mo herbage ratios;
- (iii) Copper availability predictions based on herbage Mo and S levels.

(A) Critical herbage levels of Cu, Mo, S, Fe, Zn and Cd

The ARC (1980) has calculated that the ruminant may often need a dietary copper concentration of 10 mg/kg DM to maintain an adequate copper status. If elevated levels of molybdenum and sulphur are present then copper requirements become substantially greater than 10 mg/kg DM such that in the coincident high molybdenum/high sulphur situation more than 30 mg Cu/kg DM is required by cattle to sustain normal copper metabolism (Table 7.17)

All field areas, except Onecote, supported herbage with median copper levels < 10 mg/kg DM during May 1982. This meant that any spring-born calves from copper deficient dams were likely to be susceptible to bovine hypocupraemia at this time. As the 1982 grazing season proceeded only Stow-on-the-Wold and Masham continued to support herbage with low copper levels, that were attributable to low soil copper status.

Herbage molybdenum levels in excess of 3 mg/kg DM are generally agreed to markedly reduce copper availability to the ruminant through the formation of cupric thiomolybdate in the digestive tract. Herbage samples from Glastonbury and Onecote areas consistently contained molybdenum concentrations in excess of 3 mg/kg DM which was attributed to molybdeniferous soils in both areas. The well known teart pastures of Somerset contained the highest levels of molybdenum due to their higher soil molybdenum status and greater molybdenum availability.

The total sulphur content of English pastures commonly ranges from 1,000-4,000 mg/kg DM (Whitehead, 1966a). Over the latter half of this range, impairment to copper metabolism has been found to occur, irrespective

	TOTAL COPPER REQUIREMENT (mg/kg DM)			
	Low Mo/Low S	High Mo/Low S	Low Mo/High S	High Mo/High S
Beef cattle	11	14	20	36
Dairy cattle	9	12	17	30
Suckler cows	7	10	14	25
Pregnant ewes	8	10	14	26
Growing lambs	5	7	10	18

TABLE 7.17 Table to show the total copper requirements of ruminants with various levels of molybdenum and sulphur in the diet

Low Mo - 1 mg/kg DM

High Mo - .5 mg/kg DM

Low S - 2000 mg/kg DM

High S - 4000 mg/kg DM

(Taken from MAFF (1982) Nutrition Chemistry Department, Leeds)

of molybdenum content (Suttle, 1974b). This is thought to be due to the formation of insoluble and unavailable cupric sulphide in the rumen. All the herbage samples contained sulphur levels at the upper end of the normal range or substantially in excess of it. Sulphur is likely to be important therefore, in the aetiology of bovine hypocupraemia in all of the field areas. The pastures of Onecote and Atherstone consistently contained higher sulphur levels than the other four areas, which could not be related to elevated soil sulphur concentrations. This may be attributable to an atmospheric input of sulphur as SO_2 in these two industrialised areas. A map (Figure 7.6) produced by Warren Spring Laboratory (1979), shows SO_2 levels to be highest in mid/northern England thus lending support to the above theory.

Since the Industrial Revolution man's increasing demand for energy has been met largely through the combustion of coal and oil, resulting in the release to the atmosphere of many millions of tonnes of sulphur, from its ancient natural reservoir in fossil fuels. Fossil fuel combustion is the largest form of atmospheric sulphur pollution, accounting for an estimated 85% of man-made emissions (Robinson and Robbin, 1970). The short atmospheric lifetimes of sulphur compounds result in its deposition largely within 3,000 km from the emission source. Dry deposition of SO_2 is probably the major means of sulphur transport from air to land in the U.K. Wet deposition is rather less important. In the U.K. as a whole, the dry:wet deposition ratio is 2.5:1, although it can range from 6:1 in urban areas to 1:1 in remote rural areas (Department of the Environment, 1976).

The importance of atmospheric SO_2 to the nutrition of plants cannot be over-emphasised. Fowler (1976) estimates that atmospheric SO_2 supplies 25% of the sulphur requirement of crops grown in rural areas of the U.K. Olsen (1957) reports that as much as 50% of cellular sulphur in sulphur-deficient plants may be obtained from the atmosphere. Dry deposition of SO_2 in the Atherstone area of Warwickshire is therefore likely to be a major factor in the elevated herbage sulphur levels recorded there. All the occurrences of bovine hypocupraemia investigated in this area were down-wind from the nearby Hams Hall power station. The elevated levels of sulphur in Onecote herbage could also be a direct result of SO_2 deposition, the SO_2 originating from the Stoke-on-Trent area.

Herbage iron concentrations in excess of 500 mg/kg DM are considered



ANNUAL ARITHMETIC MEAN
SO₂ CONCENTRATION
($\mu\text{g m}^{-3}$)

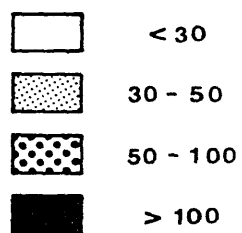


FIGURE 7.6 Distribution of sulphur dioxide over Great Britain
(redrawn from Warren Spring Laboratory, 1979)

to have similar inhibitory effects on copper metabolism as 5 mg/kg DM molybdenum (Bremner et al., 1983). Few of the unwashed herbage samples contained this level of iron, making iron-induced bovine hypocupraemia unlikely in the areas surveyed. Concentrations of iron in excess of those normally found in perennial ryegrass, nevertheless do occur as a result of soil contamination, and could perhaps exacerbate a copper deficiency. May herbage iron levels in Brayford and Onecote frequently exceeded 350 mg/kg DM - the level at which inhibition of copper utilisation first occurs. It is plausible that in these cases, elevated herbage iron levels enhances the probability of hypocupraemia, early in the grazing season. It is also possible that other factors present in soil-contaminated herbage further inhibit copper metabolism. The soil's physico-chemical properties could result in the occlusion of copper from the gastro-intestinal tract (Suttle, Alloway and Thornton, 1975). Recent research (Suttle, Abrahams and Thornton, 1984) has also indicated that soil ingestion could impair copper absorption by trapping sulphur as heavy metal sulphides, such as FeS, and releasing the sulphides in the acid abomasum.

Critical levels of zinc and cadmium in herbage have been set at 200 mg/kg DM and 3 mg/kg DM respectively (ARC, 1980). All the herbage samples analysed had background concentrations of both elements - herbage zinc contents did not exceed 100 mg/kg DM over the grazing season, and cadmium levels were so low (i.e. < 1 mg/kg DM) that many fell below the detection limit (< 0.06 mg/kg DM). Neither zinc or cadmium-induced bovine hypocupraemia are therefore implicated in any of the six field areas. Antagonism by these elements are likely to be localised, at sites of mineralisation or smelting, and unlikely to be of significance in the widespread occurrence of bovine hypocupraemia in England.

(B) Critical Cu:Mo herbage ratios

The toxicity of any particular level of dietary molybdenum is affected by the ratio of dietary copper to dietary molybdenum. Even with very high molybdenum intakes, the clinical signs can be alleviated by high levels of copper. Although it is clear that the Cu:Mo ratio is important, there is little agreement on a critical ratio.- Multimore and Mason (1971) suggested a Cu:Mo ratio of 2:1, whilst Alloway (1973) proposed a ratio closer to 4:1. Cu:Mo dietary ratios have been calculated for May, July and September herbage and are presented in Tables 7.18-7.23. Boxplots,

SAMPLE	MAY	JULY	SEPTEMBER
1	6.3	5.8	7.0
2	11.5	8.6	11.3
3	5.5	7.4	9.3
4	2.1	2.5	3.4
5	10.1	7.4	10.7
6	10.2	7.1	7.9
7	8.5	6.2	11.2
8	7.3	-	12.3
9	-	5.7	5.4
10	6.2	9.1	10.2
11	12.5	9.0	8.3
12	4.5	3.4	7.3
13	6.5	5.6	6.3
14	7.2	7.5	6.6

TABLE 7. 18 Cu : Mo ratios for May,
July and September herbage in Brayford,
North Devon

SAMPLE	MAY	JULY	SEPTEMBER
15	0.4	1.1	1.7
16	0.7	0.3	0.3
17	0.9	2.0	2.6
18	1.1	1.4	0.9
19	0.5	0.6	0.7
20	2.1	3.5	3.0
21	1.2	1.4	2.7
22	3.0	2.3	3.4
23	2.1	1.7	1.8
24	1.7	1.5	1.7
25	2.9	2.3	2.0
26	1.7	2.4	2.9
27	0.7	1.0	1.6
28	3.1	3.0	2.9
29	1.2	1.4	1.8

TABLE 7. 19 Cu : Mo ratios for May,
July and September herbage in
Glastonbury, Somerset

SAMPLE	MAY	JULY	SEPTEMBER
30	4.8	-	7.5
31	4.4	4.0	4.3
32	4.8	6.9	9.4
33	4.3	-	3.5
34	5.5	7.8	9.4
35	7.4	8.5	4.4
36	4.1	4.8	4.4
37	4.8	3.5	2.9
38	5.5	6.5	12.0

TABLE 7.20 Cu : Mo ratios for May,
July and September herbage in Stow-
on-the-Wold, Gloucestershire

SAMPLE	MAY	JULY	SEPTEMBER
39	5.6	4.3	8.0
40	5.3	7.1	6.9
41	6.5	5.2	7.2
42	8.1	5.5	11.6
43	7.5	8.4	10.2
44	6.4	6.1	9.8
45	6.0	4.2	5.1
46	5.2	4.6	5.7
47	5.9	4.2	-
48	7.7	6.2	10.8
49	4.4	7.6	7.6
50	3.6	5.1	6.1
51	8.4	4.4	8.8
52	4.3	5.0	4.5

TABLE 7.21 Cu : Mo ratios for May,
July and September herbage in
Atherstone, Warwickshire

SAMPLE	MAY	JULY	SEPTEMBER
53	2.4	2.1	2.6
54	2.9	2.0	1.1
55	2.7	-	1.9
56	3.8	5.0	2.6
57	-	5.5	2.9
58	3.4	1.6	-
59	3.2	3.2	2.3
60	0.8	-	-
61	4.4	3.8	2.9
62	3.4	3.4	3.6
63	4.5	5.0	4.8
64	2.8	2.6	2.4
65	2.0	1.1	2.4
66	2.9	2.2	3.0
67	2.9	2.1	1.8

TABLE 7. 22 Cu : Mo ratios for May,
July and September herbage in Onecote,
Staffordshire

SAMPLE	MAY	JULY	SEPTEMBER
68	5.0	4.0	3.4
69	5.7	3.9	5.5
70	3.2	5.2	2.9
71	4.8	2.8	2.9
72	4.1	3.9	-
73	8.7	6.1	5.9
74	3.4	2.5	2.5
75	6.2	5.6	5.0
76	6.9	5.7	-
77	6.1	4.1	6.3
78	5.9	5.0	6.5
79	6.8	4.5	6.2

TABLE 7.23 Cu : Mo ratios for May,
July and September herbage in Masham,
North Yorkshire

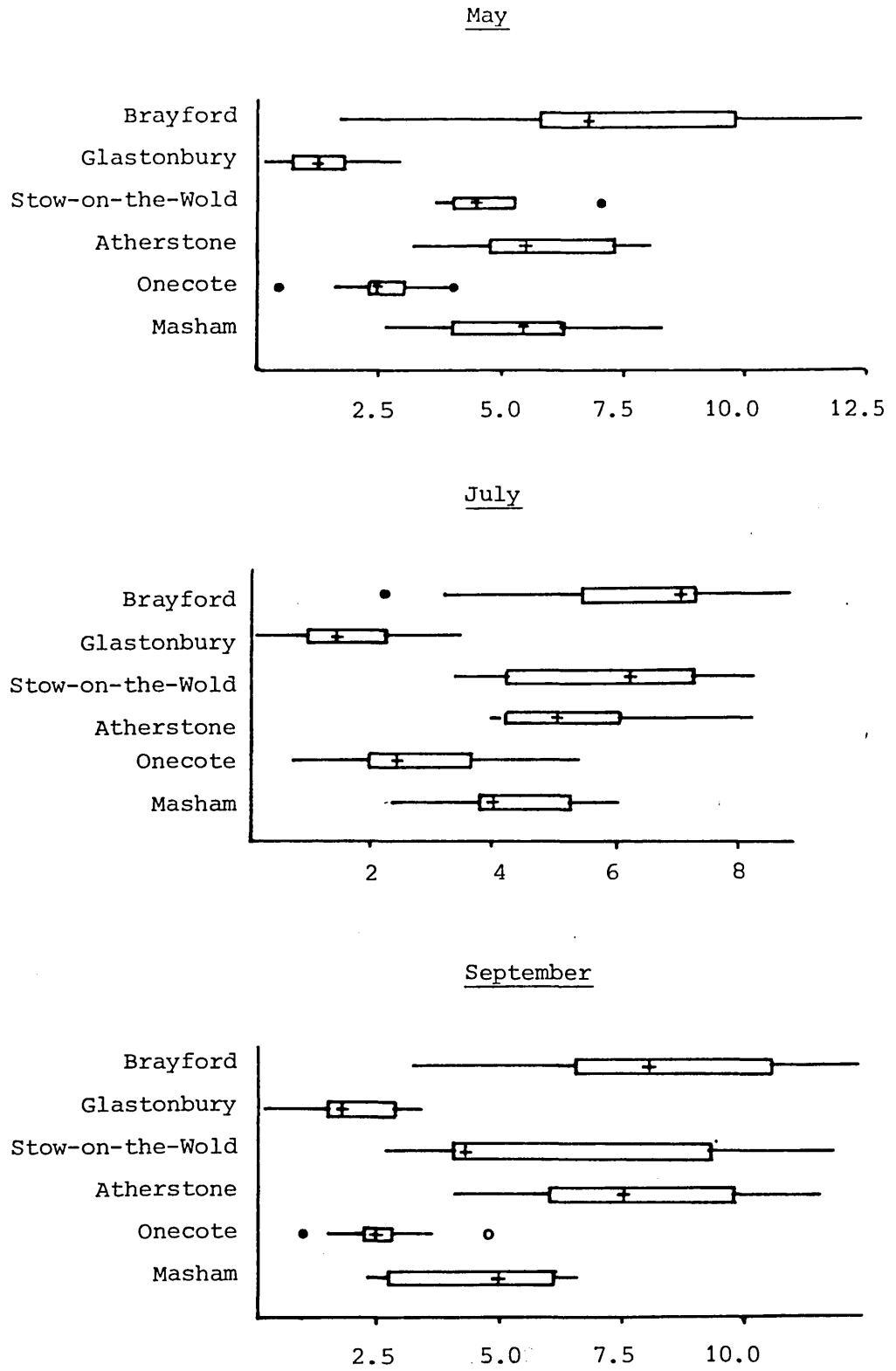


FIGURE 7.7 Boxplots of Cu:Mo ratios in May, July and September herbage

summarising these data are presented in Figure 7. 7.

From the tables and boxplots, it is apparent that Cu:Mo ratios of less than 4:1 were obtained for herbage from Glastonbury and Onecote. Ratios of less than 2:1 were common for Glastonbury herbage. Even though the copper concentrations exceeded the ARC (1980) minimum of 10 mg/kg DM they were insufficient to counteract the effect of such high molybdenum concentrations. According to Table 7.17, the MAFF (1982b) copper requirement is more of the order of 30 mg/kg DM. As these levels were not observed in Glastonbury and Onecote herbage at any time during the grazing season, it is likely that molybdenum-induced bovine hypocupraemia would pose a serious problem in both areas.

Sporadic occurrences of Cu:Mo ratios of less than 4:1 were observed in a few herbage samples outside the Glastonbury/Onecote areas. These are attributable to localised pockets of molybdeniferous soil (sample 37); herbage (samples 70, 71 and 74); or to copper depleted soils and herbage (sample 4). Cattle grazing these areas might be expected to exhibit molybdenum-induced bovine hypocupraemia even though it might not be well recognised in the area as a whole.

(C) Copper availability predictions based on herbage Mo and S levels

The interrelationships between copper, molybdenum and sulphur is of paramount importance when discussing copper deficiency in cattle because of the combined effect these elements have on the availability of dietary copper. Equations predicting ruminant copper availability from molybdenum and sulphur levels in semi-purified diets and fresh herbage (Suttle and McLauchlan, 1976; and Suttle, 1983b) have been derived. The equation for fresh grass -

$$A_{Cu} = 5.72 - 1.297S - 2.785 \log_e Mo + 0.227(Mo.S)$$

(where A_{Cu} is copper availability (percent) and Mo is transformed to natural logarithms) may be applied to herbage with $Mo < 6$ mg/kg DM and $S < 4,000$ mg/kg DM. Copper availability was thus calculated for all herbage samples where appropriate. The results are presented in Tables 7.24-7.29. Where molybdenum and sulphur levels were in excess of 6 and 4,000 mg/kg DM respectively, the equation was not applied and an 'M' or 'S' has been inserted

SAMPLE	MAY	JULY	SEPTEMBER
1	1.83 (0.14)	S	S
2	2.32 (0.21)	S	S
3	S	S	S
4	0.70 (0.03)	0.73 (0.05)	S
5	1.65 (0.17)	S	S
6	1.86 (0.17)	S	S
7	S	S	S
8	1.01 (0.08)	-	S
9	-	S	S
10	2.09 (0.17)	S	S
11	2.77 (0.28)	S	S
12	1.63 (0.11)	S	S
13	2.14 (0.17)	S	S
14	1.47 (0.15)	S	S

TABLE 7.24 Copper availability in May,
July and September herbage in Brayford,
North Devon

M - Herbage molybdenum concentrations in excess of 6 mg/kg DM

S - Herbage sulphur concentrations in excess of 4,000 mg/kg DM

MS - Herbage molybdenum and sulphur concentrations in excess of 6 and 4,000 mg/kg DM respectively

Copper availability is in %. Estimated levels of dietary available copper (in parentheses) are in mg/kg DM

SAMPLE	MAY	JULY	SEPTEMBER
15	M	MS	-
16	M	M	MS
17	M	S	S
18	0.66 (0.03)	M	0.99 (0.05)
19	M	MS	MS
20	S	S	S
21	M	MS	S
22	0.42 (0.03)	S	S
23	0.78 (0.08)	MS	MS
24	M	M	MS
25	0.62 (0.05)	0.50 (0.05)	S
26	MS	S	S
27	MS	MS	MS
28	0.90 (0.08)	S	S
29	M	MS	MS

TABLE 7.25 Copper availability in May,
July and September herbage in Glastonbury,
Somerset

SAMPLE	MAY	JULY	SEPTEMBER
30	1.68 (0.1)	-	S
31	2.39 (0.14)	1.61 (0.10)	S
32	1.78 (0.11)	S	S
33	2.29 (0.13)	-	S
34	1.78 (0.13)	1.32 (0.12)	S
35	1.93 (0.17)	S	S
36	1.50 (0.11)	0.55 (0.05)	S
37	1.61 (0.12)	0.57 (0.04)	S
38	2.73 (0.17)	S	S

TABLE 7. 26 Copper availability in May, July and September herbage in Stow-on-the-Wold, Gloucestershire

SAMPLE	MAY	JULY	SEPTEMBER
39	1.08 (0.08)	S	S
40	S	S	S
41	S	S	S
42	S	S	S
43	S	S	S
44	2.14 (0.16)	0.95 (0.09)	S
45	S	S	S
46	0.50 (0.05)	S	S
47	1.76 (0.10)	S	-
48	1.48 (0.14)	S	S
49	1.05 (0.08)	S	S
50	0.69 (0.06)	0.52 (0.02)	S
51	S	S	S
52	0.67 (0.06)	S	S

TABLE 7. 27 Copper availability in May, July and September herbage in Atherstone, Warwickshire

S - Herbage sulphur concentrations in excess of 4,000 mg/kg DM

Copper availability is in %. Estimated levels of dietary available copper (in parentheses) are in mg/kg DM

SAMPLE	MAY	JULY	SEPTEMBER
53	0.5. (0.05)	S	S
54	S	S	MS
55	S	-	S
56	S	S	S
57	-	S	S
58	0.48 (0.05)	S	-
59	S	S	S
60	MS	-	-
61	S	S	S
62	S	S	S
63	-	S	S
64	S	S	S
65	MS	MS	MS
66	0.51 (0.05)	0.76 (0.09)	S
67	0.50 (0.06)	S	S

TABLE 7. 28 Copper availability in May,
July and September herbage in Onecote,
Staffordshire

SAMPLE	MAY	JULY	SEPTEMBER
68	1.63 (0.10)	S	S
69	0.99 (0.07)	S	S
70	1.35 (0.08)	S	0.40 (0.03)
71	1.03 (0.10)	0.19 (0.02)	0.37 (0.03)
72	-	S	-
73	S	S	S
74	S	0.47 (0.04)	S
75	S	S	S
76	-	S	-
77	1.48 (0.10)	S	S
78	S	S	0.87 (0.08)
79	S	S	S

TABLE 7. 29 Copper availability in May,
July and September herbage in Masham,
North Yorkshire

Note : Copper availability is in %. Estimated levels of dietary available copper (in parentheses) are inmg/kg DM

M - represents herbage Mo concentrations in excess of 6 mg/kg DM

S - represents herbage S concentrations in excess of 4,000 mg/kg DM

MS - represents herbage Mo and S concentrations in excess of 6 and 4,000 mg/kg DM, respectively

in the tables to indicate that the herbage data were out-of-bounds for molybdenum and sulphur respectively. The absorption coefficients were multiplied by the appropriate herbage copper level to obtain an estimate of herbage-available copper in mg/kg DM. These data are in parentheses.

From the tables of predicted copper availability, it is apparent that the prediction equation was frequently inappropriate. Sulphur levels exceeded 4,000 mg/kg DM in July and September herbage samples from most areas, and molybdenum exceeded 6 mg/kg DM in May, July and September herbage from Glastonbury. In the relatively few cases where herbage-available copper has been calculated, it is consistently less than the minimum range of 0.38-0.48 mg/kg DM suggested by ADAS (cited in Givens and Hopkins, 1978), therefore indicating widespread bovine hypocupraemia at all sites.

Givens and Hopkins (1978) working in Nidderdale (an area close to the Masham sites) calculated a mean herbage-available copper level of 0.33 mg/kg DM, using the prediction equation originally derived for semi-purified diets (Suttle and McLauchlan, 1976). Recalculation of Givens and Hopkins' data, using the more appropriate equation for fresh herbage, gives a herbage-available copper level of 0.11 mg/kg DM which is considerably less than their original value, and much more consistent with the data presented in Table 7.29 for Masham herbage.

Final conclusions on causes of bovine hypocupraemia in Brayford, Glastonbury, Stow-on-the-Wold, Atherstone, Onecote and Masham.

Application of the three criteria in A, B and C has indicated that bovine hypocupraemia should be expected in all field areas, attributable to low herbage copper contents, low Cu:Mo herbage ratios, high herbage molybdenum, sulphur and iron concentrations, and low levels of herbage-available copper.

In Stow-on-the-Wold and Masham areas, an absolute copper deficiency is suspected due to consistently low levels of herbage copper (< 10 mg/kg DM) over the grazing season. Interference to copper metabolism from molybdenum and iron is not anticipated, as these elements were within the normal range for herbage. Sulphur levels in excess of 4,000 mg/kg DM, particularly in July and September, could exacerbate the absolute copper deficiency.

In Glastonbury and Onecote areas, a molybdenum-induced copper deficiency is clearly suspected, due to consistently high levels of molybdenum (>3 mg/kg DM) in pasture herbage samples and low (<4) Cu:Mo herbage ratios. In Onecote, copper metabolism could be further antagonised by elevated levels of sulphur and iron in the herbage.

A sulphur-induced bovine hypocupraemia is suspected in Atherstone due to elevated herbage sulphur levels, particularly in September (5,300-6,900 mg/kg DM). Copper, molybdenum and iron levels are all within the normal range, such that antagonisms from these elements are not suspected. The high herbage sulphur levels may be attributable to dry deposition of SO_2 from the nearby power station.

Causal factors of bovine hypocupraemia in Brayford are more difficult to define. Herbage copper levels are adequate, and molybdenum levels are low. Elevated iron concentrations due to soil contamination of herbage are evident, and could perhaps account for the bovine hypocupraemia recorded in the area. It has been suggested (Suttle, Abrahams and Thornton, 1984) that soil ingestion impairs copper absorption to sheep by trapping sulphur as heavy metal sulphides such as FeS, and releasing sulphides in the acid abomasum. Herbage sulphur levels, in excess of 4,000 mg/kg DM, favour this mechanism in Brayford.

7.10 EXTRAPOLATIONS OF CAUSAL FACTORS TO OTHER HYPOCUPRAEMIC AREAS

In Section 5.2 hypocupraemic areas were grouped on a basis of their similar geochemistries, and causal factors of bovine hypocupraemia in some groups were hypothesised. An absolute copper deficiency was suspected in areas of low stream sediment copper levels, e.g. North Yorkshire, Sussex, Lincolnshire, Cumbria and Gloucestershire ; and a molybdenum-induced copper deficiency was suspected in areas of high stream sediment molybdenum e.g. Somerset, Northampton, Warwickshire, Leicestershire and West and South Yorkshire. Follow-up fieldwork in Masham and Stow-on-the-Wold has shown the low stream sediment copper concentrations to be reflected in the low soil and herbage copper contents. Bovine hypocupraemia due to an absolute copper deficiency may therefore be suspected in the other areas with low stream sediment copper concentrations. Similarly, follow-up field work in Glastonbury and Onecote has shown the high stream sediment molybdenum levels to be reflected in the molybdeniferous soils and herbage of these

areas. Molybdenum-induced bovine hypocupraemia was therefore suspected in the other areas with high stream sediment molybdenum. Elevated stream sediment cadmium levels were not reflected in the soils and herbage of Onecote, making antagonisms to copper metabolism from cadmium unlikely.

The lack of stream sediment geochemical data for sulphur made it impossible for extrapolations on sulphur-induced bovine hypocupraemia to be made. Nevertheless, farms down-wind from power stations and in industrialised areas might be expected to exhibit a sulphur-induced bovine copper deficiency.

Iron-induced bovine hypocupraemia is dependent on the amount of soil ingested from soil contaminated herbage, and as such, cannot be related to geochemistry. Occurrences of iron-induced bovine hypocupraemia cannot therefore be extrapolated to other areas with similar geochemistries as Brayford.

7.11 THE PREDICTION OF BOVINE LOW BLOOD COPPER STATUS FROM SOIL AND HERBAGE PARAMETERS

It was an original aim of this thesis to investigate the possibility of deriving an improved country-wide prediction of the incidence of bovine hypocupraemia, using soil and herbage data instead of stream sediment predictors. With this objective in mind, veterinary surgeons were contacted for bovine blood copper data from all the farms sampled. This information, however, proved to be unreliable owing to the considerable influence copper supplementation has had in recent years, in raising blood copper levels. Unfortunately, the blood copper values did not reflect the severity of bovine copper deficiency at the farm level. Consequently, the data originally collected from farmers (Appendix 1) was considered as an alternative, if somewhat subjective, approach in defining indices of severity. An attempt was then made to predict these indices from soil and herbage geochemical parameters using ridge regression. Not surprisingly, this proved statistically unreliable and the exercise was aborted. Clearly, more reliable veterinary data is required. Also, numbers of soil and herbage samples, far in excess of those collected here (i.e. several thousand) are necessary to derive reliable predictions. Ideally, trace element maps for agricultural use should be based on the analysis of soil or vegetation, sampled at a density sufficiently intense, to account for differences in

parent material or soil type. In Britain however, such an approach has proved impractical because of the cost and time required in taking the numerous closely spaced samples needed to represent the many units related to complex geology, soil type and topography. Improvements to the original country-wide prediction of bovine hypocupraemia using soil and herbage parameters therefore seems impractical and uneconomic at the present time.

CHAPTER 8

SUMMARIES, CONCLUSIONS AND SUGGESTIONS FOR FURTHER WORK

8.1 SUMMARIES

(a) Chapter 2

Copper deficiency in grazing livestock has been recognised for some forty years. It occurs as a simple deficiency due to the low copper content of the diet, or as a conditioned deficiency due to the presence of excessive molybdenum, sulphur, iron, zinc or cadmium dietary levels. The condition manifests itself in a variety of clinical symptoms which result in reductions in milk and beef yields. Although there are adequate methods of prophylaxis and therapy available, there has been a resurgence of the condition in recent years. This has been attributed, in part, to changes in grassland and stock management which have led to a reduction in copper content of the sward, more intensive grazing and the production of more rapidly growing calves with greater demands for copper. The general decline in the copper status of livestock has been conservatively estimated to cost £4.8 million p.a., through losses in production. Dietary factors affecting copper availability are clearly important if copper status is to be improved and losses avoided. The amounts and forms of elements in soil; botanical composition of the sward; maturity of the plant; season of the year; lime and fertiliser application, and water regime of the soil are the most important factors to be considered. Because of the complicated relationships between copper, molybdenum and other dietary antagonists in the diet, it is difficult to define critical levels, above or below which, effects on copper metabolism occur. This has partly been overcome by the development of equations for the prediction of copper availability from molybdenum and sulphur concentrations in the diet. Further equations describing the effects of iron, cadmium, zinc and soil ingestion on copper availability are still required. The application of stream sediment geochemistry to delineating areas of suspected subclinical bovine hypocupraemia has played a significant role in combating molybdenum induced copper deficiency in various parts of Great Britain.

(b) Chapter 3

The extent and seriousness of the resurgence of bovine hypocupraemia had to be established. Blood copper was chosen as the diagnostic criteria^{0.5} for determining the existence of bovine hypocupraemia in England owing to the accessibility of fairly standardised data. Thresholds were set at 0.5 mg Cu/l for acute deficiency and 0.6-0.8 mg Cu/l for marginal deficiency. Two maps showing the incidence of acute and marginal deficiencies for the period 1977-1980 inclusive, were compiled from these data. A further map was plotted, based on the locations of administered copper injections, to determine more information on treatment and preventative measures. It was apparent from all the maps that losses due to the cost of diagnoses, treatment, prevention and subclinical losses in yield were considerable. Some regions exhibiting bovine hypocupraemia could be related to high levels of stream sediment molybdenum, low levels of stream sediment copper, or a combination of the two. Other areas (in north Devon, Warwickshire, Lancashire, Yorkshire and Cumbria) could not, hence the involvement of elements other than molybdenum and copper were suspected.

(c) Chapter 4

Polygonal areas of relatively uniform geology, exhibiting bovine hypocupraemia were defined, and ridge regression analysis was performed using veterinary and geochemical data for each area. This multiple regression technique, ideally suited to ill-conditioned data, was applied with the low blood copper incidence rate as the dependent variable and the stream sediment data as the 23 independent predictor variables. The subset of predictors comprising the most statistically reliable regression were Cu, Mo, Sr, Cd, Cr, Al, K and As. These were used to compile a map showing the estimated incidence of bovine low blood copper status in England and Wales. This map successfully predicted many well-established areas of copper deficiency, in addition to delineating other not so well known areas. The potential of ridge regression as a technique for predicting animal trace element disorders from geochemical data is evident.

(d) Chapter 5

The causes of bovine hypocupraemia were then investigated on a farm level, to determine the significance, if any, of the stream sediment predictors. Soil and herbage sampling on farms with hypocupraemic stock was carried out on six objectively-selected areas of England (Brayford, Glastonbury, Stow-on-the-Wold, Atherstone, Onecote and Masham). These areas had a wide range of geological, pedological and topological environments. Topsoils and subsoils were sampled on one occasion, whilst pasture herbage was collected in May, July and September to enable variations in trace element content to be detected.

(e) Chapter 6

Soil samples underwent nitric acid-perchloric acid digestions followed by ICP determinations of Cu, S, Fe, Zn, Sr, Cr, Ca, Cd, K, Al and Mn. Soil molybdenum was determined colorimetrically, and As by magnesium nitrate ashing, hydride generation and AAS. Loss on ignition as a measure of organic matter content, and pH were determined by standard methods. Herbage samples also underwent nitric acid-perchloric acid digestions followed by ICP determinations of Cu, Mo, Fe, Zn, Sr, Cr, Ca, Cd, Al and Mn. A predigestion in fuming nitric acid and magnesium nitrate ashing were necessary for S determination in herbage, followed by hydride generation and AAS for As. Analytical quality control showed the results to have acceptable precision and accuracy, and negligible error from contamination.

(f) Chapter 7

The main features of the soil and herbage data were summarised in boxplots which enabled simple comparisons between areas to be made. Trace element variations in soil samples were explained in terms of differences between soil series and the parent material from which they were derived. Element means for each area were compared to published data for British soils. Soil copper levels on Stow and Masham farms for example, were less than the British mean of 20 ppm. This was attributed to the coarse-grained, copper-depleted parent material from which the soils were derived. Soil molybdenum levels on the Onecote and Glastonbury farms far exceeded the national mean of 1ppm. This was again attributed

to underlying geology; in this case marine black shales of Carboniferous and Jurassic ages. Some elements like sulphur, arsenic, chromium and zinc showed little overall variation between areas. Others, particularly calcium, showed wide ranges of concentration. Glastonbury, and to a lesser extent Stow farms, had soils enriched in calcium attributable to their calcareous bedrock. Consequently these soils also had the highest pH values of all.

The vertical distribution of elements in the soil profile was discussed in terms of topsoil:subsoil ratios; ratios greater than 1 indicated topsoil enrichment, and those less than 1, topsoil depletion. In this context, sulphur, zinc, manganese and calcium were consistently enriched in the topsoil - sulphur, zinc and manganese because of organic matter degradation in the zone of plant enrichment near the surface, and calcium because of lime application. A trend towards topsoil depletion of most elements was detected in the poorly-drained gleys on Onecote farms.

Seasonal variations in the trace element content of herbage were discussed and reasons for differences between areas considered. Seasonal increases in the copper, molybdenum and sulphur contents were detectable, although herbage copper levels did not exceed the ARC (1980) minimum requirement of 10 mg/kg DM on farms in Stow and Masham (low levels of soil copper and a high pH regime in Stow, were held responsible). Conversely, herbage molybdenum exceeded the ARC (1980) critical level of 3 mg/kg DM on farms in Glastonbury and Onecote, attributed to molybdeniferous soils in both areas and a high soil pH regime in Glastonbury. The effects of soil contamination on herbage composition were discussed. Herbage levels of iron, aluminium, arsenic and chromium, owing to their low relative accumulation ratios provided a good index of soil contamination. This was greatest in Brayford in May and September, and was attributed to short grass, soil splash and ruminant poaching at these times of the year.

Robust correlations were calculated between soil and herbage variables for May, July and September data, and significant correlations were discussed from pedological, chemical and biochemical aspects. The correlations of soil pH and calcium with herbage molybdenum, copper, zinc and manganese were significant owing to the dramatic effects these

two parameters have on plant uptake. Also, aluminium, potassium and iron correlated with copper, zinc, strontium and calcium in soils, indicating their association with the cation exchange sites and iron oxides in clays.

Bivariate regression analysis showed total soil data to be of little use in predicting plant content. Herbage molybdenum was the only element to be predicted with any degree of confidence from soil data. Log-transformed relative accumulation ratios and soil data were found to provide better linear models. As the factors affecting plant uptake are complex, multivariate ridge regression was used to determine the best predictors of herbage copper, molybdenum and sulphur levels: the ridge solutions for predicting herbage copper were found to be more helpful than the bivariate models, owing to the inclusion of more parameters, accounting for a greater proportion of the variation. Little difference was detectable between bivariate and multivariate models for predicting molybdenum owing to the sole importance of soil molybdenum as a strong positive predictor. Herbage sulphur predictions were poor owing to the lack of appropriate predictor variables.

Possible causal factors of bovine hypocupraemia were discussed on the basis of three criteria: (1) critical herbage levels of Cu, Mo, S, Fe, Cd and Zn; (2) Cu:Mo herbage ratios; (3) ruminant Cu availability predictions. From this analysis, an absolute copper deficiency was suspected in the cattle on farms in Stow and Masham owing to consistently low herbage copper levels throughout the grazing season. Antagonism from molybdenum was suspected to cause a molybdenum-induced bovine hypocupraemia in Glastonbury and Onecote - herbage molybdenum levels were consistently elevated and Cu:Mo ratios were low. Predicted ruminant copper availability was also less than the lowest recommended level. Antagonism from sulphur was suspected on farms in Atherstone where herbage copper levels were adequate and molybdenum levels low. The high levels of herbage sulphur were attributed to the dry-deposition of SO_2 from the nearby power station. The causal factor of bovine hypocupraemia on Brayford farms was more difficult to determine. Herbage copper levels were sufficiently high, and molybdenum and sulphur levels sufficiently low, that antagonisms of this sort were not suspected. Herbage iron levels however, were generally higher than elsewhere, indicating a possible iron-induced copper deficiency. Antagonisms from

cadmium and zinc were not suspected on any of the farms sampled in the course of this research.

Extrapolations were made concerning the causes of bovine hypocupraemia in the other hypocupraemic polygons. Absolute copper deficiency was suspected in areas of North Yorkshire, Humberside, Sussex, Kent, Cumbria, Gloucestershire, Oxfordshire and Lincolnshire. Molybdenum-induced bovine hypocupraemia was suspected in parts of Northamptonshire, Warwickshire, Staffordshire, Leicestershire, Derbyshire and Somerset and sulphur-induced copper deficiency was expected on farms in close proximity to power stations and in parts of northern England where SO_2 levels reach a maximum.

Finally the prediction of bovine low blood copper status from soil and herbage data was considered. Unfortunately, inadequate veterinary data and the very large numbers of soil and herbage samples required, made this prediction impossible.

8.2 CONCLUSIONS

(a) The resurgence of bovine hypocupraemia in recent years has led to a high incidence country-wide. This is likely to be of considerable economic importance through subclinical losses in yield, increased susceptibility to infection and delays in maturity. Areas previously not recognised as having hypocupraemic stock have been clearly associated with bovine hypocupraemia.

(b) Improvements in veterinary data collection are essential - more systematic, unbiased records are required. The use of more reliable biochemical criteria to determine the existence of copper deficiency is also necessary.

(c) Some areas with bovine hypocupraemia have been related to the occurrence in stream sediments of high concentrations of molybdenum, low concentrations of copper or a combination of the two. A previously unrecognised association of sulphur and iron with the occurrence of bovine hypocupraemia in areas outside those affected by the molybdenum - copper interaction has been demonstrated in the field.

(d) Ridge regression is clearly useful for the reliable prediction of animal disease incidence from ill-conditioned data. The improvements to veterinary data collection suggested above, and the inclusion of more relevant geochemical parameters - by replacing stream sediment data with soil-plant information - would further strengthen statistical predictions of this type. The benefit of using such techniques to gain an understanding of agricultural problems could be considerable.

(e) Soil and herbage studies have proved invaluable in determining geochemical causes of bovine hypocupraemia at the farm level. Local extrapolations to regions of similar geochemistry enabled causes of bovine hypocupraemia to be proposed for other areas.

(f) The elevated concentrations of sulphur in herbage are almost certainly of considerable importance in the aetiology of bovine hypocupraemia in Britain - particularly in industrialised areas.

(g) The significance of all the elements in the original stream sediment prediction equation to the aetiology of bovine hypocupraemia cannot be quantitatively determined in this study. This is because a lack of reliable veterinary data and adequate numbers (i.e. several thousand) of soil and herbage samples at present preclude establishing any better country-wide prediction of the incidence of bovine hypocupraemia, than that derived here. Improvements to the prediction could only be achieved at considerable expenditure of time and cost, which might prove uneconomic.

8.3 SUGGESTIONS FOR FURTHER WORK

(a) Soil aspects

(i) Improved methods for measuring the availability of various forms of copper and molybdenum in soils should be developed. This would enable more direct links to be made between soil status and animal disease incidence.

(ii) More emphasis should be given to sulphate-sulphur determinations in soils as total-soil sulphur yields little information on plant-available sulphur.

(iii) At the soil-plant interface more research is required on sward

selection and its response to various soil management regimes, such that sward copper levels are increased.

(b) Animal aspects

(i) Work on the prediction of copper absorbability from natural food-stuffs in cattle should be encouraged. Quantitative effects of copper antagonists such as iron, soil, cadmium and zinc should be included in this study.

(ii) New methods of improving copper status by dietary supplementation and fertiliser application warrant further study.

(iii) Improvements to existing biochemical criteria for assessing copper status in the ruminant is required - further studies on the timing and the consequences of loss in activity of caeruloplasmin, superoxide dismutase, lysyl oxidase and metallothionein in copper-depleted ruminants, are necessary.

(c) General aspects

(i) Multidisciplinary collaboration in dose response trials is to be recommended in various areas of Britain to gain a comprehensive set of soil, pasture and animal data. Responses to supplementary copper could be used to ascertain better estimates of the economic importance of bovine hypocupraemia.

(ii) A comprehensive veterinary data bank is required on animal disease incidence. More work is consequently needed to improve the VIDA II system so as to reduce the number of limitations currently inherent in it.

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APPENDIX 1

TABLE TO SUMMARISE FARMING ENTERPRISE, SEVERITY OF BOVINE HYPOCUPRAEMIA
AND THE ADMINISTRATION OF COPPER SUPPLEMENTATION ON THE SITES SAMPLED

Sample No.	Beef	Dairy	Severity	Supplementation
1	Y	N	1	Y
2	Y	N	1	Y
3	Y	N	1	Y
4	Y	N	1	Y
5	N	Y	1	Y
6	N	Y	1	Y
7	N	Y	1	Y
8	N	Y	2	Y
9	Y	N	2	Y
10	Y	N	1	Y
11	N	Y	0	N
12	N	Y	1	Y
13	N	Y	2	Y
14	Y	N	1	Y
15	N	Y	2	Y
16	Y	N	2	Y
17	Y	N	2	Y
18	Y	N	2	Y
19	N	Y	2	Y
20	Y	N	2	Y
21	N	Y	2	Y
22	N	Y	1	Y
23	N	Y	2	Y
24	N	Y	2	Y
25	N	Y	2	Y
26	Y	Y	2	Y
27	Y	Y	2	Y
28	N	Y	1	Y
29	N	Y	1	Y
30	N	Y	2	Y
31	N	Y	2	Y
32	N	Y	2	Y
33	Y	N	1	Y
34	Y	N	1	Y
35	Y	N	2	Y
36	N	Y	2	Y
37	Y	N	1	Y
38	Y	N	1	Y

Note: N = No, Y = Yes, 0 - 2 indicates increasing severity of bovine hypocupraemia.

APPENDIX 1 (continued)

Sample No.	Beef	Dairy	Severity	Supplementation
39	N	Y	2	Y
40	N	Y	2	Y
41	N	Y	1	Y
42	N	Y	2	Y
43	N	Y	1	Y
44	N	Y	1	Y
45	N	Y	2	Y
46	N	Y	2	Y
47	N	Y	1	Y
48	N	Y	1	Y
49	N	Y	1	Y
50	N	Y	1	Y
51	N	Y	1	Y
52	N	Y	1	Y
53	N	Y	2	Y
54	N	Y	2	Y
55	N	Y	2	Y
56	N	Y	1	N
57	N	Y	1	Y
58	N	Y	1	Y
59	N	Y	2	Y
60	N	Y	2	Y
61	N	Y	1	Y
62	N	Y	2	Y
63	N	Y	1	N
64	N	Y	2	Y
65	N	Y	1	N
66	N	Y	2	Y
67	N	Y	2	Y
68	Y	N	2	Y
69	Y	N	1	N
70	Y	N	1	N
71	Y	N	1	N
72	Y	N	1	N
73	Y	N	1	Y
74	N	Y	1	Y
75	Y	N	2	Y
76	N	Y	1	Y
77	N	Y	1	Y
78	N	Y	1	Y
79	N	Y	1	Y

Note: N = No, Y = Yes, 0 - 2 indicates increasing severity of bovine hypocupraemia.

REAGENTS

Perchloric acid - 60%

Nitric acid - 70%

Hydrochloric acid - 6M

EQUIPMENT

Test tubes and racks

Deep heating block with automatic timer

Whirli-mixer

PROCEDURE

- (a) Weigh each sample (0.25g, 105°C, -80 mesh) into a clean, dry numbered test tube.
- (b) Weigh standard and duplicate samples, and leave empty test tubes at random intervals for blank determinations.
- (c) Add 4 ml nitric acid to each test tube.
- (d) Add 1 ml perchloric acid to each test tube.
- (e) Place the test tubes in the cold heating block and raise the temperature to 150°C $\pm$ 5°C over 2 - 3 hours. Leave at this temperature until copious evolution of fumes cease.
- (f) Increase the temperature to 185°C $\pm$ 5°C and transfer the tubes to wire racks when the residue is dry.
- (g) Allow the tubes to cool. Add 2 ml hydrochloric acid to each tube.
- (h) Place the tubes on a shallow heating block at 60°C and leave for one hour. Transfer the tubes to wire racks and allow to cool.
- (i) Add 8 ml DIW to each tube and whirli-mix thoroughly.
- (j) Allow the residue to settle for a minimum of four hours before introducing the solution to the ICP.

APPENDIX 2a. Nitric acid - perchloric acid attack for the determination of total Cu, S, Fe, Zn, Cd, Al, K, Ca and Mn in soils
(Applied Geochemistry Research Group unpublished method)

REAGENTS

Nitric acid - 70%

Perchloric acid - 60%

Hydrochloric acid - 6M

EQUIPMENT

Conical flasks (250 ml) with watch glass covers

Hotplate

Graduated plastic tubes (10 ml)

PROCEDURE

- (a) Weigh each sample (2 g dried, milled) into a clean, dry numbered conical flask (250 ml).
- (b) Weigh standard and duplicate samples and leave blank flasks at random intervals.
- (c) Add 40 ml nitric acid to each flask, cover with a watch glass and set aside in a fume cupboard overnight.
- (d) Place the covered flasks on a hotplate and warm gently until frothing ceases.
- (e) Allow the flasks to cool and add 3 ml perchloric acid.
- (f) Replace the flasks on the hotplate, remove the watch glasses and cautiously heat just to dryness.
- (g) Allow the flasks to cool, add 2ml hydrochloric acid and 2 - 3 ml DIW to each flask. Warm gently until the residue dissolves.
- (h) Transfer the cooled solution to graduated test tubes and dilute to volume with the washings from the flasks.
- (i) Allow the residue to settle for about four hours before ICP analysis.

APPENDIX 2b. Nitric acid - perchloric acid attack for the determination of total Cu, Mo, Fe, Zn, Cd, Al, Ca and Mn in herbage.
(Applied Geochemistry Research Group unpublished method)

REAGENTS

Nitric acid - 70%

Perchloric acid - 60%

Hydrochloric acid - 6M

Reducing solution - 75g citric acid, 150g ascorbic acid
diluted to 2ℓ with DIW.

Potassium iodide - concentrated (500g KI in 500ml DIW)

Dithiol solution - (20ml ethanol + 3g zinc dithiol + 40ml DIW
+ 20g sodium hydroxide + 10ml thioglycollic acid. Mix. Dilute -
to 500ml with DIW. Add 500ml potassium iodide. Mix. Keep cold.

Toluene

EQUIPMENT

As for nitric acid -perchloric acid attack (Figure 6.1)

Whirli-mix, diluter, borosilicate test tubes, zipette, glass
tubes, mechanical shaker, Gilford 300N

PROCEDURE

- (a) Nitric acid - perchloric acid attack to stage (f) in Figure 6.1.
- (b) When cool leach with 10 ml 6M hydrochloric acid.
- (c) Whirli-mix the contents and allow to settle overnight.
- (d) With a diluter, take up 5 ml of the liquid and wash into a borosilicate test tube with 4 ml of reducing solution and leave for 5 - 10 minutes.
- (e) Add 2 ml concentrated potassium iodide solution by zipette.
- (f) Add 1 ml dithiol solution by zipette and stand for 10 minutes.
- (g) Add 3 ml toluene by zipette.
- (h) Stopper the test tube and shake mechanically for 2 minutes.
- (i) Centrifuge at 2000rpm for 2 minutes. Decant the toluene layer into glass tubes.
- (j) Leave the tubes for 30 minutes.
- (k) Calibrate the Gilford 300N with reagent blanks and standard molybdenum solutions.
- (l) Measure the absorption of the toluene samples using the Gilford.

APPENDIX 2c. The determination of molybdenum in soils colorimetrically

(Applied Geochemistry Research Group unpublished method)

REAGENTS

Magnesium nitrate saturated solution - $140\text{g Mg(NO}_3)_2 / 100\text{ ml}$

Potassium iodide - 0.2%

Hydrochloric acid - concentrated

EQUIPMENT

Pyrex beakers (50 ml)

Plastic tubes

Clingfilm

Epindorf pipette

PROCEDURE

- (a) Weigh each sample (0.25 g, 70°C, -80 mesh) into a clean, dry numbered 50 ml Pyrex beaker.
- (b) Add 1 ml saturated magnesium nitrate solution by Epindorf pipette to each sample.
- (c) Shake each sample to ensure all the soil particles are wetted.
- (d) Place in a drier overnight set at 100°C to dry completely.
- (e) Transfer to a muffle furnace and leave at 450°C for four hours.
- (f) Remove from furnace and allow to cool.
- (g) Add 5 ml concentrated hydrochloric acid . Seal the top with clingfilm and leave overnight on the shaker.
- (h) Add 5 ml 0.2% potassium iodide, transfer to plastic tubes and shake to homogenise.
- (i) Leave overnight to settle before presenting the solution for hydride generation and arsenic determination.

APPENDIX 2d Magnesium nitrate ashing for the determination of arsenic in soils

(Pahlavanpour, Pullen and Thompson, 1980)

REAGENTS

Fuming nitric acid
 Magnesium nitrate - 2%
 Hydrochloric acid - concentrated
 Potassium iodide - 0.2%

EQUIPMENT

Pyrex beakers (50 ml) with watch glass covers
 Sand bath
 Clingfilm
 Plastic tubes
 Epindorf pipette

PROCEDURE

- (a) Weigh herbage sample (0.5g, dried, milled) into clean, dry 50 ml Pyrex beakers.
- (b) Add 1 - 2 ml DIW to moisten
- (c) Add 5 ml fuming nitric acid and immediately cover with watch glasses.
- (d) Allow to stand overnight.
- (e) Add 2 ml magnesium nitrate by Epindorf pipette. Mix
- (f) Place the beakers on a sand bath maintained at 120 - 130°C. Evaporate to dryness such that a yellow/brown residue remains.
- (g) Place the beakers in a cool muffle furnace and raise the temperature to 450°C. Maintain this temperature for 2 - 3 hours.
- (h) Allow the beakers to cool. Leach with 5 ml concentrated hydrochloric acid and cover with clingfilm.
- (i) Take 0.5 ml aliquot for sulphur analysis. Make this up to 5 ml with DIW ready for S determination by ICP.
- (j) Add 4.5 ml potassium iodide to the remaining 4.5 ml and decant into tubes for analysis by AAS.

APPENDIX 2e Magnesium ashing for the simultaneous determination of arsenic and sulphur total concentrations in herbage.
 (Leech et al., 1982 - unpublished)

SAMPLE	SOIL SERIES	Cu	Mo	S	Fe	Zn	Sr	Cr	Al	Ca	Mn	As	K	pH	OM
1	Highweek	23	1.8	760	3.9	119	56	25	7.3	2100	1320	14	1.5	5.7	10.4
2	Loxhore	16	1.4	740	3.0	68	38	30	4.9	1200	1360	14	1.0	5.3	10.8
3	Highweek	32	1.5	580	3.9	62	75	20	7.0	1500	2600	12	1.6	5.5	8.1
4	?	3	1.1	690	0.8	15	29	16	1.9	3400	134	5	0.5	5.8	14.8
5	Highweek	32	1.7	570	5.2	121	55	19	8.0	1430	2100	24	1.7	5.4	8.4
6	Highweek	28	1.1	680	4.5	73	85	16	8.1	3200	2500	18	1.7	6.0	10.8
7	Highweek	22	1.2	860	4.0	130	43	33	6.3	2900	1590	23	1.3	5.8	13.3
8	Highweek	19	1.0	520	3.3	99	24	34	5.7	2800	1070	24	1.1	6.2	7.9
9	Dartington	6	1.1	550	2.9	24	40	22	4.7	2000	2200	7	1.1	5.9	10.7
10	Highweek	24	1.1	450	4.7	89	65	16	6.7	1810	870	16	1.4	5.7	4.8
11	Highweek	17	1.2	660	2.6	68	15	30	4.8	3000	380	12	1.0	5.6	9.0
12	Highweek	18	8.7	640	4.9	145	86	18	8.0	2400	2300	23	1.6	6.0	12.1
13	Highweek	23	0.9	620	3.8	90	31	24	6.5	2800	1130	14	1.4	5.9	9.9
14	Highweek	23	0.9	520	3.6	63	76	22	7.0	2800	1800	11	1.6	5.9	8.6

APPENDIX 3a. Topsoil data from Brayford, North Devon

Note: Cu, Mo, S, Zn, Sr, Cr, Ca, As and Mn are in ppm, and Fe, Al and K are in %

SAMPLE	SOIL SERIES	Cu	Mo	S	Fe	Zn	Sr	Cr	Al	Ca	Mn	As	K	pH	OM
15	Evesham	54	41.3	730	3.5	109	99	4.4	5.4	6.6	830	15	1.0	8.0	9.4
16	Evesham	65	30.2	950	4.2	142	98	5.2	6.2	5.2	770	15	1.2	7.6	12.1
17	Butleigh	48	6.5	900	3.4	94	111	4.1	5.8	5.0	770	12	1.2	7.8	11.2
18	Sedgemoor	26	12.5	9800	2.3	84	420	2.9	3.6	4.3	1060	14	0.7	7.0	44.2
19	Midelney	65	38.0	1150	4.3	181	82	4.8	6.8	1.7	520	14	1.4	7.4	13.8
20	Charlton Bank	38	6.9	970	4.0	173	77	4.6	6.5	0.8	380	14	1.1	6.4	10.4
21	Turbary Moor	-	-	-	-	-	-	-	-	-	-	-	-	6.1	37.2
22	Charlton Bank	38	4.1	820	3.0	99	66	2.6	4.6	2.7	760	11	1.0	7.5	12.0
23	Long Load	54	9.0	730	3.7	128	122	2.5	5.8	3.2	520	11	1.4	7.9	9.4
24	Sedgemoor	45	13.2	1670	4.3	114	103	6.2	6.8	1.3	350	13	1.2	5.9	26.4
25	Somerton	58	7.0	980	3.7	105	177	3.3	5.2	5.6	990	11	1.4	7.9	10.5
26	Worcester	23	5.1	540	3.2	109	47	2.7	4.7	2.0	680	11	1.5	7.8	6.5
27	Sedgemoor	19	11.4	11600	1.8	61	280	3.9	2.7	3.0	540	11	0.4	5.6	58.4
28	Somerton	45	10.4	810	4.5	101	58	4.9	5.7	2.1	1030	21	1.1	7.8	9.9
29	Evesham	38	10.3	380	2.9	77	117	2.2	4.7	9.2	710	11	1.0	8.1	5.5

APPENDIX 3b. Topsoil data from Glastonbury, Somerset

Note: Cu, Mo, S, Zn, Sr, Cr, Mn and As are in ppm and Fe, Al, Ca and K are in %

SAMPLE	SOIL SERIES	Cu	Mo	S	Fe	Zn	Sr	Cr	Al	Ca	Mn	As	K	pH	OM
30	Denchworth	10	1.9	640	3.1	78	37	4.1	5.2	0.5	220	12	4.0	6.2	8.2
31	Sherbourne	19	1.8	710	3.3	107	73	4.7	2.9	6.1	730	39	3.9	7.7	7.8
32	Denchworth	18	1.8	840	4.1	101	53	3.3	7.0	0.6	270	17	7.4	6.8	7.0
33	Sherbourne	14	4.0	480	5.8	91	140	5.3	5.6	9.4	860	93	6.5	8.1	7.2
34	Waltham	13	2.2	380	3.7	65	28	3.6	3.6	0.3	940	22	4.0	5.9	6.4
35	?	16	1.7	730	3.7	79	35	4.1	3.9	3.2	1050	12	5.6	6.9	11.6
36	Sherbourne	29	3.0	640	4.2	133	48	5.2	3.8	2.3	580	44	3.9	7.4	10.3
37	Sherbourne	11	6.2	870	4.9	79	54	5.8	3.4	3.5	570	79	4.0	8.0	7.8
38	?	16	1.8	640	3.8	78	47	3.7	6.3	0.6	240	22	6.9	6.4	9.6

APPENDIX 3c. Topsoil data from Stow-on-the-Wold, Gloucestershire

Note: Cu, Mo, S, Zn, Sr, Cr, Mn and As are in ppm and Fe, Al, Ca and K are in %

SAMPLE	SOIL SERIES	Cu	Mo	S	Fe	Zn	Sr	Cr	Al	Ca	Mn	As	K	pH	OM
39	Reclaimed	29	3.3	1310	2.5	85	55	4.0	5.5	5900	850	10	7.6	6.5	11.0
40	Merevale	48	8.4	740	4.2	127	51	4.4	5.7	5000	1160	30	10.8	6.3	6.6
41	Shifnal	13	1.2	240	1.3	47	17	3.1	1.6	1710	590	6	3.1	6.8	3.0
42	Shifnal	13	1.1	230	1.6	57	19	2.1	2.1	1320	610	4	3.7	6.0	3.1
43	Shifnal	14	0.7	250	1.5	56	19	1.9	2.1	1370	430	5	3.7	6.5	3.0
44	Gatacre	20	0.9	580	2.2	81	31	4.4	3.0	3200	610	7	4.8	6.0	7.2
45	Baxterley	20	1.7	590	2.3	130	37	3.1	3.6	3200	330	8	6.1	6.1	8.1
46	Bardsey	17	1.1	440	2.1	81	33	2.4	3.3	2900	410	7	4.8	6.0	6.2
47	Baxterley	14	1.0	410	2.4	69	37	3.7	3.9	3100	550	6	6.4	6.5	5.4
48	Dodmoor	18	1.0	460	2.9	75	42	3.0	4.1	3900	690	8	8.0	7.0	5.8
49	Shifnal	14	1.5	270	2.3	65	32	2.7	3.6	3000	530	7	7.0	7.1	3.6
50	Dodmoor	10	1.7	250	1.8	57	24	2.4	2.7	2300	440	6	4.9	6.9	2.5
51	Whimble	18	0.9	370	2.3	99	27	2.7	3.3	2600	510	8	7.3	-	3.9
52	Bardsey	58	2.8	860	2.8	129	46	2.5	5.1	3500	810	11	7.5	6.0	10.7

APPENDIX 3d. Topsoil data from Atherstone, Warwickshire

Note: Cu, Mo, S, Zn, Sr, Cr, Ca and As are in ppm and Fe, Al and K are in %

SAMPLE	SOIL SERIES	Cu	Mo	S	Fe	Zn	Sr	Cr	Al	Ca	Mn	As	K	pH	OM
53	B/D/B	21	6.8	710	2.1	90	36	3.3	3.3	2600	90	15	3.4	5.5	10.9
54	Onecote	24	6.2	740	1.5	86	58	2.9	4.6	4700	77	16	5.9	5.9	13.3
55	Wilcox	22	4.8	880	1.3	110	44	3.6	3.2	3200	89	13	4.0	5.5	13.1
56	Malham	31	5.4	560	3.0	148	28	4.8	4.1	4700	1500	14	6.1	6.8	9.2
57	Bodden	28	5.1	720	2.5	136	29	3.5	3.4	3800	620	14	4.4	6.2	9.6
58	Ticknall	28	6.9	560	2.4	68	47	2.8	4.7	4300	99	16	4.7	6.4	9.1
59	Dale	38	12.2	1030	3.3	200	62	4.3	5.1	5400	230	42	4.4	5.5	13.4
60	Dale	69	17.6	840	3.8	220	132	7.0	5.6	10700	730	4	7.3	7.0	9.3
61	Brickfield	16	2.7	660	1.7	66	23	3.0	2.4	3900	154	9	2.9	6.7	8.5
62	B/D/B	20	3.5	770	2.0	79	33	2.8	3.9	3200	154	9	5.6	5.8	11.6
63	B/D/B	18	4.4	530	2.9	89	32	3.4	4.3	4000	870	10	5.3	6.4	8.4
64	Dale	54	0.7	760	3.1	330	66	5.8	4.8	6000	430	47	6.0	5.9	12.6
65	Dale	64	-	690	3.6	210	63	4.6	4.8	11100	240	50	5.9	6.9	12.2
66	Brickfield	32	12.5	650	3.3	110	36	3.7	3.5	3700	450	19	4.0	6.4	9.2
67	Heapy	21	5.6	320	3.0	140	53	2.8	5.1	3500	310	14	6.5	5.9	9.0

APPENDIX 3e. Topsoil data from Onecote, Staffordshire

Note: Cu, Mo, S, Zn, Sr, Cr, Ca and As are in ppm and Fe, Al and K are in %. B/D/B/ is Brickfield, Dale, Bardsey series.

SAMPLE	SOIL SERIES	Cu	Mo	S	Fe	Zn	Sr	Cr	Al	Ca	Mn	As	K	pH	OM
68	Dunkeswick	7	0.8	600	1.5	29	25	1.5	2.3	2800	250	6	2.5	6.5	13.9
69	Dunkeswick	8	1.0	550	1.9	48	28	1.9	2.9	2900	360	5	3.1	6.5	9.1
70	Wilcocks	13	1.3	540	2.3	82	21	2.3	2.3	2600	580	6	2.7	6.8	6.3
71	?	11	1.8	500	2.6	110	30	2.8	2.7	3300	840	7	2.7	6.6	7.0
72	Dunkeswick	13	1.5	580	2.8	93	63	3.3	2.8	13300	840	7	3.2	7.7	8.5
73	Wilcocks	12	1.2	580	2.6	79	35	2.5	3.8	2500	490	6	3.8	6.2	8.9
74	?	23	1.6	600	2.3	69	31	2.2	3.4	3500	470	6	3.4	6.5	10.4
75	Wick	12	2.4	740	1.7	45	22	2.2	2.2	2900	360	7	1.9	6.3	11.9
76	?	16	1.7	720	2.6	71	25	2.5	3.3	1350	360	8	2.1	5.7	9.7
77	E. Keswick	11	1.1	600	2.0	80	27	2.0	2.0	4000	540	6	2.1	6.7	9.1
78	E. Keswick	13	1.6	1090	1.7	86	34	2.1	2.4	4700	220	6	2.6	6.1	14.9
79	Wick	8	1.6	620	1.7	63	30	1.8	3.1	1990	460	6	3.2	6.1	8.7

APPENDIX 3f. Topsoil data from Masham, North Yorkshire

Note: Cu, Mo, S, Zn, Sr, Cr, Ca and As are in ppm and Fe, Al and K are in %

SAMPLE	SOIL SERIES	Cu	Mo	S	Fe	Zn	Sr	Cr	Al	Ca	Mn	As
1	Highweek	9.4	1.5	4033	403	37	14.1	1.6	637	6633	253	.22
2	Loxhore	11.0	1.1	4267	217	45	15.2	0.9	221	4333	480	.14
3	Highweek	13.7	1.4	4700	289	51	12.7	1.3	441	5367	247	.12
4	?	6.6	2.4	4667	184	41	8.6	1.1	120	6700	160	.12
5	Highweek	11.1	1.2	4467	275	39	10.9	1.2	271	6167	104	.16
6	Highweek	10.4	1.3	4200	313	35	13.2	1.4	467	5633	135	.22
7	Highweek	9.8	1.2	4633	290	47	13.9	1.2	267	6533	131	.17
8	Highweek	10.2	1.0	5000	196	38	9.2	0.9	147	5700	110	.15
9	Dartington	10.8	2.0	4500	134	42	12.1	1.1	85	5900	157	.07
10	Highweek	11.6	1.4	4933	356	42	15.0	1.3	360	6100	262	.19
11	Highweek	11.6	1.2	4833	331	42	9.7	1.3	412	5567	148	.18
12	Highweek	9.2	1.9	4333	173	34	12.7	0.9	154	7567	161	.12
13	Highweek	8.8	1.5	4300	232	41	13.3	1.1	245	5533	98	.19
14	Highweek	11.4	1.6	4500	197	37	15.0	0.9	240	6833	171	.11

APPENDIX 3g Mean herbage data from Brayford, North Devon

Note : All concentrations are in mg/kg DM

SAMPLE	SOIL SERIES	Cu	Mo	S	Fe	Zn	Sr	Cr	Al	Ca	Mn	As
15	Evesham	11.5	13.8	4000	137	36	13.7	0.8	113	7333	24	.11
16	Evesham	13.6	37.8	3533	338	40	14.9	1.2	381	7900	46	.15
17	Butleigh	10.4	6.1	4367	142	38	15.3	0.9	114	7933	28	.08
18	Sedgemoor	6.5	5.7	3700	176	24	76.0	0.8	165	6900	79	.17
19	Midelney	10.9	18.5	4333	192	37	18.0	1.3	281	7167	34	.12
20	Charlton Bank	8.6	3.1	4867	433	41	22.6	2.5	526	6133	121	.23
21	Turbary Moor	11.3	6.8	5333	214	42	26.3	1.0	206	7567	96	.10
22	Charlton Bank	8.2	2.8	4967	150	32	12.3	0.9	123	6900	30	.12
23	Long Load	13.1	7.3	4567	307	40	20.3	1.2	323	7833	40	.14
24	Sedgemoor	11.7	7.4	3867	253	43	27.0	1.3	254	5900	144	.13
25	Somerton	9.6	4.1	4200	193	35	29.0	1.0	145	10667	27	.29
26	Worcester	10.8	4.9	5867	192	39	10.9	1.0	147	5833	43	.15
27	Sedgemoor	7.6	6.9	5600	188	39	75.0	0.9	170	9733	139	.49
28	Somerton	8.1	2.7	4000	232	31	10.9	1.1	225	6633	47	.15
29	Evesham	9.4	6.6	4067	149	37	11.9	1.0	112	7767	27	.11

APPENDIX 3h Mean herbage data from Glastonbury, Somerset

Note : All concentrations are in mg/kg DM

SAMPLE	SOIL SERIES	Cu	Mo	S	Fe	Zn	Sr	Cr	Al	Ca	Mn	As
30	Denchworth	7.6	1.3	3933	183	34	12.3	0.9	187	7350	74	.14
31	Sherbourne	6.5	1.5	3467	164	30	9.8	1.3	109	9600	34	.13
32	Denchworth	8.7	1.3	4567	197	35	17.4	0.8	194	7700	35	.11
33	Sherbourne	6.7	1.8	3450	171	27	10.4	0.7	89	8350	61	.13
34	Waltham	9.0	1.2	4133	208	39	11.7	1.4	204	6867	266	.22
35	?	9.7	1.5	4133	158	39	10.7	1.0	114	6633	90	.10
36	Sherbourne	8.6	1.9	3733	267	35	10.0	1.1	181	7433	65	.22
37	Sherbourne	7.6	2.1	3667	203	34	9.6	1.1	118	7967	51	.27
38	?	10.1	1.3	4800	187	40	11.9	1.0	154	7633	80	.17

APPENDIX 3i Mean herbage data from Stow-on-the-Wold, Gloucestershire

Note : All concentrations are in mg/kg DM

SAMPLE	SOIL SERIES	Cu	Mo	S	Fe	Zn	Sr	Cr	Al	Ca	Mn	As
39	Reclaimed	10.8	2.0	6100	144	42	9.8	1.0	111	6333	198	.18
40	Merevale	11.9	1.9	6267	298	43	11.4	1.3	324	5967	88	.21
41	Shifnal	10.8	1.7	5200	260	45	5.3	1.2	196	5133	78	.21
42	Shifnal	11.4	1.3	5200	243	51	8.5	1.1	198	5133	129	.17
43	Shifnal	11.4	1.3	5167	223	46	7.5	1.1	166	5767	90	.24
44	Gatacre	10.9	1.4	4200	221	53	8.7	1.3	179	5767	121	.16
45	Baxterley	13.8	2.7	4833	250	52	9.7	1.2	261	5700	124	.25
46	Bardsey	12.4	2.4	4933	337	55	10.7	1.5	225	6100	107	.28
47	Baxterley	7.4	1.5	5850	124	37	10.0	1.0	132	7250	70	.14
48	Dodmoor	10.3	1.3	4700	264	38	10.3	1.0	249	5833	60	.19
49	Shifnal	11.1	1.7	4800	155	37	10.5	0.9	107	7800	49	.12
50	Dodmoor	10.9	2.2	4167	163	37	10.8	1.0	124	7400	58	.14
51	Whimble	10.4	1.5	5633	181	37	10.5	1.2	146	5300	74	.17
52	Bardsey	11.6	2.5	4933	283	45	10.0	1.2	333	5267	96	.23

APPENDIX 3j Mean herbage data from Atherstone, Warwickshire

Note : All concentrations are in mg/kg DM

SAMPLE	SOIL SERIES	Cu	Mo	S	Fe	Zn	Sr	Cr	Al	Ca	Mn	As
53	B/D/B	11.2	4.6	4567	265	54	7.2	1.6	303	5733	360	.30
54	Onecote	9.5	5.6	5333	118	45	8.5	1.1	85	5767	232	.22
55	Wilcox	10.7	4.7	5900	198	56	8.2	0.9	213	5750	335	.19
56	Malham	11.3	3.1	5733	235	40	6.5	1.4	205	7200	83	.21
57	Bodden	10.7	3.1	4767	170	46	7.1	1.1	104	6500	90	.14
58	Ticknall	9.7	4.3	4600	118	37	7.2	1.0	65	6900	56	.16
59	Dale	13.5	4.7	5300	266	69	13.9	1.4	251	6033	238	.30
60	Dale	7.8	9.5	4100	184	36	13.8	1.3	94	7600	40	.25
61	Brickfield	13.1	3.6	4967	184	44	5.6	1.0	144	5700	121	.18
62	B/D/B	10.5	3.0	5200	277	48	7.5	1.3	281	6133	133	.20
63	B/D/B	10.7	2.3	5333	275	40	6.7	1.4	289	7533	110	.20
64	Dale	13.1	5.0	5500	263	64	9.5	1.6	350	5933	140	.31
65	Dale	12.6	7.1	4867	203	49	8.7	2.2	788	5967	130	.21
66	Brickfield	12.3	4.6	4067	114	46	6.7	0.9	51	5033	110	.11
67	Heapy	10.8	4.9	4600	133	45	7.5	1.0	95	5933	227	.15

APPENDIX 3k Mean herbage data from Onecote, Staffordshire

Note : All concentrations are in mg/kg DM

: B/D/B is Brickfield, Dale, Bardsey series.

SAMPLE	SOIL SERIES	Cu	Mo	S	Fe	Zn	Sr	Cr	Al	Ca	Mn	As
68	Dunkeswick	7.3	1.8	4100	136	48	4.6	1.2	69	4533	139	.11
69	Dunkeswick	7.4	1.5	4467	133	43	6.1	0.8	81	4833	114	.13
70	Wilcocks	8.1	2.2	4033	147	43	6.7	1.4	85	4533	103	.10
71	?	8.9	2.7	3667	160	32	11.8	0.9	105	5433	119	.08
72	Dunkeswick	10.7	2.6	5000	194	37	21.4	1.4	58	7800	87	.14
73	Wilcocks	10.3	1.5	4933	243	48	9.9	1.4	208	5933	184	.14
74	?	10.5	3.8	4233	194	40	7.9	1.1	183	6033	83	.12
75	Wick	11.1	2.0	5267	157	44	7.0	1.0	83	5267	167	.11
76	?	10.4	1.9	4500	236	57	10.0	1.6	190	5100	298	.13
77	E. Keswick	6.7	1.3	4000	200	40	14.8	1.2	130	5200	74	.12
78	E. Keswick	9.0	1.5	4100	209	40	13.8	1.3	138	5467	66	.13
79	Wick	10.6	1.9	5167	147	48	10.4	1.2	82	4867	273	.12

APPENDIX 31 Mean herbage data from Masham, North Yorkshire

Note : All concentrations are in mg/kg DM

APPENDIX 4

PAPERS PUBLISHED DURING THIS RESEARCH PROJECT1 **Incidence of bovine copper deficiency in England and the Welsh borders**

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TWO previous studies have led to the publication of maps showing the incidence of bovine hypocupraemia in Britain (Allcroft 1952, Allcroft and Uvarov 1959, Thomson 1971, Thomson and others 1972). These are of limited use today because both surveys were conducted before 1975, when the occurrence of bovine hypocuprosis started to increase dramatically. This increase was sustained until 1979 when a small decline was noted, possibly caused by awareness of the problem and control measures (Lewis 1980).

As the initial part of a research programme to assess the importance of geochemical factors in present day copper problems in livestock, a detailed survey has been conducted, based on the copper content of whole blood samples from cattle analysed by the veterinary investigation service over the period 1977 to 1980 inclusive. The advantages of using blood copper as an index of the copper status of cattle outweigh the disadvantages, in that it provides a reliable, widely used diagnostic technique with an abundance of data for most of Britain. Data were compiled from records of the Central Veterinary Laboratory, Weybridge, and the veterinary investigation centres at Shrewsbury and Sutton Bonington. The area covered was restricted to England and the Welsh borders; data for Wales were not included as they were not of a strictly comparable form.

The method used for compiling the map (Fig 1) was to take the lowest blood copper value in a herd, providing it fulfilled the following criteria: (a) there was more than one sample from the herd; and (b) the values were less than or equal to 0.08 mg copper/100 ml blood. Cattle with blood copper values in the range 0.06 to 0.08 mg/100 ml were considered to be marginally deficient, and those with levels of 0.05 mg/100 ml or less to be deficient. Owing to the confidential nature of the data the exact locations of farms with hypocupraemic cattle could not be used; the eight-figure British national grid reference of the approximate centre of the enclosing parish has been recorded instead. Over the period 1977 to 1980 cattle in 4088 herds with blood copper values of 0.08 mg/100 ml or less satisfied the above criteria. Of these, 1748 had blood copper values of 0.05 mg/100 ml or less.

From the map it is apparent that copper deficiency in cattle continues to pose a serious problem to agriculture. Although hypocupraemia may precede weight loss and other clinical symptoms of hypocuprosis by several months (Mills 1979), the wide ranging spread of low blood copper levels must surely be taken as a warning. Bovine hypocuprosis has been

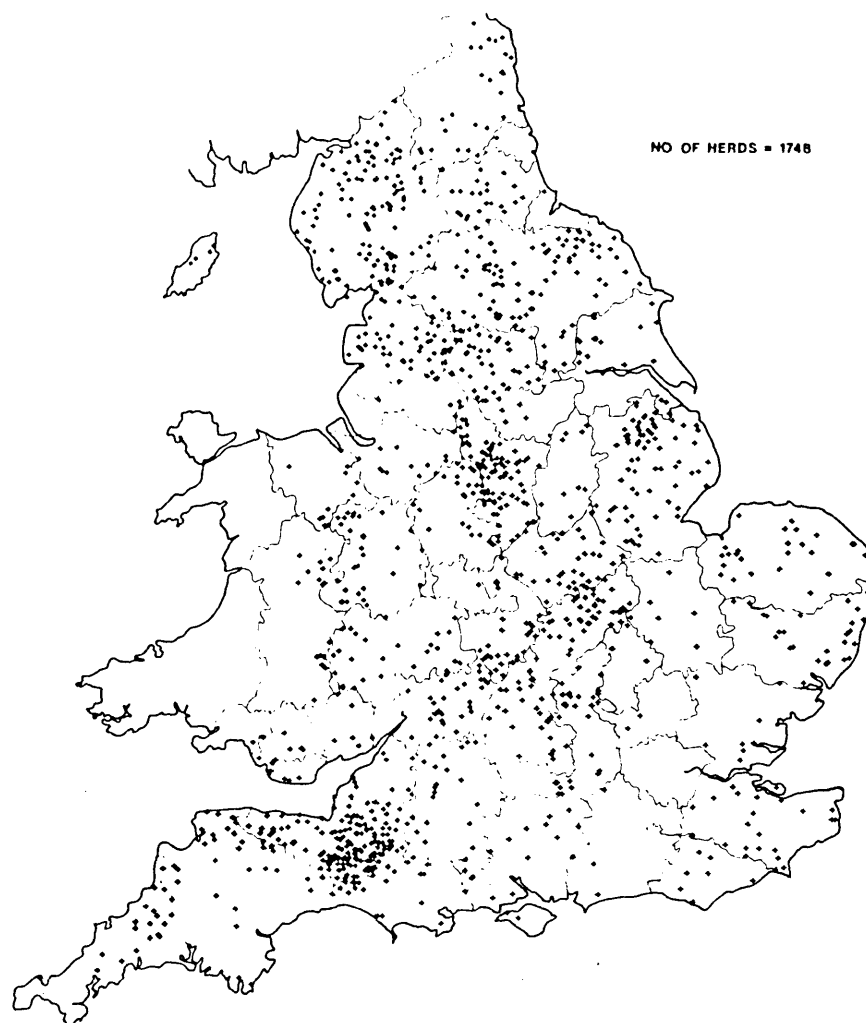


FIG 1: Distribution of bovine hypocupraemia in England and the Welsh borders, 1977 to 1980. (Blood copper levels of 0.05 mg/100 ml or less)

recognised in Britain for 30 or more years (Allcroft and Parker 1949, Jamieson and Allcroft 1950) and effective treatments are readily available, including the addition of copper salts to the diet, oral dosing with copper compounds, the provision of mineral supplements or parenteral injection with a suitable proprietary copper complex.

The low blood copper herds recorded were spread throughout most of the cattle populated regions of England and the Welsh borders, but there were obvious foci of concentration, particularly in parts of Somerset, Derbyshire, Lincolnshire and Cumbria. The cause in some of these areas was suggested to be excess dietary molybdenum. Molybdenum induced copper deficiency in cattle has previously been recognised and studied in Somerset, Dorset, the southern Pennines, Gloucestershire and Warwickshire, where the cause of the problem was traced to underlying molybdenum-rich rocks, the marine black shales of Jurassic and Carboniferous eras (Thornton and Webb 1980). These areas include the well known teart pastures of Somerset, where the source of molybdenum is the lower beds of the Lower Lias formation (Ferguson and others 1943, Lewis 1943, Le Riche 1959). Previous geochemical studies have shown that molybdenum-rich rocks underlie about two million acres of agricultural land in England and Wales (Thornton and Webb 1979). However, it is apparent from the map presented that several hypocupraemic regions fall outside the molybdeniferous areas, and so must be attributed to other causes including a low intake of copper, further multi-element interactions involving copper and management effects.

Maps showing the regional distribution of 24 elements including copper, molybdenum, cadmium and zinc, have been published in the Wolfson Geochemical Atlas of England and Wales (Webb and others 1978). These data will provide

an excellent opportunity to make comparisons with those for copper deficiency, particularly in the less well recognised areas of bovine copper deficiency, shown by this present study, and to examine the possibility of other elements being involved. A programme of rock, soil and pasture herbage analysis will be conducted in a selection of these areas by the applied geochemistry research group over the next two years.

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APPENDIX 4 (cont.)

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6.5 THE INCIDENCE OF BOVINE HYPOCUPRAEMIA IN ENGLAND AND WALES AND ITS RELATIONSHIP WITH GEOCHEMISTRY

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As the initial part of a research programme to assess the importance of geochemical factors in livestock production, a survey has been conducted on the copper (Cu) content of whole blood samples from cattle analyzed by the Veterinary Investigation Service over the period 1977-1980 inclusive. The area covered was restricted to England and the Welsh borders; data for Wales were not included, as they were not of a strictly comparable form, being based on plasma rather than on whole blood analysis.

Some 4 088 herds were found with blood Cu levels ≤ 0.8 mg/l; of these, 1 748 herds had levels ≤ 0.5 mg/l. Their distribution was mapped and the hypocupraemic herds were shown to be spread throughout most of the cattle-populated regions of England and the Welsh borders. There were obvious foci, however, particularly in parts of Somerset, Derbyshire, Lincolnshire and Cumbria. The causal factor in some areas, like Somerset, Dorset, Southern Pennines, Gloucestershire and Warwickshire, has been attributed to an excess of dietary molybdenum (Mo) from the underlying Mo-rich rocks — marine black shales of Jurassic and Carboniferous age (Thornton and Webb, 1980). Previous geochemical studies have shown that Mo-rich rocks underlie some 2 million acres of agricultural land in England and Wales (Thornton and Webb, 1979). However, it is apparent that several hypocupraemic regions fall outside the molybdeniferous areas and, hence, must be attributed to different causal factors, including a low intake of Cu, further multi-element interactions involving Cu, as well as management effects.

"Smoothed" geochemical data from the Wolfson

Geochemical Atlas of England and Wales were obtained for 22 of the most severely affected regions of the country (Webb, Thornton, Thompson, Howarth and Lowenstein, 1978). A Ridge regression analysis, in preference to ordinary least squares regression, was then undertaken to establish the best geochemical predictors of bovine hypocupraemia. The Ridge regression removed the effect of correlations between the independent variables and so enabled reliable regression coefficients to be obtained. The first run of the program utilized geochemical data for 23 elements. Successive runs enabled the data set to be reduced and the regression equation to become more statistically sound. The optimum regression equation was achieved with Mo, Cd, Sr, Cr and Al as the best positive, and Cu, K and As as the best negative predictors of bovine hypocupraemia (Table 6.5.1). This equation was then used to predict the incidence rate of bovine hypocupraemia throughout England and Wales which was plotted on a grey-scale map. Whilst the prediction map is based solely on stream sediment data and therefore only approximates to metal content in the rock, soil, plant and, hence, the animal, the indicated results are very sensible. Many of the areas predicted to be severely affected by bovine hypocupraemia have already been recognized as such by the veterinary profession. It is hoped to improve on the prediction equation with follow-up field work. This is currently being undertaken on farms in Devon, Somerset, Gloucestershire, Warwickshire, Staffordshire and North Yorkshire. It is hoped, from the soil and herbage samples collected, to determine the pathways and quantities of metals transferred from the soil to the

APPENDIX 4 (cont.)

Bovine Hypocupraemia in Relation to Geochemistry

TABLE 6.5.1

The most statistically significant solution to the Ridge regression analysis

Solution	Predicting elements	R ²	F-ratio
	Al, Ca, Fe, K, Mg, Si, Ga, Sc, Cu, Pb, U, Ba, Co, Cr.		
1	Ni, Mn, Sn, Sr, Li, Mo, As, Zn, Cd	Unavailable	Unavailable
2	Al, K, Si, Ga, Cu, Cr, Sr, Mo, As, Zn, Cd	.7455	2.6625 ^{NS}
3	Al, K, Ga, Cu, Cr, Sr, Mo, As, Cd	.7505	4.0107*
4	Al, K, Ga, Cu, Sr, Mo, As, Cd	.7390	4.6008**
5	K, Ga, Cu, Cr, Sr, Mo, As, Cd	.7323	4.4451**
6***	Al, K, Cu, Cr, Sr, Mo, As, Cd	.7704	5.4528**
7	Al, Cu, Cr, Sr, Mo, As, Cd	.7556	6.1819**
8	Al, K, Cu, Sr, Mo, As, Cd	.7385	5.6485**
9	K, Cu, Cr, Sr, Mo, As, Cd	.7515	6.0555**
10	Al, K, Cu, Cr, Sr, Mo, Cd	.7497	5.9898**
11	Cu, Mo, Cd	.5858	8.4874**
12	Cu, Sr, Mo, Cd	.6247	7.0740**
13	Cu, Sr, Mo, As, Cd	.6679	6.4367**

^{NS} — not significant* — significant at F_{.05}** — significant at F_{.01}*** — The most statistically significant solution to the Ridge regression analysis, as indicated by the R² and F-ratio values.

animal via the pasture herbage. With an improved regression equation, the prediction map might be of use in determining whether farms lie in a high, medium or low risk area. Appropriate precautions can then be taken and a possible loss in production avoided.

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