TROPICAL SOIL-PLANT INTERACTIONS IN RELATION TO MINERAL IMBALANCES IN GRAZING LIVESTOCK

BY

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Work forming part of the requirements for the degree of Doctor of Philosophy of the University of Nairobi.

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DECLARATION

1. I declare that this thesis is of my own composition and, apart from the acknowledged assistance, is a record of my own research. The material has never been presented before to the University of Nairobi or any other establishment for an academic award.

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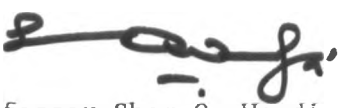

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ABSTRACT

1. A survey of the literature revealed that mineral imbalances were likely to arise in livestock grazing tropical pastures; that a variety of techniques involving soil, plant or animal measurements could be used to assess imbalance but none could be singularly relied upon; that no comprehensive study of mineral imbalance had been made in Western Kenya.

2. One hundred and thirty-five samples of soil and herbage were collected from 84 farms in Bungoma and Trans Nzoia Districts; sites were classified in terms of their geology, topography, altitude and management type and herbage by species. Soils were assayed for extractable calcium (Ca), phosphorus (P), iron (Fe), aluminium (Al), cobalt (Co), manganese (Mn), copper (Cu), molybdenum (Mo) and zinc (Zn), and for total selenium (Se). Herbages were analysed for the same elements plus magnesium (Mg), sulphur (S) and silicon (Si) as total concentrations on a dry matter (DM) basis.

3. Forage analysis revealed low mean concentrations for most elements, notably P (1.35g/kg DM), Ca (1.48g/kg DM), S (1.46g/kg DM), Cu (4.0mg/kg DM) and Se (97ug/kg DM). Comparison with the Agricultural Research Council (1980) nutrient requirements suggested that 25-98% of the herbage samples were inadequate with respect to these elements.

4. The analysis of species 'effects' showed that both Kikuyu and Rhodes grasses were superior to Napier grass and Nandi setaria with respect to the concentrations of Ca, Mg, Se, P and S. However, the advantage of Kikuyu grass was offset by the likely occurrence of deleterious compounds and presence of higher levels of Mo which, with S, may inhibit Cu utilization in the ruminant. The legumes lucerne and sweet potato vines generally had a higher mineral content which may offer an alternative to supplementation of animal diets.

5. Low herbage concentrations of Ca, Mg, S and P were not confined to particular bedrocks, indicating that geology has a negligible effect on the distribution of the macro-mineral deficiencies in grazing livestock in the survey area. Opposing influences of geology on the trace element composition of soil and herbage, resulted in poor prediction of herbage composition form the soil data. The trace elements most affected by geology were Cu and Se, and Se deficiency is expected on soils overlying igneous granites and alluvial deposits.

6. Low herbage phosphorus concentrations were obtained at low altitudes (< 5000ft above sea level); these were attributed to fixation by high exchangeable Fe and Al levels in the soils and low pH. Variations in altitude were associated with far larger changes in extractable mineral concentrations in the soil than

in herbage and trends were often in opposite direction for Co, Al, Mn and Fe. Higher altitude herbages were richer in S, Ca, Cu, Fe, Mg, Mo and Zn and low in Se and Mn.

7. The prevalence of herbage mineral deficiencies was not attributable to differences in landscape profiles or management: good management may be nullified by overriding soil properties of low pH and geology, and the strong effects of plant species and altitude.

8. The evaluation of a variety of soil extraction methods showed that availability in vitro may not be a good predictor of herbage composition in the survey area. The poor soil-herbage relationships were attributable to inconsistencies in the way in which extraction methods simulated soil conditions at the root surface.

9. Strategies and priorities have been suggested for further investigations concentrating on blood and tissue analysis and response to supplementation in young, growing animals. In this way any constraints on livestock production can be identified and alleviated.

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CHAPTER ONE

INTRODUCTION

The greater understanding of the nutritional requirements and genetic potential of milk and meat producing animals has resulted in changes in the breeds of livestock used and the recognition of the long-term economic benefits obtainable from good pasture in the tropics. This had led to much poor and unproductive land being improved and reclaimed, and to the use of a variety of pasture species obtained by selective plant breeding techniques. With the increased ability to control disease, many of the limitations of livestock production from pasture are being overcome, but some still need comprehensive research. Amongst these factors is the problem of providing the correct balance of minerals in the animals' diet and it is feared that suboptimal performance and growth among grazing livestock are frequently related to an imbalance in mineral supply. Balch and Moir (1983), in summing up proceedings of the International Symposium on Herbivore Nutrition in the Tropics and Subtropics said that: "Mineral deficiencies and excesses are of special importance to tropical and subtropical herbivores. Research is needed at all levels from plants, through metabolism in the gut flora, to metabolism in the animal".

In certain tropical regions it has been reported that mineral deficiencies, imbalances and toxicities are severely inhibiting livestock production (McDowell, 1976; Conrad and McDowell, 1978). Grazing livestock usually do not receive mineral supplementation except for common salt and must depend almost exclusively upon forages for their requirements. Only rarely,

however, can forages completely satisfy all mineral requirements - deficiencies are either prevalent or anticipated. For example, in the 1974 Latin American Tables of Feed Composition (McDowell et al, 1977), borderline or deficient levels of certain elements were noted for many forages; the number of countries in which they were reported were 13 for Co cases, 14 for Cu and Mg, 10 for S, 24 for P, and 8 for Sodium (Na) and Zn (McDowell, 1976).

Plants derive their mineral elements from the soil in which they grow, and the amount of any element in the soil is related to the content of the underlying rock from which the soil is formed. The rocks forming the earth's crust are therefore the ultimate source of minerals to the grazing animal with soil acting as the entry point to the food chain; this is reflected by the fact that many naturally occurring mineral imbalances in livestock are associated with specific regions and are directly related to rock and soil characteristics. However, in describing the complex nature of the soil-plant-animal relationship, Mitchell (1972) considered that apart from geology effects, several factors may have an influence on mineral availability to the grazing animal; these factors include botanical composition (especially with regard to type and variety of plant species), soil moisture availability and management. Suttle (1987a) identified the complex actions and interactions of plant and animal factors which impinge upon absorption, retention and function of mineral nutrients in

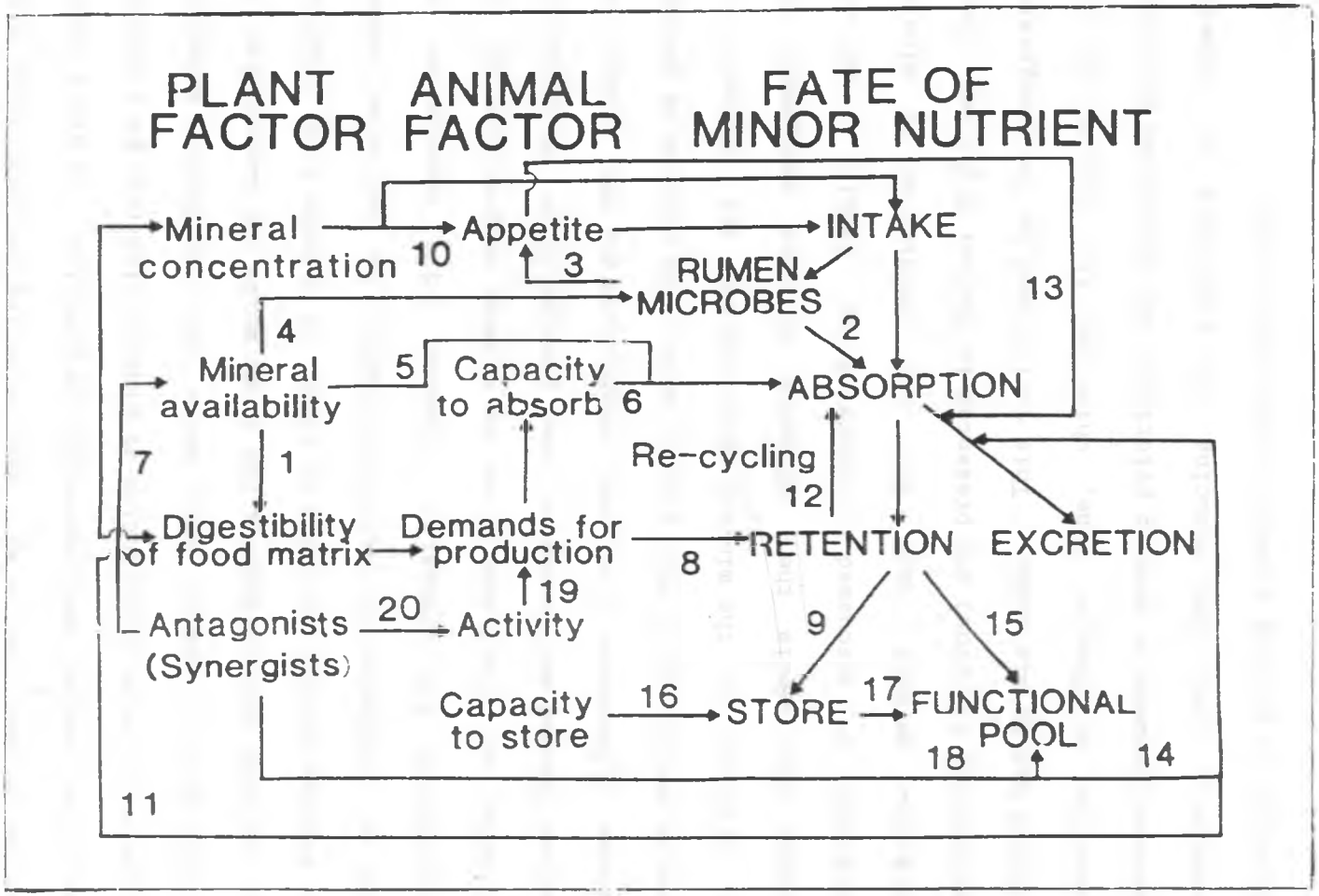
grazing animals (Figure 1) and concluded that the importance of particular factors and interactions varies from nutrient to nutrient, from feed to feed and from one herbivore to another.

In tropical Africa, few reports have been published giving extensive information on the extent of mineral imbalance, and in Kenya fears that grazing livestock production may be hampered by mineral deficiencies in certain areas have received attention in recent pasture and animal production meetings. Priority research strategies include mineral mapping on a district basis (International Service for Agricultural Resarch, ISNAR, 1985). More work is therefore required to clarify the mineral status of grazing livestock and to establish factors responsible for influencing the risks of mineral imbalance.

The objectives of the work presented in this thesis were (1) to carry out a survey on the mineral status of tropical forages in the Mt Elgon area of Western Kenya (ie Bungoma and Trans Nzoia Districts), so as to identify those elements that may be most limiting to grazing livestock production and how the anticipated problems might be distributed in the area; (2) to establish the factors contributing to the risks of mineral imbalance and how they might be controlled; and (3) to establish whether soil analysis could be used to predict and assess the anticipated problems.

The description and discussion of the project undertaken is preceded by a review of the literature concerned with mineral elements in animal nutrition (Chapter Two). A general overview

Fig. 1 Illustration of Complex Actions and Interactions of Plant and Animal Factors in Determining Absorption, Retention and Function of Minor Nutrients in Herbivores.*



*This figure shows that of the available plant nutrients absorbed by the herbivore, part is retained as a functional pool and body reserve while the rest is excreted. The retained fraction can be remobilized not only to sustain demands for production, antagonistic stress and dietary deficiency but also to restore appetite for optimal feed intake.

of the importance of mineral imbalance to grazing livestock is followed by a detailed discussion of the methods commonly used in assessing the incidence and impact of mineral deficiencies and toxicities, their respective advantages and disadvantages, and the ultimate choice of forage screening for predicting areas where the risks of imbalance might be prevalent. A brief review of the world-wide distribution of mineral disorders in livestock is also given. Chapter Three deals with the general materials and methods used throughout the study while the description of the herbage results obtained is given in Chapter Four, together with a discussion of their relationship to published animal requirements for minerals. Chapter Five reports on the influence of plant species on the mineral composition of the sampled forages and discusses the possibilities of exploiting species differences in mineral composition for the control of mineral imbalances in the survey area. The effects of geology and altitude on the mineral distributions are discussed in Chapter Six, while the influence of farm management and topography is discussed in Chapter Seven. Results of the soil extraction methods used for the prediction of mineral availability to plants are presented in Chapter Eight and their relative merits discussed. This is followed by the Integrating Discussion in Chapter Nine, which uses the results of all previous Chapters to develop priorities and strategies for first determining and then reducing the prevalence of mineral imbalances in grazing animals in the survey area.

CHAPTER TWO

IMPORTANCE OF MINERAL IMBALANCE TO LIVESTOCK PRODUCTION

2.1 GENERAL INTRODUCTION

Undernutrition is commonly accepted as the most important limitation to grazing livestock production in many parts of the world. Lack of sufficient energy and protein is often responsible for suboptimum cattle and sheep production. Numerous investigators, however, have observed that cattle sometimes deteriorate in spite of an abundant feed supply (Davis, 1951; Russell and Duncan, 1956; Sutmoller et al, 1966; Read et al, 1986). Mineral deficiencies or imbalances in soils and forages have long been held responsible for low production and reproduction problems among grazing cattle in the tropics. Cattle grazing forages in severe P-, Co- and Cu-deficient areas are even more limited by lack of these elements than by lack of either energy or protein. Research especially in tropical regions has shown that mineral supplementation increases calving percentage by 20-100%, growth rates from 10-25%, and reduces mortality significantly (McDowell and Conrad, 1977).

At least fifteen mineral elements are nutritionally essential for ruminants. There are seven major or macrominerals - Ca, P, potassium (K), Na, chlorine (Cl), Mg and S; and eight trace or microminerals - Fe, I, Zn, Cu, Mn, Mo, Co and selenium (Se). By definition, the major elements are present in the body in large quantities, while essential trace elements are those required in very small amounts for optimum body function

(Cotzias, 1967; Mertz, 1974) but they are equally important in livestock nutrition and each deficiency can lead to lowered productivity and clinical disease.

The newly discovered essential elements - chromium (Cr), vanadium (V), nickel (Ni), tin (Sn), Si and arsenic (As) have been reviewed by Underwood (1977) and McDowell et al (1978). The practical significance of the newer trace elements for ruminants has not been demonstrated since evidence for essentiality has been based almost exclusively on growth effects in "laboratory" animals receiving highly purified diets in controlled environments (Underwood, 1981).

In specific regions, toxic concentrations of Cu, fluorine, Mn, Mo, Fe or Se in pasture limit grazing livestock production (Rosenfield and Beath, 1964; Kubota et al, 1967; Thomson et al, 1972; Bingley and Anderson, 1972). Additional toxicity hazards may arise by contamination of ruminant diets with As, lead (Pb), cadmium (Cd), mercury (Hg) and Al.

In the context of this thesis, the major concern is with deficiency and to a lesser extent toxicity problems in grazing livestock. The major elements plus Cu, Co, Se, Mo, Zn, I, Fe, Mn and Si have so far been found to be of agricultural significance causing clinical disease or limiting productivity in the deficient or toxic state (Mills, 1983). Iron has never been reported to produce deficiency problems in grazing livestock (Underwood, 1977) and the occurrence of Mn related

problems is still in dispute (Egan, 1972; Hartmans, 1974; Underwood, 1981). As knowledge on these minerals is vast and the voluminous literature is constantly being updated, it would be impossible to review each element exhaustively here. The following short review is therefore restricted to consideration of the major elements Ca, P, Mg and S, and the trace elements Cu, Co, Se, Zn and Mo.

2.2 MINERAL FUNCTIONS IN ANIMALS AND THE GEOGRAPHICAL DISTRIBUTION OF MINERAL IMBALANCE PROBLEMS

In the body, minerals tend to (a) play a structural role in the skeleton and other connective tissue; (b) function as osmotic and ionic regulators in body fluids; and (c) be present as integral parts of enzymes. Problems of diverse nature can therefore accrue from an imbalanced supply of minerals to livestock.

Specific clinical symptoms of mineral imbalance may occasionally be useful in diagnosing deficiency, but by the time these are apparent the deficiency or excess is usually in an advanced stage (eg in P deficiency, Little, 1982). With P, as with most mineral deficiencies, borderline or subclinical cases do not produce symptoms specific to a single mineral. The use of biochemical criteria of availability from the feed and within the animal or the response of the animal to supplementation may provide the best diagnosis of deficiency. Each mineral or narrow group must, however, be considered separately because of distinctive features.

2.2.1 The Major Elements

(a) Calcium and Phosphorus

Ninety-nine percent of the body's Ca is found in the skeleton, and it is in the bones that the classic signs of Ca deficiency are to be found. Calcium in bones is present in the form of the mineral hydroxyapatite, and it is this mineral that provides strength and density; inadequate supply of Ca results in easy fracture and deformation of the bones. At times of insufficient supply of Ca, grazing animals react by redistributing skeletal Ca reserves by a remodelling process involving resorption of bone from mineralized sites and deposition of new bone at the points of growth (Suttle, 1983a). It may thus be many months before characteristic signs of severe deficiency - skeletal and dental abnormalities, lameness and depressed growth, rickets in young and osteomalacia in older animals - become visible. Dietary Ca concentrations < 2g/kg dry matter (DM) are likely to cause these disorders (Long et al, 1957). Acute calcium deficiency ('milk fever') occurs in parturition in dairy cows because of the sudden increase in Ca requirement for milk production but in sheep the onset of deficiency is more gradual because of the higher relative demand for late foetal growth (Suttle, 1983a).

Seventy-five percent of P in the body is found in the bone. Although this predominance is less than that of Ca, the skeleton

is still the major repository for this mineral and therefore it is also the skeleton which reveals the consequences of P deficiency (Little, 1984; Read et al, 1986). Phosphorus is also present in the bone mineral, hydroxyapatite in a ratio of 1:2.2 with Ca. The consequences of dietary P deficiency on the skeleton are generally the same as those of Ca deficiency causing rickets in the growing animal. In addition, severe P deficiency in animals fed low P diets can cause loss of appetite and depraved appetite, manifested in the chewing of bones of dead animals and other debris, as in 'pica' - the wasting disease which occurs worldwide in cattle and sheep (Russell and Duncan, 1956; Butler and Jones, 1973). Loss of appetite may provide some protection from P deficinecy by reducing both maintenance and production needs (Suttle, 1987a).

In South Africa, P supplementation studies pioneered by Theiler in the 1930's (see Van Niekerk, 1978) during his investigations into the cause of botulism ('lamsiekte') and bovine aphosphorosis ('stywesiekte') established that P deficiency was responsible for cattle exhibiting subnormal growth and reproduction and a depraved appetite or "pica" evidenced by bone chewing. Other countries reporting death from botulism as a result of bone chewing include Senegal (Calvet et al, 1965) and Brazil (Torkarnia and Dobereiner, 1973). In areas of Piaui (Brazil) an estimated 2-3% of approximately 100,000 cattle die annually of botulism. Under conditions of extreme P shortage, cattle may go for 2 or 3 years without

producing a calf or even coming into oestrus (Phillips, 1956). In P-deficient areas, if a calf is produced, cows may not come into a regular oestrus again until body P levels are restored, either by feeding supplementary P or by cessation of lactation.

(b) Sulphur

Sulphur, like nitrogen (N), is essential for microbial synthesis because it contributes to microbial S-containing amino-acids. Failure to meet the microbial needs for S may depress the rate of digestion in the rumen (Bray and Hemsley, 1969; Kennedy and Siebert, 1973) with consequent depression of feed intake. The problems involved in assessing ruminant requirements for S are therefore similar to those outlined for N. Both organic and inorganic S in the diet can be degraded to sulphide in the rumen and subsequently incorporated into the S-amino acids of microbial protein, although S may enter microbial protein by other routes. The interdependence of S and N requirements of the rumen micro-organisms and the serious effects on rates of digestion when S is limiting has led to the recommendation of degradable S/degradable N ratio in the determination of S requirements for ruminant livestock (ARC, 1980).

Sulphur supplementation will most likely be needed to meet the requirements of ruminants when poor quality roughages are grown on S-deficient soils or feeds containing non-protein (and therefore non-sulphur) N sources are fed (Whanger, 1972). A

recent review summarized four cattle S supplementation trials in which control diets contained between 0.04 and 0.10% S (Miles and McDowell, 1983, cited by McDowell et al, 1984). Food intake by sulphur-supplemented cattle increased by 7-260% and milk and meat production by 6-400%. Evidence presented by Paladines (1984) has shown that S deficiencies appear to be frequent in savanna-type soils of the tropics. In some instances, responses to S-fertilization have been obtained. Experiments using Stylosanthes guianensis showed that both intake by herbivores and digestibility of the forage increased when this species was grown in soils fertilized with superphosphate containing 12% S (Hunter et al, 1978).

(c) Magnesium

Hypomagnesaemia and the associated tetany is a metabolic disorder encountered in both grazing adult ruminants and calves reared too long on milk without access to other foods. The disorder is due primarily to dietary inadequacy of utilizable Mg. Susceptibility to grass tetany is increased in older ruminants because of the decreased ability to mobilize skeletal Mg with increasing age (Chico et al, 1973). Grass tetany generally occurs among cattle grazing grass or small-grain forages in cool, wet weather. Clinical tetany is endemic in some countries but generally affects only a small proportion of cattle (1-2%). Losses of up to 3% can be found in dairy or beef herds (Grunes et al, 1970; Molloy, 1970; Mansfield et al,

1975; Baker and Gould, 1976). In some individual herds in some years losses may be as high as 20% (Underwood, 1981).

In Scotland, Butler(1963) detected hypomagnesaemia in clinically normal cows after turn out in spring in > 40% of the herds. Although not characterized by death, incidence of non-clinical hypomagnesaemia is far greater than clinical tetany, and the economic consequences of lowered production may be significant (Netherlands Committee on Mineral Nutrition, NCMN, 1973) and may include decreased milk and beef production (Grunes et al, 1970; Grunes and Mayland, 1975).

The prevalence of hypomagnesaemic tetany has been associated with decreased forage availability of Mg caused by antagonizing effects of Al (Dennis, 1971; Mayland and Grunes, 1979) and K (Kemp and t'Hart, 1957; Kemp et al, 1961; Butler, 1963; Suttie and Field, 1969). Provision of commercial Mg-containing mineral supplements may be of little benefit because (1) they contain inadequate quantities of Mg to protect against tetany during susceptible periods, and (2) provision of such supplements to normal animals during non-susceptible periods is useless as a prophylactic measure, since additional Mg is not stored to provide a depot of readily available Mg for subsequent emergency use (McDowell et al, 1984). However, in southeastern USA, a complete mineral mixture with 25% MgO (14% Mg) has been effective in preventing grass tetany in beef cattle (Cunha, 1973).

2.2.2 Trace Elements

(a) Physiological Mode of Action

Trace elements act primarily as catalysts in enzyme systems in the cell, although some such as Si have been found to have a structural role in connective tissue and the organic matrix of the skeleton (Underwood, 1977; Mills, 1983). Their catalytic roles vary from weak ionic effects on enzyme systems to highly specific (metalloenzyme) associations where the metal is firmly bound to protein in a fixed molecular ratio. Generally, the metal in a metalloenzyme complex is specific and cannot be replaced by another element without loss of enzyme activity. Exceptions to this however exist: *in vitro* it has been found that Zn in carboxypeptidase A can be replaced by Co (Vallee, 1971) without enzyme deactivation. Cations with similar ionic radii, electronic configuration and electronegativity are often interchangeable. Manganese, Ni, Fe and Co for example, have all been shown to activate arginase *in vitro* (Georgievskii, 1982).

Body fluids and tissues also contain metalloprotein complexes that have no catalytic action. These function in transport, absorptive and storage roles with other ions and molecules, eg the role of Fe in haemoglobin associated with the transport of oxygen from the lungs to tisues. The majority of trace elements function in the body in a wide range of active organic compounds but Co and I differ in that each also functions as an integral part of a single compound, Co in cobalamin and I as part of the hormone thyroxine. Both of these compounds and

therefore Co and I are nevertheless involved in a range of metabolic processes in the body. Table 2.1 gives the metabolic functions of some important enzymes and metalloproteins associated with some trace elements of agricultural importance.

Table 2.1. Metabolic Functions of Some Enzymes and Metalloproteins Associated with the "Agriculturally Important" Trace Elements

Element	Enzyme/Metalloprotein	Function
Cobalt	Glutamate mutase	Oxygen transfer
	Methyl malonylCoA mutase	Succinate formation
	Methyl transferases	Methyl transfer
Copper	Ceruloplasmin	Fe utilization and storage
	Cytochrome oxidase	Terminal oxidation
	Cytocuprein	Superoxide dismutation
	Haemocuprein	Storage
	Lysyl oxidase	Collagen cross-linking
	Mitochondrocuprein	Storage
	Superoxide dismutase	Dismutation of superoxide free radical
	Tyrosine-3-monooxygenase	Hair and skin pigmentation
Iron	Catalase	Protection against H_2O_2*
	Cytochromes	Electron transfer
	Haemoglobin; Myoglobin	Oxygen transport
	Haemosiderin	Storage
	Succinate dehydrogenase	Carbohydrate oxidation
	Transferrin	Storage
	Xanthine oxidase	Purine metabolism

* ie Protecting tissue damage by hydrogen peroxide

Table 2.1 (continued)

Iodine	Thyroxine	Growth control
Manganese	Arginase	Urea formation
	Deoxyribonuclease	DNA formation
	Glycosyl transferase	Glycoprotein synthesis
	Oxaloacetate carboxylase	Oxaloacetate formation
	Pyruvate carboxylase	Pyruvate formation
	Superoxide dismutase	Dismutation of superoxide free radical
Molybdenum	Aldehyde oxidase	
	Sulphide oxidase	Sulphate oxidation
	Xanthine oxidase	Purine metabolism
Selenium	Glutathione peroxidase	Peroxide removal
Zinc	Alcohol dehydrogenase	Alcohol metabolism
	Alkaline phosphatase	Phosphate ester hydrolysis
	Arginase	Urea formation
	Carbonic anhydrase	CO_2 formation
	Carboxypeptidase	Protein metabolism
	Collagenase	Collagen formation
	Cytocuprein	Superoxide dismutation
	DNA and RNA polymerase	DNA and RNA formation
	Superoxide dismutase	Dismutation of superoxide free radical
	Thymidine kinase	Thymidine metabolism

(Source: Suttle, 1983a)

(b) Trace Element Deficiencies and their Impact on Animal Production

A suboptimal dietary intake of a trace element will lead to a deficiency of that element at the site of its enzymic catalysis and, consequently the functioning of the related metabolic pathway will be impaired. Suttle (1983a) describes the changes

in a three phase sequence of depletion, deficiency and disorder. Depletion occurs when the diet fails to maintain the trace element status of the body. During this period the body may bring into action certain protective mechanisms such as mobilization of body stores, an increase in the efficiency of absorption, a decrease in endogenous loss or a decrease in the concentration of the element in a product. Eventually, a deficiency state is reached where homeostatic controls fail and biochemical changes begin to occur, upsetting the elemental distribution required for maximal physiological activity.

Disorder occurs when the biochemical signs of dysfunction become evident as a result of impairment of metabolic pathways. Clinical signs of disorder may not occur for some time after the onset of cellular dysfunction. During the preceeding lag phase animals are said to suffer subclinical or borderline deficiency. Although there are no manifestations of disease, the animals do not thrive well and may have a reduced ability to cope with extra demands put on the body by infectious disease, pregnancy, rapid growth or stress factors. The time period over which a subclinical deficiency will persist will depend on the intensity of the deficiency, the duration of the deficit and the size of the initial reserves of the element. Despite the absence of clinical symptoms, pathological changes are occuring within the animals' body during this period. Histological changes have been reported to occur in skeletal, cardiovascular and intestinal tissues (Mills et al, 1976) and in the tissue of the

pancreas (Fell et al, 1985) in cattle of low copper status not showing clinical signs of deficiency. In cases where reserves of an element do not exist, such as with zinc, or they cannot be mobilized rapidly, the depletion, deficiency and disorder phases become superimposed and the animal suffers a sudden or acute deficiency. Table 2.2 summarizes the common symptoms observed in grazing animals as a result of inadequate (dietary) intake of some important trace elements.

(i) Copper

Copper is involved in a variety of physiologically important reactions (Mills, 1983) and the enzymic roles depicted in Table 2.1 provide typical examples. The Cu enzyme cytochrome oxidase, intimately associated with cellular organelles involved in ATP synthesis, occupies a key position. If its activity is sufficiently reduced by Cu deficiency, a wide range of synthetic reactions dependent upon ATP suffer in consequence. Copper deficiency also depresses the activity of the enzyme tyrosine 3-monooxygenase (Morgan and O'Dell, 1977) situated at the point of divergence of sequences leading either to synthesis of noradrenaline or of the pigment melanin. The element is again involved at intermediate steps in both sequences. Defects in the noradrenaline branch can be expected to have a wide variety of metabolic consequences while those in steps leading to melanin synthesis are clearly implicated in the diagnostically significant changes in pigmentation of the hair and wool of Cu-deficient livestock. The role of Cu in the enzyme, lysyl

Table 2.2 Main Symptoms Observed in Grazing Ruminants as a Result of Trace Element Deficiency.

Element	Animal Species	Symptoms
Cobalt	Lambs and Calves	Anorexia; Loss of weight; Listlessness; Emaciation; Anaemia; Blanched mucous membrane; Pale, fragile skin; Diminution of lactation; Birth of weak lambs and calves.
Copper	Lambs	Inco-ordination of hind quarters; Spastic paralysis with demylination of central nervous system (CNS).
	Sheep	Depigmentation and loss of wool crimp; Anaemia; Depressed growth; Listlessness and weakness; Bone disorders; Scouring; Dehydration; Delayed or depressed oestrus. Susceptibility to infection.
	Cattle	Depressed growth; Depigmentation of hair; Bone disorders; Anaemia; Stiff gait; Fibrosis of myocardium; Diarrhoea.
Iodine	Cattle and sheep	Retarded growth; Thyroid hyperplasia; Decreased metabolic rate; Hair and wool loss; Reproductive failure; Birth of weak, dead or hairless young; Reabsorption, abortion and stillbirth; Irregular or suppressed oestrus in cattle.
Selenium	Lambs and calves	Acute or chronic myodegeneration in cardiac or skeletal muscle; Myoglobinuria; Stiff and stilted gait; Arched back.
	Sheep and cattle	Subclinical growth loss; Unthriftiness; Impaired reproductive function; Mortality.
Zinc	Cattle and sheep	Anorexia; Retarded growth; Bone disorders; Stiffness of joints and hock swelling; Skin problems; Inflammation of mucous membrane of nose and mouth; Decreased wound healing; Change in hair and horn; Delayed sexual maturity, sterility and loss of fertility.

(Sources: Underwood, 1977, 1981; Howell, 1983; Suttle, 1988).

oxidase, secreted by cells involved in synthesis of the elastin component of connective tissues, typifies situations in which the loss of activity of a single metal-dependent enzyme has important implications for the integrity of tissues as diverse as the capillary bed, ligaments and tendons (Harris et al, 1980). Reduced superoxide dismutase activity will affect cell antioxidant systems, catecholamine metabolism can be altered by decrease in dopamine- -hydroxylase and loss of ferroxidase activity of ceruloplasmin may change Fe metabolism. Prohaska and Heller (1982) concluded that the hypertrophy and abnormal functioning of hearts from Cu-deficient animals could arise from changes in at least three of the above enzymes, resulting in alterations in the elastic properties of muscle, cardiac catecholamine depletion and defective energy metabolism.

Deficiencies of Cu affect grazing livestock throughout extensive regions of the world including the tropics. Copper deficiencies in ruminants occur, as with Co, mainly under grazing conditions, gross signs of the deficiency being rare when concentrates are fed (Ammerman, 1970). The majority of the world reports are concerned with a "conditioned" Cu deficiency where normal amounts of Cu (6-16mg/kg DM) are inadequate because of forage constituents such as Mo, S and other factors which block the utilization of copper (Russell and Duncan, 1956). Copper deficiencies usually occur when forage Mo exceeds 3 or the Cu level is below 5mg/kg DM (Cunha et al, 1964). Ward (1968) categorized Cu deficiency in four groups: where the feed

contained (1) high levels of Mo (> 20mg/kg DM), (2) low Cu but significant amounts of Mo (ratio of 2:1), (3) insufficient Cu (< 5mg/kg DM) and (4) normal Cu and low Mo, with high levels of soluble protein. The last situation results from increases in the amounts of sulphide produced in the rumen from fresh pasture, thus resulting in unavailable Cu sulphide formation (Dick et al, 1975). Clinical signs of Mo toxicity are generally similar to those of Cu deficiency (Suttle, 1988). Both Mo toxicity and Cu deficiency are generally corrected by providing additional Cu in the animal's diet. In severe Mo-toxic areas, injections of Cu compounds are often the preferred method of administration, since the primary site of interaction of Cu with Mo is the gut (Suttle and Field, 1974). By contrast, a Cu content in the feed of 20mg/kg DM can cause chronic Cu poisoning, when combined with very low levels of Mo and S (Todd, 1969).

The geographical distribution of copper deficiency problems, due to suboptimal herbage Cu content shows wide variations; in Australia (Bennets et al, 1948; Lee, 1951), New Zealand (Cunningham, 1946) and Scotland (Mitchell, 1974), the deficiency has been associated with sandy and/or organic soils. In other countries including the Tropics (McDowell et al, 1983) and the Soviet Union (Kovalsky, 1972), the primary cause of Cu deficiency in some areas has not been identified: it is possible that some cases may arise from simple soil and herbage deficiency and others from interfering elements such as Cd, Zn

and Fe but with the Cu-Mo-S interaction being the most prevalent. Beeson and Matrone (1976) observed that soils often associated with Cu-Mo problems are peats, muck soils and other poorly drained soils with high organic matter contents. Thornton (1977), assessing the occurrence of acute Cu-Mo problems on molybdeniferous clays in England and Wales, and Kubota (1977) in USA both mention the additional criteria of a high soil Mo content (> 20mg/kg) and a neutral to alkaline soil reaction. On other soils with Mo levels of 2-20mg/kg, a more gradual Cu deficiency is induced: grazing ruminants are frequently found to be in a subclinical Cu status showing no obvious outward symptoms of hypocuprosis (Thornton and Alloway, 1974; Thompson and Todd, 1976; Thornton, 1977). In New Zealand the clinical Cu deficiency condition known as "peat scours" has been associated with soils of Mo content of 5-10mg/kg but in these soils Cu levels are sub-normal (Cunningham, 1954).

(ii) Cobalt

Cobalt deficiency occurs only in ruminants (Underwood, 1971) and responds completely to the Co-containing, anti-pernicious anaemia factor, vitamin B12. The severity of deficiency varies with the animal species. Sheep require higher dietary levels, due partly to the low efficiency of production of vitamin B12 from Co by the rumen micro-organisms and to the low efficiency of absorption of the vitamin (Smith and Marston, 1970a). Severe deficiency in sheep leads to severe and progressive loss of

appetite and ultimately to death from inanition. In less affected animals there is anaemia, anorexia, leading to depressed productivity and unthriftiness. Young animals are particularly susceptible and symptoms appear when reserves are exhausted; not all animals within a herd or flock are unaffected.

Co deficiency is not uncommon in grazing livestock expecially in the Tropics (NCMN, 1973). Signs are not specific and it is often difficult to distinguish between a deficiency of Co and parasitism or malnutrition due to low intake of calories and protein. However, Co-deficient cattle respond quickly to cobalt treatment, recovering appetite, vigour and weight. Subclinical deficiencies or borderline states are more common than clinical conditions and are characterized by low production rates; they may often go unnoticed thereby resulting in great economic losses to the ruminant livestock industry (Latteur, 1962). Incidence of deficiency can vary greatly from year to year, from an undetectable mild deficiency to an acute stage. In Australia, flocks of sheep were unaffected in five years, whereas in another 8 years clinical signs of variable severity of Co deficiency were encountered (Reuter, 1975b).

The essentiality of vitamin B12 is due to two enzymes that need the vitamin as coenzyme (Table 1.1): firstly is for the conversion of propionate via L-methylmalonic acid (MMA), to succinate in energy-yielding reactions catalyzed by methyl malonyl-CoA mutase (Smith and Marston, 1971); secondly for the

formation of methionine from homocysteine and 5-methyl tetrahydrofolate in a reaction catalyzed by methyl transferase which permits the recycling of methionine following loss of labile methyl group.

(iii) Selenium

Symptoms of Se deficiency vary in severity from general unthriftiness, scouring and infertility in mature animals to severe White Muscle Disease (or Nutritional Muscular Dystrophy, NMD) which is more prevalent in growing lambs and calves. NMD has occasionally been reported in older lambs in New Zealand and Scotland, and in cattle in Canada and Australia (Jenkins and Hidiroglou, 1972; Judson and Obst, 1975). The occurrence of deficiency is somewhat complicated by the vitamin E status of the livestock, since Se and vitamin E share the role of protecting tissues from oxidative stress (ARC, 1980; Chesters and Arthur, 1988). Selenium deficiency in mature cattle has been associated with retained placentae (Trinder et al, 1969), infertility (MacPherson et al, 1988) and increased susceptibility to mastitis (Smith et al, 1984).

The functional role for Se in animals is as part of the enzyme glutathione peroxidase (GSHPx). The activity of this enzyme responds rapidly to alterations in dietary Se intake and can provide one of the best links between the intake of a trace element and its functional adequacy (Hafeman et al, 1974).

(iv) Zinc

Zinc deficiency is rare in the grazing animal, but may occur if there are high concentrations of interfering elements such as Ca, Cu, Fe and Cd. Zinc responsive conditions have been reported in sheep and cattle in British Guiana (Legg and Sears, 1960), Holland (Grashuis, 1963), South Australia (Egan, 1972) and Greece (Spais and Parpasteriadis, 1974) and more recently in the USA (Mayland et al, 1980), Western Australia (Masters and Somers, 1980) and the Sudan (Mahmoud et al, 1983): they have not been associated with any specific soil type.

(c) Trace Element Imbalances Related to Toxicity in Grazing Animals

All trace elements are toxic if ingested or inhaled at sufficiently high levels and for long enough periods, but for some the required concentrations can occur under natural dietary conditions. When absorption of an element is in excess of requirement and homoestatic mechanisms fail, malfunction of certain body processes and clinical disorders may occur. Toxicity may be chronic or acute (Suttle, 1983a). In chronic toxicity there is frequently no correlation between the signs of toxicity and dietary concentrations of the element. The time course is dependent on many factors, including the species and breed of the animal (Woolliams et al, 1982; Allen et al, 1983), the storage capacity in the animals body for the element, the physiological and elemental status of the animal prior to exposure (Davies et al, 1977; Campbell and Mills, 1979) and the intensity of the toxicity.

Interactions between trace elements and other components in the diet, both organic and inorganic, can be extremely complex when toxicities are involved (Davies, 1979).

Copper poisoning can occur in sheep when a pasture high in Cu but low in Mo and S is ingested (Dick and Bull, 1945). Addition of Mo and S to concentrate diets can reduce absorption and accumulation of Cu and prevent toxicity (Hogan et al, 1968; Suttle, 1977). The occurrence of Cu toxicity can also be affected by high intakes of other elements such as Fe, Zn and Cd (Bremner, 1979) and grazing of plants containing hepatotoxic alkaloids (Underwood, 1977).

Of the essential trace elements under consideration, cases of toxicities in grazing livestock have been reported for Cu, Zn, Mo, Se and As (Howell, 1983). Non-essential trace elements such as Pb can also produce toxicity symptoms in grazing livestock when present as natural or man-made environmental contaminants (Stewart and Allcroft, 1956; Harbourne and Watkinson, 1968; Thornton and Kinniburgh, 1978; Lloyd, 1983). Table 2.3 gives a brief summary of the main disorders arising in ruminants through dietary toxicities of some trace elements important in animal production. Acute Mo toxicity has presented nutrition problems in livestock in the "teart" soil area of Somerset, England (Ferguson et al, 1943; Lewis, 1943).

Table 2.3 Symptoms observed in Grazing Ruminants as a Result of Toxic Intakes of certain Trace Elements

Element	Symptoms
Copper	Chronic: Loss of appetite; Thirst; Apathy; haemolytic icterus; haemoglobinuria; Jaundice; Dyspnea and spasms; Death from hepatic coma. Acute: Abdominal pain; Diarrhoea.
Molybdenum	Growth retardation; Loss of weight; Diarrhoea (cattle); Harsh staring and discoloured coat; Wool defects; Joint abnormalities; Connective tissue changes, lifting and haemorrhage of periosteum and muscle insertions; Osteoporesis; Reproductive problems.
Selenium	Cattle: Dullness and lack of vitality; Loss of appetite; Coat roughness; Soreness and sloughing of hooves; Stiffness and lameness; Joint erosion; Blindness; Abdominal pain; Diarrhoea; Salivation; Teeth grinding; Paralysis; Anaemia; Disturbed respiration; Death. Sheep: Loss of appetite; Decreased rate of weight gain; Loss of weight.
Zinc	Parakeratosis; Salivation; Anorexia; Loss of condition; Diarrhoea; Dehydration or subcutaneous oedema; Profound weakness.
Arsenic Lead	Anaemia; Diarrhoea, Constipation; Temor; Hyperexcitability; Ataxia; Death.

(Sources: Underwood, 1977, 1981; Howell, 1983)

Cattle scour severely soon after being turned out to graze pastures on these soils. Although the condition can be alleviated by Cu supplementation, the problem is not simply one of Cu deficiency as the Cu status of the cattle remains normal (Dick, 1969). Such problems arise on outcrops of mineralized clay and shale which are naturally high in many elements, in

many parts of the world including Ireland, Europe, USA and New Zealand, and cattle are far less tolerant of high Mo levels than sheep (Ward, 1978).

Ruminants are generally considered to be extremely tolerant of high Co intakes. However, Dickson and Bond (1974) have listed field cases of Co toxicity in cattle that have arisen from incorrect usage of Co supplements either in the form of pasture topdressing, treated water, licks or ruminal bullets. Clinical signs of toxicity are not specific and tend to be similar to those of deficiency. By contrast, Se toxicity problems are widespread. Allaway (1968) has defined the general characteristics of high and low Se soils which may cause problems of Se imbalance in livestock. He concludes that toxic soils tend to be alkaline and derived from sedimentary rocks while deficient soils are acidic and commonly derived from igneous material, although some alkaline soils formed from low-Se rocks may also be deficient. Toxicity problems may also stem from grazing of Se-accumulator species. Reports from Ireland (Fleming, 1982), the USA (Kubota and Allaway, 1972), Australia (McCray and Hurwood, 1963) and India (Arora et al,1975) give examples of Se-nutrition problems on high-Se (30-3000mg/kg) soils with pasture levels averaging 5-500mg/kg DM. Selenium has a strong tendency to complex with heavy metals and toxicities can be ameliorated by As, Hg, Ag, Cu and Cd. Zinc toxicity leads to depraved appetite, decreased weight gains, decreased feed efficiency, pica and excessive salt consumption

and wood chewing (Ott et al, 1966; Allen et al, 1983). It has not been reported under grazing conditions but soils can contain excessive amounts of Zn particularly in areas of Pb/Zn mineralization where mining has occurred. In Great Britain, Zn toxicity has been recorded in plants in such areas but no reports of animal problems are as yet on record (Thornton and Webb, 1980). A few cases of Zn toxicity occurring under natural grazing conditions in both sheep and cattle have been reported (Davies et al, 1977; Allen et al, 1983), but in all cases the condition was precipitated by environmental contaminants and not excessive intakes from plant material or soil.

2.3 ASSESSING INCIDENCE AND IMPACT OF MINERAL IMBALANCES IN GRAZING LIVESTOCK

There are three principal ways of assessing the incidence and impact of mineral imbalances in grazing livestock: (1) Use of clinical symptoms, (2) Use of biochemical data from soil, plant and animal (blood or tissue) in relation to established criteria of normality, and (3) Use of supplementation trials to check response. The associated problems have been discussed in recent papers (Suttle, 1980; Suttle, 1986b; Suttle, 1987a; Langlands, 1987; Suttle, 1988).

2.3.1 Using Symptoms as a Diagnostic Aid

Table 2.2 gave the symptoms commonly attributed to some trace element deficiencies. The symptoms are rarely specific and

a comparison with those caused by corresponding toxic intakes (Table 2.3) shows that the same symptoms can arise when the dietary intake of an element is excessive. Table 2.4 gives the observed effects of excessive (Mo) and insufficient (Co, Cu, Mn, Se and Zn) intakes of some trace elements on clinical symptoms.

Table 2.4 Reported Effects of Trace Element Deficiency or Toxicity on Clinical Symptoms*

Symptom	Element					
	Co	Cu	Mo	Mn	Se	Zn
Inappetance	+++	+	+			+
Lower Liveweight Gain	+	±	+	+	+	+
Bone Defects		+	+	+	+	+
CNS Defects	-	+		+		+
Hair/Wool Changes	+	+	+			+
Scouring		±	+			
Anaemia	+	+				
Reproduction	±	±	+	+	±	+

(Source: Phillipo, 1983)

*Effects enhanced (+) or depressed (-).

Tables 2.4 confirms that symptoms overlap and may not be a useful diagnostic aid in mineral imbalance assessments. For example, the occurrence of anaemia gives little clue to the element involved or the physiological or biochemical function

primarily impaired, because anaemia is a characteristic of Fe, Cu and Co deficiencies and of Zn, Mo and Se toxicities. The functional and structural disorders apparent to the clinician or the pathologist are merely the final expressions of defects arising at different points in a chain of metabolic events or even in a quite different chain of events.

A further problem with reliance on clinical symptoms is that the same deficiency does not always give the same symptom. Deficiencies of multireactive trace elements lead to complex clinical symptoms as effects of depletion will not be uniform for all enzymes. The reactivity of some enzymes will decline rapidly whilst that of others will be strongly conserved. Mills et al (1976), for example, found a differential decline in the activity of Cu dependent enzymes when they experimentally induced copper deficiency in calves. The effects of decline of different enzyme activities will depend on the duration and severity of the deficiency and the physiological development during that time. It is therefore not surprising that clinical and biochemical consequences of a trace element deficiency often vary widely between different species of animals and within the same species at different stages of physiological growth and under different environmental conditions. Symptoms of acute deficiency are often very different from those seen with a longer term, gradual chronic deficiency, possibly because of differences in the order in which the activities of different enzymes are impaired (Suttle, 1983a).

In must be borne in mind that not all deficiencies occur in isolation: some cases of dual deficiencies have been reported, notably "Coast disease" (Cu and Co; Reuter, 1975b).

Non-specific symptoms of Co deficiency such as debility and starvation can be confused with "ill-thrift" syndromes unrelated to mineral disorders. Australian workers have had trouble diagnosing Co deficiency in "Coast disease" due to confounding symptoms of internal parasitism and undernutrition of protein (Reuter, 1975b). Similarly, subclinical Cu deficiency can lower the wool quality of otherwise healthy sheep (Reuter, 1975b) but other tests are needed to confirm areas of marginal deficiency because many nutritional and non-nutritional factors affect wool strength.

The failure to conclusively assess incidence and impact of mineral imbalances from diagnostic records of visual symptoms has led to use of alternative procedures. The next sections examine the application of soil, plant and animal data in relation to biochemical criteria in the assessment task.

2.3.2 Using Soil Analysis Data and Reconnaissance Techniques

A soil is formed from a parent material derived from rock and therefore has a mineral composition similar to that of the bedrock. Determination of the chemical composition of soils therefore often offers alternatives for predicting mineral imbalances in grazing animals in particular localities. An example is provided by Watkinson (1983) who has used soil data

to depict Se deficient areas in New Zealand. Deficient areas have been associated with particular geological formations (Hartmans, 1970), climate and its effect on leaching and weathering processes (Pfander, 1971; Langlands et al, 1981b), and with soil drainage conditions (Latteur, 1962; Pfander, 1971; Miller et al, 1972; Mitchell and Burridge, 1979). Such information has been of great use in assessing livestock mineral deficiencies in tropical and sub-tropical regions of Australia (Egan, 1975; Loneragan, 1975), Brazil (Conrad et al, 1980) and USA (Kiatoko et al, 1982).

(i) Factors Influencing Soil Mineral Composition

The mineral content of any parent material reflects the elemental distribution that occurred during the formation of the earths crust. Solidification of igneous rock from the molten silicate magma began with the formation of magnesium alumino-silicate-rich ultrabasic and basic rocks such as gabbros. This was followed by formation of intermediate rocks such as andesite and finally acid rocks such as granites. The elemental composition of the various igneous formations was controlled by the major mineral types deposited at the time and the amount of isomorphous substitution by trace elements that occurred. The ability of trace elements to enter crystal lattices or spatial networks of the different minerals as they crystallized is related to the chemical properties of the elements such as charge, ionic radii (Goldschmidt, 1937) and

electronegativity (Ringwood, 1955). Goldschmidt (1958) proposed that the ultrabasic and basic rocks either incorporated or occluded into their lattices trace metals from the magma with ionic radii close to those of Fe and Mg, such as Co, Cr, Ni and Zn. Rocks formed subsequently crystallized from a magma gradually depleted in bioessential trace elements.

For sedimentary rocks, formed through hydrological and glacial weathering of igneous rocks, the trace element content depends not only on the basic minerals from which they were derived, but also on the ease with which the minerals were weathered. The more readily weathered igneous rocks tend to form layered clay or mineral-rich argillaceous parent materials such as shales. These contain considerable amounts of the bioessential trace elements both incorporated into the crystal lattice and adsorbed onto mineral surfaces. The more resistant types of igneous rocks and minerals formed arenaceous bedrocks such as sandstones, which have lower contents of trace elements. The calcareous limestones and dolomites also tend to have low bioessential element contents (Mitchell, 1964; Beeson and Matrone, 1977).

The effect of metamorphism on mineral distribution have not been widely studied. The results that have been reported show that metamorphic rocks, such as slates, schists and gneiss produced by the effects of extreme pressure and temperature, tend to have elemental contents similar to their parent sedimentary rocks, but the mode of occurrence of the trace elements is often modified (Engel and Engel, 1960).

The earth's crust is composed mainly of igneous rock (95%) and a small amount of sedimentary rock (5%) (Mitchell, 1964). Sedimentary formations, however, tend to be concentrated at the earths surface and are the most important and widespread parent materials of the agricultural soils of the world (Beeson and Matrone, 1976). Of the sedimentary formations, 80% are shales, 15% sandstones and 5% limestones.

Physical, chemical and biological weathering of any parent material leads to the formation of soil in an integrated and continuous process. The influences of parent material on the chemical composition of the soil are further modified during formation by the effects of climate, relief and organisms, and the length of time that these factors have interacted (West, 1981). During the soil formation process, elements are released from the various minerals forming the parent material and become distributed in different groupings based on factors such as bonding, extractability and mobility. Essentially, a very small fraction remains free in solution; part remains dispersed within primary rock-forming minerals; the remainder is held with varying degrees of binding, by electrostatic attraction, covalent bonding, chelation or within secondary minerals and organic matter (Burridge et al, 1983). It is likely that each element will be present in many of these forms and that dynamic equilibrium will exist between each form and the soil solution. Continuous interchange of nutrient ions in the soil solution and those adsorbed onto soil particles and organic matter will occur (Quirk and Posner, 1975).

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More recent pedogenic processes such as leaching, gleying, podzolization and surface organic matter accumulation and soil properties such as reaction (pH) and redox potential will further influence both the total amount and forms of trace elements in the soil. The main result may be the vertical redistribution of the trace elements between the varying horizons of the soil profile (Berrow and Mitchell, 1980; Swaine and Mitchell, 1960).

(ii) Assessment of Soil Mineral Status

Soil analysis can indicate the need to apply deficient elements at establishment or sowing time. Conclusions of this kind have been arrived at by examining the "total" or "extractable" elemental concentrations. Except where the concentration of an element in soil is very low or very high, total levels are poorly correlated with plant requirements (Reuter, 1975b). Therefore, most assessments are based on availability, obtained by extraction and equilibration of soil with chemical reagents. The procedures used tend to be empirical due to an imcomplete understanding of the soil/ soil solution/plant system, and due to the fact that most tests are calibrated in glasshouse experiments where conditions may differ from those in the field. As a result, extractable soil element concentrations correlate poorly with plant uptake due to wide variations in soil properties including pH (Beckwith, 1963; Reith, 1965; Fleming, 1973; Loneragan, 1975) organic matter

(Schnitzer and Khan, 1972) uptake capacity of plant species (Allaway, 1968; Fleming, 1973; Reid et al, 1970), action of microbes (Henderson and Duff, 1963; Bromfield, 1978). Inter-element interactions, some due to effects of added fertilizers, also affect the soil-plant relationship (Reuter, 1975a,b; Giordano and Mortvedt, 1972; Murphy and Walsh, 1972).

Plants are the final arbiter of the amounts of mineral elements which are "available" in the soil. Availability can therefore be assessed by measurement of plant composition and relating this to the elemental content of the soil. However, as mentioned previously the quantity of any element that is absorbed depends on both the plant considered and soil micro-climate conditions at the time of sampling. An arbitrary assessment of availability is therefore made by measuring the amount of a soil element removed by a chemical extractant.

The choice of extractant used depends on both the element being measured and the nature of the soil (Mitchell, 1971; Scott et al, 1971; West, 1981). The "critical" soil concentration, below which deficiency can be expected, is estimated either by regression analysis or by separating deficient and non-deficient soils into classes on the basis of plant analysis (Mitchell et al, 1957). The precision of the "critical" value is indicated by the variance of the data used for calibration which is usually wide. On their own, soil tests separate extremely deficient soils from adequately supplied soils (eg Watkinson, 1983) but leave a "grey" area between these extremes (Reuter, 1975a).

(iii) Use of Geochemical and Regional Reconnaissance Techniques

The increasing awareness that quite subtle variations in both absolute and relative abundances of minor and trace elements in soils play a major part in determining the thriftiness of plants and animals has generated considerable interest in regional variations in soil chemistry. A major problem is posed by the fact that soils are capable of profound variations, both vertically and laterally, even when developed over homogenous parent material. Consequently, in many areas the density of sampling required to confidently describe the chemistry of soil by direct sampling is prohibitive. The solution is to switch attention to some indirect method of sampling which will indicate an average composition of the soils of a relatively large area with the minimum number of samples. There are four basic methods: First, geological maps may provide some information on the gross distribution of elements in the parental materials from which soils are derived, but they are not an adequate answer because many soils of agricultural importance are transported; it is common for soils of different chemical and physical character to develop even over homogenous parental rock within a small area. Furthermore, many rock types show considerable chemical variation and, in any case, detailed chemical data are rarely available for map areas of significant size. Nevertheless, mineral deficiencies or toxicities in grazing livestock can be predicted by use of systematic mapping

survey techniques or regional reconnaissance especially in the tropics. Analysed Se and Co levels of USA soils have been related to Se- and Co-responsive diseases (Kubota, 1968; Kubota et al, 1967). Similar mapping techniques based on forage and soil analysis have been undertaken for Ca and P in Brazil and Se in Venezuela (McDowell et al, 1984). Egan (1975) reported that the sampling and analysis of stream-bed sidements have revealed areas of hitherto unsuspected Mo-induced Cu deficiency in sheep and cattle, Mn deficiency in cattle and Co deficiency in sheep. Cobalt and/or Cu deficiencies of grazing ruminants, depicted by low concentrations of these elements in the liver, have been identified in specific Brazilian regions and have been associated with deficient levels in the soils (Torkarnia and Dobereiner, 1973). Deficiencies of P were discovered by mapping in Venezuela (Chico and French, 1959) and in Africa, regional reconnaissance has also been used to identify problem areas. Van der Merwe (1967) and Boyazoglu (1973) reported Cu, Zn and Co to be the most likely deficiencies in the soils of South Africa.

Secondly, remote sensing by aircraft or spacecraft on the basis of colour or reflectivity variation induced in plants by variations in soil chemistry can be used. The disadvantage is that this method is limited mainly to areas nurturing a very restricted variety of plants, whether natural or cultivated, and not all plants show significant colour or reflectivity responses to chemical characteristics of the soil which retard their

growth or that of animals or other plants introduced to the area (Joyce, 1975). Thirdly, airborne scintillometer surveying based on radiation from uranium (U) and K has been demonstrated as a reconnaissance technique for mapping soils in the USA. The method probably has considerable potential for rapid assessment of the homogeneity and patterns of soil types in a survey area, but it does nothing towards assessing the abundance of elements other than U and K (Joyce, 1975).

Finally, stream sediment geochemistry offers the widest scope for agricultural reconnaissance. Low density sampling of stream sediments is a widely practised and successful method of prospecting for concealed mineral deposits. The method is based on the premise that the chemistry of a sediment sample approximates to the average composition of the soils within the catchment area upstream of the sampling point. Proof that stream sediment geochemistry can be adapted for agricultural purposes is afforded by publications emanating from the Applied Geochemistry Research Group at Imperial College, the most impressive examples being those concerning Cu deficiency and Mo poisoning in the UK (Thornton et al, 1969; Thomson, et al, 1972; Thornton et al, 1972).

2.3.3 Using Forage Plant Composition to Assess Mineral Imbalance in Grazing Animals

Herbage analysis has been successfully used for assessing the mineral status of pastures (Mitchell et al, 1957) in relation to disorders in grazing ruminants on a specific or regional basis

(Cunningham, 1960; Kubota et al, 1967; Hogan et al, 1971) and for estimating the mineral supply of conserved feeds. Critical dietary concentrations or recommended standards below or above which imbalances can be expected in livestock are available (eg NRC, 1976, 1978, 1980; ARC, 1980; Underwood, 1981; Suttle, 1983b) and some data for tropical conditions have been given by McDowell (1976) and McDowell et al (1982).

Concentrations of minerals are dependent upon the interaction of a number of factors, including soil, plant species, stage of maturity, yield, pasture management and climate. Most naturally occurring mineral deficiencies in grazing animals are associated with specific regions but large variations in mineral content of different plant species growing on the same soil have been reported. In Kenya 58 grasses grown on the same soil had Ca, 0.09-0.55% DM and P, 0.05-0.37% DM (Dougall and Bogdan, 1958).

It is generally accepted that herbs and legumes are richer in a number of mineral elements than grasses (Fleming, 1973). For most minerals, accumulator plant exist which contain extremely high levels of a specific mineral (Malyuga, 1964; Schuttle, 1964). Therefore the mineral status of the sward will depend on its botanical composition and species analysis is important.

Variations in plant composition with stage of growth occur. The decline in mineral content of plants as senescence sets in is associated with reduced translocation to the root system (Tergus and Blue, 1971). Phosphorus deficiency is more common than Ca deficiency in grazing animals because P content of

forage is more sensitive to senescence, falling rapidly than forage Ca with plant age. Forage Ca is relatively insensitive to senescence and paddock roughage can constitute a major source of Ca for animals receiving cereals as a supplement in times of drought.

The nutritional value of a pasture vis a vis a mineral may be influenced by organic nutrients present. Both Se and vitamin E protect cell membranes from damage by peroxide and other active radicals and some manifestations of Se insufficiency (eg White muscle disease in sheep and cattle) are moderated by vitamin E. Dietary components such as polyunsaturated fatty acids are readily metabolized to peroxides and aggravate the deficiency condition if not first hydrogenated in the rumen (Chesters and Arthur, 1988; Sanders, 1988). Selenium insufficiencies are typically observed on fertilized pastures on acid soils in high rainfall regions. Forage Se concentrations < 50 ug/kg DM are regarded as low (ARC, 1980) and may result in muscular dystrophy, stiffness in the limbs, inability to stand, sudden deaths, depressed growth and wool production and infertility in domestic ruminants. Dietary vitamin E is provided by a number of tocopherols and tocotrienols. Vitamin E concentrations also vary widely within plants and decline when pastures mature and senesce, or if forages are conserved and stored. Sheep and cattle grazing green pastures for most of the year are more likely to be Se than vitamin E deficient (Langlands, 1987).

Disadvantages of forage element analyses in assessing the mineral adequacy for grazing livestock include:

(i) Uncertainty of samples representing what livestock consume: This complication arises from the observation that grazing animals tend to select particular species during periods of pasture abundance. There is recorded evidence that grazing ruminants tend to favour higher protein, more highly digestible fractions from the pasture available (Egan, 1975).

(ii) Difficulty of estimating forage intake: Published mineral requirements (eg ARC, 1980) are usually converted into dietary concentrations by dividing by DM intake, which in turn is determined by the animal's energy requirement. For a particular class of animal, DM intake is directly proportional to the metabolizable energy (ME) required by the animal but inversely related to the metabolizability (q) of the diet. DMI intakes are therefore hard to predict but are an unescapable determinant of mineral intakes. Furthermore, an increase in q would result in a higher mineral requirement (in concentration terms) (ARC, 1980) since more production is sustainable on the more energy-dense diet.

(iii) Variation in the availability of forage elements: This is brought about by interactions between dietary components. For example, as mentioned above, manifestations of Cu deficiency described in Table 2.2 can be brought about by Mo toxicity (Table 1.3) and can be further complicated by high levels of dietary S (Dick et al, 1975) through formation of insoluble

copper thiomolybdates. Hence in the interpretation of forage composition data, the significance of the dietary Cu concentration must be considered in relation to both Mo and S concentrations (Ward, 1968). The New Zealand Society of Animal Production (1983) presented a table showing the relationships between Mo and S concentrations and the health of sheep and cattle. Herbages containing < 3mg Cu/kg DM and no Mo were described as indicative of "simple Cu deficiency", while those containing 5mg Mo/kg DM and 5 (sheep) or 9 (cattle) mg Cu/kg DM were described as "Mo in excess; Cu inadequate". The role of K in augmenting the severity of hypomagnesaemic tetany has already been mentioned previously.

(iv) The possiblity of soil-contaminated forage samples.

Problems of soil contamination of forages sampled for mineral assessment became realized through soil ingestion studies with grazing animals in many parts of the world. Research carried out in New Zealand (Healy and Ludwig, 1965; Healy, 1967, 1968a), Australia (Arnold et al, 1966) and Scotland (Field and Purves, 1964) has established that animals can ingest large quantities of soil while grazing. The majority of this soil is ingested accidentally along with the herbage as soil adhering to plant roots pulled up during grazing, as earthworm casts and as soil adhering to plant leaves following rain splash, trampling by animals or wind blow. By far the most common form of unavoidable soil ingestion is soil contamination of herbage tops; this presents problems during field sampling of herbage

for measurement of the true trace element status of the grazing sward not only as a predictor of the trace element status of a soil but also for the grazing animal (Metson et al, 1979; Reid et al, 1970; Cherney et al, 1983).

For elements such as Cu and Mo where plant contains about as much as the soil, slight soil contamination may not be a problem; but the situation can be serious for elements that are more abundant in the soil such as Fe, Al and sometimes Mn, and for those elements whose concentrations in the soil are several orders of magnitude greater than plant levels, such as Co and Ni. Total soil concentrations of the latter elements are typically about 100 times as much as the plant levels, although this correlation may be dependent on plant species and conditions of soil pH, soil moisture and temperature. For example, at a plant Co concentration of 0.075mg kg^{-1} DM, 0.05% contamination by a soil containing 60mg/kg as total Co will increase the herbage Co status from the deficiency level (< .08mg kg^{-1} DM) according to the ARC (1980) standards to dietary adequacy (0.105mg kg^{-1} DM).

The degree of soil contamination (and ingestion) varies depending on forage availability and has been reported to: (i) be seasonally dependent (Nolan and Black, 1970); (ii) increase with paddock stocking rate (Healy, 1968b; Langlands et al, 1982; Cherney et al, 1983); (iii) be higher on poorly-drained, weak- structured soils (Healy and Ludwig, 1965; Arnold et al, 1966) as well as soils with greater population of surface

earthworm casts (Healy, 1973); and (iv) be greatly reduced by good grazing management practices (Kirby and Stuth, 1980; Fries et al, 1982).

2.3.4 Use of Biochemical Indices (Markers) in Animals

Problems relating to the anticipation and elimination of poor production due to mineral imbalances in grazing livestock were recently examined by Suttle (1986a, 1988). The analysis of animal blood or tissue was generally considered to be more reliable than the visual symptoms approach or at the very least is an important supplement to it. Tissue samples may be collected from the main site of mineral storage eg liver or from blood or other tissues or fluids such as saliva and milk. These may be analyzed for the mineral itself, a metabolite (eg serum MMA for Co), or biologically active forms such as enzymes, hormones and vitamins. Some analyses can be useful for contemporary mineral intake (eg plasma Se), others reflect body reserves (eg liver Cu), while others indicate changes in normal metabolic processes (eg serum MMA as an index of Co deficiency). Nutrients in circulation are readily accessible to the sampler but are often under homeostatic control, and concentrations do not reflect mineral status except in times of crisis. Other nutrients are incorporated into red blood cells during haematopoiesis and concentrations in whole blood reflect intake over an extended period.

The change from mineral depletion to deficiency is often characterized by biochemical changes which can be monitored and often precede the appearance of clinical disorders. Suttle (1986a) has described these changes and attributed them to four phases of: depletion, deficiency (marginal) dysfunction and disease. Homeostatic control is most influential at the deficiency or dysfunction stages before clinical lesions can be identified.

Another approach to detecting the mineral contribution to metabolic or nutritional disorders using animal data is the "Compton Metabolic Profile Test" (Payne et al, 1970), which attempts to identify abnormalities in the composition of blood. Samples are analyzed for packed cell volume, haemoglobin, glucose, serum urea, albumin, total protein, Ca, Cu, Fe, K, Mg, Na and inorganic P. This test was developed in England for dairy herds when equipment for automatic clinical chemistry became available and at least holds open the possibility of non-mineral causes of ill-health. The profile has however been criticized on a number of grounds (eg Adams et al, 1978; Wolff et al, 1978) including cost and restricts the number of trace elements covered.

(i) Calcium, Phosphorus and Magnesium

Concentrations of Ca, P and Mg are not always useful as determinants of deficiency. Plasma Ca is regulated homeostatically, is sensitive to changes in albumin concentrations and declines only in severe deficiency or

suddenly at parturition. The latter condition is not associated with delays in adjusting the mechanisms for increasing the mobilization of bone Ca but rather their incapacity to provide sufficient Ca to meet the increased demand from the mammary gland. Normal values for plasma Ca range from about 80-120mg/l.

The buffering capacity of the skeleton is illustrated by the fact that a 50kg sheep and a 400kg cow contain about 0.5 and 5.6kg Ca and 0.32 and 3.2kg P respectively (New Zealand Soc. Anim. Prod., 1983). As mentioned previously, more than 99% of the Ca and 75% of the P are located in the bone, and in the sheep up to 40% of the P (Benzie et al, 1959) can be depeleted; Ca and P are preferentially reabsorbed from spongy bone, and the vertebrae and ribs are more sensitive than the long bones to changes in Ca and P status.

It might appear that the Ca and P status of ruminants would be monitored better by taking bone than by taking plasma samples. However, bone composition is relatively constant during depletion and the Ca:P ratio remains about 2:1 whether Ca or P is deficient (Field et al, 1968): the main effect of depletion is to reduce total mineral content, which cannot be monitored by biopsy. Biopsy techniques for P studies were initially described by Benzie et al (1959) and Little (1972) for sheep; Little (1984) and Read et al (1986) have extended them to cattle in the assessment of P reserves. These studies have shown that P concentration in total fresh ribs is not always a sensitive criterion of the body P reserves. A variety of sites

and criteria may be used: measurement on a biopsy sample of the 12th rib, thicknesses of compact bone and osteod tissue on the endosteal surface and the ratio of cortical bone area to total area, calculated from midshaft dimensions of the metacarpal or metatarsal. Gartner et al (1982) showed that the choice of biopsy method affected the conclusions drawn about P status. A further disadvantage of bone biopsy techniques is that they are not specific for detecting inadequate intakes of dietary P; cattle receiving a diet containing adequate P but insufficient protein show similar demineralisation (Siebert et al, 1975). Commonly therefore, plasma inorganic P is used to assess the extent of P imbalance although it is at its most sensitive in young growing animals (Suttle, 1987b).

Storage of magnesium in the adult ruminant is about 0.3-0.5g mg/kg liveweight; about 70% is located in the skeleton and 25% in the skeletal muscle (New Zealand Soc. Anim. Prod. 1983). Some bone Mg is available as a reserve which is mobilized independently of Ca and P particularly in young animals and Mg depletion can be assessed by Ca:Mg ratio in rib biopsy or post mortem samples (Smith, 1959). If insufficient Mg is mobilized to supplement dietary sources, plasma Mg concentration falls from a normal value of 18-30mg/l in response to a decline in Mg intake (or availability) or to an increased Mg demand at the initiation of lactation.

The urine is the principal means by which Mg absorbed in excess of the animal's requirement is excreted. Kemp et al

(1961) regarded Mg content of urine as a better indicator of Mg status than serum Mg because it responds more rapidly to a change in available Mg supply; they regarded a daily excretion of 1g/day as low while > 3g/day was considered to be adequate.

Plasma Mg is, however, the most widely used index of Mg status: it is more reliable than plasma Ca or P although some animals with equally low concentrations succumb to tetany while others remain clinically normal (Suttle,1987a).

(ii) Copper

The liver is the main storage site for body Cu and can be used as an index for assessing the Cu status of grazing animals (eg Tartour, 1975). Three disadvantages of using the liver for diagnosing copper imbalance are: (1) the specimen is not easily obtainable - samples can only be collected by biopsy or by sacrificing the animal; (2) variability between animals in liver Cu concentration is high; (3) concentrations can fall to very low levels before health is affected and the threshold value associated with ill-health is ill-defined (Suttle, 1986a). Therefore other animal assessments are preferred and the most common is plasma Cu.

Most plasma Cu is associated with the enzyme ceruloplasmin, and depressed concentrations of plasma Cu or ceruloplasmin indicate depleted Cu reserves. Ceruloplasmin is less sensitive to contamination, but there is no suitable reference standard and relationships between plasma Cu concentration and ceruloplasmin activity are usually derived (Lorentz and Gibb,

1975). Normal plasma Cu varies between 0.6 and 1.2mg/l and values < 0.5mg/l are indicative of a low Cu status. As with liver Cu, the diagnostic criteria for plasma Cu are not well-defined and a two tier interpretation with a marginal category between 0.2 and 0.6mg/l (Suttle, 1986a) has been suggested.

Most erythrocyte Cu is associated with the enzyme superoxide dismutase (SOD) which is incorporated at erythropoiesis and is retained until the erythrocyte is turned over at about 3 months. Erythrocyte Cu reflects the quantity of Cu absorbed over an extended period as the animal becomes deficient. However, the usefulness of SOD assay for Cu deficiency diagnosis is still under investigation (Suttle and McMurray, 1983; Suttle, 1986a).

Other tissues which can be assayed include hair in cattle (Suttle and McMurray, 1983; Kellaway et al, 1978) and wool (Woolliams et al, 1983) in sheep. Care and standarized procedures are needed to clean the fibre to remove contaminants but not the fibre constituents.

(iii) Cobalt and vitamin B12

As previously mentioned, the only known functions of Co arise from its place at the centre of the corrin ring of two cobalamin (Cbl) molecules, methyl- (Me) and adenosyl- (Ado) Cbl, which have contrasting functions in the body. MeCbl acts as a co-enzyme to methionine synthetase and is linked to folate metabolism using Me tetrahydrofolate as the Me group recipient.

Deficiencies of this co-enzyme can therefore impair methionine synthesis and the bioavailability of folate and cause formiminoglutamic acid (FIGLU) to appear in urine. AdoCbl enables propionate to be used for gluconeogenesis via succinate and the tricarboxylic acid cycle, acting as co-enzyme to Me malonyl CoA isomerase. Insufficiency causes MMA to accumulate. The anorexia and anaemia (Table 2.2) which are successive debilitating consequences of deficiency may reflect the dysfunction of first AdoCbl, then Mecbl. Loss of appetite has also been linked to increased accumulation of MMA and the disturbance of rumen fermentation (McDonald and Suttle, 1986; Rice et al, 1989); while anaemia may reflect the role of Mecbl in deoxyribonucleic acid synthesis and red cell maturation (Chanarin et al, 1981).

The Co concentration of some tissues reflect Co intakes but unlike vitamin B12 reserves confer no known physiological benefit and are less sensitive indicators of the ruminants' ability to survive on Co-deficient pastures than vitamin B12 assays. Vitamin B12 concentrations in plasma/serum and liver are frequently determined for assessment of Co status of grazing animals. Suttle (1986a) has reported that serum vitamin B12 concentrations below 188 picomol/l are indicative of functional deficiency in sheep whereas cattle with values between 38 and 76 picomol/l may be only marginally deficient.

The biochemical confirmation of deficiency from the assay of plasma vitamin B12 in cattle is more difficult than in sheep for

a number of reasons. Firstly, plasma vitamin B12 concentrations do not appear to show the same response to Co supplementation as they do in sheep. Research has shown that oral doses of Co ten times higher on a bodyweight basis than those which increase plasma vitamin B12 in sheep (Field et al, 1988) are ineffective in cattle (N.F. Suttle, Personal Communication); and during periods of insufficient dietary Co supply, plasma vitamin B12 can fall to very low levels (< 100pg/ml) (eg Reid and McQueen, 1985) before cattle fail to thrive though there are exceptions (eg Duncan et al, 1986). Secondly, the assay of vitamin B12 either by microbiological or the more favoured radioisotope binding method, is susceptible to interference from non-specific binders (Wright et al, 1982; Millar et al, 1984). Thirdly, the transport proteins for vitamin B12 in plasma, the transcobalamins, show quantitative differences as well as seasonal anomalies (Millar et al, 1984) which may influence vitamin B12 assay by certain methods. The results of assays of vitamin B12 in bovine plasma are therefore likely to vary substantially between laboratories. Assays of the analogue component by difference, using specific and non-specific binders, may not always be quantitative.

There is little information on liver vitamin B12 concentrations in cattle or their relationship with plasma vitamin B12. Diagnostic thresholds for sheep have only been extrapolated to cattle: thus values < 0.10mg/kg fresh liver have been taken to indicate moderate to severe deficiency and

values > 0.19mg/kg have been regarded as normal. In Australia, the validity of the lower limit (ie 0.10mg/kg) has been confirmed for grazing calves (Winter et al, 1977).

The diagnosis of Co disorders from the presence of abnormal metabolites in the blood and urine is well established in sheep and non-ruminants but has only recently been extended to cattle. Quirk and Norton (1988) found that heifers grazing pastures low in Co (0.036mg/kg DM) remained healthy and excreted no MMA or FIGLU in urine, despite low plasma vitamin B12 concentrations (96pg/ml). After calving, milk yield was unaffected but low vitamin B12 concentrations in the milk (42-86pg/ml) caused depletion of plasma vitamin in their calves (59-74pg/ml) and growth retardation, which was accompanied by increased urinary excretion of FIGLU. They concluded that MMA was less reliable than FIGLU as an index of dysfunction but their method for MMA was relatively insensitive (sensitivity 30umol/l). However, concentrations of MMA in the plasma > 5umol/l may offer a guide to diagnosis of functional vitamin B12 deficiency (Suttle, 1986a; Rice et al, 1987).

(iv) Selenium and Vitamin E

Animal Se status is often assessed from the Se content of whole blood, plasma or erythrocytes, or GSHPx activity in whole blood or erythrocytes. Most blood Se in sheep and cattle is located in erythrocytes and Se concentrations and GSHPx activities are closely correlated, particularly when Se

concentrations are low (Suttle, 1986a). The enzyme has the advantage as a diagnostic aid that it is the biologically active form of Se and activity can be determined spectrophotometrically or by a simple spot test (Langlands et al, 1980). Another advantage is that preliminary digestion of the sample with perchloric acid is not required. The main disadvantages are that GSHPx enzyme assay procedures are difficult to standardize and may therefore lack comparability between laboratories (Langlands et al, 1980). There are also various methods for reporting results: in blood, GSHPx activities can be reported as units per g of haemoglobin, per ml of cells or per ml of whole blood, each at a range of assay temperatures from 25-37^{o}C. Activities in plasma are low, unstable and sensitive to haemolysis.

Use of blood Se concentrations involves a lengthy fluorimetric assay or assay of volatile anhydrides by AAS: neither method is easy to perform routinely with consistent results.

Additional evidence of response to Se supplements may be needed in order to effectively diagnose Se-responsive conditions because vitamin E also determines what is adequate. However, responses to Se supplementation can be expected to vary with the animal's demand for Se, which in turn will vary with age, species and physiological state and with composition of the diet. For example, Sheppard et al (1984) reported that in New Zealand where vitamin E status is likely to be high,

Se-responsive conditions occurred in lambs when blood Se concentrations fell below 10ug/l. Lamb deaths occurred when blood concentrations were < 5ug/l. The generally accepted threshold for diagnosing Se deficiency is however much higher at 50ug/l. Cattle appear to be more tolerant of a low Se status than sheep. In New Zealand, attempts to solve Se deficiency problems by top-dressing pastures with sodium selenate appeared to be successful (Watkinson, 1983).

(b) Using Responsive Conditions in Absence of Clinical Signs

There is abundant evidence of improved wool production and lamb growth rate in large areas of Australia where increased amounts of Cu and Co have been supplied through the herbage or by animal supplementation, even though clear deficiency signs had not been recognized (eg Reuter, 1975b). Studies of supplementation in the field have also revealed areas where female reproductive performance may be improved by specific trace elements. Thus, in cattle, responses in terms of reduced returns to service have been recorded following Mn supplementation (Wilson, 1965). In pen studies, Underwood and Somers (1969) have shown the Zn requirement for testicular development in young male sheep to be greater than that for rapid body growth. Though not yet carried into the field, observations on reproductive efficiency imply that large areas of Australian soils support plants with a Zn content which is less than required for normal testicular development.

For all elements the surest diagnosis is often an improvement in growth or health in response to a specific supplement (Suttle, 1986a) although the adoption of preventive measures should be prompted by biochemical evidence of marginal deficiencies in animals (rather than soils or pastures) although economic responses may not necessarily follow (MacPherson, 1987; Phillipo, 1983). There are problems associated with mineral supplementation and these were summarized by McDowell and Conrad (1977) with particular reference to situations in tropical regions. They include (1) insufficient chemical analyses and biological data to determine which minerals are required and in what quantities, (2) lack of mineral consumption data needed for formulating supplements, (3) inaccurate and/or unreliable information on mineral ingredient labels, (4) supplements that contain inadequate amounts or imbalances, (5) standardized mineral mixtures that are inflexible for diverse ecological regions (eg supplements containing Se distributed in an Se toxic region), (6) farmers amending mixtures recommended by the manufacturer (eg mineral mixtures diluted 10:1 and 100:1 with additional salt, and (7) difficulties concerned with transportation, storage and cost of mineral supplements. As mineral toxicities are always a risk of ad hoc supplementation, the chemical analysis of feeds to determine which minerals are required and in what quantities is a priority step before

supplementation can be considered. Supplementation programmes to check responses can be extremely expensive when the limiting elements have not been identified; hence the purpose of present study.

CHAPTER THREE

MATERIALS AND METHODS

3.1 SAMPLING OF SOILS AND PASTURE

One hundred and thirty-five soil and herbage samples from as many sites on 84 farms covering the Mt Elgon region in Kenya were collected during 1987 and 1988. The selection of areas and sampled sites was based on the following criteria:

1. Areas were situated in regions where soils had been weathered directly from the underlying rock or any drift was of local origin.
2. Areas were covered by the Survey of Kenya geological maps allowing correct identification and description of the area geology.
3. Livestock production was prevalent in the area, whether good or poor.
4. Sampling sites were situated on agricultural land or open moorland under permanent pasture being grazed by livestock.
5. Sampling sites included the entire altitude range of the region (4000-8000 feet above sea level) to reflect any effect of altitude on the mineral element distribution.
6. Sampling sites covered areas of varied topography. Information on whether the landscape profile was uniform, gradational or contrasting was recorded.

Sampling Procedures

Samples were collected in mid-field positions away from roads (allowance of at least fifty metres), hedges and buildings to avoid contamination or spoilt land. Consistency of cleanliness was further checked by dividing the entire sampling team into two groups of ten and assigning one group exclusively to undertake the herbage sample collection and packing, ahead of the soil sampling team. Samples were not taken close to trees or tracks within the field and pylon lines were avoided. At least three sampling points each representing a 4x4 metre square were chosen at random depending on the farm size and nature of the landscape profile (whether uniform, gradational or contrasting).

Soils (0.30cm) were sampled by auger at ten random positions within the square to give a representative sample of about 8kg. The combined sample was mixed thoroughly and sub-sampled by the quartering procedure (Hesse, 1971). The soil was spread uniformly over a sheet of polythene and divided into four equal portions. The portions marked one and four were discarded and the remaining portions mixed together, spread out again and reduced to half by the same procedure. This process was repeated until a sample of representative bulk (1kg) was obtained. The samples were placed in clean paper bags and stored in a cool dry place until return to the dust-free laboratory for air-drying.

Pasture samples were cut at hay stage from the same soil sampling sites (using stainless steel sickles) at several points within the square. They were mixed and sub-sampled to give a representative sample of about 1.5kg fresh weight. Information on the botanical composition was recorded for each sub-sample. The samples were placed in clean mini-grip polythene bags and stored in a clean, dust-free place prior to oven-drying. For the fodder grasses (eg Napier) a 5-10kg sample was cut at 8-10 week maturity, chopped into smaller pieces and sub-sampled to give a 1.5kg fresh weight representative sample for oven-drying. Table 3.1 gives the sampling frequency in relation to botanical composition of the collected herbage.

3.2 SAMPLE PREPARATION

3.2.1 Soils

Samples were stored in clean paper bags under cool, dry conditions. Soils were air-dried at room temperature (<26^{o}C) for three days. Once dry, the soils were disaggregated with a porcelain pestle and mortar and passed through a 2mm (80 mesh) nylon seive. The <2mm fraction was retained and divided into three sub-samples. One portion was oven-dried at 70^{o}C for one hour for Se and Co analyses, another was oven-dried at 105^{o}C for one hour prior to general element analysis, and the remainder was retained without further drying for pH determination and for analytical comparisons in the event of contamination of the other portions. Oven-dried samples were homogenized in an agate Tema mill.

TABLE 3.1 Sampling Frequency in Relation to Botanical Composition of Collected Herbage*

Sampled Herbage Species (Botanical Name)	Classification	No. of Sites/samples	% of Total Samples
Napier grass (Pennisetum purpureum)	Fodder	39	28.9
Rhodes grass (Chloris gayana)	Ley Pasture	45	33.3
Nandi Setaria (Setaria sphacelata)	Ley Pasture	9	6.7
Nandi Setaria + Rhodes grass Mixture 50:50	Ley Pasture	13	9.6
Kikuyu grass (Pennisetum clandestinum)	Ley Pasture	9	6.7
Natural grass and assorted single species	Mixed Pasture	20	14.8
TOTAL		135	100

* The nature, identification and characteristics of the species including details of average nutritive value in relation to animal performance and production were described by Bogdan (1977). Data regarding site location, topography, land usage and any other relevant information for all farms sampled are given in Appendix I. The assorted single species were classified under mixed pasture because they were poorly represented on the farms visited.

3.2.2 Herbage

Herbage samples were dried in a forced-drought oven at 70°C for forty-eight hours. They were ground in a Wiley Laboratory Mill and sieved through 0.5mm stainless steel screen. They were sub-sampled into three portions: one was reserved for general

element analysis without further drying; the second portion was oven-dried at 105°C for three hours for the X-ray fluorescence determination of S and Si, and the rest was stored for analysis of Co and Se.

3.3 REAGENTS AND GLASSWARE

3.3.1 Chemicals

Chemicals were of Analar, Aristar, Pronalys or HPLC grade. They were obtained from normal laboratory suppliers, BDH, Koch-Light, Sigma, May and Baker and Aldrich Chemical Company, United Kingdom.

3.3.2 Glassware

All glassware were washed thoroughly in alkaline laboratory detergent (Teepol), soaked in 3M nitric acid for forty-eight hours and rinsed thoroughly in distilled de-ionized water (DIW) before use, to leach out any trace element contamination. Plastics were soaked in detergent and rinsed thoroughly in distilled DIW.

3.4 DETERMINATION OF SOIL pH

The pH of each soil sample was measured in distilled DIW according to the methods of Hesse (1971) and Black (1965). A 20g aliquot was shaken with 50ml of water for one hour in a reciprocating shaker. The pH of the 1:2.5 soil-water suspension was measured using a standard glass electrode and pH meter after standing for twenty minutes.

3.5 ASSESSMENT OF EXTRACTION PROCEDURES FOR PREDICTION OF AVAILABILITY OF NUTRIENTS IN THE SOILS

Different extractants were selected for the evaluation of available Fe, Al, Mn, Zn, Ca, Cu, Mo and P. Twelve different soil samples were chosen to represent different bedrocks (Table 3.2) and the various solutions used for extraction tests are given in Table 3.3.

The soil-to-solution ratios used were 1:5 for extraction of available Al and Cu; 1:10 for Ca, Mn, Zn, P and Fe; and 1:15 for Mo. For Mo, the sample aliquots were shaken with the extracting solution on a reciprocating shaker for a period of sixteen hours, while for extraction of all the other elements, samples were shaken for a period of one hour. Table 3.4 gives the breakdown of the various methods tested for the extraction of a specific element under study. All extracts were filtered (Whatman No.40) and the filtrates retained for analysis.

Choice of extraction methods for the prediction of mineral availability to plants was based on recovery data coupled with good reproducibility between replicate determinations for the twelve representative samples. The recoveries and methods chosen for batch analysis are given in Table 3.5 while the methods used for chemical analysis are described in Section 3.8.

TABLE 3.2. Samples Chosen for Extraction Tests

Sample No.	Description of Soil Bedrock	Herbage Species
12	Local drift (Alluvium/Black Valley and sandy soils)	Kikuyu grass
14	Metamorphic gneisses	Rhodes grass
27	Soft volcanics (Mt. Elgon series)	Rhodes grass
50	Soft volcanics	Rhodes grass
78	Local drift (Alluvium/Black Valley and sandy soils)	Rhodes grass
84	Metamorphic gneisses	Nandi setaria
109	Igneous (granitic soils)	Natural grass
121	Metamorphosed sedimentary	Napier grass
126	Metamorphosed sedimentry	Kikuyu grass
130	Sedimentary: sandstone + grits	Nandi/Rhodes
132	Igneous (granitic)	Rhodes grass
135	Sedimentary: sandstone + grits	Napier grass

3.5.2 Extraction of Available Cobalt

Cobalt was extracted following the methods recommended by Scott et al (1971) and West (1981). Sample aliquots were shaken with 10ml of 0.5M acetic acid (pH 2.5) for sixteen hours on a roller shaker. The extracts were filtered (Whatman No. 40) and the filtrates analyzed for Co as described in Section 3.8.2. For extract concentrations less than 0.1ug/ml, fresh sample aliquots (3g) were re-extracted with 10ml of the extracting solution and filtered. The extraction efficiency was assessed by recovery checks.

TABLE 3.3 Test Solutions used for Extraction Studies

Method	Extract (aqueous)
A	1M Ammonium acetate, pH 7.0
B	1M Ammonium acetate, pH 4.8
C	1M Ammonium acetate in 0.05M hydrochloric acid, adjusted to pH 3
D	1M potassium chloride (KCl)
E	0.5M acetic acid
F	0.1M hydrochloric acid in 0.0125M sulphuric acid
G	Ethylenediamine tetraacetic acid (EDTA) 0.05M adjusted to pH 7 with ammonia
H	1M Ammonium oxalate adjusted to pH 3
J	Hot distilled DIW
K	0.5M sodium bicarbonate pH 8.5

3.5.3 Extraction of Total Selenium

Selenium was extracted by refluxing the soil samples first with nitric acid (HNO_3), followed by phosphoric acid (H_3PO_4) according to the procedures of Hemsted et al (1972) and MAFF (1981). A 0.5g sample was heated under reflux for fifteen minutes first with 5ml of HNO_3 (SG 1.42) then with 5ml H_3PO_4 (SG 1.74). The contents were allowed to cool, diluted to 25ml with distilled DIW and mixed thoroughly. 5ml of the clear supernatant was evaporated to about 2ml, cooled and mixed with

TABLE 3.4. Distribution of Test Methods for Extraction of Each Element (excluding cobalt and selenium)

Element	Method of Extraction										Literature Source
	A	B	C	D	E	F	G	H	J	K	
Phosphorus	*				*	*				*	Williams (1950) Nelson et al (1953); Olsen et al (1954); McDowell et al (1982)
Calcium) Manganese)	*				*	*					Weir & Sommer (1948); Blume & Smith (1954); Boken (1958); Nelson et al (1959); Metson (1961); Scott et al (1971).
Copper	*					*	*				Cheng & Bray (1953); Fiskell & Westgate (1955); Hesse (1971); Suttle et al (1975); Clayton & & Tiller (1979); McDowell et al (1982)
Molybdenum	*					*		*	*		Grigg (1953), Lowe & Massay (1965); Gupta & MacKay (1966); West (1981); McDowell et al (1982)
Iron		*	*			*					Olson & Carlson (1950); Jackson (1958); McDowell et al (1982)
Aluminium	*	*		*							Coleman et al (1959); McLean et al (1959); Pratt & Blair (1961); Hesse (1971); Black (1965)
	A	B	C	D	E	F	G	H	J	K	

* Method tested

TABLE 3.5. Mean Recoveries (%) of Added Elements from Soils and Choice of Extraction Methods[+]

Element	Extraction Method[*]									
	A	B	C	D	E	F	G	H	J	K
Phosphorus	57 (42-72)				72 (65-79)	92* (89-95)				88 (72-104)
Molybdenum	93* (88-98)					84 (72-90)		90 (80-99)	97 (98-105)	
Copper	91 (86-96)					96* (91-101)	93 (85-101)			
Zinc	80 (74-86)				84 (76-92)	90* (96-95)				
Manganese	92* (83-101)				95 (80-110)	90 (72-108)				
Calcium	96* (91-101)				72 (61-83)	94 (77-111)				
Aluminium	93 (77-109)	104 (84-121)		97* (90-104)						
Iron		85* (79-91)	78 (62-94)			95 (78-112)				

[+] Values in brackets indicate the ranges obtained for twelve duplicate determinations.

[*] Method chosen for batch analysis.

0.3g potassium persulphate. The mixture was boiled gently to expel residual HNO_3, 2ml of 6M hydrochloric acid (HCl) was added and the mixture refluxed for thirty minutes in a Waterbath at 90°C to reduce Se VI to Se IV. Finally, 5ml of 0.2M HCl was added and refluxing continued for a further fifteen minutes. The solution was cooled and retained for chemical analysis. Blanks and calibrating standards containing 0-0.1ug Se were digested similarly with 1ml HNO_3 and 1ml of H_3PO_4 under reflux, respectively, in accordance with the sample procedure but omitting the dilution step. Selenium was analyzed as described in Section 3.8.9.

3.6 PROCEDURES FOR THE DIGESTION OF HERBAGE PRIOR TO EXTRACTION OF MACRO AND TRACE ELEMENTS

Digestion methods fall into one of two main classes, ie dry ashing and wet digestion. In the former oxidation is accomplished by heating the sample to a relatively high temperature, usually between 400°C and 700°C when atmospheric oxygen serves as the oxidizing agent. Chemical compounds may sometimes be added to aid the process. In wet digestion the temperature is much lower, liquid conditions are maintained throughout and the oxidation is carried out by oxidizing agents in solution. However, both classes of methods suffer from several drawbacks involving losses of trace element by volatilization, fixation or other mechanisms (thus leading to lower recoveries) on the one hand, and accretions from the reagents or apparatus (ie contamination) on the other.

The nature of the problem often varies from one element to another. A multi-element study of the type proposed requires establishment of the fact that the digestion stage does not introduce appreciable errors.

For the purposes of this investigation eight wet digestion and four dry ashing procedures were compared for the batch determination of Fe, Cu, Zn, Mn, Mo, Mg, P, Al and Ca in herbage samples represented by two of the grass species, Napier grass and Nandi setaria. Copper and Mo were chosen for recovery studies for two reasons: (1) the very low levels of the two elements normally found in herbage, and (2) previous investigations (Gorsuch, 1959; Chesters, 1983) have established problems in the analysis of the two elements.

3.6.2 Digestion Procedures Tested

Unless otherwise stated concentrated reagents were used, viz: HNO_3, $HClO_4$ (SG 1.54); sulphuric acid (H_2SO_4 SG 1.84), H_3PO_4, HCl (SG 1.18), and hydrogen peroxide, H_2O_2 (100 volumes). Tables 3.6 and 3.7 show the different procedures tested in this study.

For wet extraction, all sample aliquots (2g) were digested in 250ml conical pyrex flasks while the dry ashing procedures were carried out in high-form porcelain crucibles in a temperature-controlled muffle furnace. In each procedure, distilled DIW (2ml) was treated for the blank. Digests were, unless stated otherwise, finally extracted by boiling with 2M HCl (15ml) for five minutes before diluting to 25ml with the

acid. For recovery studies samples were spiked with 10ug of Cu and Mo in a standard solution mixture and the contents left to stand overnight at 40°C before digestion. With the wet oxidation procedures, 250ml conical flasks were used to give some measure of refluxing. In all the procedures, it was vital to increase the digestion temperature slowly, especially with the HNO_3 mixtures where rapid increase in temperatures can result in excessive frothing and rapid distillation of the acid.

TABLE 3.6. Test Methods for the Wet Digestion of Herbage Samples*

Method	Digestion Mixture	Matrix of Working Standards+	Literature Sources
1	$H_2SO_4/HClO_4$	10% H_2SO_4	Modified from Cummins et al (1964)
2	$HNO_3/H_3PO_4/H_2O_2$	8% H_3PO_4	Reamer & Veilloi (1981)
3	HNO_3/H_2O_2	8% HNO_3	Analytical Methods Committee (1979)
4	$HNO_3/HCl/H_2O_2$	8% HNO_3	Modified procedure of Hall & Gupta (1969)
5	$HNO_3/HClO_4$	10% $HClO_4$	Watkinson (1966) Olson et al (1975)
6	HNO_3/H_2SO_4	10% H_2SO_4	Kapel & Komaitis (1979)
7	$HNO_3/HClO_4/H_2SO_4$	8% H_2SO_4	Eden & Green (1940) Milner & Whiteside (1984)
8	$HNO_3/HClO_4/H_2SO_4 + Na_2MoO_4$	8% H_2SO_4 + .08% aq Na_2MoO_4	Cummins et al (1964)

+ All matrix dilutions were made with 2M HCl (Grace and Wilson, 1972).

* Precautionary steps:

Method 1 Samples were wetted with 4ml water and heated with 20ml of 50% H_2SO_4 in $HClO_4$ until fumes of H_2SO_4 were produced and volume of digest had decreased to about 3ml. The digests were then extracted with 2M HCl.

Method 2 Samples were first treated with HNO_3 (14ml) overnight at room temperature. After complete dissolution of the herbage at 150°C, H_3PO_4 (4ml) was added and heating continued. H_2O_2 (2ml) was added dropwise and contents heated until digest cleared and final volume of about 3ml remained. HCl extraction followed.

Method 3 Samples were digested with 14ml HNO_3 and 6ml H_2O_2 according to Method 2 above.

Method 4 HCl was substituted for $HClO_4$ in the Hall and Gupta (1969) procedure. Samples were digested with a mixture of HNO_3 (14ml) and HCl (2ml) at 150°C until 5ml of liquid remained. H_2O_2 (4ml) was added slowly and digestion continued until 2ml of digest remained before HCl extraction.

Method 5 Samples were predigested overnight at room temperature with HNO_3 (20ml). They were then heated at 150°C for one hour. $HClO_4$ (4ml) was added and contents heated at 200°C until white fumes appeared. Heating was continued for a further 30 minutes before the final HCl extraction.

Method 6 Samples were digested with a mixture of HNO_3 (16ml) and H_2SO_4 (4ml) first at 150°C for one hour and then at 200°C until fumes of H_2SO_4 were produced. Heating was continued until volume of digest was reduced to 3ml before HCl extraction. Charring was prevented by addition of small amounts of HNO_3.

Method 7 After overnight predigestion with HNO_3 (14ml), samples were heated at 150°C for one hour. H_2SO_4 (2ml) and $HClO_4$ (4ml) were added and digestion continued at 200°C until white fumes were produced and volume of digest remaining was 3ml. The digests were then extracted with 2M HCl. Charring was prevented by dropwise addition of HNO_3.

Method 8 Samples were first wetted with 1ml of 2% aqueous sodium molybdate (Na_2MoO_4 $2H_2O$) before digestion according to procedures of method 7 above. All flasks were covered with watchglasses to prevent possible ejection of digest (Ewan et al 1968). This digestion procedure requires maximum attention as sudden violent reactions have been reported. Digestion times were however reduced by more than one hour compared with Method 7.

TABLE 3.7. Methods Tested for the Dry Ashing of Herbage Samples*

Method	Digestion Procedure	Matrix of Working Standard	Literature Sources
9	Dry ashing at 500°C with HNO_3 aid	4% HNO_3 in 20% HCl	Isaac & Johnson (1975)
10	Dry ashing with $Mg(NO_3)_2$	8% $Mg(NO_3)_2$ in 2M HCl	Siemer & Hageman (1975) Reamer & Veilloi (1981)
11	Dry ashing with Na_2MoO_4	0.4% aq Na_2MoO_4. $2H_2O$ in 2M HCl	Modified procedure of Cummings et al (1964)
12	Dry ashing with NH_4VO_3	0.16% NH_4VO_3 in 2M HCl	Modified procedure of McNulty (1947)

Method 9 Samples were first ashed at 500°C for 3 hours. The residues were reacted with 50% HNO_3 (4ml) and evaporated to dryness at 120°C. The crucibles were returned to muffle furnace and heated for a further one hour at 500°C. Final ash was extracted with 50% HCl (10ml) and diluted to 25ml with water.

Method 10 Samples were heated at 50°C overnight with 2ml of saturated magnesium nitrate [$Mg(No_3)_2$] before ashing at 450°C for five hours. Water (5ml) was added and contents boiled with 6M HCl (5ml) for five minutes before diluting to 25ml with 2M HCl.

Method 11 As for Method 10 with the substitution of 5% aqeous Na_2MoO_4 $2H_2O$ (2ml) for the $Mg(NO_3)_2$.

Method 12 As for Method 10 with the substitution of 2% ammonium vanadate (NH_4VO_3) (2ml) for the $Mg(NO_3)_2$.

3.6.3 Digestion Procedure Employed Prior to Determination of Total Herbage Cobalt

Cobalt was extracted according to the wet ashing procedure of Watkinson (1966) using $HNO_3/HClO_4$ mixture. Nitric acid (5ml) was added to 0.5g sample aliquots contained in 4"x1" borosilicate glass vials. After overnight pre-digestion at room temperature the samples were brought to boiling on a heating block set at 150°C. After one hour the temperature of the block was increased to 200°C and $HClO_4$ (1ml) added to the digests. Heating was continued until fumes of $HClO_4$ appeared and thereafter digestion allowed to proceed for a further 30 minutes. After cooling 4ml of water was added and the contents boiled for one minute, cooled and retained for Co determination.

3.6.4 Digestion Procedure Employed Prior to Determination of Total Herbage Selenium

Selenium VI extraction and subsequent reduction to Se IV was based on the procedures of Watkinson (1966) and Olson et al (1975) with some modifications. Samples were wet-ashed with $HNO_3/HClO_4$ mixture and Se VI reduced to Se IV with 6M HCl at 90°C under reflux.

A 0.5g sample was allowed to react overnight with HNO_3 (5ml) in a 4"x1" borosilicate glass tube. The tubes were placed on the digestion block and its temperature raised gradually to 180°C. After one hour at 180°C, $HClO_4$ (2ml) was added and

heating continued until fumes of $HClO_4$ appeared. After thirty minutes following the appearance of the fumes, the digests were allowed to cool, 2ml water was added and the contents boiled to expel traces of nitrogen oxides. The digests were cooled and 6M HCl (2ml) added. The tubes were transferred to a water bath and contents refluxed at 90°C for thirty minutes. Water (4ml) was added and the extracts cooled and retained for analysis.

Blanks and standards for calibration containing 0-0.1ug Se were digested and treated as for the samples.

3.6.5 Treatment of Excessively Coloured Extracts

Some wet-ashing procedures for herbage samples and certain soil extracting solutions such as ammonium acetate may yield coloured extracts due to incomplete oxidation and/or solubilization of organic compounds in the sample matrix. The intensity of the coloured solutions may adversely interfere with the determination of the elements by chemical methods such as spectrophotometry (eg P and Mo) and polarography (eg Cu). It was therefore desirable to decolorize such solutions without introducing contamination or additional dilutions. This was achieved by use of pure activated charcoal which was purified by the following procedure.

Portions of laboratory grade activated charcoal (100g) were continuously leached with three 500ml portions of 20% HCl by shaking well and allowing to stand overnight before filtering (Whatman No.1). The residue was washed with

additional 250ml of 20%HCl and filtered. 25ml-portions of the filtrate were tested for presence of phosphate-$P_{..}$ by the vanado-molybdate method (section 3.8) and if the tests were positive the leaching procedure was repeated until the leachate absorbance reading did not exceed that of the blank (20%HCl). The residue on the filter paper was washed thoroughly with distilled DIW until the filtrate was free of chloride ($AgnO_3$ drop test). The purified charcoal was finally dried at 80°C for 16 hours and stored.

Each of the coloured extracts and blanks were shaken for twenty seconds with 0.3g of the purified activated charcoal and the contents allowed to stand for two hours at room temperature. The extracts were then filtered (Whatman No. 1) and the clear colourless filtrates retained for analysis.

3.7 INSTRUMENTATION METHODS

Determination of the concentrations of the macro and trace elements in the samples was carried out by one of the following techniques.

3.7.1 Flame Atomic Absorption Spectrometry (AAS)

Unless otherwise stated a Perkin-Elmer Model 2340 atomic absorption spectrometer fitted with a background correction accessory and appropriate burners for air/acetylene, and nitrous oxide/acetylene flame systems was used. Instrumental sensitivity calibrations were performed as needed following recommended procedures (Perkin-Elmer, 1976).

3.7.2 Flameless Atomic Absorption Spectrometry (FAAS)

A varian Model AA1475 atomic absorption spectrophotometer with a graphite furnace attachment was used. An inert gas system comprising nitrogen and argon at a flow rate of 3 l/minute was used for furnace clean-up at each stage of the analysis. The graphite tube atomizer used was Model GTA-95.

3.7.3 Differential Pulse Anodic Stripping Voltammetry (DPASV)

The instrumentation comprised a Princeton Applied Research (PAR) Model 174A Polarographic Analyzer connected to a Houston Omnigraphic Recorder Model 2000 (Houston Instruments, Texas, USA). The potentiostatic control used was a PAR three-electrode system consisting of the hanging mercury drop as the indicator electrode, platinum wire as the auxiliary electrode and saturated calomel as the reference electrode.

3.7.4 Molecular absorption spectrometry

Colorimetric determinations were performed on a Perkin-Elmer Lambda 3 uv/vis spectrophotometer using 10mm pathlength quartz cells and background correction system.

3.7.5 X-ray fluorescence spectrometry (XRF)

A semi-automatic Phillips all-vacuum X-ray spectrometer Model PW1540 with a 1kw constant-potential generator and chromium anode operating at 60kV, 32mA was used, in combination with a

gas-flow proportional counter, coarse collimator and pentaerythritol (PE) crystal for wavelength dispersion.

3.7.6 Fluorometry

An AMINCO Fluoro-colorimeter Model J4-7440 (American Instruments Company) with a wavelength range of 250-650nm was used for obtaining fluorometric measurements. The excitation source was a GE No. F4 T4/BL, 4W uv lamp capable of producing steady radiation at 369nm.

3.7.7 Hydrogen-ion Activity

A Radiometer Titrator (Copenhagen) type TTT1 and Pye Unicam Model 391 pH meters with glass electrodes were used for all pH measurements.

3.8 CHEMICAL ANALYTICAL METHODS

3.8.1 Determination of Phosphorus

Phosphorus in solution as phosphate was determined by reacting it with excess molybdate ions in the presence of ammonium metavanadate in acid medium to form a yellow-coloured complex. The intensity of the colour, attributed to the substitution of oxyvanadium and oxymolybdenum radicals for oxygen of phosphate (Jackson, 1958), was measured spectrophotometrically at 470nm. In comparison with the molybdenum blue procedure of Murphy and Riley (1962), this method was found to be of slight advantage because of its wider working

range of phosphorus concentrations (0-20mg/l P) and its lesser susceptibility to iron III and silicon IV interference which necessitate additional extraction steps (Jintakanon et al, 1975).

To 5ml of standard solution or unknown extract containing up to 0.2mg PO_4-P was added 60% perchloric acid (0.5ml) followed by 1ml of stock vanado-molybdate reagent (a 50:50 mixture of 0.25% ammonium metavanadate in 33% nitric acid and 5% w/v aqueous ammonium molybdate). The contents were mixed, diluted to 10ml with distilled DIW and shaken thoroughly on a vortex mixer. The intensity of the coloured solutions was measured absorptiometrically at 470nm. This procedure was a modification of the method recommended by Hesse (1971).

3.8.2 Determination of Cobalt

Cobalt in the soil extracts and herbage digests was determined by one of two methods. The first involved complexing the Co with 2-nitroso-1-naphthol, extracting the complex into methyl isobutyl ketone (MIBK) and measuring its absorption signal by FAAS (Hagemann et al 1975; Simmons, 1975). This work was kindly undertaken with the help of the Trace Element Department of the East of Scotland College of Agriculture, Edinburgh.

10ml of extract was mixed with 20% sodium citrate (12ml) and 1ml of hydrogen peroxide, followed by 1ml of 1% 2-nitroso-1-naphthol in glacial acetic acid. The contents were

mixed and allowed to stand for thirty minutes before extracting with MIBK (3ml). The organic layer was retained for analysis at 240.7nm. Calibrating standards containing 0-0.18ug Co were used. For herbage these were digested in accordance with the sample procedure (section 3.6.3) while for soils they were made by dilution of a BDH standard stock solution (1mg Co/ml) using 0.5M acetic acid.

The second method was suitable for soil extracts containing Co concentrations greater than 0.1mg/l. The solutions were analyzed directly by conventinal atomic absorption spectrophotometry using a lean oxidizing air/acetylene flame at 240.7nm. A correction was made for matrix effects by deducting absorbance at 238.8nm. This work was performed on an Instrumentation Laboratory Model 151 atomic absorption spectrophotometer with a 10cm slot burner.

3.8.3 Dermination of Sulphur and Silicon

Sulphur and Si in herbage samples were determined by the XRF spectrometric procedure described by Evans (1970). This work was done at the Edinburgh Research Station of the MaCaulay Land Use Research Institute, Scotland.

Samples were dried in an oven at 105^{o}C for three hours and cooled in a desiccator. 0.3g aliquots were then made to 2.00g with pre-dried Whatman Standard grade cellulose and the mixture quantitatively transferred to a steel grinding cylinder. The mixture was ball-milled (Glen Creston Mill, Stanmore, England)

with three pestles for ten minutes. The finely powdered homogeneous mixture was transferred to a sample holder (Research and Instruments Co., London, England) and compressed into a disc at an exertion pressure of five tons per square inch for ten minutes. The discs were stored in a desiccator until required for analysis. For quantitative determination the absolute ratio method of XRF measurement was used along with the instrumental conditions outlined in Section 3.7.5.

Certified plant reference materials were used as external standards for instrument calibration and quantitative analysis. Interferences causing common systematic errors of absorption, enhancement, particle size variation and density effects (Jenkins and de Vries, 1967; Carr-Brion and Payne, 1970) were minimized by grinding and pelleting the standard materials with cellulose in accordance with the sample procedure.

The emission wavelengths used for analysis were 5.372A^{o} and 7.125A^{o} for S and Si respectively. The reference materials used, their certified S and Si contents and typical count rates and linear regression data obtained for quantitative analysis are given in Table 3.8.

3.8.4 Determination of Aluminium

Calibrating standards and blanks in the working concentration range 0-50mg Al/l were used. For soil extracts, standards were made in appropriate extracting solutions (methods A,B,D in Table 3.3) while for herbage digests these were prepared in 2M HCl.

TABLE 3.8. Standard Plant Reference Materials Used for XRF Calibration and Quantitative Analysis

Reference Material	Sulphur		Silicon		
	mg/kg DM	XRF Typical Count Rate (Sec)	mg/kg DM	% Silica Equivalent	XRF Typical Count Rate
Heather	1178	8085	5100	1.09	5119
Nordus	1768	12973	22500	4.81	28166
Clover	1844	13604	-	-	-
Orchard Leaves	1800	13910	-	-	-
Lolium perene	2133	16005	5300	1.13	6219
Faeces	2343	17742	37200	7.96	41991
Kale	16025	131208	200	0.04	587
Regression Equation:	Count = 1682 + 8.29C (r = 1.00) where C = concentration (mg/kg DM)		Count = 222 + 1.15C (r = 0.998)		

Aluminium was determined by AAS at 309.3nm using a rich nitrous oxide/ acetylene flame (Price, 1972). Except for KCl soil extracts, all solutions (5ml) were made 1% with respect to K^+ ions by mixing with 0.1ml of 19.6% KCl before analysis to prevent ionization interference.

3.8.5 Determination of Calcium and Magnesium

Test solutions, calibrating standards and blanks were brought to the desired working concentration range of 0-10mg/l by further dilution. For soil extracts, dilutions were made in appropriate extracting solutions (methods A,E,F in Table 3.3) while for herbage the dilutions were made with 2M HCl.

Calcium and Mg were determined by AAS at 422.7nm and 285.2nm, respectively using an oxidizing air/acetylene flame (Price, 1972). 5ml aliquots of all solutions were made 0.1% with respect to lanthanum by mixing with 0.1ml 27% lanthanum III chloride heptahydrate in 0.1M HCl to prevent interference due to refractory compound formation.

3.8.6 Determination of Zinc, Iron and Manganese

Test solution, calibrating standards and blanks were futher diluted to give a suitable working concentration range of 0-2mg Zn/l, 0-5mg Fe/l and 0-3mg Mn/l, respectively. For soil extracts the dilutions were made with the appropriate extracting solutions (Methods A,B,C,E,F as shown in Table 3.3). The herbage digests were diluted with 2M HCl.

The working solutions were atomized directly for the determination of Fe at 248.3nm (Allan, 1959), Zn at 213.9nm (Allan, 1961a) and Mn at 279.5nm (Allan, 1959) by AAS using an oxidizing air/acetylene flame.

3.8.7 Determination of Copper

Copper in the extracts and digests was determined by two different analytical techniques - AAS (Allan, 1961b) and DPASV using procedures previously established (Wandiga and Jumba, 1982) for acid digests.

3.8.7.1 Copper Analysis by AAS

Calibrating standards and blanks in the working range of 0-5mg Cu/l were prepared by dilution with appropriate extracting solutions for soils (Methods A,F,G in Table 3.3), and 2M HCl for the herbage digests. All solutions were then atomized directly and copper analyzed at 324.8nm using an oxidizing air/acetylene flame. By this method, extractable soil Cu, recovery studies, interferences caused by matrix composition of herbage digests, and effect of signal enhancers were determined using the instrumentation described in Section 3.7.1; while herbage digests were analysed using an Instrumentation Laboratory Model 151 atomic absorption spectrophotometer.

Recoveries of Added Copper from Herbage Digests

(a) Wet Ashing Procedures:

Appendix II gives the percent recoveries of Cu (10ug) added to herbage samples before digestion with the various mixtures. The following observations were made:

(i) Recoveries ranged from 63% with $HClO_4/H_2SO_4$ mixture to just under 99% when $HNO_3/HClO_4$ mixture was used. In general low recoveries were obtained with procedures involving H_2SO_4 and H_3PO_4 (63-78%), using the direct calibration curve method. Analysis of the two grass species gave similar results.

(ii) Determination of Cu by the standard addition procedure led to a general improvement in the recoveries, indicating a possibility of matrix inference in the analytical procedure.

(iii) Extraction procedures with $HNO_3/HClO_4$ (Method 5) and $HNO_3/HClO_4/H_2SO_4$ (Method 7) proved to be excellent and trouble-free. They gave high recoveries that were reproducible for routine determination of Cu. A slight disadvantage with Method 7 is the formation of insoluble sulphates with elements like Ca but this was avoided by ensuring that digests did not evaporate to dryness and all residues were extracted by boiling with excess 2M HCl.

(iv) Digestion with $HNO_3/HClO_4/H_2SO_4$ in the presence of molybdate (Method 8) gave good recoveries that were suitable for routine determination of Cu. The results obtained were in good agreement with those of Methods 5 and 7. However, this

procedure was much faster to use but was unsuitable for the determination of Mo. Digests obtained with this procedure were much clearer and colourless but great care was required as the reactions were vigorous and potentially hazardous.

(v) Digestion with a mixture of HNO_3 and H_2SO_4 (Method 6) gave improved recoveries for Cu with the standard addition method but recovery of Mo was poor when compared with Methods 7 and 8. The procedure was however very slow and maximum care was required to prevent charring. The presence of H_2SO_4 imposes the limitations mentioned in part (iii) above and yields solutions with very high viscosities that are unsuitable for determinations by atomic absorption.

To establish the causes of the variations in copper recoveries, interference studies were carried out using a 5mg Cu/l standard solution. It was observed that:

(1) The absorbance signal was seriously depressed when analyte solutions contained matrix species at an extent equivalent to or greater than 0.075% w/v sodium molybdate (Method 8) and 0.5% w/v potassium dihydrogen phosphate. Previous investigations (Gorsuch, 1959) established that large losses of Cu during digestion were primarily due to formation of phosphosilicate glass in which the metal ions were trapped. Phosphorus pentoxide is one of the few glass-forming oxides that is 4-coordinated and could be expected to form a stable compound with Cu.

This problem can be avoided by ensuring that digests are not evaporated to dryness so that the only disadvantage with procedures involving H_3PO_4 and/or H_2SO_4 eg Method 2 is the high viscosity of the resultant extracts.

(2) The addition of ferric chloride to digests did not improve sensitivities in the analytical procedure by atomic absorption. Recoveries of added Cu with and without the presence of phosphate did not change either, with the addition of Fe III: this is contrary to previous observations (Price, 1972) and this suggests that the lower recoveries obtained with Methods 1, 2, 6 may not be caused by interference of phosphate at concentrations normally found in plants.

(3) The incorporation of organic solvents like ethanol or butanol in the sample digests to an extent of 10% v/v resulted in an enhancement of the absorbance signal by 17-40%. This was of advantage especially in the analysis of solutions with very low Cu levels or high viscosities (eg Methods 2 and 6) since the solvents improve sample uptake at the nebulization stage.

(b) Dry Ashing Methods

The following observations were made from the results in Appendix II:

(i) Recoveries in general varied from 80-102% with higher values obtained using the standard addition method. Differences between the various methods were not significant.

(ii) The use of HNO_3 (Method 9) seldom improved the recoveries, although clean ash, readily soluble in dilute acid was produced.

The HNO_3 was not added until the material was well ashed to avoid deflagration and serious losses in presence of appreciable amounts of carbon.

(iii) The use of $Mg(NO_3)_2$, molybdate and vanadate did not increase the recovery of Cu from the two grass species.

Conclusions and Choice of Ashing Procedure for Herbage

The low recoveries obtained with the dry ashing procedures were probably attributable to losses due to volatilization and retention which can be serious when dealing with batch analysis of survey samples. Hence dry ashing procedures were not employed. The three wet oxidation procedures (Methods 5, 7 and 8) were equally well suited to the determination of the elements in herbage. The merit of the molybdate procedure (Method 8) for the determination of Cu was further confirmed by using polarographic techniques as summarized below. However, Method 7 ($HNO_3/H_2SO_4/HClO_4$ procedure) was selected for the batch determination of Al, Fe, Mn, Mo, Zn, Ca and P; while Method 5 ($HNO_3/ HClO_4$) was used for the wet extraction of Cu and Mg prior to AAS determination.

3.8.7.2 Copper Determination by Differential Pulse Anodic Stripping Voltammetry (DPASV)

The DPASV method of analysis is based on a conventional two-stage procedure. Oxygen-free samples are first electrolyzed in KCl-buffered medium and the various metals in solution

deposited for a pre-set period of time onto a hanging mercury drop electrode (HMDE) to form a mercury amalgam by a process of reduction at a fixed potential more negative than that of the test species being determined. At the end of the deposition, quiescience time (15 seconds) is allowed for the solutions to equilibriate and the stripping step started in the differential pulse mode. The current due to the re-oxidation of the deposited species is measured and presented in the form of peak-shaped curves. The peak current is proportional to the concentration of the test species hence quantitative analysis can be achieved easily, usually by the standard addition technique to compensate for matrix effects. The test solution is spiked with known volume of standard solution of the element and the procedure repeated. Concentrations are calculated using the equation of Meites (1965) for polarographic analysis, which requires the use of the two peak currents (or peak heights) obtained with and without the addition of standard solution to the test sample.

The method required the following special reagents:

(a) Potassium chloride solution, 1M:

A solution of 7.455% w/v aqueous KCl was prepared and purified by electrolysis at constant potential (-1.5v) for sixteen hours to remove traces of metal contaminants, using mercury pool and platinum as electrodes and the polarographic analyzer as the potentiostat.

(b) Ammonium Chloride Solution, 3M:

A 16.1% w/v solution of ammonium chloride was made in distilled DIW and purified by electrolysis at constant potential using the same procedure as for the KCl above.

(c) Ultra-pure Ammonia Solution:

This was prepared by isothermal distillation following the procedure previously described by Wandiga and Jumba (1982), as follows: One litre of stock ammonia solution (specific gravity 0.91) in a pyrex beaker was placed in a large vacuum desiccator beside another beaker containing 400ml of distilled DIW and the two systems allowed to equilibraite at 40^{o}C over a period of four weeks. Aliquots of this solution (20ml) were diluted to 50ml with distilled DIW and standardized against dilute (0.1M) H_2SO_4 by titration. The molarity of the original distillate was determined and used in the preparation of buffer below.

(d) Ultra-pure HCl Solution:

The above prodedure was repeated using concentrated HCl (SG 1.18) in place of stock ammonia. The distillate was standardized against 0.1M sodium hydroxide solution and the calculated molarity used in the preparation of buffer and all pH adjustments.

(e) Ammonia/Ammonium Chloride Buffer, 1M:

The ultra-pure ammonia and the 3M ammonium chloride solutions were mixed in correct proportions to make 1M of each in the mixture and the pH adjusted to 9.0 using the ultra-pure HCl obtained above.

(f) Hydroxylamine solution:

A 20% w/v aqueous solution was made using distilled DIW and the purity checked polarographically for trace metal contaminants.

Experimental Procedure for Polarographic Analysis

Solution aliquots (5ml) were pipetted into the polarographic cell and mixed with 1ml of 1M KCl. Hydroxylamine solution (0.1ml) was added and the contents adjusted, with the aid of a pH meter, to pH between 5.5 and 7.0 using the ammonium chloride buffer and ultra-pure HCl. The solution was then diluted to 10ml with distilled DIW to make it 0.1M with respect to KCl as supporting electrolyte. The solution was deoxygenated by bubbling nitrogen through for fifteen minutes. The nitrogen was purified by bubbling "white spot" tank nitrogen first through 1% vanadium II chloride in 10% HCl to absorb oxygen, and then through the supporting electrolyte (0.1M KCl) to wash and equilibriate it before reaching the polarographic electrolysis cell. Copper in the solution was deposited under steady stirring conditions and under nitrogen onto the HMDE (of

constant drop size) at an initial (deposition) potential of -450mV for two minutes and stripped at negative potentials close to 0mV (vs SCE). After obtaining duplicate peaks for the unknown copper concentration the test solution was spiked with 50ul of a 10ug Cu/ml standard solution. The solution was deaerated for two minutes and the deposition and stripping processes repeated in duplicate, preferably at the same current sensitivity. The peak heights were measured and recorded.

A blank (5ml) was mixed with 1M KCl (1ml) and hydroxylamine solution (100ul) and the pH adjusted to 6.0 as for the sample above. The solution was deaerated for fifteen minutes and subjected to the deposition and stripping processes above using the current sensitivity as for the test solution. At least three voltammograms were obtained. The peak heights were measured and the mean value calculated. All spiked and unspiked sample peaks were corrected for the mean blank peak height and the net values used in the calculation of Cu concentrations in the digests. Copper was calculated from the equation (Meites, 1965)

$$Cu = \frac{i_1 \, v \, Cs}{i_2 v + (i_2 - i_1) V}$$

where Cu = the determined Cu concentration in ug/ml

v = volume of standard solutin added (ml)

Cs = concentration of standard solution used for spiking, in ug/ml

i_1 = peak height (or peak current) due to copper in the test solution corrected for blank value

i_2 = spiked peak height (or peak current)

V = original volume of sample solution taken (ml)

The above experimental procedure was adopted after a series of test studies involving peak current resolution as a function of pH of test solution; Fe III and Se VI interferences with the stripping process and resultant peak signal; and peak current as a function of concentration to check whether the system obeyed the Ilkovic Equation for polarographic analysis as well as confirm the overall instrumental calibration. The tests revealed the following:

(a) Maximum peak current resolution was maintained between pH 5.5 and 7.0. The peak current decreased sharply beyond pH 7.0 possibly due to complexation of Cu ions in the solution matrix. Table 3.9 compares the results obtained in the analysis of a sample digested by two different ashing procedures that showed good recoveries of added Cu (Appendix II).

(b) A serious interference due to presence of high levels of Fe III. The peak current of the spiked digests decreased as the Fe III concentration increased. Addition of 100ul of the hydroxylamine solution suppressed this interference (up to 1000ug Fe III) attributed to the participation of iron in the re-oxidation of deposited Cu.

TABLE 3.9. Effect of pH on Peak Current Resolution*

pH of Cell Solution	Peak Current (uA) Corrected for Blank Reading	
	Method 7 ($HNO_3/HClO_4/H_2SO_4$)	Method 8 ($HNO_3/HClO_4/H_2SO_4/Na_2MoO_4$)
1.0	2.6	2.5
2.4	2.9	2.8
3.0	3.5	3.7
4.0	4.0	3.8
5.1	4.4	4.6
5.6	4.9	5.1
6.0	5.2	5.5
7.0	5.0	5.1
8.1	1.0	1.2
9.0	0.1	0.4

*5ml of sample solution was spiked with 50ul of 10ppm Cu standard solution before analyzing at the various pH values. Each datum is a mean of three scans.

(c) Addition of L-ascorbic acid to test solutions did not change the peak current resolution trend hence Se VI interference was negligible at the concentrations present in the grass sample digests.

(d) The determination of Cu by DPASV was not affected by the molybdate used in Method 8 ashing procedure.

(e) The standard addition method resulted in linear calibration curves which obeyed the Ilkovic Equation at Cu concentrations of up to 0.1ug/ml. One regression analysis gave the Pearsons correlation coefficient value of 99.50%.

(f) Analysis of ten samples comprising five grasses and five commercially compounded feeds gave Cu levels ranging from 2.50 - 16.1mg Kg^{-1} DM by DPASV and 3.13 - 16.36mg kg^{-1} DM by the AAS method. Good agreement between the two methods was obtained: the regression equation was

$$Cu(DPASV) = 0.986\ Cu(AAS) - 0.367,\ r = .9846$$

where Cu is in mg kg^{-1} DM.

(g) Digests containing non-detectable Cu levels (by AAS method) could be conveniently analyzed by DPASV without a preconcentration step and detection limits of 0.5-1ug/l were obtained; these were much lower than those found by AAS (eg 60ug/l) whose sensitivities were much lower (0.15ug/l). The

DPSAV method was not used routinely because it is time consuming but was held in reserve for use with samples of very low Cu content.

3.8.8 Determination of Molybdenum

Molybdenum in extracts and digests was determined spectrophotometrically by a modification of the method described by Bingley (1959; 1963). Each test sample solution (10ml) was adjusted to pH1 with 7M ammonium hydroxide or 10% HCl using a pH meter. 0.25ml of Fe III solution (10% w/v ferric ammonium sulphate in 2% H_2SO_4) was added and the volume adjusted to 25ml with distilled DIW. 50% w/v potassium iodide (0.25ml) was added and the mixture allowed to stand for 10 minutes with occassional swirling. Iodine liberated was then discharged by dropwise addition of 10% sodium thiosulphate before addition of 0.25ml 50% aqueous tartaric acid followed by 2ml of 10% thiourea. Dithiol reagent (2ml of 2% 4-methyl 1,2-dimercaptobenzene in 1% sodium hydroxide) prepared according to the procedure of Bingley (1959) was added, the contents mixed and allowed to stand for thirty minutes. The complexed Mo was extracted by shaking with 5ml of isoamyl acetate (boiling point range 136-142^{o}C) for thirty seconds. The organic layer was separated and centrifuged for five minutes before measuring the molecular absorption intensity of the green Mo-dithiol complex at 680nm using the acetate in the reference channel. Calibrating standard solutions containing 0-10ug Mo were made to 10ml with distilled

DIW and treated in accordance with the sample procedure starting with the pH adjustment stage.

Recoveries of Molybdenum from Herbage Extracts: Choice of Digestion Procedures

The wet ashing procedures produced variable recoveries of added Mo (Appendix II). The procedures involving H_2SO_4 with HNO_3 or $HClO_4$ gave values that were particularly erratic and extremely low, contrary to the findings of Gorsuch (1959).

As was similarly observed in Section 3.8.7 (Cu recoveries) the extraction procedures involving $HNO_3/H_2SO_4/HClO_4$ and $HNO_3/HClO_4$ (Methods 5 and 7) proved to be excellent for molybdenum extraction, and gave reproducible results, in good agreement with Evans et al (1950), Johnson and Arkley (1954), Gorsuch (1959) The dry ashing methods generally gave high recoveries (mean 95.7%) except the procedure involving $Mg(NO_3)_2$ (Method 10) which produced erratic values. Use of NH_4VO_3 in the ashing procedures (Method 11) did little to enhance the efficiency of extraction of herbage Mo. For batch determinations, Method 7 ($HNO_3/HClO_4/H_2SO_4$) was selected.

3.8.9 Determination of Selenium

The two major considerations for accurately determining Se in biological material are the need for measurement of sub-microgram amounts and the avoidance of losses during

digestion of the samples, especially those of plant origin. Wet ashing techniques involving $HNO_3/HClO_4$ (Watkinson, 1966; Olson et al 1975) and $HNO_3/HClO_4/H_2SO_4$ (Analytical Methods Committee, 1979) can give satisfactory recoveries of added Se and complete extraction of endogenous Se from the organic matrix. A number of sensitive methods for determining the element in the digests have been reported. Kelleher and Johnson (1961) proposed a combined spectrophotometric and isotope dilution (SID) method to compensate for unavoidable losses. Handley (1960) described an x-ray fluorescence (XRF) procedure and neutron activation analysis (NAA) was reported by Bowen and Cawse (1963). Other methods include gas-liquid chromatrography (GLC), atomic absorption by hydride generation and spectrophotometric techniques (Siemer and Hagemann, 1975; Analytical Methods Committee, 1979). However procedures based on SID, XRF, NAA, GLC and atomic absorption mostly require lengthy analytical and calibration steps, and are expensive and sophisticated.

Most routine Se determinations are based on spectrophotometric reactions involving chelation and/or extraction. These can be conventiently divided into three classes: the first involves the formation of complexes in which the Se bonds either partially or completely to S ligands. Typical reagents in this class include 2-mercaptobenzoic acid and 2-mercapto-benzothiazole (Cresser and West, 1968). The second class involves the reduction of Se IV to the elemental form often with the simultaneous production of a coloured product and includes

the well known Se sol and iodine methods (Boltz, 1958), the oxidation of phenylhydrazine-p-sulphonic acid to the diazonium ion followed by coupling with 1-naphthylamine (Kirkbright and Yoe, 1963), the oxidation of NN diphenylhydrazine (Mushrova and Sushkova, 1967), and a few catalytic reactions (Kawashima et al 1977). The third class involves the formation of piazselenols in which Se as selenous acid reacts with aromatic o-diamines to produce compounds containing the selenodiazol five-membered ring system which can be quantitatively measured either absorptiometrically or using fluorometric techniques. Typical reagents in this class are 3,3-diaminobenzidine (Cheng, 1956; Watkinson, 1960; Dye et al 1963), 1,2 diaminonaphthalene (Parker and Harvey, 1962) and 2,3-diaminonaphthalene, DAN (Parker and Harvey, 1962; Watkinson, 1966).

In selecting the DAN-fluorometric method (Watkinson, 1966) for batch analysis of samples in the present study, two observations were taken into consideration:

(a) Methods in the first and second classes are often subject to serious interferences from other ions in the solution matrix resulting in sensitivities of eg 0.005 - 0.063ug Se(Bera and Chakrabartty, 1968; Cresser and West, 1968) which are an order of magnitude lower than those obtained by typical fluorometry, (0.002ug Se in 5ml cyclohexane; Parker and Harvey, 1962).

(b) Parker and Harvey (1962) made detailed studies of the reactions between selenous acid and aromatic o-diamines. By comparison of the luminescence of their piazselenol products they showed that by far the most sensitive reagent for the determination of Se is DAN with which it forms 4,5-benzopiazselenol. This compound displays a brilliant lime-green fluorescence when excited at 369nm in either cyclohexane or dekalin, and has marked advantages over the 3',4'-diamino diphenylpiazselenol formed by reaction of Se with excess 3,3' diaminobenzidine. Sensitivities better than 0.002ug Se and detection limit of 0.044ng/g have been obtained (Brown and Watkinson, 1977) using automated techniques. Recent modifications reported by Hemsted et al (1972), Olson et al (1975), Analytical methods Committee (1979) and Bayfield and Romalis (1985) were evaluated before adopting the modified Watkinson (1960) procedure described below.

Reduction of Se VI to Se IV

The preliminary requirement for Se determination by piazselenol formation is that the element must be reduced to the Se IV form from the Se VI present in the digests prior to complexation with DAN. Reduction methods that have been used include boiling for twenty minutes with 10% v/v (1.2M) HCl (Watkinson, 1966; Olson et al 1975) or 4M HCl (Analytical Methods Committee, 1979); heating in a boiling waterbath with

6M HCl (Hemsted et al 1972); and reduction at unspecified temperature with 6M HCl (Hall and Gupta, 1969).

The use of 10% HCl yielded erratic results with recoveries of added Se fluctuating between 61% and 95%. The discrepancies were attributed either to incomplete reduction or losses of Se as the volatile chloride during the twenty-minute heating. It was also possible that differences in residual volumes of digests caused irregular Se reduction as a result of varying HCl concentrations in the diluted digests.

Further tests to improve the reduction efficiency were therefore carried out in triplicate under varied conditions using 0.1ug Se for digestion and

(i) larger standardized volumes (3ml) of digests in all vials before reduction to ensure constant final HCl concentration.

(ii) Constant complexation pH of 1.5.

(iii) Constant reduction temperature of 90^{o}C.

(iv) 6M HCl and 8M HCl were tested using different volumes.

(v) Variable reduction times.

The results given in Table 3.10 show that complete reduction was achieved with 1.5ml 6M HCl. Volumes larger than 1.5ml did not increase the fluorescence and no losses of Se due to apparent volatilization were observed. For batch analysis a larger volume of 2ml was used to offset differences in the Se concentrations from one sample to the other. With 8M HCl volumes > 2ml a slight decrease in intensity was observed.

Table 3.10 Effect of HCl-reduction Volume and Concentration on Fluorescence Intensity of 0.1ug Se

Volume of HCl (ml)	Relative Fluorescence Intensity (Arbitrary Units)	
	with 6M HCl	with 8M HCl
0.5	47.5	49.0
1.0	50.0	51.0
1.5	54.0	55.0
2.0	54.5	53.5
2.5	54.0	54.0
3.0	54.0	50.0
4.0	53.5	50.0
5.0	52.0	45.0

Complete reduction was effected after 15 minutes beyond which no significant increase in the fluorescence intensity was observed (Table 3.11). However, because of the wide range of Se concentrations expected in the samples, a thirty minutes reduction period was used subsequently. No appreciable losses of Se were observed during reduction periods even in excess of one hour at 90^{o}C in the temperature-controlled waterbath.

Table 3.11. Effect of Reduction Period on the Relative Fluorescence Intensity

Reduction time (Min) (with 2ml 6M HCl)	Relative Fluorescence Intensity
3	46
5	51
10	56
15	57
20	55.5
25	56
30	56
40	56.5
60	56.5
75	55.5
90	54

Control of Complexation pH

The importance of attaining correct acidity for piazselenol complex formation after acid digestion was recognised by Hall and Gupta (1969) and led to the routine use of formic acid in buffer mixtrues of pH 2.0 by MAFF (1981). Grant (1981) found that the optimum pH range was 1.2 - 1.6 but no advantage was found in using buffers. There is general recognition that regulation of pH in the range of 1-2 is required for complexation (Lott et al, 1963; Watkinson, 1966; Brown and Watkinson, 1977; Hasunuma et al, 1982; Bayfield and Romalis,

1985) and this has been achieved either by pH meter or by use of indicators. However, careful pH adjustments may be nullified by the subsequent addition of DAN reagent in acid solution unless buffers have been incorporated (Hall and Gupta, 1969; Allaway and Cary, 1964; Moreno-Dominguez et al 1983).

In the present study the titrimetric use of indicators such as cresol red and methyl orange did not give satisfactory day-to-day reproducibility as standard Se fluorescence values in some cases were only 65% of those obtainable by other methods. In agreement with the findings of Grant (1981), no advantage in terms of analytical precision was found in the use of buffers during pH adjustment. Where samples contained Se concentrations of only 0.01ug/g, the use of a pH meter for precise pH control was particularly important. While the Analytical Methods Committee (1979) recommended a controlled pH of 1.8 it was easier to control pH at 1.5; the resultant difference in fluorescence intensity did not affect the method sensitivity.

Source of DAN

The source of DAN was important. One source (Sigma Chemical Co Ltd) gave high background fluorescence even after re-extraction in cyclohexane. DAN obtained from Aldrich Chemical Co gave low background fluorescence and was used throughout the study.

Use of Sodium Sulphate

Hemsted et al (1972) suggested the use of anhydrous sodium sulphate to remove interfering substances in the organic extract prior to fluorometry. In the present study inconsistent reproducibilities and fluctuations in fluorometric measurements of duplicate samples and standards attributed to presence of water in the organic extract were eliminated by the addition of sodium sulphate which also aided in the flocculation of interfering suspended particles such as silica and insoluble metal salts and hydroxides. The interfering deposits could however be less prominent in the determination of Se in materials such as serum and blood plasma.

Procedure Employed for Batch Determination of Selenium

Selenium in the soil and herbage digests was determined by modification of the fluorometeric method of Watkinson (1966). Se IV was complexed with DAN reagent at pH 1.5. The 4,5-benzopiazselenol complex was extracted into cyclohexane before measuring its fluorescence with excitation at 369nm and emission reading at 525nm.

To the digests and standards containing Se IV, distilled DIW (20ml) was added followed by 2ml of stabilizer solution (a solution of 0.5M diammonium EDTA in 5% aqueous hydroxylammonium chloride). The pH of the mixture was adjusted to 1.5 with 7M NH_4OH and/or 10% HCl using a pH meter and the volumes added were noted. The contents were quantitatively transferred to 8" x 1"

pyrex glass tubes with two 5ml portions of distilled DIW. All solutions were then adjusted to same volume. Herbage (pH-adjusted) extracts with excessive silica deposits were filtered through glass wool previously washed and rinsed several times firstly with 1M $HClO_4$ and then with distilled DIW. The tubes were placed in a water bath at 50°C for five minutes adding 5ml of DAN reagent (0.1% DAN hydrochloride in 0.1M HCl). The contents were heated for twenty minutes at 50°C in diffused light and cooled to room temperature. Cyclohexane (5ml) was added and the benzopiazselenol complex extracted by shaking vigorously for thirty seconds. The aqueous layer was discarded and the organic phase swirled with 0.3g anhydrous sodium sulphate prior to taking fluorescence measurements. The readings for the calibrating standards were corrected for the blank value and plotted against amount (ug) of Se to give linear calibration curves which were then used to determine the concentration of Se in the samples. These steps gave excellent calibration curves ($r = 0.995$) and low coefficients of variation (2.0-3.1%).

With the procedure described above two grass species, napier grass and Nandi setaria were spiked with different amounts of Se, digested in accordance with the method given in Section 3.6.4 and analyzed. The results presented in Table 3.12 show that satisfactory recoveries were obtained.

Table 3.12. Recovery of Added Selenium from Plant Material

ug Se added	% recovered	
	Napier grass	Nandi setaria
0.01	89.0	103.0
0.02	97.5	96.4
0.04	93.0	98.0
0.06	105.0	96.0
0.08	98.7	100.0
0.12	96.0	99.2

3.9 STATISTICAL ANALYSIS

The herbage/soil data from the 135 sites were classified according to various factors - eg division or farm of origin, management, altitude, geology, landscape profile and soil pH (Appendices I, III-V) and herbage samples were classified by species. The dataset was unbalanced in that some factors were poorly represented numerically. An initial approach to the study of the data was to calculate the arithmetic mean for each of the herbage/soil elements for all levels of a particular factor by averaging over the other factors using conventional one-way analysis of variance. While this provided a useful summary of the data, significant differences between factors may result from correlations with other factors particularly for poorly

represented classes. Complete analysis required a more robust statistical model. for analyzing unbalanced data called Residual Maximum Likelihood (REML) described by Patterson and Thompson (1971; 1975), Harville (1977) and Robinson (1987). This work was undertaken at the Scottish Agricultural Statistics Service, University of Edinburgh.

The application of a simple REML model to the survey data allowed the disentangling of the effects of the various factors. The original dataset was transformed (by taking logarithms) to ensure approximate normality and to simplify the relationships between the factors. For each soil element the model consisted of fitting five independent factors (management, landscape, altitude, area, and geology). It was assumed that the effect of division and farm (Appendix I) was random. The REML model fitted to the herbage data was very similar except that an additional factor, species was added.

The interpretation of the REML output consisted of checking the "estimated components of variance" and the "mean effects". The estimated components of variance provided a breakdown of the total variance in terms of the division, farm and residual components. Spatial variation in the data was indicated by a large combined division and farm component relative to the residual component. For each of the factors the REML output gave a "mean effects" and also a matrix of "standard errors of differences between pairs" to facilitate student's t-tests between unequally represented classes. The appropriate degrees of freedom were also given.

In addition to data analysis by the REML model, the batch results were occasionally subjected to conventional correlation and regression tests to establish relationships between the measured parameters.

3.10 CONTOUR MAPPING

A mapping technique (Simpleplot Mark II, 1982; Copyright: Bradford University Research Limited) was used to assess soil and herbage mineral distribution by contour plotting. This work was also kindly undertaken at the Scottish Agricultural Statistics Service.

CHAPTER FOUR

MINERAL COMPOSITION OF HERBAGE IN RELATION TO RUMINANT REQUIREMENTS

4.1 INTRODUCTION

Estimation of mineral requirements of animals of different classes producing at different rates is usually made using a factorial approach (ARC, 1980). The factorial method assesses requirements in two stages. Firstly, the net requirement is calculated from estimates of the accretion and secretion of the element made during growth, pregnancy and lactation, including inevitable losses from the body (endogenous losses). Secondly, the dietary requirement is calculated by dividing the net requirement by a factor that represents the proportion of the dietary mineral that is absorbed as assessed from metabolism experiments. In detail:

(i) Net minimum endogenous requirement (E) is the inevitable loss of the element from the body in faeces and urine

(ii) Net requirement for body growth (G) is the daily retention of the element at the specified rate and stage of growth

(iii) Net requirement for pregnancy (P) is the daily retention of the element in the foetus and adnexa at the specified stage of pregnancy

(iv) Net requirement for lactation (L) is the daily secretion of the element in milk at the specified yield

(v) Total net requirement (N) is the sum of all the requirements above (ie N = E + G + P+ L)

(vi) Dietary requirement is the net requirement divided by the absorption coefficient, A, ie N/A = (E + G + P + L)/A

(vii) Absorption is defined as the amount of a mineral supplied in the diet that enters the body from the gut, ie apparent absorption plus the net faecal endogenous excretion. The coefficient of absorption is the amount absorbed divided by the amount ingested.

Factorial estimates of requirement in g/day can be converted to dietary concentrations using assumed dry matter intakes (DMI) (ARC, 1980). Of the various components of the factorial estimate, the biological variation in coefficient of absorption is likely to be of greatest significance, as it affects the calculation of dietary requirements for each individual function. For example, the absorption of Ca by ruminants is affected by their physiological status and is largely independent of dietary characteristics; while for P, the excretion into the gut varies substantially with dietary supply and must be measured separately for each determination of coefficient of absorption. The factorial method has been widely used for macro-minerals and ARC (1980) extended the method to Cu and Zn. For other trace elements, requirements were assessed by

the presence or absence of clinical or subclinical abnormality at given dietary trace element inputs.

Different authorities have published different estimates of mineral requirements and those published by ARC (1980) were generally lower than previously published values. They not only represent the most rigorous test of dietary adequacy available but also provide threshold levels as a guide for control of nutritional problems arising from excessive intake by the animal (ie toxicity).

The first step in characterising the mineral status of the sampled forages from the Mt Elgon region and, by inference, the soils which support them will be to compare the range of values recorded with the ARC (1980) standards for deficiency and excess of each element.

4.2 RESULTS

Complete results of the batch analysis of herbage are presented in Appendix III. The following histogram representation shows the class concentration frequencies of the data for Ca, P, S and Mg (Fig. 4.1); Al and Si (Fig. 4.2); Cu and Mo (Fig. 4.3); Zn, Se and Co (Fig. 4.4); and Fe and Mn (Fig. 4.5).

4.2.1 Calcium, Phosphorus, Sulphur and Magnesium

Results for the essential macro-elements Ca, P, Mg and S are summarised in Table 4.1.

Fig. 4.1 Herbage Concentration Ranges for Calcium, Phosphorus, Magnesium & Sulphur

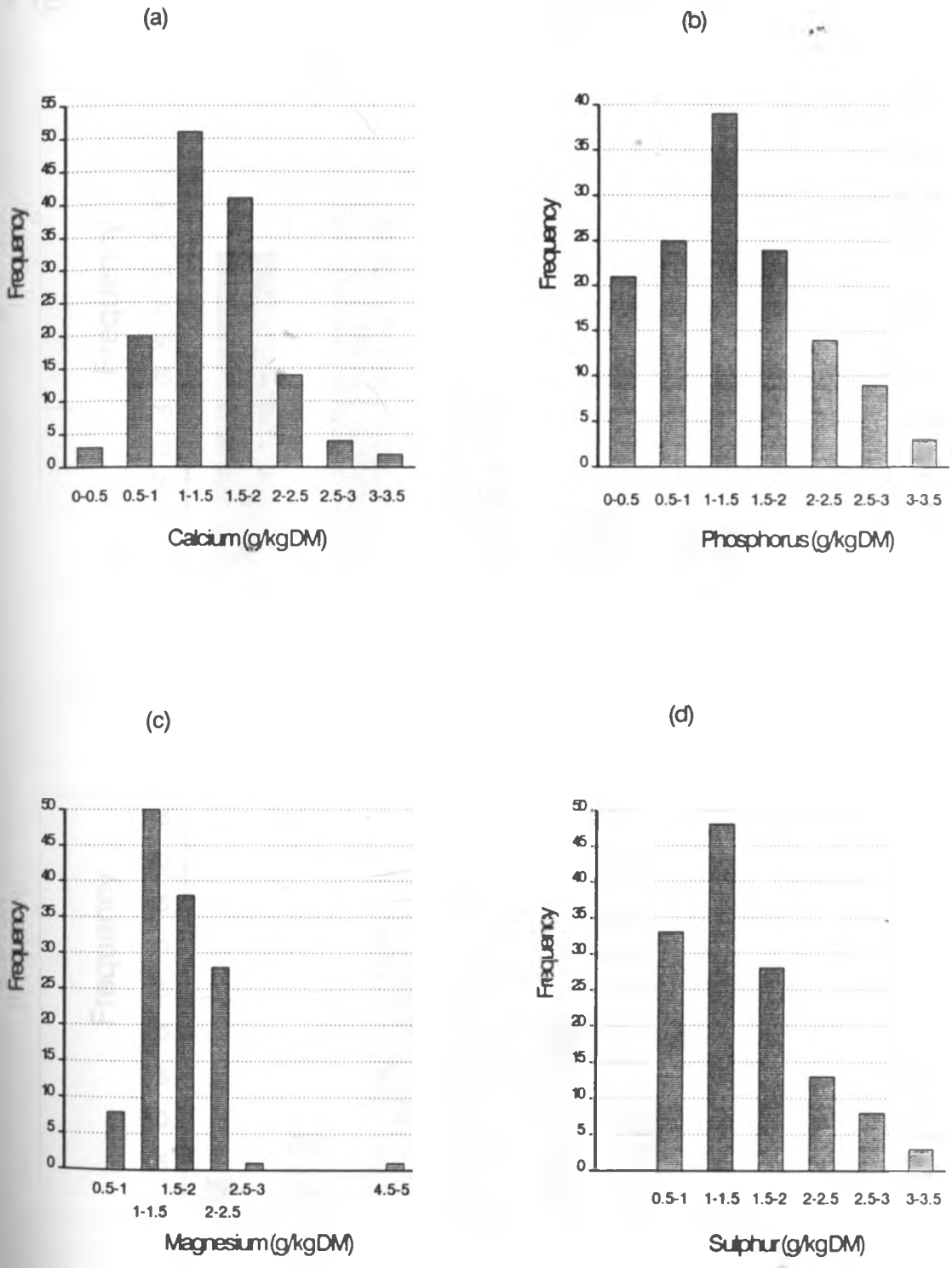


Fig. 4.2 Herbage Concentration Ranges for Aluminium and Silicon

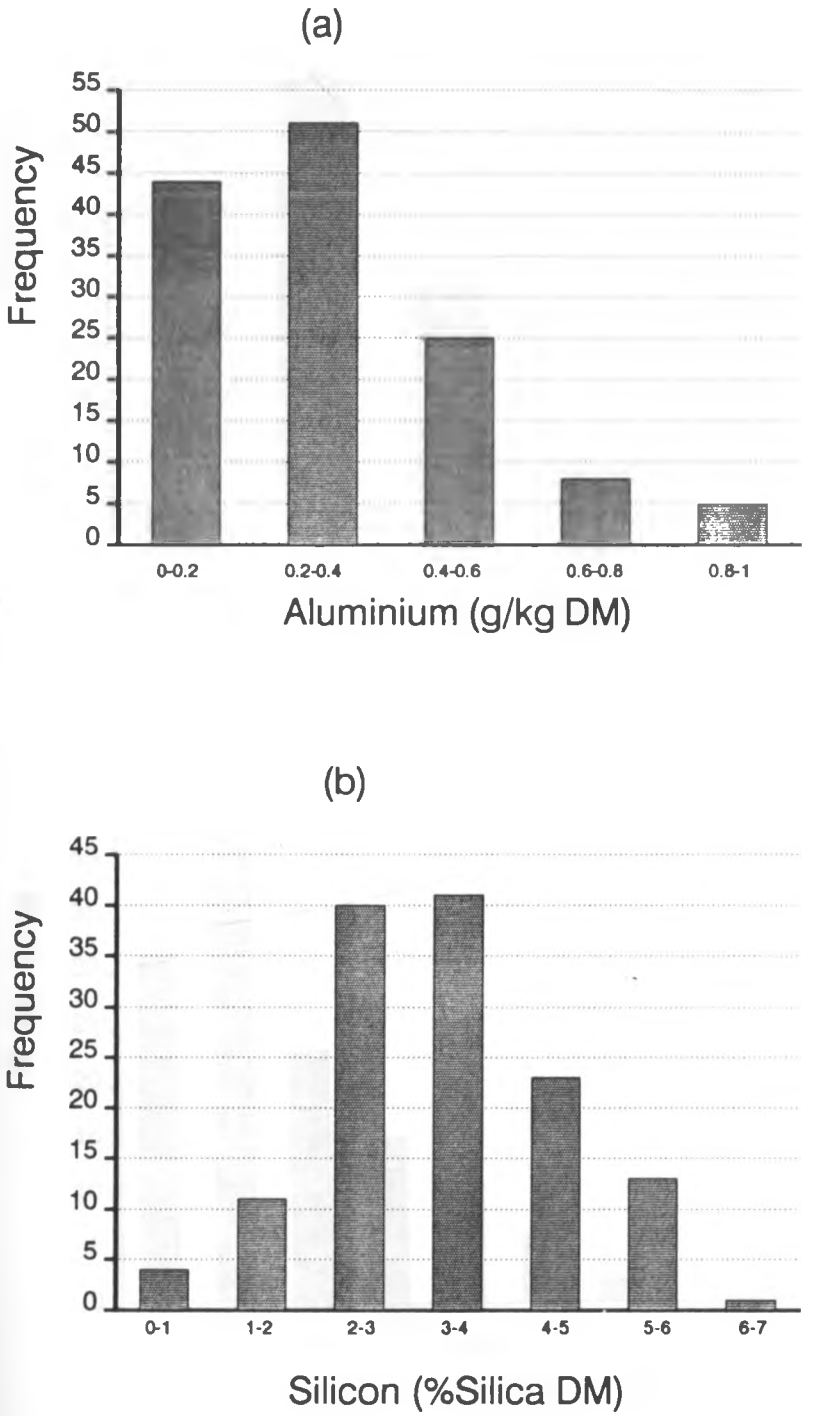
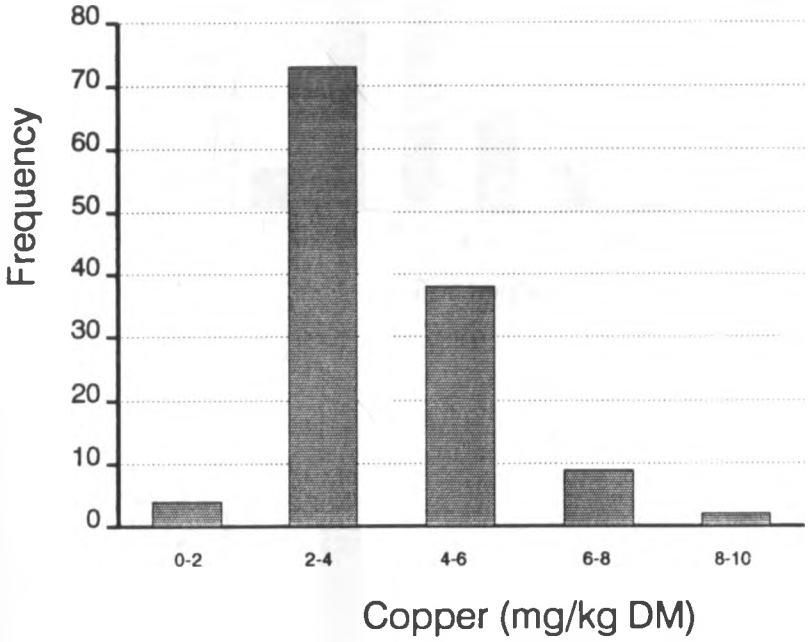


Fig. 4.3 Herbage Concentration Ranges for Copper and Molybdenum



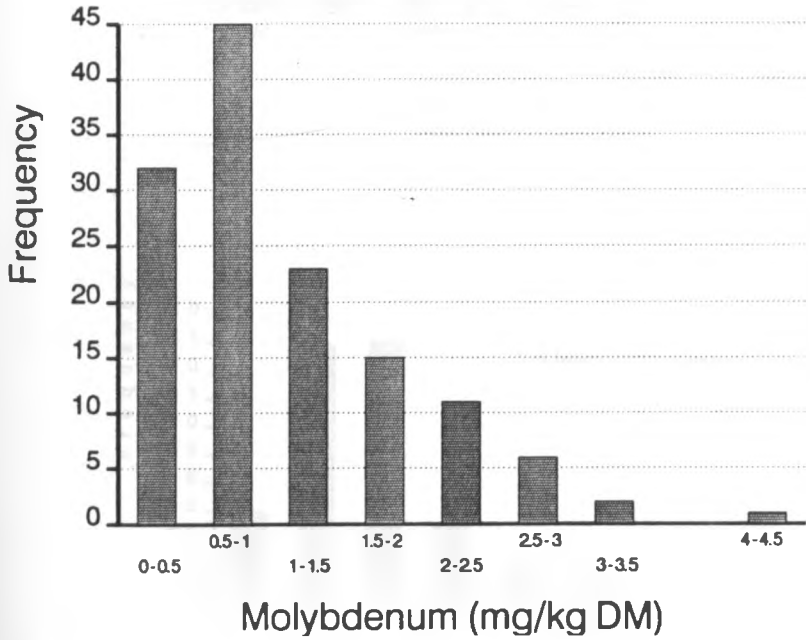
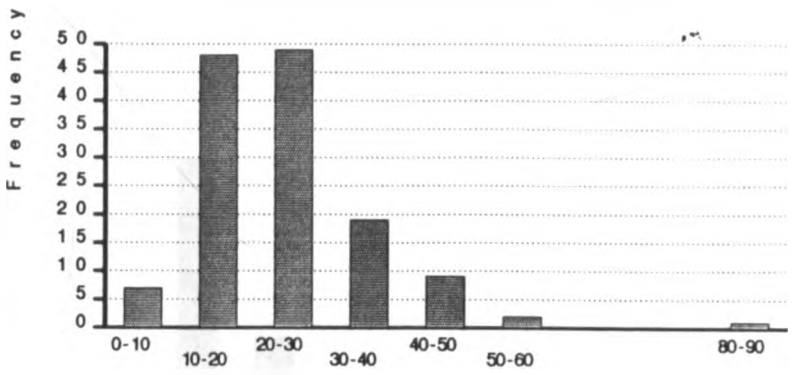
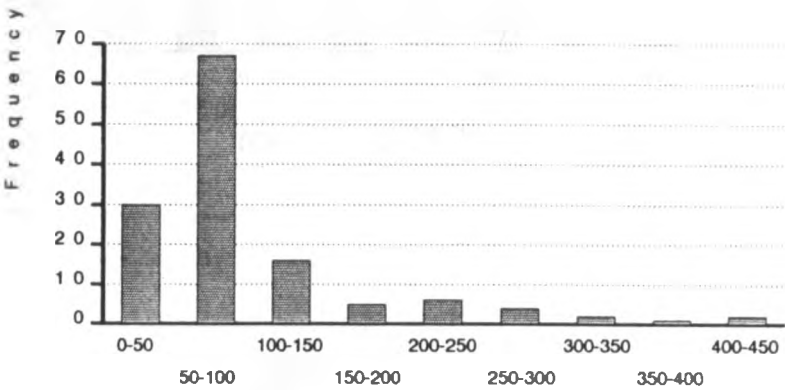


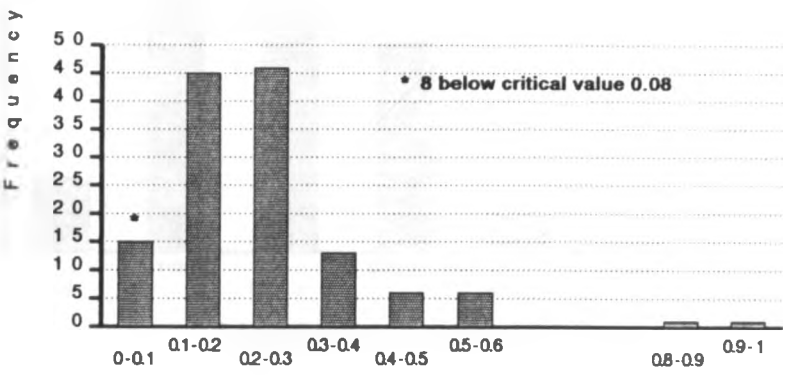
Fig. 4.4 Herbage Concentration Ranges for Zinc, Selenium & Cobalt



Zinc (mg/kg DM)



Selenium (ug/kg DM)



Cobalt (mg/kg DM)

Iron (g/kg DM)

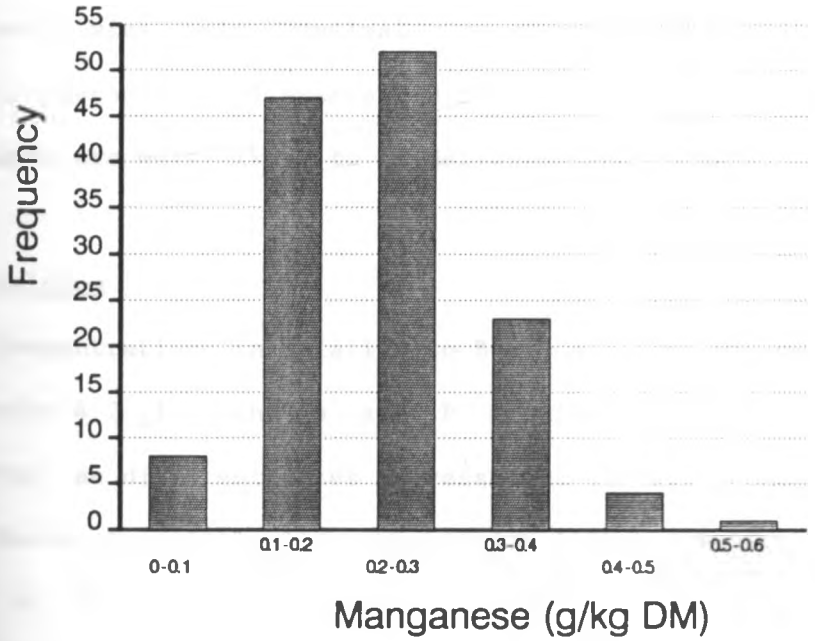

Frequency
55
50
45
40
35
30
25
20
15
10
5
0
0-0.1
0.1-0.2
0.2-0.3
0.3-0.4
0.4-0.5
0.5-0.6
Manganese (g/kg DM)

Fig. 4.5 Herbage Concentration for Iron and Manganese

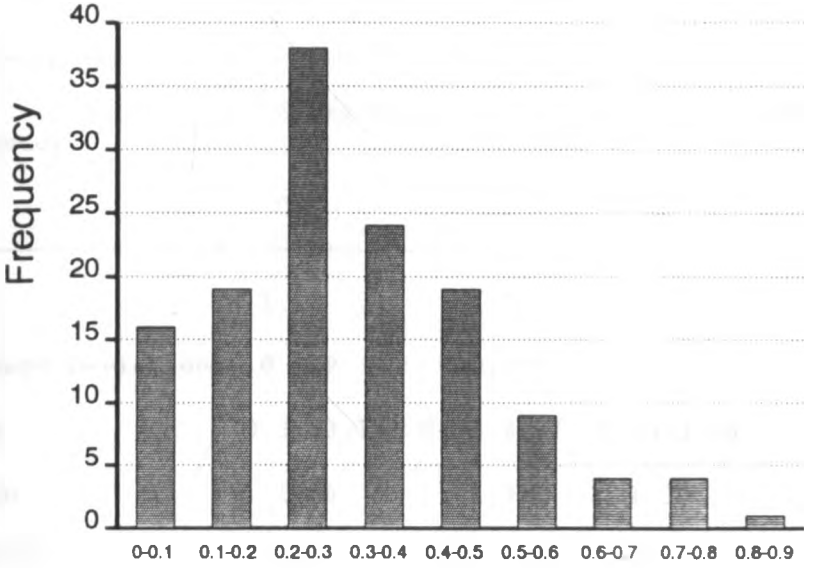


Table 4.1. Summary of the Herbage Concentrations of Calcium, Phosphorus, Sulphur and Magnesium Compared with Standard Requirements for Ruminants.

Parameter	Element Concentration (g kg^{-1} DM)			
	Ca	P	S	Mg
Mean	1.48	1.35	1.46	1.61
Standard Deviation	0.522	0.758	0.594	0.530
Range	0.33-3.18	0.04-3.47	0.50-3.08	0.57-2.56
Median	1.44	1.32	1.30	1.57
ARC (1980)	2.0-6.7	1.1-3.4	1.0	1.0-2.1

The mean concentrations of the four macro elements were surprisingly close (1.35 to 1.61g/kg DM) and the coefficients of variation wide, being maximal for P (56%) and minimal for Mg (33%). Because requirements for the macro elements differ, some elements are more likely to be deficient than others.

(a) Calcium

(i) Concentrations in Relation to Beef Cattle Requirements

Table 4.2 gives the Ca and P requirements of beef cattle gaining at different rates expressed as dietary concentrations for diets of a particular energy value (60% metabolizability) as set by ARC (1980). According to this table dietary levels of calcium of 1.7 to 2.8g/kg DM will support beef cattle but

without any liveweight gains. The Ca concentrations found in herbage samples were therefore generally deficient with a mean of 1.48g kg^{-1} DM and median of 1.44g/kg DM. The results summarized in Table 4.1 showed extreme fluctuations with concentrations ranging from 0.33 - 3.18g kg^{-1} DM. According to data in Appendix III, 24% of the herbage samples analyzed had concentrations between 1.7 and 2.8g kg^{-1} DM; while only 3% had Ca levels above 2.8g kg^{-1} DM. Thus the majority of the samples (ie 73% or 98 out of the 135 sites sampled) had Ca levels below the 1.7g kg^{-1} DM minimum maintenance requirement. It can be concluded from these results, therefore, that most of the herbages analyzed in this survey were grossly deficient in Ca for growing beef cattle which require 3.1-5.2g Ca/kg DM.

(ii) Lactating Cows

Table 4.3 gives the Ca requirements for lactating cows expressed as dietary concentrations (for 60% metabolizability) for different breeds giving different milk yields. According to data obtained in this study (Appendix III) the Ca concentrations found were also inadequate to support milk production even at the 10kg/day level.

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Table 4.2. Calcium and Phosphorus Requirements of Beef Cattle, Expressed as Dietary Concentrations.[1]

LW+ (kg)	LW Gain (kg)	Dry Matter intake (kg/day)	Requirements*			
			Daily Ca	Dietary Ca	Daily P	Dietary P
100	0	1.4	2(2)	1.7	1.5(0.6)	1.1
	0.5	2.1	12(8)	5.5	6.2(4.5)	2.9
	1.0	3.2	21(15)	6.7	11 (8.3)	3.4
200	0	2.3	5	2.0	3.1	1.3
	0.5	3.3	14	4.3	7.8	2.4
	1.0	4.7	24	5.2	13.0	2.7
300	0	3.1	7	2.3	4.6	1.5
	0.5	4.3	17	4.0	9.3	2.2
	1.0	6.0	26	4.3	14	2.3
	1.5	8.8	35	4.0	19	2.1
400	0	3.8	9	2.5	8.3	2.2
	0.5	5.2	19	3.6	15	2.8
	1.0	7.3	28	3.8	21	2.9
	1.5	10.6	38	3.6	27	2.6
500	0	4.4	12	2.7	10	2.3
	0.5	6.1	21	3.5	17	2.7
	1.0	8.5	30	3.5	23	2.7
	1.5	12.2	40	3.3	29	2.4
600	0	5.0	14	2.8	12.5	2.5
	0.5	6.9	23	3.4	19	2.7
	1.0	9.6	34	3.5	25	2.6
	1.5	13.8	43	3.1	32	2.3

‡ LW Liveweight
From ARC (1980)
* Daily requirements in g; Dietary requirements in g/kg DM; values in parenthesis are the requirements of milk fed calves.

Table 4.3 Calcium and Phosphorus Requirements of Lactating Cows, Expressed as Dietary Concentrations

Breed	Milk Yield kg/day	Dry Matter* Intake	Requirements+ Ca		P	
			Daily	Dietary	Daily	Dietary
Friesian[1]	0	5.0	14	2.8	2.5	2.5
	10	9.4	31	3.3	28	3.0
	20	14.0	48	3.4	44	3.1
	30	18.8	64	3.4	59	3.1
Ayrshire[2]	0	4.4	12	2.7	10	2.3
	10	9.0	29	3.2	27	3.0
	20	13.7	46	3.4	43	3.1
	30	18.6	63	3.4	59	3.2
Jersey[3]	0	3.8	9.5	2.5	8	2.2
	10	9.0	30	3.3	29	3.2
	20	14.5	50	3.5	51	3.5

* kg/day, q=0.6
1 600kg LW; 36.8g fat/kg milk
2 500kg LW; 38.6g fat/kg milk
3 400kg LW; 29.0g fat/kg milk
\+ Daily requirements in g; dietary requirements in g/kg DM

(iii) Pregnant Cattle

Recommendations for pregnant cattle of three different breeds (Friesian, Ayrshire and Jersey) have been set (ARC, 1980) to meet requirements at different periods of pregnancy (Table 4.4). The dietary concentrations all fall in the range 2.8-3.3g/kg DM (at 60% metabolizability). Examination of the data in Appendix III showed that only 3% of the herbage samples met the ARC (1980) requirements for Ca (values > 2.8g kg^{-1} DM). This therefore shows that the herbage collected in this study was

largely deficient in Ca with respect to requirements of pregnant cattle.

Table 4.4 Calcium and Phosphorus Requirements for Pregnant Cattle, Expressed as Dietary Concentrations

Breed, etc	Weeks before Term	Dry matter* Intake, q=.6	Mineral requirements	
			Ca	P
Standard	12	5.1	3.0	2.5
40kg Calf	8	5.7	3.1	2.6
	4	6.6	3.2	2.6
	0	8.3	3.1	2.4
Friesian	12	5.8	3.1	2.6
42kg Calf	8	6.3	3.2	2.7
	4	7.3	3.3	2.6
	0	9.0	3.2	2.5
Ayrshire	12	5.0	2.9	2.5
32kg Calf	8	5.4	3.0	2.5
	4	6.2	3.1	2.5
	0	7.5	3.1	2.4
Jersey	12	4.2	2.8	2.3
24kg Calf	8	4.5	2.9	2.4
	4	5.1	2.9	2.4
	0	6.1	2.9	2.3

* kg/day

(iv) Sheep

Table 4.5 gives the dietary allowances of Ca for various categories of sheep contrasted with the mean and median concentrations found in the herbage.

Examination of data in Appendix III revealed that the herbage Ca concentrations with respect to sheep requirements were

grossly inadequate, with 18% of the samples giving very low values (< 1g/kg DM): 36% of the samples met the Ca requirements for pregnant ewes, but only 8% satisfied the requirements for growing or lactating sheep. These results, in general, indicate that the herbage sampled in this study was of low quality in relation to ovine requirements for Ca.

Table 4.5 Dietary Requirements for Calcium and Phosphorus for Sheep Contrasted with Herbage Concentrations+

Element	Recommended Allowance*			Herbage Composition		
	Growth	Pregnancy	Lactation	Mean	Median	Range
Calcium	2.9-4.5	1.6-3.0	2.8-3.0	1.48	1.44	.33-3.18
Phosphorus	2.1-3.8	2.4-3.3	3.9-4.4	1.35	1.32	.04-3.47

* From Suttle (1983b)
+ All values expressed in g/kg DM

(b) Phosphorus

The P requirements for most classes of ruminants are less than those for Ca so that a smaller proportion of the herbage samples are nominally deficient. Nevertheless, Tables 4.2 to 4.5, which present requirements for P as well as Ca, when compared with the P concentrations in herbage (Tables 4.1 and 4.5) show the majority of samples to be deficient for production (Fig. 4.1b) with some exceedingly low in P (4% had P levels < 0.15g/kg DM). The data in Appendix III show that only 14.1% of

the samples had P levels above 2.3g/kg DM, the level which is adequate to maintain cows but without any milk yield (Table 4.3); while only 13.3% (P levels > 2.3g/kg) met the minimum requirements for pregnant cattle. For growing beef cattle, the herbage concentrations were again extremely low in relation to requirements. The majority (90%) of the samples had levels which could not support any liveweight gains at all.

For sheep, only 17% of the herbage samples had P levels > 2.1g/kg DM, the minimum requirement for growing lambs (Table 4.5): 10% of the samples had levels which met the requirement for pregnant ewes but none had concentrations that satisfied the P requirements for lactating sheep.

Calcium:Phosphorus ratios

According to ARC (1980) standards, Ca:P ratios in ruminant diets should fall within the safe range of 1:1 to 2:1. The maximum ratio of 2:1 was chosen because it is roughly the ratio of their respective concentrations in bone (Chapter Two) and exaggerates the nutritional importance of the ratio. However, abnormal ratios may be of significance if the diet is low in Ca or P (ARC, 1980). Table 4.6 gives the frequency distribution of Ca:P ratios in relation to the range of herbage Ca and P concentrations from Appendix III.

Table 4.6 Frequency Distribution of Ca:P Ratios in Herbage Samples in Relation to Individual Concentration Ranges.

Ca:P Ratio	% of samples	Ca concentration (Range)	P concentration (Range)	Remarks*
0-1	39.2	0.41-2.74	0.68-3.47	1
1-2	34.8	0.46-3.18	0.41-2.30	2
2-3	11.9	1.11-3.03	0.41-1.16	3
3-5	7.4	0.33-2.39	0.10-0.64	
>5	6.7	0.74-1.96	0.04-0.29	4

*1 Except for two values, P concentrations were above 1g kg^{-1} DM.

2 Lower range P values were also associated with low Calcium values so that resultant ratios were usually > 1.

3 Only two samples in this class had P concentrations above 1g kg^{-1} DM. The rest of the concentrations fell below 1g kg^{-1} DM.

4 The highest ratio found was 18.5 and was associated with the lowest Ca and P levels in this class.

The summary results in Table 4.6 show that only 34.8% of the herbage had Ca:P ratios which fell in the range of 1-2 recommended by ARC (1980). The majority of the samples (39.3%) had very low ratios ranging from 0.2-1.1, while ratios > 2 were obtained in 25.9% of the herbages. These observations indicate a prospect of Ca/P antagonism exacerbating a P deficiency in livestock raised in the surveyed area because high ratios are often associated with low P concentrations. The low ratios

were, however, associated with relatively high concentrations of both elements.

(c) Magnesium

Concentrations of Mg in the herbage samples ranged from 0.57-4.74g/kg DM with a median of 1.57 and mean of 1.61 ± 0.530g/kg DM (Table 4.1). The distribution (Fig. 4.1c) showed that 6% of the samples had levels below 1g/kg DM while the majority (71%) had concentrations in the range of 1-2g/kg DM.

According to ARC (1980), standard Mg requirements vary according to the category of livestock being considered. For beef cattle gaining at different rates, dietary Mg concentrations of 1.0-2.1 g/kg DM are required. For pregnant cattle, the minimum standard requirements range from 1.4g/kg DM just before term to 2.0g/kg DM at twelve weeks pregnancy. For lactating cows the requirements vary only slightly with the type of breed but fall in the range 1.4-2.1g/kg DM.

From the data presented in Appendix III for the individual farms, it was observed that 94% of the samples met the minimum ARC (1980) requirements for beef cattle compared to 63.5% for pregnant cows, while 63.5% and 42% of the herbages satisfied the Mg requirements for lactating Jersey and Friesian/Ayrshire cows, respectively. These results suggest that Mg deficiency could be a problem in pregnant (on 36.5% of the sampled sites) and lactating cows (on 36.5% to 58% of the sites).

Suttle (1983b) suggested that grazing sheep should be allowed more Mg than the ARC (1980) recommended. He calculated that growing sheep should be fed diets containing Mg at minimum concentrations ranging from 1.2 to 2.1g/kg DM depending on growth rate, while the dietary allowances for pregnant and lactating ewes were set, depending on term of pregnancy or milk yield, at 1.5-2.0 and 2.1-2.5g/kg DM, respectively. According to data in Appendix III, samples with Mg levels that met the minimum allowances (Suttle, 1983b) for growing ewes (ie values > 1.2g/kg DM) constituted 80% of the batch; 54% of the samples satisfied the requirements (values > 1.5g/kg DM) for pregnant ewes, while only 19% of the samples met the dietary standards for lactating ewes (ie concentrations > 2.1g/kg DM). These results point to prospect of Mg deficiency problems for grazing sheep, especially lactating and pregnant ewes in the survey area.

(d) Sulphur

The ARC (1980) recommended requirement for dietary S for ruminants is a constant 1.0g S kg^{-1} DM based on the requirements of the rumen microbes for protein synthesis. Although a mean value of 1.46g kg^{-1} DM was found in the herbage samples, there was extreme variability in the data for the individual farms (Table 4.1 and Appendix III), with some very low concentrations recorded. A quarter of the samples had S concentrations below the minimum (ARC, 1980) requirement (Fig. 4.1d); this suggests

prospects of S deficiency in livestock fed such herbages as the principle constituent of the diet. The situation could be worse for sheep, whose requirements may be higher because they recycle S less efficiently than cattle (Kennedy and Siebert, 1973; Kennedy et al 1975) and have high S requirements for wool growth.

4.2.2. Aluminium and Silicon

Table 4.7 gives a summary of the data presented in Appendix III for aluminium and silicon concentrations.

Concentrations of Al in the herbage were extremely variable as shown by the large standard deviation compared to the mean. The data for the individual farms showed that 10% of the samples had levels < 100mg/kg DM, 23% had concentrations between 100 and 200mg kg^{-1} DM while 22% had Al contents between 200 and 300mg/kg^{-1} DM. A small percentage (1.5) of the samples had concentrations above 1g kg^{-1} DM (Fig. 4.2a).

The distribution of data in Appendix III showed that nearly 90% of the herbage samples had silica levels < 5% in DM, with the majority between 1-3% in DM (Fig. 4.2b). These concentrations fall within the common ranges quoted for grasses, ie 3-4% silica DM with values as high as 6-8% in certain species (Dougall and Bogdan, 1958; Jones and Handreck, 1965; Underwood, 1977).

For both elements some high levels may be partially attributable to soil contamination.

Table 4.7 Aluminium and Silicon Concentrations

Parameter	Al* (gkg^{-1} DM)	Si+ (as % silica equivalent)
Mean	0.30	3.34
Standard Deviation	0.331	1.230
Range	0.04-0.76	0.26-6.59
Median	0.26	3.25

* Values trimmed of one outlier - 3g/kg DM
+ Silicon concentrations were converted to % silica equivalent in the DM for ease of comparison with published literature values.

4.2.3. Trace Element Composition of Herbage in Relation to Animal Requirements and Tolerances

Table 4.8 summarizes the trace element composition of the herbages from individual farms (Appendix III) in relation to the recommended dietary allowances for cattle (ARC, 1980) and sheep (Suttle, 1983b).

(a) Copper (and Molybdenum)

Although Table 4.8 shows a mean Cu level of 4.0 (± 1.46) mg kg^{-1} DM there were wide variations in Cu concentrations across the sites as shown by the range (1.8 - 9.4mg kg^{-1} DM). Data in Appendix III show that nearly all the samples (98.4%) had Cu levels below a minimum dietary allowance of 8mg kg^{-1} DM for growing and lactating cattle and sheep. Copper concentrations

in a few (3%) samples were below 2mg kg^{-1} DM, while the majority of the herbage (58%) had concentrations between 2 and .4mg kg^{-1} DM (Fig. 4.3).

TABLE 4.8. Trace Element Composition[+] of Herbage Contrasted with Standard Requirements for Cattle[a] and Sheep[b]

	Herbage Composition			Recommended Dietary Allowances	
	Mean	Median	Range	Cattle	Sheep
Cobalt	0.22	0.21	0.03-0.96	0.08-0.11	0.08-0.11
Copper	4.0	3.7	1.8 -9.4	8-20	8-11 (Mo < 1) 14-23 (Mo > 3)
Iron	0.30	0.27	0.03-2.7	0.03	0.03
Manganese	0.22	0.21	0.05-0.59	0.025	0.025
Molybdenum	1.1	0.88	0.13-4.21	-	-
Selenium	97	65.5	9-621	50	50
Zinc	23.6	22	5-87	12-35	8-21

[a] From ARC (1980)

[b] From Suttle (1983b)

[+] Cobalt, Cu, Mo and Zn in mg/kg DM; Se in ug/kg DM; Fe and Mn in g/kg DM.

The failure of the majority of the samples to meet the minimum Cu requirements given in Table 4.8 suggests that there may be widespread potential of simple Cu deficiency in grazing cattle and sheep where herbages provide the principle component of the diet.

The situation could be further complicated by the high levels of Mo found in some samples. Data for the individual farms (Appendix III) showed that 15% had concentrations > 2mg kg^{-1} DM, the level at which Cu absorption begins to be inhibited (Suttle, 1987b). The highest Mo level was 4.21mg kg^{-1} DM with a corresponding Cu concentration of 3.55mg/kg DM.

From earlier field studies on the effect of Mo upon beef cattle (Miltimore et al, 1964), Miltimore and Mason (1971) suggested a minimum Cu:Mo ratio of 2:1 as being safe for cattle. The calculated ratios for data in Appendix III were extremely variable, ranging from 0.84 to 23 with a median of 4.4 (Table 4.9). Although the ratios were obviously dependent on the Cu content of each sample, they were influenced more by the variation in Mo concentrations.

(b) Zinc

The Zn concentrations (range 5-87mg kg^{-1} DM) varied considerably between the sites. However, examination of the data in Appendix III revealed that nearly 96% of the samples (Fig. 4.4) met the minimum recommended allowance (Table 4.8) of 8mg kg^{-1} DM for growing sheep, while 90% satisfied the minimum requirements for growing cattle and pregnant sheep (12mg kg^{-1} DM). For pregnant cattle, 87% of the samples had concentrations that met the requirements while 67% and 84% met the minimum dietary allowances for lactating cattle and sheep respectively. No samples had Zn concentrations above 150mg kg^{-1} DM and

therefore Zn toxicity should not be a problem in the sampled area. In general the results show that relatively few sites would require attention with respect to Zn nutrition for satisfactory production to be realized.

Table 4.9 Class Distribution Frequencies of the Herbage Cu:Mo Ratios

	Herbage Concentrations mg/kg DM			
Cu:Mo ratio	Mo	Cu	% of samples	Remarks*
< 1	4.21	3.55	1.0	1
1-2	0.94-3.15	1.82-4.93	16.7	2
2-4	0.49-2.62	1.79-8.83	27.8	3
4-6	0.50-1.55	2.14-7.14	20.6	
6-10	0.23-0.76	2.21-5.65	15.9	
10-15	0.20-0.83	2.43-9.43	11	
15-25	0.13-0.35	2.5 -7.05	7	

*1. The lowest Cu:Mo ratio was associated with the highest molybdenum concentration.

2. Cu:Mo ratios falling below the safe limit of 2:1 were found in nearly 17% of the samples. These low ratios were associated with low herbage copper levels in the range 1.82-4.93mg/kg DM.

3. Only three molybdenum values were above 2mg kg^{-1} DM and were associated with highest Cu levels.

(c) Selenium

Examination of the data in Appendix III revealed that 22% of the samples had concentrations below the minimum dietary requirement of 50ug kg^{-1} DM (Table 4.8) for both cattle and sheep (Fig. 4.4). Half of the samples had marginal levels (30-50ug kg^{-1} DM) at which Se deficiency symptoms may be determined by the level of dietary vitamin E. This therefore means that livestock on these sites could well be at risk from primary or secondary Se deficiency. Half of the samples had Se concentrations between 50 and 100ug kg^{-1} DM. Although a few samples (2%) exceeded 400ug kg^{-1} DM, the levels were far below the maximum tolerable level of 3000ug kg^{-1} DM (ARC, 1980) beyond which Se toxicity becomes evident.

(d) Cobalt

Concentrations of Co in the herbage varied across the sites, from 0.03mg-0.96mg/kg DM but the mean level indicated no great risk of deficiency. Data for the individual farms presented in Appendix III shows that only 6% of the samples had Co concentrations below the minimum ARC (1980) requirement of 0.08-0.11mg kg^{-1} DM: 69% of the samples had concentrations between 0.1 and 0.3mg kg^{-1} DM and 1% had levels above 0.4mg kg^{-1} DM (Fig. 4.4).

(e) Iron and Manganese

Table 4.8 shows that the mean herbage Fe level of 0.322g kg^{-1} DM was high in relation to the animal requirement of 0.03g kg^{-1} DM for cattle and sheep (ARC, 1980) and 98.5% of the samples analyzed (Appendix III) had Fe concentrations above the requirement (Fig. 4.5). There may however be problems of Fe excess: 13 samples had levels > 0.5g kg^{-1} DM, the concentration regarded as the maximum tolerable by ruminants (ARC, 1980).

The minimum dietary requirement for Mn was set by ARC (1980) to be 0.025g kg^{-1} DM. According to the summary results presented in Table 4.8, all the samples analyzed met this requirement (Fig. 4.5). Examination of the data given in Appendix III revealed that only 7% of the samples had Mn levels < 0.1g kg^{-1} DM; 41% had Mn levels between 0.1 and 0.2g kg^{-1} DM, while 49% had concentrations > 0.2 but < 0.4g kg^{-1} DM.

4.3 DISCUSSION

4.3.1 Calcium

The herbage Ca concentrations found in this survey were much lower than those reported for forages in temperate zones, eg 5.9 (range 3-10) g/kg DM (Adams,1974) and 4.0 (2-7) g/kg DM (L'Estrange and Axford, 1964), 5.4-6.7g/kg DM (Whitehead et al, 1978) in the UK; and 5.1 (4.2-5.7) g/kg DM in US pastures (Stillings et al, 1964); and 4.9 $\pm$ 1.3g/kg DM in tropical forages of Bolivia (McDowell et al, 1982).

The assessment of the levels of Ca in the sampled Kenyan herbages has indicated prospects of Ca deficiency disorders in cattle and sheep raised in the area. However, typical symptoms attributed to Ca deficiency may not develop because animals can tolerate low dietary concentrations. Evidence from research experiments suggests that animals adapt to low Ca diets by increasing the capacity for intestinal absorption of Ca (Dowdle et al, 1960; Kimberg et al, 1961), a phenomenon which is considered to be related to the degree of depletion of the skeleton (Nicolayson et al, 1953). More recently, Braithwaite et al (1969) and Sykes and Dingwall (1975) have shown by kinetic studies and comparative slaughter techniques, respectively, that sheep can increase the rate of intestinal absorption of Ca during lactation to offset severe skeletal depletion during pregnancy. Thus, there is a wide margin between the low dietary concentrations shown to cause clinical abnormalities by experiment and the dietary requirements which various authorities (eg ARC, 1980) recommend as the basis for ration formulation. The recommended allowances contain a margin of safety and animals apparently have their own buffering mechanisms which enable them to meet temporary shortfalls. However, the fact that the herbages collected in this study often had far less than the recommended allowances of Ca suggests that signs of ill-health may occur if there are no no opportunities for recovery, a situation more likely to occur in cattle than in sheep.

4.3.2 Phosphorus

The herbage P levels were extremely low in relation to cattle and sheep requirements; this strengthens the views of McDowell (1976) and McDowell et al (1984) that the most prevalent mineral deficiency in the world is that of P, with at least 38 tropical countries reporting such a problem (McDowell, 1976). Table 4.1 shows values as low as 0.04g/kg, which are far lower than concentrations of 0.29g/kg DM reported for South African pastures (Bisschop, 1964, cited by Read et al, 1986) and the extremely variable levels of 2.0 ± 2.3g/kg DM in forages of the Bolivian tropics (McDowell et al, 1982); yet none of the farmers reported ill-health among farm animals in the area.

The failure to observe symptoms caused by P deficiency may be related to the capacity of certain tissues of grazing animals to accumulate nutrients in excess of the requirement for growth during periods of generous dietary supply, and mobilize them during periods of shortage. In both cattle and sheep, about 80% of the labile P pool is in the skeleton (Little, 1980; Grace, 1983; Ternouth, 1989) and the bone responds to demands for P rather than variations in bone stress including variations in liveweight (Read et al, 1986). This enables the grazing animal to cope with considerable fluctuations in the growth and composition of herbage. When fed deficient diets, cattle and sheep have been observed to lose as much as 30% (Little, 1984) and 40% (Benzie et al, 1959) respectively of their bone

minerals so that their dietary requirements may remain unmet for considerable periods of time (Braithwaite, 1983a; 1983b; Read et al, 1986; Suttle, 1987b).

Skeletal reserves of P provide the most striking example of the impact of body stores in well-mineralised bone. Spence et al (1985) found that the ash content of bone increases substantially in lambs on upland pastures of Scotland between birth and 15 months of age. It follows that the ability to tolerate a deficiency will increase with age and in the adult hill ewe, 25% of the complete reserve of P in bone may be removed during a deficiency period (Field et al, 1968): thus the young growing animals are more vulnerable. Studies with pregnant sheep (Little et al, 1978) and cattle (Call et al, 1978; 1986) have revealed a tolerance of diets which are low in P by all but the lowest standard (ARC, 1980). This tolerance of 'inadequate' diets almost certainly reflects the sample size of the skeletal reserve in relation to demands for maintenance and foetal growth (Suttle, 1987b). The situation with young, rapidly growing animals is however very different; hypophosphataemia is readily induced by feeding diets deficient in P, and inorganic plasma P can readily reflect the dietary supply of P (Suttle, 1987b).

The physical form of diets fed to ruminants has also been shown to affect P absorbability and endogenous loss. Ternouth (1989) observed a positive relationship between the amount of P absorbed and lost and the amount of P ingested, and concluded

that requirements increased with dry matter intake, and when the diet consisted of coarse, long roughage. The increased demand for P may also be aggravated by variability in herbage availablity (Field et al, 1983) due to either the P remaining associated with some fraction of the diet and not being released in the gastro-intestinal tract or because the P becomes sequestered with molecules or soil particles as it passes down the tract. Such molecules include complexes of Ca, Al and Fe (Rosa et al, 1982; Valdivia et al, 1982). Braithwaite (1985) has provided some evidence that endogenous faecal P is increased as dietary P absorption is decreased when P is sequestered within the gastro-intestinal tract. These findings indicate that dietary composition of the interfering elements Ca, Al and Fe needs to be controlled for efficient P utilization, especially in situations of dietary deficiency of the mineral.

4.3.3 Calcium:Phosphorus (Ca:P) Ratios

Calcium and P are important nutrients in practical animal diets, and there is accumulating evidence that if the supply of one is only marginally adequate, increases in the amount of the other may precipitate a deficiency of the marginal mineral by reducing its absorption (Wise et al, 1963; Young et al, 1966; Field et al, 1975). Wise et al (1963) observed depressed growth rates in young ruminants fed diets with extreme Ca:P ratios (ie ratios < 1:1 and 7:1) and suggested that ruminant animals are more likely to handle higher levels of dietary Ca

than non-ruminants. More recently, Field et al (1983) studied the effect of different Ca:P ratios in the diet (varying between 0.6-3.6) and found that Ca at 3.4-5.4g/kg DM reduced the efficiency of P absorption in sheep by 18%. The interference in P availability to ruminants indicates that Ca:P ratios are more critical in diets deficient in P. In the present investigation, detrimental effects among livestock are likely to be prevalent on 40% of the sites (Table 4.6) due to interference by Ca in P absorption exacerbating the effects of the very low levels of P found in the herbage.

4.3.4 Sulphur

Examples of literature values for herbage S show wide variations in different regions of the world. Beck (1961) found concentrations ranging from 0.9-6.2 (mean 3.1) g/kg DM in Cu-deficient areas compared with 1.0-9.0 (mean 2.2) g/kg DM in pastures from areas where Cu deficiency signs had not been observed in Western Australia. Allcroft and Lewis (1956) gave a range of 2.9-9.6g/kg DM for pastures from swayback and non-swayback areas in England. In tropical Africa, Mahmoud et al (1983) recorded herbage S levels in the range 3-6g/kg DM in the Sudan. The concentrations of S obtained in the present investigation (0.9-3.1g/kg DM) show that the herbages sampled for the study were very low in S when compared with the levels found in the examples previously cited. The extent to which S deficiency per se restricts production, will, however, depend

on the adequacy of energy and nitrogen supply for microbial protein synthesis: these may be the primary factors limiting production.

4.3.5 Magnesium

The Mg concentrations obtained in the present study were relatively low in relation to requirements for ruminant nutrition and production, and prospects of Mg deficiency are high on some sites. The levels were lower than those reported by Tringle and Elliot (1975) who found a range of 1.5-2.8g/kg DM in grasses growing in soils of pH 5.5-5.8 - which is the typical range covered in the present survey. Arroyo-Aguilu and Coward-Lord (1975) found wider variations in tropical grasses (1.5-4.3g/kg DM), while Hill and Guss (1976) reported Mg concentrations ranging from 0.9-2.7g/kg DM in different forage species. Hypomagnesaemic tetany is commonly found in temperate pastures with Mg concentrations of 1-2g/kg DM (Kemp and Hart, 1957; Kemp et al, 1961; Butler and Jones, 1973). The importance of species variations will be discussed in the next chapter.

4.3.6 Aluminium

The majority of the samples (83%) had Al concentrations < 500mg kg^{-1} DM and only 5.2% had levels above those shown by Allen (1984) to cause problems in animal metabolism. Furthermore, herbage Al might be less potent than the inorganic

salts of Al that were added to synthetic diets for studies on the toxicity of Al to ruminants (Allen, 1984; Wilkinson et al, 1982). The higher herbage Al values may be partially due to soil contamination since soils are much richer in Al than pasture.

In comparison with the data obtained in the present study, the herbage levels found by Dennis (1971), Wilkinson and Studeman (1979), Allen and Robinson (1980), and Wilkinson et al, (1982) during outbreaks of hypomagnesaemic tetany are far above the levels found in the samples from the individual farms (Appendix III). A role of Al in inducing Mg deficiency in grazing ruminants in the sampled areas may therefore be considered as unlikely.

4.3.7 Silicon

The high silica concentrations in a few of the samples could influence forage digestibility, urolithiasis and tooth wear. Of these the first may be the most important under Kenyan conditions.

The digestibility of forage dry matter *in vivo* has been shown to be significantly depressed by increasing levels of silica. Smith et al (1971) found that organic matter digestibility (OMD) was related to forage content of chemical constituents (N, ash, ether extracts, cell-wall constituents, acid-detergent fiber, acid-detergent lignin and crude silica) in multiple linear regression equations. By further stepwise regression

they obtained relationships which indicated that silica decreases the digestibility of organic matter by approximately 1% for each % increase in the silica content of the forage. Van Soest and Jones (1968) reported that forage silica contents above 5% DM decreased forage OMD. Using grasses such as reed canary grass (5.4% silica) and coastal Bermuda grass (5.2% silica) they found a larger decline of 3 units of digestibility per unit of silica on average. The Si effect was modified by adding a mixture of Mg, Mn, Zn, Co and Cu to the diet suggesting that availability of minerals to sustain cellulolytic microbial activity may influence the effect of soluble silica on forage digestion by ruminants (Underwood, 1977). The difference between the results of Van Soest and Jones (1968) and Smith et al (1971) may therefore reflect differences in minerals other than Si.

Of the samples analyzed in the present survey only 11% had silica concentrations between 5 and 7% DM; this suggests few problems due to a possible reduction in digestibility and hence, availability of nutrients (including minerals) in the herbages. Furthermore, some of the elements found by Van Soest and Jones (1968) to counter the effect of Si on digestibility were present at high concentrations (eg Zn, Co, Mn).

Toothwear is principally a problem for animals grazing temperate pastures eg in New Zealand. Since sheep at least can lose their incisor teeth without losing their ability to consume forage (Field and Purves, 1964) toothwear may not have proportional effects on production.

The factors responsible for the formation of siliceous calculi include low water intake but attempts to induce them in sheep and cattle by adding silicates to the diets or by restricting water consumption have not been successful (Underwood, 1977). The period of low water consumption in Kenya is mainly in the dry, hot months of January to March and this may be the time of greatest risk. However, there is no record of clinical disorders associated with high silica intakes in the region.

4.3.8 Copper and Molybdenum

Molybdenum has long been established as an antagonist to Cu absorption and metabolism through the possible formation of insoluble Cu complexes in the gut (Dick et al, 1975; Suttle, 1975). Research in several parts of the world where Cu/Mo nutrition problems have been detected in grazing animals has established that the concentrations of the two elements in herbage can be quite variable. Table 4.10 gives typical ranges reported for some of the case studies contrasted with the results obtained in this survey: it can be seen that the mean Cu values obtained in the present study are within the ranges quoted for the forages in the problem areas. Many herbages do not meet the Cu requirements of cattle before any antagonisms are taken into account. The quantity of supplementary Cu necessary to maintain a satisfactory Cu status of these animals must also be modified according to the Mo content of forages and

Table 4.10. Copper and Molybdenum Concentrations (mg/kg DM) of Forages in Some Areas Affected by Copper Deficiency Problems Contrasted with Levels Found in this Study and Standard Requirements for Sheep and Cattle

Case Study in Affected Area	Forage Composition (Range)		Reference
	Copper	Molybdenum	
1 Present investigation	4.0(1.8-9.4)	1.1(0.1-4.2)	See App. III & Table 4.8
2 Clinically silent hypocuprosis in Australian cattle	4.3(2.4-6.7)	5.7(2.1-9.2)	Bingley & Anderson (1972)
3 Copper status of grazing cattle with elevated iron intake in New Zealand	10.7 ± 0.32	1.5 ± 0.12	Campbell et al (1974)
4 Geographical variations in forage copper and molybdenum in Canada	1.4-34.3 (majority 3-6)	1-42 (majority < 3)	Boila et al (1984a)
5 Composition of grass forage in Manitoba, Canada	5.4(1.8-12.4)	0.6(0.2-42)	Drysdale et al (1980)
6 Severity of hypocupraemia in beef cattle in Canada	6.8-10.1	1.7-5.1	Boila et al (1984b)
7 Incidence of bovine hypocuprosis in England and Wales	6.8(5-17.5)	2.3(0.8-7.2)	Thomson et al (1972)
8 Molybdenum toxicity in grazing animals in Oregon, USA	2.8-9.9	2-15	Kubota et al (1967)
9 Copper supplementation for grazing cattle	5.1(1.9-9.4)	1.61(0.3-4.0)	Boila et al (1984c)
10 Recommended allowances for sheep	8-11	Mo < 1	Suttle (1983)
	14-23	Mo > 3	
cattle	8-20		ARC (1980)

high dietary S, both of which have adverse effects on Cu availability (Suttle, 1974: 1983b). The effects of the triple interaction between Cu x Mo x S will be discussed in full in the next chapter but in the meantime it is noteworthy that 17.5% of the herbage samples had Cu:Mo ratios below those considered by some to present risks of induced Cu deficiency (ie < 2:1, Miltimore and Mason, 1971; Bingley and Anderson, 1972).

Unlike P and Ca which are deposited in the bone during periods of high dietary availability, the liver is the main pool for Cu deposition and storage in the grazing ruminant. Animals exposed to low dietary Cu intakes may therefore not show any clinical abnormalities for some time since such temporary shortfalls are met by depletion of Cu reserves in the liver. This may help explain the widespread failure to observe clinical symptoms of Cu deficiency in the survey area despite the extremely low levels of Cu found in the herbages which constitute the major component of the animal diets. Depletion occurs when the amount of Cu absorbed each day is less than that required for the growth of new tissue, the secretion of milk and the replacement of Cu excreted endogenously (predominantly via the faeces). Prolonged periods of depletion may terminate in chronic hypocuprosis (Suttle, 1987b) but only after the gradual development of low plasma Cu concentrations (hypocupraemia).

4.3.9 Zinc

Zinc concentrations in pasture herbage show wide variations in many parts of the world. Cathcart et al (1980) and Redshaw et al (1978) reported Zn concentrations ranging from 31-133mg/kg DM for pastures, 7-138mg/kg DM for grass hays, and 10-80 for lucerne hays in Canada. Grace (1972; 1973) recorded values in the range 17-70mg/kg DM for improved pastures in New Zealand, while McDowell et al (1982) reported concentrations of 25 ± 13mg/kg DM in forages of tropical Bolivia. The range of concentrations (5-87mg/kg DM) obtained in this study is much wider than in the above examples. The lower values are of particular interest in view of the conflict between the ARC (1980) recommended dietary standards for ruminants (8-35mg/kg DM) and the data of Mahmoud et al (1983) who reported Zn deficiency in sheep grazing Sudanese pastures which contained 16-27mg/kg DM, and Legg and Sears (1960) who recorded a parakeratosis skin disorder in cattle grazing forages containing 18-42mg/kg DM in Guyana.

Zinc deficiency is relatively uncommon in grazing ruminants but has been reported in grazing animals in tropical South America (McDowell et al, 1983; 1984). Deficiencies are difficult to diagnose and may be manifested by depressed intake, retarded growth and reproductive disorders, loss of hair and wool, the development of scaly skin which cracks readily, and delayed healing of wounds (Underwood, 1981). However, there is no obvious relationship between herbage concentrations and

Zn responsive conditions or onset of clinical abnormalities. Mills et al (1967) and Langlands (1987) consider that the Zn content of forage is not a reliable means of identifying responsive situations. Although sources of variation in Zn availability in the non-ruminant diet are well known, there is little corresponding information for ruminants. Another possible explanation for poor correlations between clinical deficiency and dietary Zn intake is that Zn released by tissue catabolism (associated for example with insufficient intakes of energy and protein) in animals is available to supplement dietary Zn (Masters, 1984). Such interactions highlight one of the problems in assessing the nutrient status from plant and animal tissue analysis.

4.3.10 Selenium

The large number of sites with low Se concentrations in herbage is consistent with the general observation that Se insufficiencies are common in pastures on acid soils in high rainfall regions (Langlands et al, 1981a; 1981b; Langlands, 1987). The low Se values obtained in this study compare with those of Wilkinson (1960) for New Zealand pastures (24-33ug/kg DM) and Gardiner et al (1962) for Australian grasses (10-80ug/kg DM). The low results could be attributed to the low soil pH, the poor depth of root system of the grasses. Alternatively, the high Fe content of the soils which could have bound soil Se informs unavailable to the plants, (Gardiner et al, 1962).

It is unlikely that high available S levels in the soil contributed to the low Se status. Although Se and S are closely related (competitive) elements and high soil levels of the latter may reduce plant uptake of Se (eg Hurd-Karrer, 1936) the herbages were generally low in S suggesting a soil S deficit. The danger of exacerbating the Se deficiency in correcting a S deficiency by fertilization should, however, be borne in mind. Ravikovitch and Margolin (1959) showed that the addition of gypsum at a rate of 2.3 tons per acre to seleniferous soils containing 0.5mg/kg Se reduced uptake of the element by lucerne by 60-70%. Although this rate of application is high, the results are of importance. The use of single superphosphates (which contain Ca sulphate) in Kenya is frequent and a build up of soil S under acid conditions may have contributed to the low herbage Se by antagonising absorption by the plant thereby increasing the risk of Se deficiency in some instances.

4.3.11 Cobalt

Although there is a possibility of over-estimation of herbage Co due to unavoidable trace contamination from soil or dust particles (Mitchell, 1960), ingested soil cobalt is partly available to ruminants (McDonald and Suttle, 1986; Brebner, 1988). It is suggested that the number of sites where risks of Co deficiency exist may not greatly exceed the 11% indicated by pasture analysis (Fig. 4.4).

4.3.12 Iron and Manganese

The principal problems with these two elements are those excess intakes. According to Cunningham et al (1966), depressed feed intake, rate of gain and blood haemoblogin concentration were observed in calves with a high intake of Mn and diets containing > 2g Mn kg^{-1} DM were considered suspect. Grace (1973) found that the rate of growth of young sheep was reduced by daily intakes of 400-500mg Mn. Few of the sampled pasture would provide more Mn than this when ingested at 1kg per day by sheep.

Evidence of an antagonistic interaction between Mn and Fe was obtained in studies of the Mn tolerance of lambs (Hartman et al 1955) in which a higher level of 1g Mn kg^{-1} DM greatly reduced serum Fe and prevented haemoglobin regeneration in anaemic lambs. Matrone et al (1959) estimated that as little as 0.04g Mn kg^{-1} diet can have adverse effects on Fe utilization in lambs with low tissue reserves of Fe. The high Fe concentrations in the sampled pastures may offset any effects of high Mn. Disagreement on dietary Mn concentrations that should be regarded as the maximum acceptable may be due to the Fe x Mn antagonism. If 1g Mn kg^{-1} diet DM is regarded as the threshold (Hartman et al, 1955), then all the samples analyzed in this survey may be considered safe from Mn toxicity.

The dangers of excess Fe may lie in interference in the absoption and utilization of Cu and exacerbated by elevated Fe intakes through soil ingestion either directly or from contaminated herbage (Wynne and McClymont, 1955; Campbell et al,

1974; Thornton, 1974; Suttle et al, 1975; Bremner et al, 1983; Humphries et al, 1983).

4.4 CONCLUSION

The assessment of the herbage analysis results in Appendix III has revealed potential for severe nutrition problems especially with regards to Ca, P, S and Cu deficiencies in the survey area. Further investigations into the principle causes of variation were therefore made and the next chapters underline the importance of species differences in mineral uptake and retention or accumulation (Chaper Five), geology and other variables (Chapter Six) as factors which influence the deficiency risk.

CHAPTER FIVE

INFLUENCE OF HERBAGE SPECIES ON MINERAL COMPOSITION

5.1 INTRODUCTION

The previous chapter showed that a substantial proportion of the grass samples taken from the two regions were deficient in a large number of elements. There was considerable variation in the botanical composition of the samples taken (Appendix III), and since there are species differences in macro and trace element composition (Chapter One) it was decided to examine the effect of herbage species on the prevalence of deficient, sufficient or potentially antagonistic mineral concentrations.

5.2 MATERIALS AND METHODS

Fourteen pasture species were collected in this study. They were sampled from the 135 different sites in the area under survey, as described in Appendix I. The dominant (pure stand) species were Kikuyu grass, Napier grass, Nandi setaria, Rhodes grass, and Rhodes/N. setaria mixture: these constituted 85% of the total while the rest of the samples comprised natural grass mixtures (11%) and assorted single species (4%) which included the legumes lucerne and sweet potato vines (see Tables 3.1 and 5.1). Appendix III gives the mineral composition of the individual species samples while Appendix IV relates the data to the corresponding soil chemical analysis results. The concentrations were subjected to statistical unbalanced data analysis (Patterson and Thomson, 1971; 1975; Harville, 1977; Robinson (1987) as described in Section 3.9.

5.3 RESULTS

Table 5.1 gives the derived means of the elemental concentrations in the different species. Table 5.2 gives the extractable metal concentrations in soils associated with each species and provides a check against bias in species effects arising from association with extreme soils.

Concentrations of the elements in the various species varied greatly. Numerous significant differences were detected (Table 5.3), but they were generally not influenced by the variations in soil composition at the respective sites (Table 5.4).

5.3.1 The Macro Elements

Calcium

Four-fold variations in Ca content between species, from 0.97g/kg DM in N. setaria to 3.76g/kg DM in lucerne were observed (Table 5.1). The summary given in Table 5.3 shows that concentrations in Nandi setaria were lower than in Kikuyu and native grasses ($P < .10$), Napier grass and Rhodes grass ($P < .001$), while Rhodes grass had higher levels than Rhodes/N. setaria mixture ($P < .05$).

Examination of the soil data (Table 5.2) showed that extractable Ca levels varied from 1.32me% on the natural grass sites to 1.80 and 2.21me% on the Rhodes/setaria and Kikuyu grass sites respectively. This narrow range provided no statistical differences between available Ca at the various species sites

TABLE 5.1. Species' Concentrations of the Elements (Derived Means)[a]

Element	Species Concentration[b]										
	NP	S	R	K	RS	NG	L	SD	SV	ST	SP
Aluminium	0.12	0.31	0.22	0.55	0.27	0.37	0.45	0.09	0.55	0.24	0.26
Calcium	1.24	0.97	1.69	1.37	1.37	1.38	3.76	2.77	3.10	2.16	1.74
Cobalt	0.16	0.18	0.17	0.32	0.20	0.25	0.20	0.11	0.81	0.20	0.16
Copper	4.27	3.74	3.50	5.80	3.70	4.64	9.70	5.17	8.51	3.67	2.64
Iron	0.16	0.26	0.27	0.38	0.28	0.35	0.34	0.17	0.43	0.37	0.24
Magnesium	1.68	1.38	1.50	2.11	1.57	1.86	2.01	1.71	5.53	1.48	0.94
Manganese	0.16	0.26	0.21	0.14	0.25	0.17	0.09	0.34	0.22	0.19	0.26
Molybenum	0.96	0.87	0.76	1.69	1.01	1.24	0.48	0.89	0.85	1.49	0.54
Phosphorus	1.19	1.04	1.33	1.71	1.42	1.35	2.70	2.75	1.13	1.96	1.19
Sulphur	1.10	1.32	2.01	1.75	1.47	1.51	2.10	1.78	2.57	2.24	1.51
Selenium	57	43	67	62	65	57	505	327	28	87	46
Silicon	2.77	2.21	3.18	3.23	3.89	3.27	0.28	5.02	0.25	3.55	3.56
Zinc	23	20	19	39	29	23	31	18	11	20	31

[a] Units on dry matter basis:
Al, Fe, Mg, Ca, P, in g/kg
Co, Cu, Mo, Zn in mg/kg
Se in ug/kg; Si as % silica

[b] Species Abbreviations (No. of samples):
NP Napier Grass (39)
S Nandi setaria (9)
R Rhodes grass (45)
K Kikuyu grass (9)
RS Rhodes + N. Setaria (3)
NG Natural grass (15)
L Lucerne (1)
SD Sudan grass (1)
SV Sweet potato vines (1)
ST Star grass (1)
SP Sporobolus (1)

TABLE 5.2. Mineral Composition of Soils at Species' Sites 8 (Derived Means)

Element	Species Site and Concentration[a]										
	NP	S	R	K	RS	NG	L	SD	SV	ST	SP
Al	0.95	0.99	1.05	0.95	0.93	1.03	1.05	1.02	0.80	0.72	1.14
Ca	1.72	1.52	1.76	2.21	1.80	1.32	1.42	1.66	1.56	1.75	1.32
Co	0.93	1.00	0.97	0.96	0.96	1.01	1.24	1.11	1.04	0.82	0.94
Cu	1.72	1.35	1.62	2.04	1.56	1.68	1.82	1.71	1.84	0.46	1.92
Fe	0.39	0.36	0.35	0.34	0.39	0.37	0.49	0.35	0.35	0.39	0.34
Mn	1.42	1.67	1.75	1.74	1.24	1.51	2.23	1.28	1.40	0.84	1.18
Mo	1.32	1.37	1.31	1.48	1.29	1.30	1.13	1.21	1.58	1.58	1.61
P	21.5	27	22.5	27.7	21.1	18.1	24	24	10	27.7	22.4
Se	221	241	230	271	299	192	376	209	135	225	206
Zn	2.68	2.65	2.49	2.70	2.59	2.35	2.89	2.09	2.26	2.5	2.30

[a] Al and Fe in g/kg; Co, Cu, Mo, P, Zn in mg/kg; Se in ug/kg; Ca and Mn in me%.

TABLE 5.3 Statistical Differences between Elemental Concentrations in Different Pasture Species or Mixtures[a]

Element	Level of Significance*			
	P < .10	P < .05	P < .01	P < .001
Aluminium	L : NP	K : S	S : NP	K : NP
	SV : SD	SV : NP	R : NP	NG : NP
		NG : R	RS : NP	
		K : SD	K : R	
		RS : SD		
Calcium	K : S	SD : NP	L : NP	NP : S
	NG : S	SV : NP	L : S	R : S
		SD : S	SV : S	
		R : RS		
		L : RS		
		L : NG		
Cobalt	K : RS	NG : NP	K : NP	
	SV : ST	K : S	SV : NP	
		SV : RS	K : R	
		SV : NG	SV : SD	
		SV : SP		
Copper	SP : SD	SV : NP	NP : R	
		NP : SP	K : NP	
		L : ST	L : NP	
			L : S	
			L : R	
			K : SP	
			L : SP	
			SV : SP	
Iron		S : NP	K : NP	R : NP
		RS : NP	NG : NP	
Magnesium	NP : S	NP : SP	R : S	SV : NP
	K : NP		K : SP	SV : S
	NG : S		SV : ST	SV : SP
	L : SP		SV : NG	SV : RS

Table 5.3 (continued)

Element	Level of significance[b]			
	P < 0.10	P < .05	P < .01	P < .001
Manganese	SD : NP	S : L S : NG SD : L SP : L RS : L	S : NP R : NP S : K	RS : NP
Molybdenum	K : SP	K : NP	K : R	
Phosphorus		K : NG		
Sulphur	L : NP SV : RS	SV : NP ST : NP RS : NP NG : NP SV : S	K : NP	R : NP R : S
Selenium	RS : S	R : S L : ST	L : S L : SV L : SP L : RS L : NG	
Zinc	NP : R	K : NP K : SV K : NG	K : S RS : S	
Silicon	For silica levels, concentrations in SD > S with difference significant at P < .05; while those in NP, S, R, K, ST, NG, RS, SP and SD were all significantly > L and SV at P < .001.			

[a] Key to abbreviations is given in Table 5.1. Throughout the text, native and natural grasses have been assumed to mean the same group of species mixtures. Student's t-values for all differences recorded at P < .10 were very close to those for significance at P < .05.

[b] The species named <u>first</u> had significantly higher concentrations; for example, lucerne had higher Al level than Napier grass (P< .10)

TABLE 5.4 Soil Composition Differences between Species Sites*

Element	Level of Significance		
	P < .05	P < .01	P < .001
Aluminium	-	-	-
Calcium	-	-	-
Cobalt	-	-	-
Copper	NP : S	SP : ST RS : ST NG : ST	K : ST
Iron	-	-	-
Manganese	-	-	-
Molybdenum	-	-	-
Phosphorus	NP : SV R : SV K : SV ST : SV RS : SV	S : SV	
Selenium	-	-	-
Zinc	-	-	-

* Species sites named first had significantly higher concentrations of the specified elements.

(Table 5.4) and no correlation with grass species composition; hence the herbage differences may be attributed to species effects.

Table 5.3 also shows that legumes tended to be rich in calcium: levels in sweet potato vines were higher (P < .05) than

those in Napier grass; while lucerne had higher concentrations than Nandi setaria ($P < .01$), natural grass and Rhodes/setaria mixture ($P < .05$). In addition, sweet potato vines ($P < .01$) were higher in Ca than N. setaria. However, caution must be exercised in attributing species effects when only single legume species samples were taken (this also applies to data for Sudan grass, star grass and Sporobolus).

Phosphorus

The P levels in the species were less variable than Ca levels (Table 5.1) but they still varied 2.5-fold, from 1.14g/kg DM in Nandi setaria to 2.70 and 2.75g/kg DM in lucerne and Sudan grass, respectively. The statistical test showed that P concentrations in Kikuyu grass (1.71g/kg DM) were significantly higher ($P < .05$) than those in the natural grasses (1.35g/kg DM) and this difference was not attributed to bias from the soil test results (Table 5.4). There were no other effects of botanical composition on herbage P. The soil data showed that despite the low level of P found on the sweet potato site (less than half of those in Napier, Kikuyu, Rhodes, star grass and Rhodes/setaria mixture ($P < .05$) and Nandi setaria ($P < .01$) sites), the herbage composition did not reflect that difference, thus providing possible evidence of a P-concentrating effect in the sweet potato vine.

Magnesium

Magnesium concentrations showed extreme species variation: values were lowest in the Sporobolus sample (0.94g/kg DM) and highest (5.53g/kg DM) in sweet potato vines ($P < 0.01$), with all grass species having levels < 2.2g/kg DM (Table 5.1). Several statistical differences were detected. The Nandi setaria levels (1.38g/kg DM) were lower than those of natural (1.86g/kg DM) and Napier (1.68g/kg DM) grasses ($P < .10$) and especially Rhodes grass (1.50g/kg DM) ($P < .01$), while Kikuyu grass tended to have more Mg than Napier grass (2.11 v 1.68g/kg DM: $P < .10$). Finally, the Sporobolus Mg level was lower than that of lucerne ($P < .10$), Napier ($P < .05$) and Kikuyu grasses ($P < .01$). The Mg concentration in sweet potato vines was much higher than those found in star and natural grasses ($P < .01$) and in the major species Napier grass, Nandi setaria and Rhodes/setaria mixture ($P < .01$). Mg concentrations in soils were not measured and the possiblity of confounding effects of site differences in soil Mg cannot be ruled out (the same argument applies to the next two elements, S and Si).

Sulphur

Sulphur concentrations were relatively uniform but were highest in Kikuyu and star grasses (2.01 and 2.211g/kg DM respectively) and the legume species lucerne (2.10g/kg DM) and sweet potato vines (2.57g/kg DM), and lowest in Napier and Nandi setaria (1.10 and 1.32g/kg DM respectively). The concentrations

in Rhodes grass were significantly higher (by 0.7-0.9g/kg DM) than the levels in Nandi setaria and Napier grass ($P < .001$). The mean S level found in Napier was slightly lower than the level found in lucerne ($P < .10$) and much lower than those in star grass, Rhodes/setaria mixture and natural grasses ($P < .05$) and Kikuyu grass ($P < .01$). Finally S concentrations in sweet potato vines were greater than those in Nandi setaria and Napier grasses ($P < .05$).

Silicon

Silica levels in the herbage species varied widely from very low (< 0.3% DM in the legumes) to just over 5% DM in Sudan grass. The low levels in the legumes were highly significantly different ($P < .001$) from those in Napier, Kikuyu, Rhodes, star, Sudan and natural grasses, Rhodes/N. setaria mixture, Sporobolus and Nandi setaria. However, among the grasses, the only statistical difference was provided by the N. setaria/Sudan grass comparison, the concentrations being higher in the latter ($P < .01$).

5.3.2 Aluminium and the Micro-Elements

Aluminium

Aluminium concentrations in the herbage species varied from a minimum of 0.09 and 0.12g/kg DM in Sudan and Napier grasses respectively, to 0.45g/kg DM in lucerne and 0.55g/kg DM in sweet potato vines and Kikuyu grass (Table 5.1). In contrast,

the soil Al composition showed a uniform distribution (Table 5.2) with no statistical differences across the sites (Table 5.4). Al concentrations (Table 5.3) were lower in Napier grass than in sweet potato vines ($P < .05$), Nandi setaria, Rhodes grass, the Rhodes/N. setaria mixture ($P < .01$), Kikuyu and the native grasses ($P < .001$). Rhodes grass had a lower aluminium content than natural grass ($P < .05$) and Kikuyu grass ($P < .01$); while concentrations in Kikuyu grass and Rhodes/setaria mixture ($P < .05$) were higher than those in Sudan grass. The Nandi setaria levels were also significantly lower ($P < .05$) than those found in Kikuyu grass.

Cobalt

The herbage species contained highly variable Co concentrations, ranging from 0.11 to 0.81mg/kg DM in Sudan grass and sweet potato vines respectively (Table 5.1). Kikuyu grass samples (Table 5.3) contained more Co than Rhodes/setaria mixture ($P < .10$), N. setaria ($P < .05$) and Rhodes grass ($P < .01$). Cobalt concentrations were higher in sweet potato vine than in Rhodes/setaria mixture, natural grass ($P < .05$), Sudan grass and Napier grass ($P < .01$). Cobalt concentrations in Kikuyu and natural grasses (0.35 and 0.25mg/kg DM respectively) were considerably higher than those found in Napier grass (0.16mg/kg DM) although the differences were not statistically significant. Comparison of the results in Table 5.3 with the corresponding soil assessment (Table 5.4) indicates that the

above differences were mainly attributes of the species since extractable soil Co (Table 5.2) showed a uniform distribution.

Copper

Table 5.1 shows that Cu levels in the herbage species ranged widely from 2.64 to 9.70mg/kg DM in the Sporobolus and lucerne species ($P < 0.01$), respectively, though each was represented by just a single sample. The two legume samples were superior to the grasses in Cu content. Significant differences between the well-represented grass species were detected (Table 5.3); concentrations in Napier grass were higher than those in Rhodes grass ($P < .01$) but much lower than the levels found in Kikuyu grass ($P < 01$). Sporobolus had a lower Cu level than Napier grass ($P < .05$), Kikuyu grass and sweet potato vines ($P < .01$). The Cu concentration in lucerne was higher than those of Nandi setaria and Rhodes grass ($P < .01$). The soil data (Table 5.2) showed that the star grass site was poor in available Cu and significantly differed from other sites (Table 5.4). Although the Napier grass sites had more extractable Cu than the Nandi setaria sites ($P < .05$) the significant differences summarised in Table 5.4 were not correlated to the corresponding herbage species concentrations (Table 5.3).

Iron

Iron concentrations in the herbages were highest in sweet potato vines and lowest in Napier grass (Table 5.2). Table 5.3

shows that levels in Napier grass were lower than those in Nandi setaria and Rhodes/setaria mixture ($P < .05$), Kikuyu and natural grasses ($P < .01$) and Rhodes grass ($P < .001$). In contrast, the soil data (Table 5.2) gave a narrow range of extractable Fe concentrations, from 0.34g/kg on Kikuyu grass sites to 0.49g/kg DM on lucerne sites, with no significant differences across the sites (Table 5.4).

Manganese

The herbage Mn data (Table 5.1) showed a four-fold variation, with lucerne giving the lowest concentration (0.09g/kg DM) and Sudan grass the highest (0.34g/kg DM). Table 5.3 shows that in lucerne, Mn concentrations were significantly lower than those in Nandi setaria, Sudan grass, Sporobolus and Rhodes/setaria mixture ($P < .05$); while the levels in Nandi setaria were higher than those in natural grass mixtures ($P < .05$) and Napier and Kikuyu grasses ($P < .01$). The Napier levels were also lower than those of Rhodes grass ($P < .01$) and Rhodes/setaria mixture ($P < .001$). In Table 5.2, it can be observed that although the extractable Mn varied from 0.84me% on star grass sites to 2.23me% on lucerne sites, the statistical analysis showed no significant differences between the sites (Table 5.4) that could be confounded with the above species effects.

Molybdenum

Concentrations of herbage Mo were lowest in lucerne (0.48mg/kg DM) and highest in Kikuyu grass (1.69mg/kg DM) as summarised in Table 5.1. The levels in Kikuyu grass were approximately twice as high as those in Napier grass ($P < .05$) and Rhodes grass ($P < .01$). By contrast, extractable soil Mo concentrations gave a uniform distribution across sites (Tables 5.2 and 5.4).

Selenium

Table 5.1 shows that lucerne and Sudan grass had the highest Se concentrations (505 and 327ug/kg DM, respectively) while the sweet potato vine had the lowest (28ug/kg DM) and significant differences between species were detected (Table 5.3). The Se content of Rhodes/setaria mixture tended to be higher than that of Nandi setaria ($P < .10$), while that of N. setaria was lower than that of Rhodes ($P < .05$) and lucerne ($P < .01$). The high Se concentrations in lucerne could be partly attributed to the high content of soil Se on the site (Table 5.2), but other sites did not differ with respect to Se composition (Table 5.4).

Zinc

The nearly four-fold variation in Zn content of the herbages from 11mg/kg DM in the sweet potato vines to 39mg/kg DM in Kikuyu grass (Table 5.1) again prompted an evaluation of species effects (Tabled 5.3). Napier grass tended to be higher in Zn

content than Rhodes ($P < .10$) but had lower concentration than Kikuyu grass ($P < .05$) which in turn had higher Zn levels than sweet potato vines, natural grass ($P < .05$) and Nandi setaria ($P < .01$).

5.4 DISCUSSION

The REML analysis has shown that individual species were extremely important in determining the quantities of the macro and trace elements absorbed from the soils. Table 5.5 summarises those species that were particularly high or low in each element and it is necessary to discuss their contrasting properties, how they might arise and how they might be exploited or controlled.

5.4.1. Aluminium, Iron and Manganese Toxicity

(a) Aluminium

Concentrations of Al were highest in Kikuyu grass (0.55g/kg DM) and the leguminous species lucerne (0.45mg/kg DM) and sweet potato vines (0.55mg/kg DM) but risks of Al toxicity are likely to be minimal. Even with 100% of the high-Al species in the diet, Al concentrations would be well below the 2g/kg DM required to give clinical symptoms observed by Valdivia et al, (1982) and Allen (1984). Studies have shown that dietary Al intake may be increased further by direct soil ingestion by grazing animals (Field and Purves, 1964; Healy and Ludwig, 1965; Arnold et al, 1966; Healy, 1967; Mayland et al, 1975;

Fries et al, 1982; Miller et al, 1985). However, the herbage levels found in this study may include some contribution from contaminant soils.

Table 5.5 Species with Extreme Mineral Composition

Element	Species Concentrations	
	Low	High
Aluminium	Sudan and Napier grasses	The legumes; Kikuyu grass
Calcium	N. Setaria, Napier grass	The legumes; Sudan and Star grasses
Cobalt	Sudan grass	Sweet potato vines; Kikuyu and Natural grasses
Copper	Sporobolus, Rhodes	The legumes; Kikuyu and Sudan grasses
Iron	Napier and Sudan grasses	Kikuyu and Star and Natural grasses; Sweet potato vines
Magnesium	Napier, Rhodes, N. setaria Sporobolus	Kikuyu; the legumes
Manganese	Lucerne; Kikuyu grass	Sudan grass, N. setaria, Sporoblus
Molybdenum	Lucerne, Sporobolus	Kikuyu, Star grass
Phosphorus	Setaria, Napier grass	The legumes; Star grass
Selenium	Sweet potato vines, N. setaria, Sporobolus	Lucerne, Sudan grass
Silicon	The legumes	Sudan grass
Zinc	Sweet potato vines	Kikuyu grass; Lucerne

(b) Iron

The major grass species, Kikuyu (0.38g/kg DM) and natural grasses (0.35g/kg DM) had the highest Fe concentrations, ten times higher than the recommended minimum dietary requirement (ARC, 1980) of 0.03g/kg DM. According to NRC (1980) the maximum tolerable dietary limit is 0.40mg/kg DM and therefore the above concentrations could present problems related to direct Fe toxicity such as siderosis (Hartley, 1959; Howell, 1983) or interference in Cu absorption in ruminants (Suttle et al, 1973; Campbell et al, 1974; Thornton, 1974; Bremner et al 1983; Humphries et al, 1983). Although high levels of Fe have been reported in herbage (Innes and Shearer, 1940; Jones, 1972; Campbell et al, 1974) plant-accumulated Fe may also be confounded with soil contamination. The low Fe concentrations found in Napier and Sudan (Sorghum sudanese) grasses may be attributable to the tall stemmy nature of these fodders (Bogdan, 1977) which helped to reduce contamination of the samples by soil.

(c) Manganese

The well represented species, Napier, Rhodes, N. setaria, Rhodes/setaria mixtures, Kikuyu grass and the natural grasses had high Mn concentrations (0.14-0.26g/kg DM) which were six to ten times in excess of cattle or sheep requirements of 0.025g/kg DM (ARC, 1980; Suttle, 1983b). The concentrations in the

poorly represented species were similarly in excess of ruminant requirements though few were sufficiently high to present problems related to Mn toxicity.

Geyloff et al (1959) reported that iron content in plants was negatively correlated with Mn concentrations in single species glasshouse studies. This relationship was also observed within the major grass species, their mixtures, and legumes (Fig. 5). In particular the higher level of Mn in Sudan grass, which was not related to soil exchangeable Mn (Table 5.2), could provide evidence of a possible Mn-accumulating effect, and animals whose diets comprise this species may be at risk from imbalances in iron metabolism since it was relatively low in Fe.

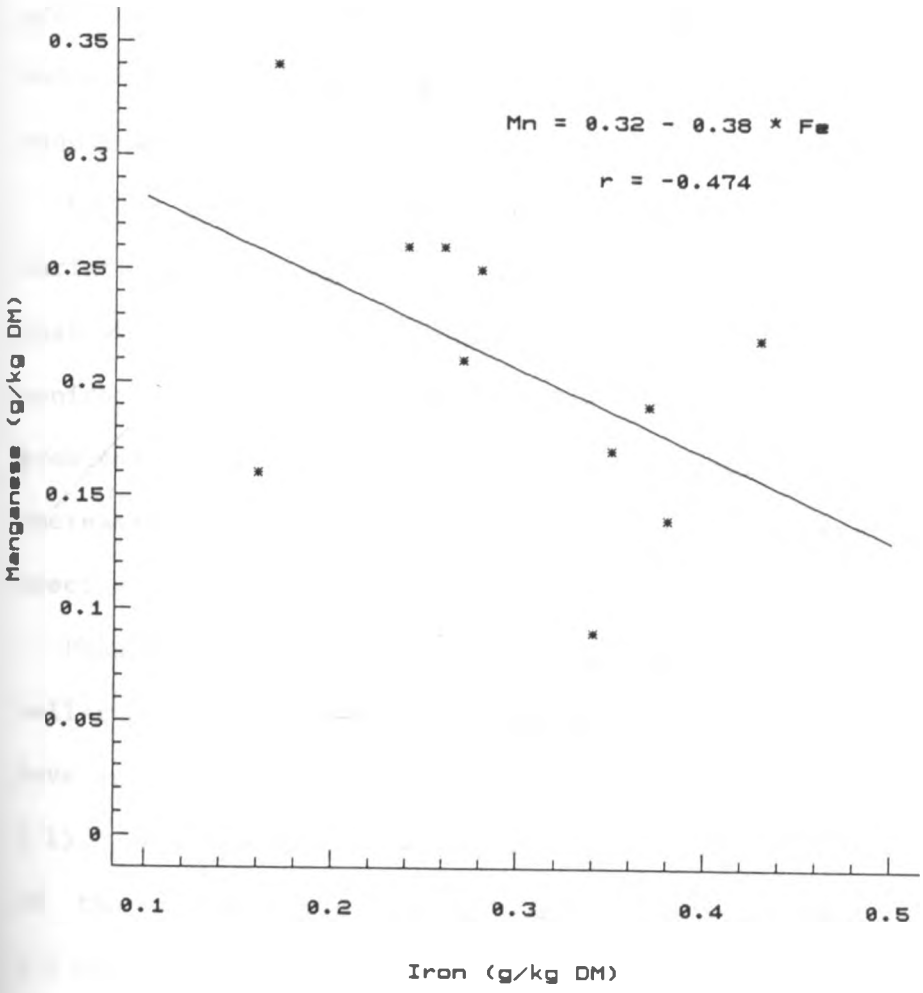
5.4.2 Macro-mineral Deficiencies

(a) Calcium, Magnesium and Phosphorus

Concentrations of the macro-elements Ca, P and Mg were all low in Napier grass and Nandi setaria. Calcium concentrations were particularly low in N. setaria (0.97g Ca/kg DM) and Napier grass (1.24g Ca/kg DM) and well below the minimum ARC (1980) dietary allowances (2.0-6.7g Ca/kg DM) for cattle and sheep (Tables 4.1 - 4.5), yet they constituted 36% of the samples collected in this study. Although the Ca concentrations in Rhodes grass, Kikuyu grass and natural and Rhodes/setaria mixtures were slightly higher than those of Napier and N. setaria, they too could not satisfy the ARC (1980) requirements for ruminants.

Fig. 5 Relationship between

Manganese & Iron in 11 Herbage Species



With regard to Mg the dominant species N. setaria and Rhodes grass (on 69% of the sampled sites) contained levels < 1.50g/kg DM and which could not meet the ARC (1980) requirements especially for pregnant and lactating cattle and sheep which need dietary levels of 1.4-2.1g Mg/kg DM. Although the other dominant species Napier, Kikuyu and Rhodes/setaria and Natural grass mixtures had Mg levels > 1.5g/kg DM, only Kikuyu grass had a concentration > 2.1g.kg DM which is consistently above the requirement.

Hull and Guss (1976) and Hacker (1982) reported wide variations in the Mg content of various species and concluded that whereas the chemical composition of forage is a convenient monitor of treatment effects for solving animal nutrition problems, plant breeding had considerable potential for increasing the mineral composition in a number of forage species.

Phosphorus levels were extremely low (< 1.5g/kg DM) in the well-represented species although Kikuyu grass again tended to have an advantage over the other species (1.71g/kg DM) (Table 5.1). All the species were grossly deficient in P in relation to the ruminant requirements (ARC, 1980) summarized in Tables 4.1-4.5.

The poorly represented species also differed in their macro-element composition. Concentrations of Ca were comparatively high in lucerne (3.8g/kg DM), sweet potato vines (3.1g/kg DM) and Sudan grass (2.8g/kg DM). Sweet potato vines

(5.5g/kg DM) also tended to accumulate Mg. Sporobolus (0.94g/kg DM) was extremely poor in Mg and since this species commonly contaminates most Rhodes grass swards, this further dilution of pasture Mg may increase the risk of deficiency. Lucerne (2.7g./kg DM) and Sudan grass (2.75g/kg DM) had high P concentrations when compared with sweet potato vines (1.13g/kg DM) and Sporobolus (1.19g/kg DM). Providing good representation, the legume species were generally richer in Ca, Mg and P. However, it is doubtful whether these relatively small species variations can be exploited to completely offset the basic problem presented by a shortage of the macro-elements in the soils sampled (Table 5.2).

(b) Sulphur

Rhodes grass (2.01g/kg DM), Kikuyu grass (1.75g/kg DM) and natural grass mixtrues (1.51g/kg DM) were the only well represented species that had S levels > 1.5g/kg DM. Concentrations in the marginal range (1.0-1.5g/kg DM) in terms of rumintant requirements (ARC, 1980) were found in Napier (1.0g/kg DM) and N. setaria (1.42g/kg DM), which constituted 36% of all samples. There is therefore a slight risk of S deficiency limiting production in these species but the risk is far less than that of Ca or P deficiency.

The requirements of pasture species themselves for S must not be forgotten. It is generally accepted that total S levels < 2.0g/kg DM or a ratio of total N to total S (N:S) wider than

15:1 is indicative of S deficiency in plants. It thus appears from the results in Table 5.1 that all the major species sampled in this study were deficient in S, possibly reflecting widespread S deficiency in the soils. Although the low S concentrations may also be attributable to a decline with plant senescence, it is possible that concentrations are higher at other times of the year.

Of the poorly represented species, lucerne (2.1g/kg DM) and sweet potato vines (2.57g/kg DM) had relatively high S levels. Sulphur has been shown to increase nitrogen fixation by legumes (Meyer and Marcum, 1980) and it is not surprising that the legumes analyzed in this study had a higher S content than the grasses.

5.4.3 Copper and its Antagonists

Copper cannot be discussed in isolation from its antagonists Mo and S (Chapter Two). Species differences in the concentrations of all three elements were obtained (Table 5.1). The Cu concentration range obtained in the major species (ie from 3.5mg/kg DM in Rhodes to 5.8mg/kg DM in Kikuyu grass) indicates that there may be Cu deficiency problems in the surveyed area since the levels are less than the standard requirements for ruminants even in the richest species, Kikuyu grass (ARC, 1980). The concentrations in the legumes lucerne (9.7mg/kg DM) and sweet potato vines (8.51mg/kg DM) compare well with that reported by Dick et al (1952) of 9.2mg/kgDM, assuming the Kenyan species were well represented.

Variations in Mo content of the major grass species were noted, with the Kikuyu grass levels (1.69mg/kg DM) being nearly double the mean levels of the other grasses: this may be sufficient to offset the advantage of Kikuyu when the inhibitory effect of Mo on Cu absorption is considered. The average S concentrations of 1.72 (range: 1.1-2.0) mg/kg DM in the major species (Table 5.1) were much lower than the levels of 1.0-9.0 (mean 2.2) g/kg DM reported by Beck (1962) in Australian tropics.

The advantage of the legumes with regard to Cu concentration will be offset by S but not Mo. The Mo concentrations found in the legumes, provided they were representative, were less than those in the grasses: this is quite surprising since generally speaking legumes are richer in Mo than grasses though less sensitive to the effect of soil pH on Mo uptake (Gladstones, 1962; Burridge et al, 1983). The low pH of the soils may be responsible for the low legume molybdenum levels.

Effects of small changes of dietary Mo and S on Cu metabolism were evident from the studies by Suttle (1978) and Whitelaw et al (1979); and more recently Suttle (1983c) obtained prediction equations which show that conservation of grass as hay diminishes the effect of Cu x Mo antagonism compared with fresh grass. The prediction equations are given below:

(a) For Hay:

$$\text{Available Herbage Cu (\% DM)} = 8.9 - 0.70 \log_e \text{Mo} - 2.61 \log_e \text{S}$$

(b) For Grass:

$$\text{Available Herbage Cu (\% DM)} = 5.72 - 1.297\ S - 2.785\ \log_e Mo + 0.227\ (Mo \times S)$$

where Mo and S concentrations are in mg/kg DM and g/kg DM respectively.

Table 5.6 gives the available Cu calculated using both equations contrasted with the individual means of Cu, Mo and S for each species (data extracted from Table 5.1). Most of the grasses were sampled at hay stage (see Chapter 3) and although the predicted availabilities are expected to lie between the values given by the two equations, they may for the most part be closer to those of the hay equation. However for Napier and Kikuyu grasses and sweet potato vine, the Grass Equation is more applicable as nearly all the sampled material was green.

The grass equation predicted values which were 2-3 times less than those for the hay equation and the differences were biggest for Kikuyu grass, star grass, Rhodes/N. setaria mixtures and natural grasses. Examination of Table 5.6 data shows that these variations were largely attributed to the high Mo and S concentrations. The Hay Equation predicted higher availability of herbage Cu in Kikuyu grass compared with Napier grass, Nandi setaria and Rhodes grass while the Grass Equation showed that Napier grass and Nandi setaria Cu availabilities were higher than those of Kikuyu grass despite the latter species' higher Cu content. This suggests that the advantage of animals grazing Kikuyu grass swards of high Cu may be offset by the parallel increases in Mo and S.

Table 5.6 Species Differences in Available Copper Calculated from Prediction Equations

Species	Mean Herbage Concentration[a]			Available Copper (mg/kg DM)	
	Cu	Mo	S	Grass Equation	Hay Equation
Napier grass	4.27	0.96	1.10	0.135	0.303
Nandi setaria	3.74	0.87	1.32	0.141	0.308
Rhodes grass	3.50	0.76	2.01	0.107	0.251
Kikuyu grass	5.80	1.69	1.75	0.127	0.383
Lucerne	9.70	0.48	2.10	0.374	0.566
Sudan grass	5.17	0.89	1.78	0.135	0.284
Sweet potato vine	8.51	0.85	2.57	0.268	0.560
Star grass	3.67	1.49	2.24	0.106	0.260
Sporobolus	2.64	0.54	1.51	0.114	0.214
Rhodes/setaria mixture	3.70	1.01	1.47	0.109	0.292
Natural grass mixtures	4.64	1.24	1.51	0.139	0.339

[a] Cu and Mo in mg/kg DM; S in g/kg DM

5.4.4 Selenium, Cobalt and Zinc

(a) Selenium

The major species had almost identical Se levels (ranging from 57ug/kg DM in Napier grass to 67ug/kg DM in Rhodes grass) except N. setaria whose concentration (43ug/kg DM) was below the standard dietary requirement for ruminants (ARC, 1980). The Se level in lucerne (505ug/kg DM) assuming there was good representation, could be of significance if this species were to be exploited for supplementation of animal diets. The level found in sweet potato vines (28ug/kg DM) was surprisingly low since legumes generally absorb more Se from the soil than grasses (Gardiner et al, 1962; Rosenfield and Beath, 1964). Possible reasons for the low sweet potato vine value may be the low soil Se concentration at the sampling site (Table 5.2) and the poor adaptability of the species to a low soil Se condition (Johnson, 1975).

(b) Zinc

Of the major species, Kikuyu grass had the highest Zn levels (39mg/kg DM) followed by Rhodes/N. setaria mixture (29mg/kg DM), Rhodes grass (19mg/kg DM), N. setaria (20mg/ kg DM) and Napier grass (23mg/kg DM). All therefore had concentrations which met a minimum dietary requirement of 8mg/kg DM for cattle and sheep (ARC, 1980; Suttle, 1983b). As for Se, the poorly represented species provided contrasting relationships with sweet potato

vines giving very low Zn levels (11mg/kg DM) compared with lucerne (31mg/kg DM). Available literature to date gives no evidence connecting a high or low Zn uptake with any botanical families or species.

(c) Cobalt

All the major species had similar Co concentrations that were above the (ARC, 1980) requirements for cattle and sheep (0.08-0.11mg/kg DM). Concentrations in Kikuyu grass (0.32mg/kg DM) were, however, almost double those of Napier and Rhodes grasses. Variations in Co contents among the poorly represented species were observed, with sweet potato vines having the highest level (0.81mg/kg DM) compared with lucerne (0.20mg/kg DM) and Sudan grass (0.11mg/kg DM). Because soil contamination can affect herbage Co estimations (Fleming 1973), species of different habit may vary in Co concentration not because of differences in accumulation but through differences in susceptibility to soil contamination eg Kikuyu and Napier grasses.

5.4.5 Explaining Species Differences in Mineral Composition

The concentrations of bioessential elements found in herbage samples is influenced by many factors other than the plant genotype.

(a) Differences Related to Distribution in the Plant and Stage of Maturity

The distribution of mineral elements within plants is not uniform. This was demonstrated by Burridge et al (1983) who found large concentration gradients between the top and bottom nodes of barley and even the closely linked leaf parts, the blade and sheath, exhibited differences. The stem was low (Table 5.7) in Mn but was similar to the sheath concentration of Cu. The distribution of elements in pasture is likely to show similar variations and some have been reported for cocksfoot (Davey and Mitchell, 1968): for example, Zn tends to be concentrated markedly in the young leaves and in meristematic tissues.

Table 5.7 Variations of Trace-element Concentrations (mg/kg DM) Between Parts of Mature Barley Plants*

		NODE			
		1 (top)	2	3	4 (bottom)
Copper:	Leaf Blade	4.6	4.5	3.1	2.4
	Leaf Sheath	1.6	1.5	1.3	1.6
	Stem	1.7	1.5	1.2	1.0
Manganese:	Leaf Blade	44	44	58	78
	Leaf Sheath	71	68	80	118
	Stem	31	24	16	10
Molybdenum:	Leaf Blade	0.43	0.31	0.19	0.19
	Leaf Sheath	0.12	0.11	0.09	0.08
	Stem	0.13	0.09	0.07	0.09

* From Burridge et al (1983)

An extreme example of within-plant variations was shown by Gardiner (1961) who found much higher ratios of Ca:P in both lupin stalks (5.6) and leaves (11.5) than in seeds (0.43); the differences were attributed largely to the higher P content in the seeds (3.7g/kg DM) than in the leaves (1.2g/kg DM) and stem (0.7g/kg DM). In the same report, Cu concentrations tended to be higher in the leaves (12.1mg/kg DM) than in the seeds (7.8mg/kg DM) and stems (3.0mg/kg DM) while Co concentrations were 4-5 times lower in the stems than in the leaves (0.42mg/kg DM). Magnesium concentrations in the seeds (1.6g/kg DM) and stems (1.9g/kg DM) were much lower than in the leaves (2.7g/kg DM). These results were later confirmed by Gladstone and Drover (1962) who, in addition, showed that Mo content was particularly low in the leaflets. With Mn exactly the opposite situation was observed: accumulation took place mainly in the leaflets and in most cases the seed contents were quite low in comparison. While the values for seeds are irrelevant to the grazed legume sward, the evidence cited above shows that the composition of the sampled plant material with respect to stem and leaf can greatly influence the overall mineral composition of survey samples. Where samples are collected at mature or post-flowering stage, the stem-to-leaf ratio will be high and give extreme elemental concentrations. The species represented in the present study differ in their growth characteristics ie in the way in which stem-to-leaf ratios (S:L) increase with maturity (Bogdan, 1977). In N. setaria and Rhodes grass the

S:L ratio increases rapidly after the flowering stage (ie 42-48d following nitrogen application under normal field conditions) when senescence sets in. In Napier grass the ratio begins to increase sharply after the 8th week of N top-dressing. N. setaria and Napier grass have higher S:L ratios than Kikuyu grass species when cut or grazed at the recommended stages. The lower concentrations of Si, Se, S, P and Ca in Napier grass and N. setaria than Rhodes grass (Table 5.1) may reflect the lower stem-to-leaf ratio in the Rhodes species. If the opal phytoliths are mainly deposited in the leaves of plants, then the Si results may provide a useful prediction of the S:L ratio within species. The consistently high concentrations of most of the elements in Kikuyu grass may be partially attributed to the higher leaf-to-stem ratio of the sampled material.

Differences in species' composition between studies may be partially related to the period of sampling ie seasonal rather than maturity effects. Mitchell and Burridge (1979) and Burridge et al (1983) have provided evidence that describes the effect of seasonal changes on whole-plant composition. According to Burridge et al (1983) the Cu concentration of field-grown barley plants declined during the year from 8.1mg/kg DM in May to 3.3mg/kg DM in September.

Van der Merwe and Perold (1967) observed a decline in N and P contents of grasses at maturity and during the dry season but found no obvious trends in trace element concentration. Under East African tropical conditions the

principle seasonal changes refer to the wet and dry periods of the year.

Patterns of change in the mineral composition of herbage may be complicated by interactions with the system of pasture management. Regrowth after irregular grazing (ley grasses) or cutting (eg Napier fodder) interrupt the growth cycle to maturity. Species variations in plant regrowth vigour made it impossible to ensure sampling at a similar stage of maturity in this as in other survey studies. There are therefore a number of factors other than plant genotype which may contribute to the species differences reported and it is important to confirm them under a wider range of pasture conditions.

(b) Differences Related to Root System

Differences in root system and in depth of root penetration to different soil horizons offer a possible explanation for some of the differences in mineral content between species. Van der Merwe and Perold (1967) observed that grass growing in close proximity to or under bushes contained higher concentrations of trace elements than those situated further away. They suggested that leaves and other debris from the deeper rooted bushes and shrubs augmented the supply of trace elements in the surface soil layer from which the shallow rooted grass species obtained their nutrients. Of the grass species represented in this study, the higher mineral concentrations found in Kikuyu grass could be attributed to the more widespread and dense root system

of this species compared with Napier, Rhodes grass and N. setaria whose root establishments are rather shallow and limited in depth of penetration and surface expansion.

(c) Differences Related to Species Characteristics

Plant varieties and species can differ genetically in their capacity to absorb mineral elements from the soil. Differences can be related to the species ability to adapt to soil conditions of moisture, pH, chemical composition and drainage (Loneragan, 1975; West, 1981). Jones et al (1963) and Jones and Handreck (1965) reported that variations in silica content of plants were associated with not only availability in the soil but also characteristics of the plant species. They found that many members of of the grass family were active accumulators of Si. Baker et al (1961) reported marked species differences among plants in the total amount of silica absorbed and subsequently secreted as phytoliths. They found Rye grass (Lolium perene) to contain 23 times as much insoluble ash as lucerne (Medicago sativa) with accompanying differences in the Si and opal phytolith content of the two species. In another study, prairie grass hay (mainly Festuca scabrella) averaged 2.92% Si (6.25% silica) compared with 0.18% Si (0.39% silica) in lucerne (Bailey, 1967). There is general agreement that grasses have higher silica content than legumes and the data presented in Table 5.1 show this ratio to be about 10:1. Table 5.1 also shows that Sudan grass with a level of > 5% silica may be a possible

accumulator of Si assuming that it was well represented in the study.

Experiments on grazing swards have shown that the Cu content of grasses scarcely varies with the Cu status of the soil, unlike Zn (Davey and Mitchell, 1968; West, 1981): and that clover is a better indicator of soil Cu status. Gladstones (1962) and White et al (1981) have provided evidence that lupin and wheat plants differ both between species and within varieties in the accumulation of Cu. There is general agreement that grasses have a lower Cu content than legumes (Beck, 1962; Gladstones, 1962) and results presented in Table 5.1 confirm this. Legumes could be exploited more in animal feeding rations in Kenya. Of the major grasses, Kikuyu grass was superior but the level was not high enough to obviate Cu supplementation.

It has been found that plants with naturally high S contents such as the Cruciferae (wild radish) absorb much more Se from soils than those low in S (Loneragan, 1975). The role of S may reflect the fact that most of the Se in common forages is present in the form of Se analogues of the S amino acids (eg selenomethionine). Among the crop plants, Hamilton and Beath (1963) found that sunflower possessed the highest Se-absorbing ability, with flaxseed, safflower seed and sugar beet absorbing and storing the least. Examination of species data in Table 5.1 shows that the Se content of grasses gave a positive linear relationship with S content (Herbage Se = 14.2 + 28.7 x

Herbage S; r = .60) which was significant at $P < .05$ (for six degrees of freedom) (Table 5.8). The legume data in Table 5.1 are included for comparison but the poor representation in the sampling exercise could not provide enough data for correlation.

TABLE 5.8. Relationship between Species Se and S Contents

Grasses			Legume and Potato		
Species	Se	S	Species	Se	S
Nandi setaria	43	1.32	Lucerne	505	2.10
Sporobolus	46	1.51	Sweet potato vines	28	2.57
Napier	57	1.10			
Natural grass	57	1.51			
Kikuyu grass	62	1.75			
Rhodes/N. setaria mixture	65	1.47			
Rhodes grass	67	2.01			
Star grass	87	2.24			

* Se in ug/kg DM; S in g/kg DM

5.4.6 Exploiting Species Differences

The use of species variation in the management of trace element deficiencies is not straightforward. For example, sweet potato vines accumulated high levels of Co and supplementation of animal diets containing high proportions of the grasses low

in Co with sweet potato vines could most likely alleviate nutrition problems due to Co deficiency. However, the sweet potato vine was also found to be particularly low in Zn, Se and P; and although its Mg concentration was high, the Al level approached antagonistic proportions that could induce grass tetany in ruminants. The Zn, Se and P levels in sweet potato vines were all below the minimum (ARC, 1980) requirements for growing cattle, pregnant and lactating cattle and sheep. In contrast, lucerne had double the P, almost triple the Zn and about 20 times the Se content of the sweet potato vines but was not particularly rich in Co (Table 5.1).

Another example of conflicting properties is given by Sudan grass. Although this species had a high Se content, its high silica was undesirable and could cause problems related to toothwear, reduced feed digestibility and urolithiasis. This conflict does not exist as far as the single sample of lucerne is concerned. If the quality of the species were to be ranked according to their silica content, then lucerne would be of superior composition compared to the grasses. The high level of Se (505ug/kg DM) in lucerne on the sampled farm shows that it might be useful for supplementing deficient ruminant diets. For example, for a 550kg cow feeding on 15kg N. setaria hay (Se level 43ug/kg DM, Table 5.1) per day, inclusion of only 1.0kg of lucerne in the diet would increase the dietary Se concentration from the marginal level (< 50ug/kg DM) to concentrations (73.8ug/kg DM) considered adequate for cattle and sheep production (ARC, 1980; Suttle, 1983b).

Since no one species can remedy all defects a number of factors must be borne in mind in deciding which species to use. They include (a) the relative severity of the deficiences (b) the availability and cost of alternative treatments (c) anti-nutritional factors and (d) miscellaneous limitations eg seed availability.

(a) Relative Severity of the Deficiencies

The results of the forage mineral composition presented in the previous chapter revealed gross deficiencies of Ca, P and Cu in relation to tabulated requirements but less problems with Mg and S and only a few sites may also require attention with regard to Se, Co and Zn deficiencies in the grazing ruminants.

While Ca deficiency can easily be produced in young growing animals and lactating dairy cows fed native low- Ca forages supplemented with concentrates, it has not been reported in grazing beef cattle even during lactation (Loosli, 1978). Therefore the more common mineral deficiency in cattle in the field which requires priority attention in W. Kenya and indeed in the world is lack of P, (McDowell et al 1984). The species differences summarised in this chapter could contribute most to the control of P and Cu deficiencies by improving the mineral content of the staple diet in the area under study.

(b) Alternative treatments

Whereas Ca deficiency can be remedied quite cheaply by the practice of soil liming and trace elements can sometimes be provided cheaply and effectively as fertilizers (Donald and Prescott, 1975), attempts to correct P deficiency by fertilizer application can, unfortunately, be cost-prohibitive. The basic problem is one of non-availability through P fixation and application of phosphate merely adds to the pool of non-available P at great cost.

(c) Deleterious factors

There are certain plant constituents of non-mineral origin which when ingested at toxic levels can be deleterious to grazing ruminants. These compounds are produced by some plant species as a defence mechanism against insect and fungal attack and in others as a mechanism for restricting grazing by herbivores thus ensuring survival of the plant. They include glycosides (eg as isoflavones, coumarin, coumestans and cyanogenic glucosides which release the toxic hydrogen cyanide on hydrolysis by enzymes), mycotoxins, saponins and simple acids and their salts (eg oxalates and nitrates). These compounds may be toxic to animals (eg nitrate and cyanide), cause subclinical losses in productivity (eg mycotoxins) or reduce mineral availability (eg oxalate).

Mycotoxins are fungal metabolites that accumulate in dead leaf litter in pasture, in endophytes of pasture grasses and in

infected grains and stubbles of certain forage species. In Kikuyu grass swards, for example, infection with Fusarium graminearum fungi has been observed, and grazing animals are susceptible to mycotoxin poisoning, mainly zearalenone (Gallagher, 1985; di Menna and Parle, 1970) and the trichothecenes (Burgess, 1985) which cause(s) disease characterized by inappetence, oral lesions, skin necrosis, haemorrhage, scouring and decreased milk production in cattle and sheep. Zearalenone is oestrogenic and at forage concentration > 16mg/kg DM can also depress reproductive performance in cattle and sheep (Barry and Blaney, 1987).

Isoflavones and coumestans are oestrogenic substances that occur in legumes and can cause reproductive disorders and infertility in grazing animals, more commonly in sheep than in cattle. Isoflavones occur typically in clovers, being high in red clover (Trifolium pratense) and moderate in other varieties such as subterranean clover (Barry and Blaney, 1987). Formononetin, the most important of the isoflavones, is not oestrogenic but can be converted to the biologically active agent, equol.

Kikuyu grass is a strong and aggressive grass which on fertile soil and under close grazing does not normally allow any other plant to grow into the established sward. However, under severe grazing or repeated cutting the penetration of clovers is more likely, and in Kenya, wild Trifolium semipilosum often found in Kikuyu grass swards can make up to 15% of the low

grazed herbage (Bogdan, 1977). This is of advantage to grazing ruminants since the clover has a high content of copper; but can be undesirable if the dietary concentration of formononetin exceeds the toxic limit.

Coumestans, the second class of compounds, are present in only small amounts in healthy plants but can increase very markedly in white clover (Trifolium semipilosum) and lucerne (Medicago sativa) in response to attack by fungi (Wong and Latch, 1971) or insects (Kain and Biggs, 1980). These compounds have been associated with reproductive ineffeciency and infertility in ewes (Coop and Clark, 1960; Smith et al, 1979). It is therefore important that control of insects and fungi in lucerne establishments, and proper management of Kikuyu grass swards are undertaken if concentrations of isoflavones and coumestans are to be kept to safe levels.

Another limitation to the heavier use of legume pastures is the presence of bloat-producing proteins. The degree of susceptibility appears not to be determined by the saponin content of the legumes (Majak et al, 1980) but by the texture, composition and microbial population of the rumen contents (Clark and Reid, 1973; Reid et al, 1975; Howarth, 1975; Majak et al, 1983). Bloat is not prevalent in cattle fed lucerne hay. When rapid growth follows a period of drought or when cattle have previously been on a poor diet, grazing lush Kikuyu grass can cause disorders in the animals expressed in abdominal swellings, inco-ordination of leg movement and other symptoms

which can result in death. Whether these disorders are linked to the presence of saponins derived from medicagenic acid, or bloat-producing protein is yet to be established but they have been recorded from subtropical areas of Australia and New Zealand (Bogdan, 1977).

Oxalate occurs widely in plants as simply a waste product in some plants, but in others it may function in structural support, Ca storage and elimination, maintenance of ionic balance, or protection against predators. High oxalate concentrations in pasture vary with species and are associated with application of K and nitrogenous fertilizers. Concentrations of oxalates can be as high as 6-10% DM in Nandi setaria and 0.5-2% in Kikuyu and Napier grass (Bogdan, 1977; Barry and Blaney, 1987). In Australia, acute oxalate poisoning was reported in cattle and horses grazing Setaria sphacelata species (Seawright et al, 1970; McKenzie, 1985), and was attributed to the low availability of Ca in this grass since most of the element was present as the insoluble oxalate (McKenzie and Schultz, 1983). However, of these species mentioned above, Kikuyu grass offers a better alternative as a source of Ca. The Ca concentration can be improved cheaply, to offset the effects of oxalates, by application of lime to the soils. Although there are no reports of oxalate accumulation in legumes, Ward et al (1979) found that about 33% of the Ca in lucerne was unavailable to cattle. Most of the oxalate (about 90%) in the grass species discussed above is present in

water-soluble forms, and is produced to maintain ionic balance with plant cations (K, Na, ammonium, Ca and Mg) and anions (nitrate, chloride, phosphate and sulphate) (Osmond, 1963; Osmond and Avadhani, 1968; Hacker and Jones, 1969; Smith, 1972), and has also been associated with the low pH of these grasses (Dougall and Birch, 1967). The main disorder associated with high oxalate intake is hypocalcaemia but there is evidence that excessive intake could also lead to urolithiasis in sheep (Sutherland, 1958; James, 1978).

Under conditions of high nitrogen supply, low light intensity, or water stress, an imbalance of anions can also lead to a possible accumulation of nitrate in the forages, especially lucerne, Kikuyu grass and the stemmy N. setaria and Napier grass (Hegarty, 1981), thus placing animals at risk from nitrate toxicity. Ingestion of sublethal doses can cause abortion, depression of lactation and digestive disturbances. The nitrate disorders are attributed to accumulation of nitrite (following nitrate reduction in the rumen) which on absorption into the blood stream may oxidize haemoglobin to methaemoglobin; this prevents the haemoglobin from being effectively used and ultimately may lead to death of the animal from lack of oxygen, a condition commonly referred to as methaemoglobinaemia.

Cyanide poisoning has been attributed to intakes of high proportions of Sudan grass, star grass, clover and Kikuyu grass (Bogdan, 1977) in the diet and is associated with heavy application of nitrogenous fertilizers. Losses due to acute

toxicity usually occur under one or more of the following conditions: (1) when the animals are hungry and forage intake is increased; (2) when the fed forage is young or actively growing and (3) when the animals have been stressed (Hegarty, 1981). The toxicity is due to interference by the free cyanide in the transportation of oxygen by blood resulting in inhibition of respiration, and may terminate in death from respiratory paralysis. In ruminants, hydrogen cyanide is metabolised to thiocyanate which is goitrogenic because it inhibits the trapping of iodide by the thyroid gland. Thiocyanate-type goitres have been observed in sheep grazing clovers (Butler et al, 1957).

A significant proportion of the S ingested by animals grazing Sudan grass species may be used to detoxify the hydrogen cyanide. Wheeler et al (1975) observed reduced liveweight gains in animals grazing Sorghum pastures with appreciable concentrations of cyanogenic glucosides and attributed them to induced S deficiency. The advantage of the higher level of S found in Kikuyu, Sudan and star grasses may therefore be offset by the possible presence of high concentrations of toxic glucosides which require a high proportion of the element for detoxification.

(d) Miscellaneous Factors

Among the grass species, Kikuyu grass could be singled out as an additional source of Zn, Se, S, P, Mg, Cu and Co; with

prospects being particularly good for Co, Mg, Zn and S supplementation. Unfortunately commercial seed is usually unavailable for Kikuyu grass and establishment is by means of stolons (Bogdan, 1977). Another problem is that unless it is meant for permanent pasture, Kikuyu grass, once established, persists as long as soil fertility is maintained, hence it is not advisable to cultivate it as a grass break in arable rotations where it becomes a troublesome weed, difficult and costly to eradicate even with the use of herbicides or repeated mechanical ploughing, as has been observed in Kenya, New Zealand, USA and India (Bogdan, 1977).

5.5 CONCLUSIONS

1. Of the dominant grass species, Napier grass and N. setaria were particularly low in S, P, Se, Ca and Mg exacerbating the natural deficiencies in the soils. Rhodes grass if well managed could be an alternative source of S, Ca, Mg and Se.

2. Among the grasses, kikuyu grass was generally richer in the bioessential elements and its wider use may minimise many deficiencies. However, its high Mo and S concentrations are of less advantage since these elements are antagonists to Cu absorption in the ruminant. Furthermore, high quality management is required to control clover composition of the sward and the other factors influencing the composition of deleterious compounds and nutritional inhibitors summarized above.

3. The two legume species samples were richer in S, P, Se, Ca, Fe, Cu, Co and Mg. The advantage of sweet potato vines over lucerne in Co and Mg contents may be offset by the extremely low Se concentration found, if this is shown to be typical by this species. Lucerne offers a better supplementation alternative because of its ease of establishment even on a large scale, provided good management is employed to control deleterious effects of fungal metabolites.

4. The Sudan grass sample had high Mn, Se, P, Cu and Ca concentrations, but its nutritive quality could be reduced by the high silica composition and low Fe content whose utilization could be subject to Mn antagonism. Sudan grass could be a possible accumulator of Se. As with Kikuyu grass, good management of Sudan grass establishments is required to control outbreaks of cyanide poisoning and other deleterious effects mentioned above.

5. The presence of Sporobolus species in Rhodes grass swards may be undesirable because the species itself is, assuming the data to be representative, very poor in mineral composition in relation to ruminant requirements.

6. The high levels of Co, Mn, Fe and Al obtained in some species in this survey may be attributed to distortions caused by traces of soil contamination.

7. The existence of species differences in mineral composition must not be overlooked in using analysis of plants for predicting the ability of the soils on which they grow to supply sufficient minerals for grazing livestock. However, extreme caution should be exercised in the general interpretation of data for the singly or poorly represented species such as lucerne, sweet potato vines and sporobolus. It is therefore suggested further that the experimental data presented in this thesis be treated as tentative pending further investigations as proposed in detail in Chapter Nine.

CHAPTER SIX

INFLUENCE OF ALTITUDE AND GEOLOGY ON MINERAL COMPOSITION OF HERBAGE AND SOILS

6.1 INTRODUCTION

The complex and varied geology of the sampled areas results in livestock grazing pastures growing on soils derived from a large number of different rock types. The areas underlain by each parent material vary considerably in size but in some places the close proximity of different parent materials means that the macro and trace element content of neighbouring soils may vary considerably within a distance of a few meters (Gibson, 1954; Miller, 1956; Sanders, 1963). Theoretically it should be possible to predict the likelihood of mineral nutrition problems affecting livestock grazing in a given area from the average elemental content of the soils derived from the parent materials present. However, few reports are available in the literature that provide information about the ranges of mineral elements present in typical agricultural soils of Kenya in relation to the underlying geology. Those reports that do exist, for example, that of Hudson (1944) consider few, if any, trace elements and have ignored soil-plant interactions. Previous studies (Reuter, 1975a; Caple et al, 1980; Langlands, 1981b) have also neglected the potentially important effects of variations in soil properties like pH which contributes to the control of mineral uptake by herbage.

Changes in altitude too could have a bearing on the mineral status of plants and the soils which support them. Unfortunately, very few studies have been reported that give

comprehensive information on altitude as a source of variation on the mineral composition of forages in relation to animal requirements. Reuter (1975a,b), Caple et al (1980) and Langlands et al (1981a,b) have associated high altitude and rainfall with a decline in Se concentrations in the blood of ruminants grazing in parts of Australia. Possible reasons given for this relationship are the influence of rainfall on mineral leaching and erosion of soils in the high altitude areas and the consequent deposition to the lower regions which may result in significant concentration gradients. Results presented in Chapter Four have indicated wide variations in the mineral composition of Kenyan herbages, and it is the objective of this chapter to investigate the possible role of both altitude and geology as factors influencing the risk of mineral deficiencies and excesses in grazing ruminants.

6.2 MATERIALS AND METHODS

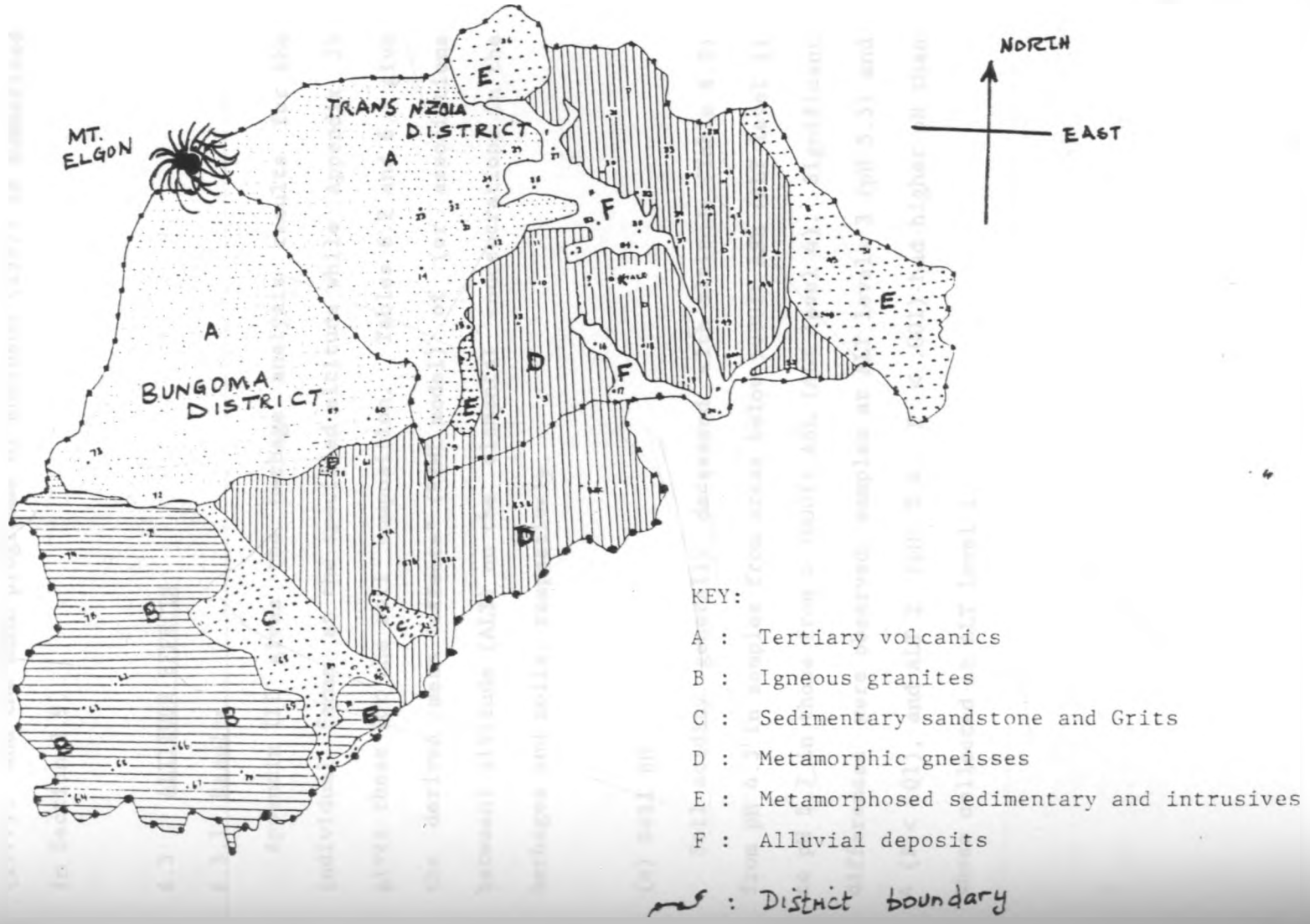
The programme of soil and herbage collection and analysis described in Chapter Three was undertaken to include topsoil sampling in drift-free areas. The 135 samples represented soils derived from a range of specific common parent materials, and from different altitude levels. The parent bedrock underlying individual farms was determined from 1:125,000 geological maps of the area published by the Geological Survey of Kenya (Gibson, 1954; Miller, 1956; Sanders, 1963). A circle, 5km in diameter delineated an area centred on the farmhouse and the principle

soil bedrock of the area as well as its altitude value were recorded (Appendix V). Altitude values were grouped into class intervals (levels) and the class frequency categorized accordingly. The distribution showed that about 16.5% of the samples were collected in the altitude range 4000-5000 feet above sea level (ft ASL), 28% in the range 5000-6000ft ASL while 4.5% came from altitudes between 7000 and 8000ft ASL. The majority of the samples (51%) were collected at altitudes between 6000 and 7000ft ASL. The position of sampled areas in relation to the solid geology of the region is shown in Figure 6.1, and a summary is given in Table 6.1 below.

Table 6.1. Distribution of Samples in Relation to Solid Geology

Soil Bedrock Type	System Classification	No. of Farms	No. of Samples	% Class Frequency
Soft volcanics	Tertiary volcanic	12	22	16.3
Igneous	Granitic (Basement)	13	18	13.3
Metamorphic	Gneisses (Basement)	36	63	46.7
Metamorphosed Sedimentary	Schists-quartz (Basement and intrusives)	5	6	4.5
Sedimentary	Sandstones and grits (Kavirondian)	6	8	5.9
Local Drift/ deposits/peat	Alluvium/Black Valley and Sandy Soils	12	18	13.3

Fig. 6.1 Distribution of sampling sites in Relation to Geology.



Samples were analyzed for the extractable mineral elements (Se alone was a total measurement) as well as soil pH, and the results subjected to unbalanced data analysis based on the procedures of Patterson and Thompson (1971; 1975) and Harville (1977), and the REML programme of Robinson (1987) as summarized in Section 3.9.

6.3 ALTITUDE EFFECTS

6.3.1 Results

Appendix III gives the herbage analysis results for the individual farms at the specified altitude while Appendix IV gives those for the soil composition. Tables 6.2 and 6.3 give the derived mean "effects" (REML model) of (or associations between) altitude (ALT) on the elemental concentrations in the herbages and soils, respectively.

(a) Soil pH

Soil acidity generally decreased with altitude (Table 6.3) from pH 4.3 in samples from areas below 5000ft ASL (ALT level 1) to pH 5.7 in those from > 7000ft ASL (ALT level 4). Significant differences were observed: samples at ALT levels 3 (pH 5.3) and 4 ($P < .01$), and ALT 2 (pH 5.4, $P < .001$) had higher pH than those collected at ALT level 1.

Table 6.2. Associations between Altitude and Herbage Mineral Composition[a]

Element	Altitude Level[b]				Significant Difference			
	1	2	3	4	P<.10	P<.05	P<.01	P<.001
Al	0.41	0.26	0.24	0.22				
Ca	1.72	1.62	1.68	2.24				
Co	0.32	0.20	0.18	0.18	1-3 1-4			
Cu	3.97	4.74	4.32	5.83				
Fe	0.23	0.27	0.24	0.41				
Mg	1.91	1.59	1.65	2.00				
Mn	0.36	0.17	0.17	0.14			1-2 1-3 1-4	
Mo	0.67	0.83	0.87	1.46				
P	0.79	1.17	1.87	3.14	3-1	4-1 3-2 4-2		
S	1.39	1.78	1.51	2.32		4-1 2-3 4-3		
Se	61	122	110	47		2-4 3-4		
Si	2.65	1.96	2.42	1.56		3-2 3-4		
Zn	18.7	19.4	22.6	31.3				

[a] Al, Ca, Mg, P, S, Mn and Fe in g/kg DM; Co, Cu, Mo, Zn in mg/kg DM; Se in ug/kg DM; Si as % Silica DM

[b] ALT level 1: 4000-5000; 2: 5000-6000; 3: 6000-7000; 4: 7000-8000ft ASL

Table 6.3. Associations between Altitude, Soil Mineral Composition[a] and pH.

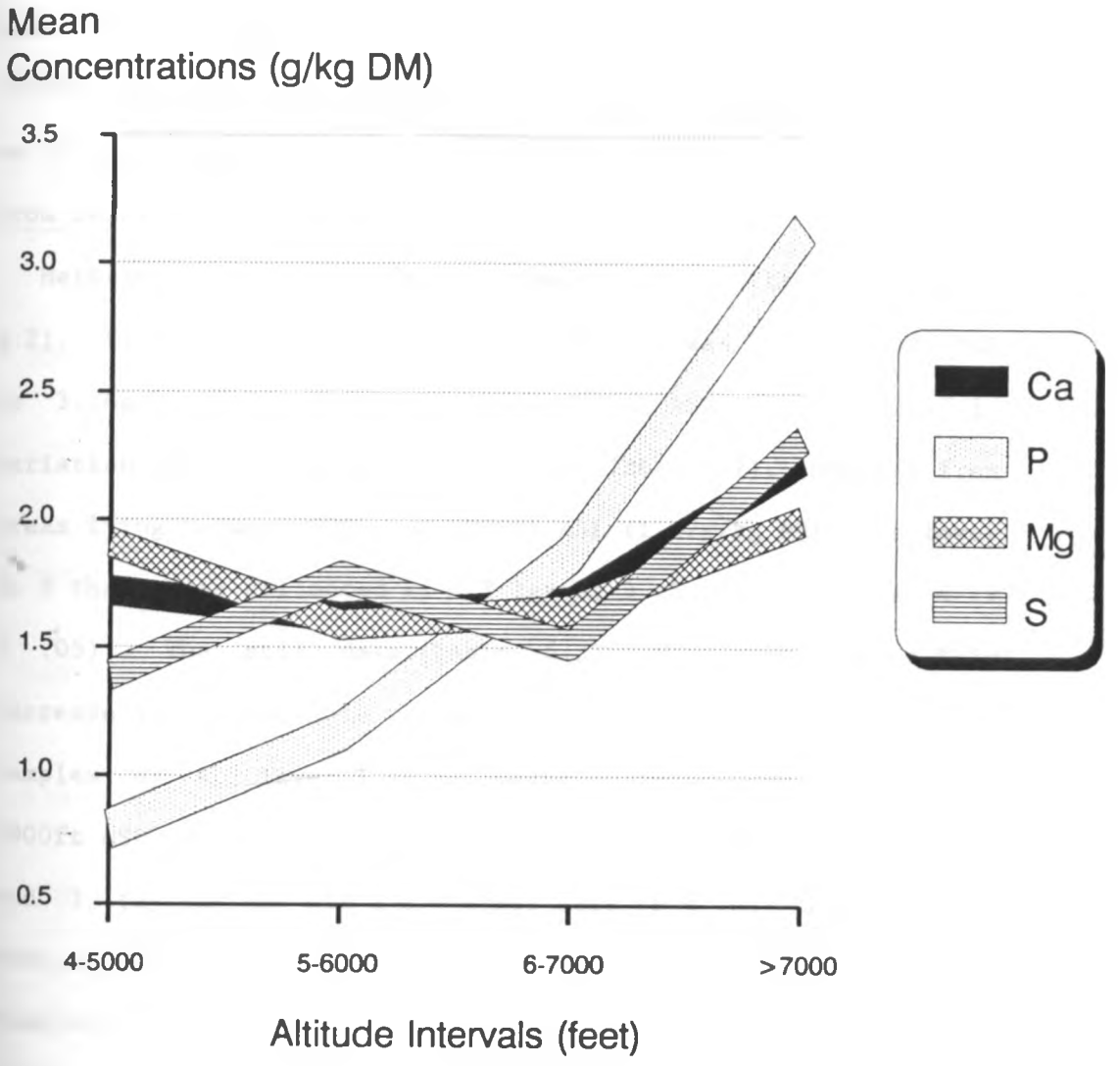
Element	Altitude level				Significant Differences			
	1	2	3	4	P<.10	P<.05	P<.01	P<.001
Soil pH	4.3	5.4	5.3	5.7			3-1 4-1	2-1
Al	0.57	0.95	1.07	1.48	3-1	4-1		
Ca	0.33	2.38	2.33	3.77		4-1	2-1 3-1	
Co	0.54	0.88	1.20	1.70		3-1 4-1		
Cu	0.77	1.33	2.14	2.44	4-1			
Fe	0.58	0.39	0.35	0.25	1-4			
Mn	0.92	1.65	1.83	1.52		2-1 3-1		
Mo	0.79	1.14	2.27	1.72			3-1	3-2
P	10.7	20.0	24.6	42.1		2-1 3-1 4-2	4-1	
Se	134	390	249	224			2-1	
Zn	1.12	2.75	3.45	3.63		2-1 4-1	3-1	

[a] Ca and Mn in me %; Al and Fe in g/kg; Co, Cu, Mo, P and Zn in mg/kg; Se in ug/kg.

(b) Calcium, Phosphorus, Sulphur and Magnesium

Calcium and Mg concentrations in the herbages (Fig. 6.2) were similar, being lowest in samples at ALT level 2 (1.62 and

Fig. 6.2 Herbage Ca, P, Mg & S Changes with Altitude

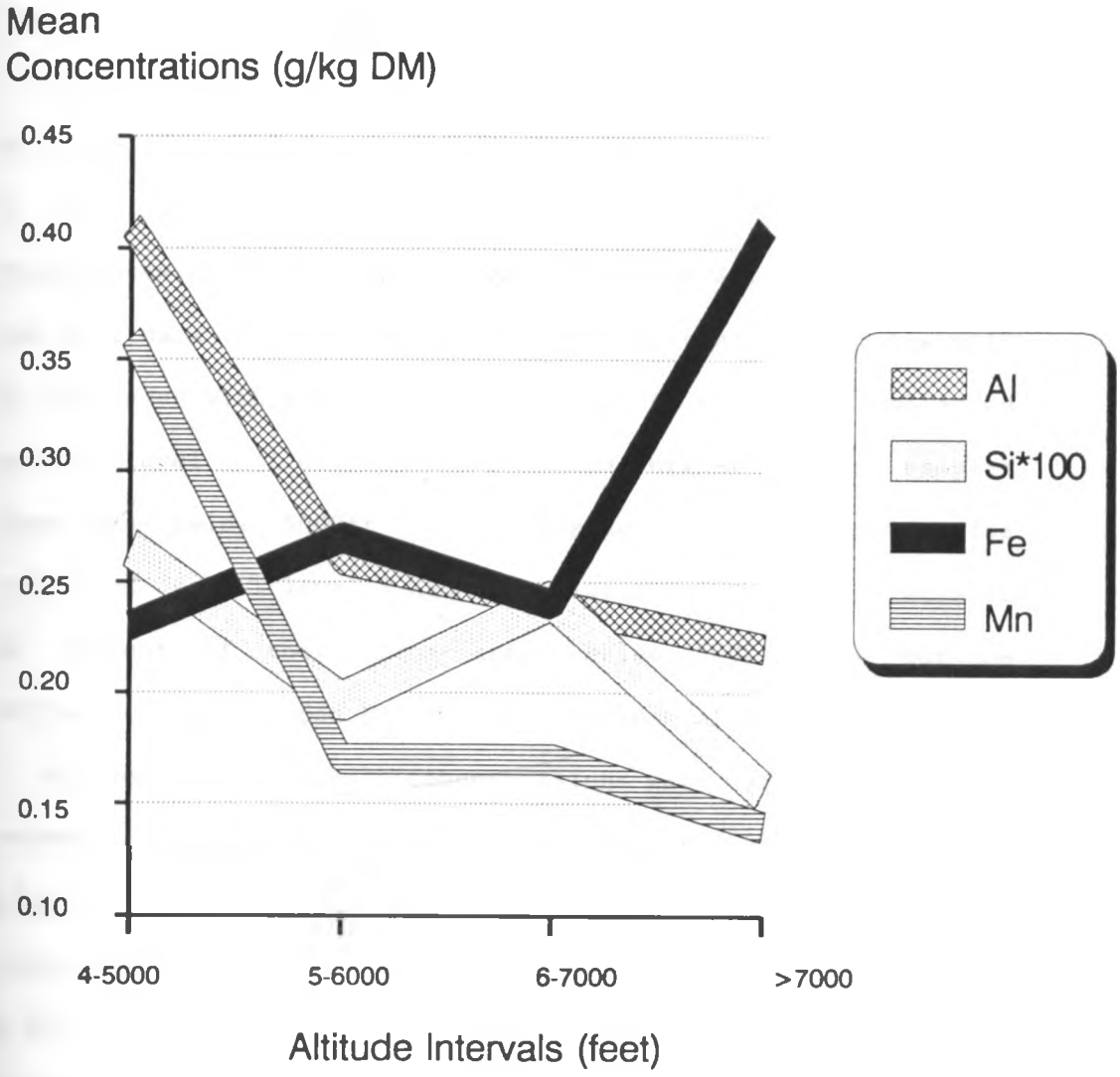


1.59 g/kg DM, respectively) and highest in those at ALT level 4 (2.24 and 2.0g/kg DM, respectively) (Table 6.2), but there were no significant differences between concentrations at the different ALT levels (Fig. 6.2). Extractable Ca from the soils showed a ten-fold increase with altitude from 0.33 me % in samples from below 5000ft ASL to 3.77 me % in those from above 7000ft ASL ($P < .05$). Samples collected at ALT levels 2 (2.38 me %) and 3 (2.33 me %) were seven times higher in Ca than those from below 5000ft ASL ($P < .01$).

Herbage P concentrations increased with altitude (Figure 6.2), from 0.79g/kg DM in samples from areas below 5000ft ASL to 3.14g/kg DM in those from above 7000ft ASL. This four-fold variation was significant at $P < .05$ (Table 6.2). Samples from areas lying between 5000 and 6000ft ASL (1.17g/kg DM) were lower in P than those collected at ALT levels 3 (1.87g/kg DM) and 4 ($P < .05$). The soil data (Table 6.3) also show a four-fold increase in extractable P with altitude, from 10.7mg/kg in samples at ALT level 1 to 42.1mg/kg in those from areas above 7000ft ASL ($P < .01$). Soil samples at ALT levels 2 (20.0mg/kg) and 3 (24.6mg/kg) had 2-2.5 times more ($P < .05$) available P than those from areas below 5000ft ASL while concentrations in samples from ALT level 4 were double those at ALT level 2 ($P < .05$).

Herbage S concentrations showed some variation with altitude, being higher ($P < .05$) in samples from areas above 7000ft ASL (2.32 g/kg DM) than in those collected at ALT levels 3 (1.5g/kg

Fig. 6.3 Herbage Al, Si, Fe & Mn Changes with Altitude



DM) and 1 (1.39g/kg DM) (Table 6.2). Concentrations in samples from ALT level 2 (1.78g/kg DM) were also significantly greater than in those sampled at ALT level 3 ($P < .05$). These variations are depicted in Figure 6.2.

(c) Aluminium, Silicon, Iron and Manganese

Concentrations of Al in the herbages tended to decline with altitude (Figure 6.3), from 0.41g/kg DM in samples at ALT level 1 to 0.22g/kg DM in those from sites above 7000ft ASL, but differences were not significant (Table 6.2). Examination of the soil data (Table 6.3), however, shows that extractable soil Al increased with altitude. Available Al in samples collected at ALT level 4 (1.48g/kg) was 2-3 times higher than in samples from areas below 5000ft ASL (0.57g/kg) ($P < .05$). Samples collected at ALT level 3 had also almost twice the extractable Al content of those from areas below 5000ft ASL but the difference only tended towards significance at $P < .05$.

Silicon (as silica) concentrations were greatest in the herbages from sites below 5000ft ASL (2.65% DM) but not significantly different from those in samples collected at the other altitudes. Concentrations in samples at ALT level 3 (2.42 % DM) were, however, significantly greater ($P < .05$) than those obtained at ALT levels 2 (1.96% DM) and 4 (1.56% DM) so that there was no consistent trend in herbage Si content with altitude (Figure 6.3).

Herbage Fe tended to increase with altitude (Figure 6.3), from 0.23g/kg DM at ALT level 1 to 0.41g/kg DM in samples obtained in areas above 7000ft ASL, but there were no significant differences (Table 6.2). By contrast (Table 6.3) extractable Fe in the soils tended to decline with altitude, from 0.58g/kg at ALT level 1 to 0.25g/kg at ALT level 4 ($P < 0.10$).

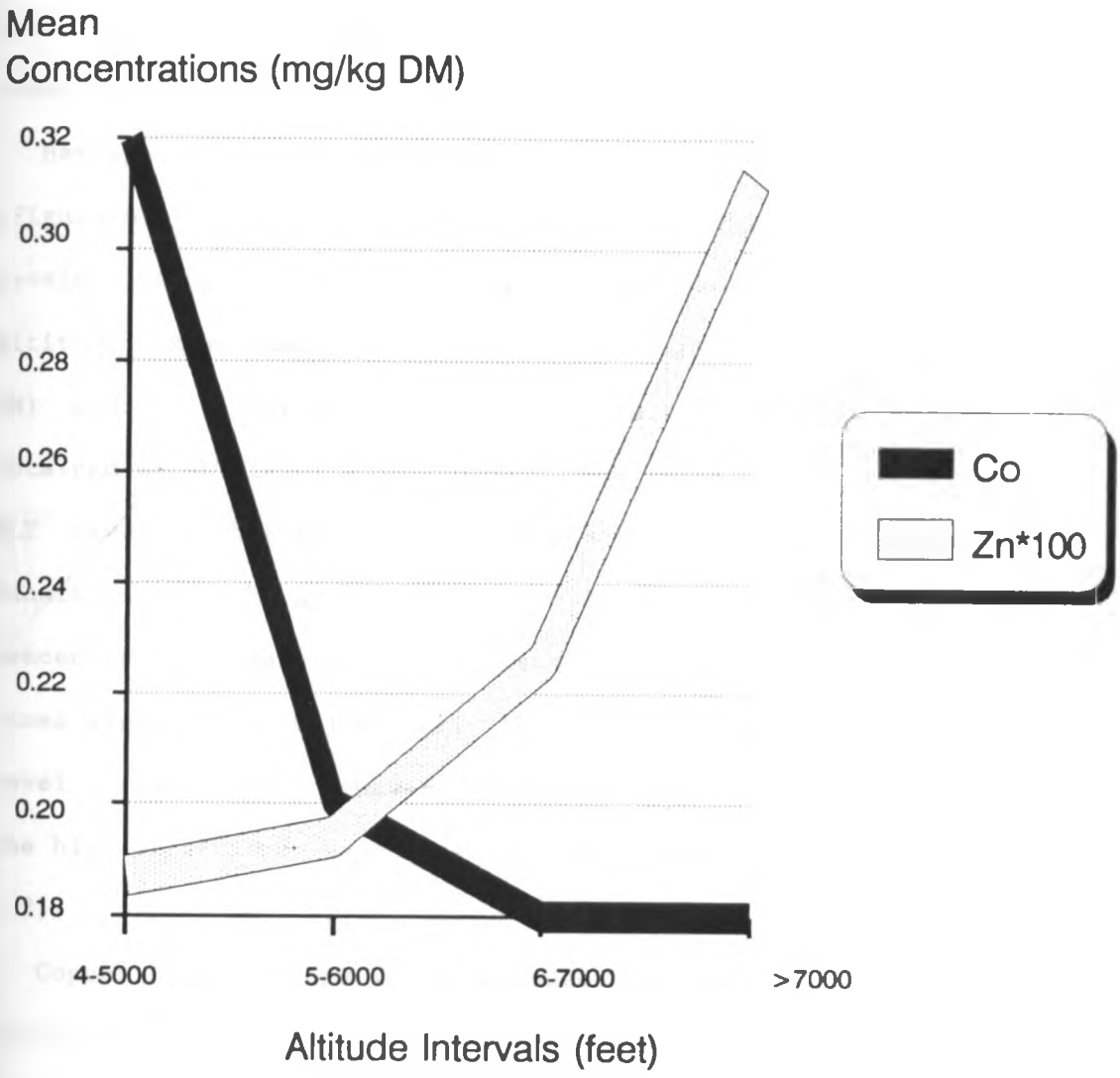
Herbage Mn declined with altitude (Figure 6.3), concentrations in samples collected at ALT level 1 (0.36g/kg DM) being higher ($P < .01$) than in those from ALT levels 2, 3 (0.17g/kg DM) and 4 (0.14g/kg DM) (Table 6.2). The soil data (Table 6.3) showed that extractable Mn increased with altitude, with concentrations in samples at ALT level 1 (0.92 me %) significantly lower ($P < .05$) than those obtained in samples collected at ALT levels 2 (1.65 me %) and 3 (1.83 me %).

Cobalt, Zinc, Molybdenum, Selenium and Copper

Mean concentrations of Co in the herbages tended to decline with altitude (Figure 6.4); those obtained in samples collected above 6000ft ASL (0.18mg/kg DM) were almost half of those in samples at ALT level 1 ($P < .10$) (Table 6.2). By contrast, exchangeable soil Co concentrations (Table 6.3) increased 2-3-fold with altitude, from 0.54mg/kg at ALT level 1 to 1.20mg/kg at ALT level 3 and 1.70mg/kg at ALT level 4 ($P < .05$).

Herbage Zn tended to increase with altitude (Figure 6.4): concentrations rose from 18.7mg/kg DM in samples at ALT level 1

Fig. 6.4 Herbage Co & Zn Changes with Altitude

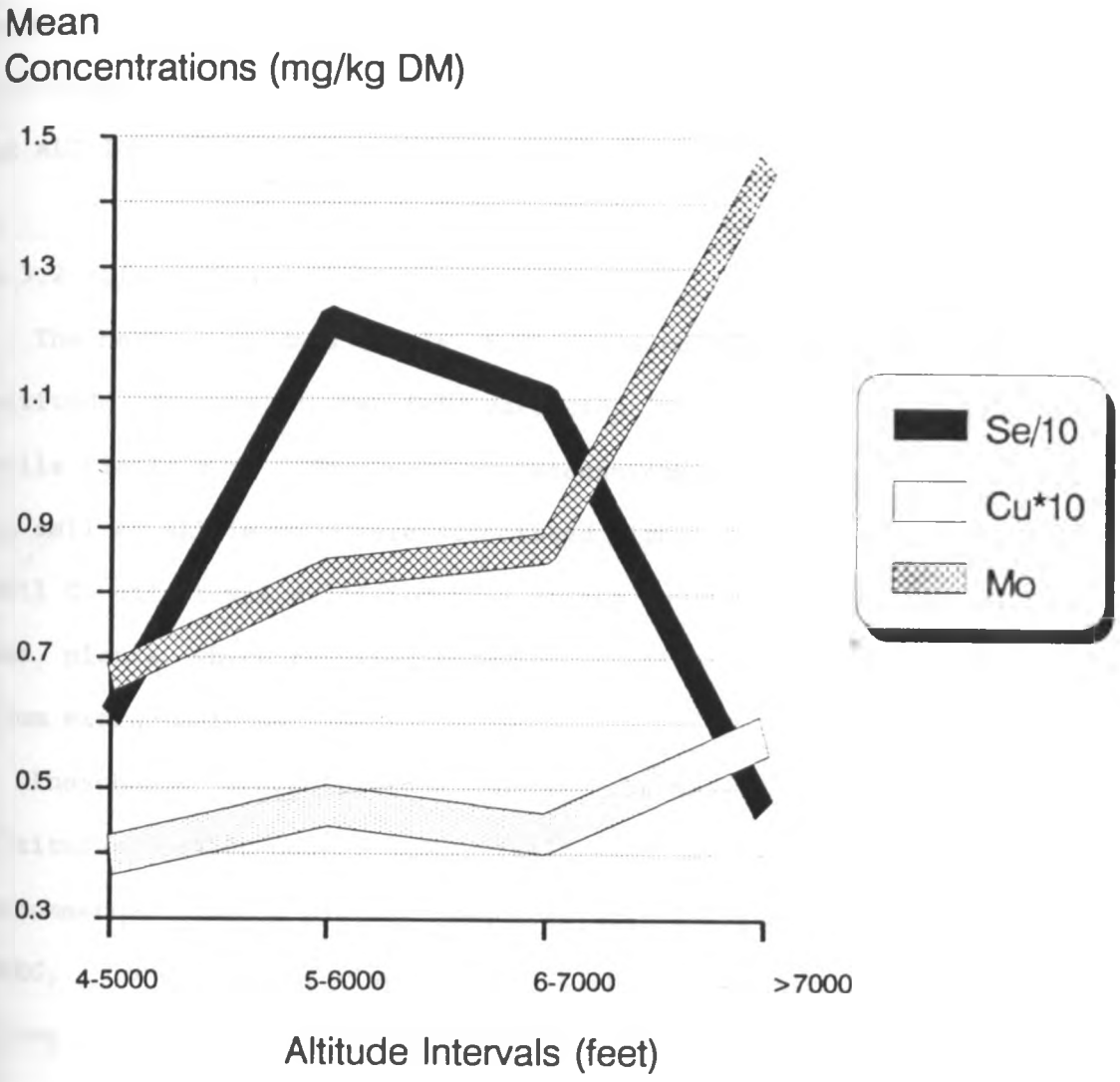


to 31.3mg/kg DM in those at ALT level 4 (Table 6.2) but gave no significant differences. Examination of the data in Table 6.3, however, showed a larger (three-fold) increase in extractable soil Zn, from 1.12mg/kg in samples at ALT 1 to 3.45mg/kg at ALT 3 ($P < 0.01$) and 3.63mg/kg at ALT 4 ($P < 0.05$). Available soil Zn in samples at ALT levels 2 (2.75mg/kg) were also higher than those at ALT level 1 ($P < .05$).

Herbage Se tended to increase with altitude up to 7000ft ASL (Figure 6.5), but the concentrations fell sharply to marginal levels (47ug/kg DM) in samples collected at the highest altitude. Mean sample concentrations at ALT levels 2 (122ug/kg DM) and 3 (110ug/kg DM) were 2-3 times higher than those obtained at ALT level 4 ($P < 0.05$), and twice those obtained at ALT level 1 but the latter difference was not significant. Examination of the data in Table 6.3 showed that total soil Se concentrations gave a similar trend with altitude, being three times higher ($P < .01$) at ALT level 2 (390ug/kg) than at ALT level 1 (134ug/kg). However, the decline in concentrations at the highest altitude was less in soil than in herbage (Table 6.2).

Copper concentations in the herbages increased slightly with altitude (Figure 6.5), from 3.97mg/kg DM in samples at ALT level 1 to 5.83mg/kg DM in those at ALT level 4 (Table 6.2) but there were no significant differences. The soil data (Table 6.3) showed a stronger trend: extractable Cu increased with altitude from 0.77mg/kg DM at ALT level 1 to 2.44mg/kg at ALT level 4 ($P < .10$).

Fig. 6.5 Herbage Se, Cu & Mo Changes with Altitude



Molybdenum concentrations in the herbages showed a two-fold increase with altitude, from 0.67mg/kg DM in samples at ALT level 1 to 1.46mg/kg DM at ALT level 4 (Figure 6.5), but the differences were not significant (Table 6.2). Extractable soil Mo (Table 6.3) clearly increased with altitude up to 7000ft ASL: concentrations at ALT level 3 (2.27mg/kg) were twice those at ALT level 2 (1.14mg/kg) ($P < .001$) and about three times those at ALT level 1 (0.79mg/kg) ($P < .01$).

6.3.2 Discussion

The herbage Ca data (Table 6.2) gave no specific trend with altitude despite the ten-fold variation in available Ca in the soils (Table 6.3). The very low exchangeable Ca concentrations in soil at ALT level 1 were associated with low soil pH, but the soil Ca effect was nullified by a strong "buffering capacity" of the plants which resulted in a poor prediction of herbage Ca from extractable soil Ca.

Phosphorus concentrations in the herbages from the lower altitudes (4000-6000ft ASL) fell the farthest below the recommended minimum dietary requirements for grazing ruminants (ARC, 1980) and the risk of P deficiency is clearly greater on sites covered by this altitude range. The soil data (Table 6.3) show parallel changes, ie extractable P increased with altitude. The low herbage P concentrations at low altitudes may reflect the fixation of soil P at low soil pH. Samples from areas below 5000ft ASL were also associated with high

extractable Fe and Al concentrations which may have contributed to the fixation of soil P (Clark, 1977).

The slight upward trend in herbage Cu concentrations with altitude was much smaller than the three-fold increase in extractable soil Cu (Table 6.3), suggesting that herbage Cu will not be well predicted by extractable soil Cu. By contrast the increase in herbage Mo with altitude (Table 6.2) may be attributable to and be predicted by the soil extractability of the element which showed a similar trend. The altitude effect on Mo may in turn be partially dependent on soil pH (Burridge et al, 1983). Poor class representation could have contributed to the inconsistency between available Mo concentrations in samples from above 7000ft ASL, and the corresponding herbage concentrations.

Herbage S concentrations in samples from below 5000ft ASL were marginal in this element in relation to ruminant requirements and may not be sufficient for protein synthesis by rumen microbes. The low S concentrations in the herbages may possibly reflect reduced availability of soil S at the lower altitudes. Alternatively they may be secondary consequences of the P deficiency at low altitudes which reduced the synthesis of herbage protein.

Bringing together three interacting elements, the effects of high altitude were similar in direction for Mo, S and Cu. The prediction of available Cu in the herbages using the equations described in Chapter Five indicated that the effects on Mo and S

tend to counteract the improvement in total herbage Cu with altitude; and neither the Grass nor the Hay equation gave significant differences between availabilities at the different altitudes (Table 6.4).

Table 6.4. Prediction of Copper Availability at Different Altitudes*

Altitude Level	Herbage Concentrations			Available Cu	
	Cu	Mo	S	Grass Equation	Hay Equation
1	3.97	0.67	1.39	0.131	0.330
2	4.74	0.83	1.78	0.173	0.409
3	4.32	0.87	1.51	0.138	0.358
4	5.83	1.46	2.32	0.145	0.449

*Cu and Mo in mg/kg DM; S in g/kg DM

Cobalt concentrations gave opposing trends. While herbage Co tended to decline with altitude, extractable Co in the soils clearly increased with altitude. These opposite trends may be explained in part by the fact that Co is not an essential nutrient for plants. The upward trend in soil Co availablility at higher altitudes cannot be attributed to soil pH with which it normally shows an inverse relationship (Burridge et al, 1984).

The tendency for herbage Zn to increase with altitude was slight compared with the three-fold increase in extractable soil Zn and

once again the plant is acting as a buffer. Although extractable soil Zn concentrations were low at low altitudes, the herbage composition suggests that there may be few cases of Zn deficiency since the values were above the minimum dietary requirement for growing cattle (ARC, 1980) and sheep (Suttle, 1983a).

Except for sites situated above 7000ft ASL which provided herbages marginal in Se (47ug/kg DM), all samples were sufficient in this element for ruminant growth and production. Gardiner and Gorman (1963), Caple et al (1980) and Langlands et al (1981b) observed a decline in ruminant blood Se with altitude in Australia and associated the concentration gradient with low herbage Se at higher altitudes. Caple et al (1980) and Langlands et al (1981b) reported that every 100mm increase in rainfall resulted in a decrease in ruminant blood Se by 0.033ug/ml, and that deficiencies were prevalent in acid soil areas receiving over 785mm of precipitation. Although current rainfall figures for W. Kenya were not readily available, the data recorded over a 35-year period (Sanders, 1963) show that the area under survey receives on average 45-60 inches (1140-1525mm) of rainfall annually which is far above the level associated with Se deficiency in Australia. Furthermore, the data show a slight decrease in rainfall to the east suggesting that the low selenium concentrations found in herbages at the lower altitudes in the west could be associated with heavier rainfall and low soil pH. The data presented in Table 6.2

suggests the association between altitude and herbage Se was not straightforward. Herbage concentrations were highest in the mid-altitudes and declined at both extremes and the total soil Se showed a similar trend suggesting a straigthforward effect of geology (next section). Alternatively, a high exchangeable soil S at high altitude may have reduced Se uptake by the plant.

The fact that the increase in herbage Fe and decline in herbage Mn with altitude was accompanied by clear, opposing trends in soil availability is consistent with claims that the plant nutrition of these two elements is subject to a mutual antagonism (Geyloff et al, 1959). Although there is a similar antagonism in animals (Hartman et al, 1955; Matrone et al, 1959) the high levels of herbage Mn in samples from the lower altitudes are unlikely to present problems in Fe metabolism because even at their lowest (0.23g Fe/kg DM at ALT level 1), herbage Fe concentrations are far in excess of ruminant requirements (ARC, 1980). However, samples from ALT level 4 tended to have high Fe concentrations which could be antagonistic to Cu absorption, especially at the very low concentrations of available herbage Cu found. The Fe concentrations at maximum altitudes (0.41g/kg DM) were close to the maximum tolerable level of 0.50g/kg DM (ARC, 1980) for ruminants and intake may be increased further by soil ingestion (see Chapter Five).

Aluminium provides yet another example of opposing effects of altitude on herbage and soil (Tables 6.2 and 6.3). The

association between altitude and extractable soil Al could reflect the composition of bedrocks underlying the more mountainous areas above 7000ft ASL.

6.3.3 Conclusions

1. Herbages from the lower altitudes (< 5000ft ASL) were low in phosphorus and tended to be highest in Al. Phosphorus deficiency in grazing animals in these areas may be aggravated by high intakes of herbage Al. The poor P status of the herbages may be due to the low soil pH which reduced the extractable concentrations in soil (Table 6.4).

2. Variations in altitude were associated with far larger changes in extractable mineral concentrations in the soil than in herbage and trends were often in opposite directions, notably for Co, Al, Mn and Fe. This "buffering capacity" of plants suggests that the chosen measure of elemental status in soils may not be a good predictor of deficiency problems in pasture crops or grazing animals (see Chapter Eight).

3. The high altitude herbages, assuming good sample representation had the highest concentrations of S and to a lesser extent Ca, Cu, Fe, Mg, Mo and Zn. They were however clearly lower in Mn and Se. The higher S and Mo concentrations at higher altitudes offset the effect on Cu by reducing its availability.

4. Altitude effects could be confounded with geological and climatic influences such as rainfall and the associated leaching and erosion of soils.

6.4 EFFECTS OF GEOLOGY

6.4.1 Results

The effects of geology on soil pH and mineral composition are shown in Tables 6.5 (herbages) and 6.6 (soils).

(a) Variations in Soil pH

Massive differences were observed between the six bedrock formations (Table 6.6): soil pH was highest in soils from igneous granitic bedrock (pH 6.1) and lowest in those from metamorphosed sedimentary material (pH 4.4) ($P < .001$). Igneous soils also had higher pH than those from tertiary volcanics and sedimentary sandstones and grits, alluvial deposits and metamorphic gneisses (pH 5.0-5.3) ($P < .01$). The pH of soils from tertiary volcanics was higher than that of soils overlying sedimentary sandstones and grits ($P < .05$) and metamorphosed sedimentary bedrocks ($P < .01$). Finally, soils derived from metamorphosed sedimentary were more acidic than those from sedimentary sandstones and grits ($P < .05$) and metamorphic gneisses ($P < .01$).

Table 6.5. Effect of Soil Bed Rock on Herbage Mineral Composition*

	Soil Bed Rock Type						Significant Differences		
	Tertiary Volcanics	Igneous (Granitic)	Metamorphic Gneisses	Metamorphosed Sedimentary	Sedimentary Sandstone	Alluvial Deposits	P<.10	P<.05	P<.01
Al	0.33	0.15	0.23	0.35	0.36	0.28	5-2		
Ca	1.71	1.89	2.12	1.40	1.98	1.78	-		
Co	0.35	0.13	0.17	0.26	0.25	0.18	1-5	1-2	1-6
Cu	4.60	4.70	6.18	4.19	4.46	4.07	-	3-6	
Fe	0.38	0.22	0.25	0.26	0.34	0.27	-		
Mg	1.95	1.47	1.80	1.68	1.88	1.95	-		
Mn	0.20	0.14	0.16	0.22	0.25	0.24	-	5,6-2 5-3	
Mo	0.93	1.02	0.71	1.19	0.96	0.80	-	-	
P	1.53	1.36	1.43	1.71	1.54	1.62	-		
S	1.60	1.77	1.96	1.65	1.59	1.76	-		
Se	76	44	133	125	85	50	4-2 3-6	4,5-6	3-2
Si	1.96	1.40	1.96	2.76	2.41	2.44	3-2	4,5,6-2	-
Zn	19.2	24.0	30.2	19.4	21.5	22.5	-		

* Concentration units as given in Table 6.2

Table 6.6. Effect of Soil Bed Rock on Soil Mineral Composition[a] and pH.

	Soil Bed Rock Type						Significant Differences		
	Tertiary Volcanic (1)	Igneous (Granitic) (2)	Metamorphic Gneisses (3)	Metamorphosed Sedimentary (4)	Sedimentary Sandstone (5)	Alluvial Deposits (6)	P<.10	P<.05	P<.01
Soil pH	5.3	6.1	5.3	4.4	5.0	5.0	1-6 6-4	2-1 1-5 5-4	1,3-4 2-3,5,6
Al	1.06	0.80	0.80	1.08	1.03	1.05	-		
Ca	2.93	2.07	3.08	1.14	1.05	0.82	-	-	1-6
Co	1.11	1.01	1.42	0.74	0.85	0.96	-	-	
Cu	3.15	0.74	1.05	2.13	1.83	1.29	1-5	1-2,3,6	
Fe	0.15	0.41	0.57	0.37	0.44	0.46	-	2-1	3,4-1
Mn	1.72	1.15	1.63	1.57	1.32	1.31	1-5		
Mo	1.34	1.42	1.51	1.43	1.30	1.24	-		
P	22.7	33.2	24.1	16.2	20.0	18.2	2-4		
Se	203	350	270	197	211	197	-		
Zn	2.84	2.85	3.42	2.31	2.24	1.67	3-6		

[a] Units same as those given in Table 6.3

(b) The Macro-elements: Calcium, Phosphorus, Magnesium and Sulphur

The different bedrocks generally had little or no effect on the macro-mineral composition of herbages (Table 6.5). Calcium tended to be lower in herbages associated with metamorphic sedimentary bedrock (1.40g/kg DM) than in those overlying metamorphic gneisses (2.12g/kg DM). By contrast, the soil data (Table 6.6) showed a nearly four-fold variation, with lowest extractable Ca in samples from the alluvial deposits (0.82 me %) compared with the metamorphic gneisses which had the highest (3.08 me %): only one significant difference was obtained, that between the compositions of soils from tertiary volcanics (2.93 me %) and alluvial deposits ($P < .01$). Phosphorus concentrations in the herbages tended to be low in samples overlying igneous granitic bedrocks (1.36g/kg DM) than in those associated with metamorphic sedimentary material (1.71g/kg DM). By contrast, the soil data showed a trend towards higher extractable P in the former group (33.2 v 16.2mg/kg: $P < 0.1$).

Herbages associated with the tertiary volcanic and alluvial deposit areas had slightly higher Mg concentrations (1.95g/kg DM) than those obtained from igneous granitic bedrock formations (1.47g/kg DM). Herbage S concentrations were also uniformly distributed, with a narrow range from 1.60g/kg DM in samples from both sedimentary sandstones and grits and tertiary volcanic bedrocks to 1.96g/kg DM in metamorphic gneisses.

(c) Aluminium and silicon

Herbage Al varied for the different bedrocks (Table 6.5). Concentrations in samples from igneous granitic rocks (0.15g/kg DM) were less than one half of those in samples from sedimentary sandstones and grits (0.35g/kg DM) and tertiary volcanics (0.33g/kg DM). However, only the igneous:sedimentary sandstone difference was significanct ($P < .05$). Table 6.6 shows that soils derived from igneous granitic and metamorphic gneisses were also low in extractable Al but the differences were not significant.

Herbage Si concentrations showed a two-fold variation (Table 6.5), and were lower in samples from igneous granitic (1.4% silica DM) than in those from metamorphosed sedimentary associations (2.76% DM), sedimentary sandstones and grits (2.41% DM) and alluvial deposits (2.44% DM) ($P < .05$).

(d) The Trace Elements

Herbage Fe concentrations were not affected by geology (Table 6.5) but the soils (Table 6.6) showed a 4-fold variation in extractable Fe, being lower in samples from tertiary volcanic areas (0.15g/kg) than in those from igneous granitic (0.41g/kg) ($P < .05$), metamorphic gneisses (0.57g/kg) and metamorphosed sedimentary bedrocks (0.37g/kg DM) ($P < .01$); and sedimentary sandstones and grits (0.44g/kg) and alluvial deposits (0.46g/kg) ($P < .001$).

Manganese concentrations in the herbages were more variable than in soils: values were lower in herbage samples from igneous granites (0.14g/kg DM) than in those from sedimentary sandstones and grits (0.25g/kg DM) and alluvial deposits (0.24g/kg DM) ($P < .05$). Concentrations in herbage samples collected on sedimentary sandstones and grits were significantly higher ($P < .05$) than those from metamorphic gneisses (0.16g/kg DM). The soils (Table 6.6) gave a narrow range in extractable Mn, from 1.15me % in samples from igneous granites to 1.72me % in those from tertiary volcanics with no significant differences.

The herbage Cu concentrations (Table 6.5) varied from 4.07mg/kg DM in samples collected on alluvial deposits to 6.18mg/kg DM in those overlying metamorphic gneisses and the two extremes were significantly different ($P < .05$). By contrast the soil data (Table 6.6) showed low extractable Cu concentrations in metamorphic gneisses (1.05mg/kg): values were significantly higher in soils from tertiary volcanics (3.15mg/kg) than in those from igneous granites (0.74mg/kg DM) and alluvial deposits (1.29mg/kg DM) ($P < .05$).

Molybdenum concentrations in the herbages gave a narrow range, from 0.71mg/kg DM in samples from metamorphic gneisses to 1.19mg/kg DM in those from metamorphosed sedimentary but there were no significant differences. The narrow range was probably due to the uniform concentrations of extractable Mo obtained in the soils (1.32-1.51mg/kg DM) which also gave no significant differences despite the wide variation in soil pH (Table 6.6).

Concentrations of herbage Co showed a nearly three-fold variation, from 0.13mg/kg DM in samples from the igneous granitic bedrock group to 0.35mg/kg DM in those associated with the tertiary volcanics (Table 6.5). These extremes were statistically different ($P < .05$). The soil Co composition showed a two-fold variation (0.74-1.42mg/kg DM) with the igneous granitic group again lowest but there were no significant differences (Table 6.6).

Mean Se concentrations in the herbages showed a three-fold variation with geology; they were significantly lower in samples collected on igneous granites (44ug/kg DM) than in those from metamorphic gneisses (133ug/kg DM) ($P < .01$). Con- centrations in samples from alluvial deposits (50ug/kg DM) were significantly lower than in those from metamorphosed sedimentary (125ug/kg DM) and sedimentary sandstones and grits (85ug/kg DM) ($P < .05$). The soil data (Table 6.6) showed that total Se varied between 197ug/kg in samples from alluvial deposits and metamorphosed sedimentary to 350ug/kg in those from the igneous granitic bedrocks but there were no significant differences.

Herbage Zn concentrations gave a narrow range, from 19.2mg/kg DM in samples from tertiary volcanic areas to 30.2mg/kg DM in those from metamorphic gneisses but there were no significant differences. The soil data (Table 6.6) also showed high Zn concentrations on metamorphic gneisses (3.42mg/kg) which were double those obtained in samples from alluvial deposits (1.67mg/kg DM) but the difference only tended towards significance ($P < .10$).

6.4.2 Discussion

Despite the 2-3-fold variations in extractable soil Ca, P, Fe and Zn, the different geologies had little effect on the herbage concentrations of these elements. This, like the altitude data indicates that plants have buffering mechanisms which nullify differences in soil composition. However, data in Tables 6.5 and 6.6 show differences between the effects of altitude and geology. While the low concentration of extractable P in the metamorphosed sedimentary group was associated with low soil pH, herbage P was maintained at a high level. This may indicate that the method for measuring available P is particularly inappropriate for soils of metamorphosed sedimentary origin (see also Chapter Eight).

The concentration of Se in soil cannot be used to predict animals at risk from Se deficiency because concentrations in soil and herbage do not correlate (Tables 6.5 and 6.6). Parent materials may, however, affect the quantity of available Se. Soils derived from igneous rocks are believed to be low from assessments of Se status of grazing animals (Gardiner, 1962; Blaxter et al, 1963; Johnson, 1975; Caple et al 1980; Langlands et al 1981b). The igneous granitic areas (see Figure 6.1) are situated on the lower slopes of Mt Elgon allowing scope for confounding effects of altitude (and rainfall) (Davies and Watkinson, 1966; Langlands et al, 1981b). The higher pH of the soils from igneous granitic bedrock may have contributed to

the low herbage Se concentrations by promoting fixation of the element by soil Fe. Well-drained igneous granitic bedrock may restrict the proportion of soil Se available for plants (Johnson, 1975).

The Co content of herbages from igneous granitic bedrocks was nearly three times less the concentrations found in samples from tertiary volcanics and one half of those in samples from metamorphosed sedimentary and sedimentary sandstones and grits. These differences could be attributed to reductions in plant uptake caused by high soil pH as well as differences in soil drainage (Burridge et al, 1984).

Another example of the "buffering" effect of herbages on differing soil compositions is provided by Cu. Tables 6.5 and 6.6 show that none of the differences between soil concentrations on the bedrock formations was reflected in the herbage composition. Indeed high herbage Cu concentrations (4.76 and 6.18mg/kg DM) were associated with soils of lowest available Cu (0.74 and 1.05mg/kg DM, respectively). This opposing trend provides further evidence that extractable soil Cu may not be a suitable predictor of herbage Cu in the survey area.

Finally, there was a partial correlation between the effects of geology on Mn in soil and herbage in that igneous granitic bedrock was lowest in extractable Mn and supported herbage low in that element. There was no such relationship for the metamorphic gneisses.

6.4.3. Conclusions

1. Uniform but low Ca, S, P and Mg contents were recorded in the herbages from all soil bedrocks and geology will probably have little effect on the distribution of macro-mineral-deficiencies in grazing livestock.

2. Geology often had opposing influences on the trace element composition of soil and herbage resulting in poor prediction of herbage composition from the soil data. The most affected minerals were Cu and Se, which among the trace elements covered, are anticipated to present most problems related to deficiency.

3.Areas with anticipated Se deficiency problems may be localized on igneous granites (all of which are situated in Bungoma) and alluvial deposits (most of which are in Trans Nzoia District) as depicted in Figure 6.1. These areas may be associated with heavily leached, well-drained soils.

4. It is anticipated that extractable element concentrations in soils overlying the different bedrocks might in addition be influenced by a number of soil properties including the types of parent minerals present, soil texture, cation exchange capacity, soil pH, organic matter content and soil drainage and moisture, as discussed in detail in Chapter Two.

CHAPTER SEVEN

MISCELLANEOUS FACTORS AFFECTING MINERAL COMPOSITION

7.1. INTRODUCTION

Factors such as crop husbandry and area physiography can influence the mineral composition of plants (Davies and Watkinson, 1966b; Hemingway, 1967; Fleming, 1973; Johnson, 1975; Hannam and Reuter, 1977; Reddy et al, 1981; Langlands et al, 1981b). For example, according to Davies and Watkinson (1966b), mixed forages from a low Se soil frequently contained less Se from superphosphate-fertilized areas than when mono-calcium phosphate was used. They attributed the differences to the increase in clover content (low Se) due to sulphate application, as well as dilution of absorbed forage Se as a result of higher yields obtained.

In Australia, Langlands et al (1981a; 1981b) found low blood selenium in cattle grazing in the Pastures Protection Board areas of northern New South Wales and attributed this to low herbage Se. These workers reported that prevalence of the deficiency problem was localized, and was associated with soils from contrasting and gradational profiles.

In Kenya, fertilizer use especially superphosphate and N application, is common in compliance with recommendations of the Agricultural Extension Service in view of the fact that soils may be grossly deficient especially in N and P, a common feature in the tropics (Paladines, 1984). Furthermore, the area under present survey has complex physiography comprising a variety of topographies that include differing landscapes (Gibson, 1954; Miller, 1956; Sanders, 1963). Since wide

variations were found in the mineral composition of herbages (Chapter Four), it is the objective of this chapter to examine the role of farm management and landscape profiles as possible factors contributing to the variation.

7.2 MATERIALS AND METHODS

Three landscape profiles and three management levels were created to facilitate analysis of the effects of topography and farm management on both soil and herbage mineral composition and its distribution in relation to deficiencies and/or excesses in ruminants grazing in the survey area. The 135 sampling sites (Chapter Three) were therefore categorized into groups depending on whether they were situated in localities with uniform, gradational or contrasting profiles (Appendix I); and whether they were on poorly, fairly, or well-managed farms (Appendix V). The rating of farm management was an arbitrary one. In addition to evidence of good animal husbandry and pasture maintenance, the classification of a well-managed farm was based on practices that have been reported to influence mineral composition of plants such as fertilizer N and superphosphate application (Davies and Watkinson, 1966b; Johnson, 1975; Reddy et al, 1981), different species types and varieties established on the farm, and evidence of soil conservation.

The resultant class distribution frequency showed that 36.3% of the samples came from profiles with uniform landscapes, 44.4% were obtained from gradational landscapes, while 19.3% were

collected from contrasting profiles. With regard to management type, 57% of the materials were sampled on well-managed farms; 15% on farms with fair management and 29% were from poorly-managed sites.

Samples were subjected to chemical determination (Chapter Three). The results, presented in Appendix III (herbage) and Appendix IV (soils), were subjected to unbalanced data analysis using the REML statistical model (Patterson and Thompson, 1971; 1975; Harville, 1977; Robinson, 1987) as described in Section 3.9.

7.3 RESULTS

7.3.1 Effects of Landscape Profile

Table 7.1 gives the results of the effects of topography on herbage and soil mineral composition as well as soil pH. The mean concentrations show uniform distributions (ie narrow variations) and suggest that landscape profile (LSP) had little or no effect on soil pH (range 5.1-5.2) and on all mineral elements in both herbage and soils, except herbage Fe which gave the only significant difference.

Concentrations of Fe in the herbages declined from 0.35g/kg DM in samples from uniform profiles to 0.24g/kg DM in those from gradational landscapes. This 1.5-fold variation was significant ($P < .05$). Herbage samples from uniform LSP also tended to be higher in Al, S, Co, Cu and Mo compared with sample concentrations obtained from contrasting profiles. The three

Table 7.1 Effect of Topography on Herbage and Soil Mineral Composition and Soil pH*.

Parameter	Herbage			Soil		
	Uniform	Gradational	Contrasting	Uniform	Gradational	Contrasting
Soil pH	-	-	-	5.2	5.1	5.2
Aluminium	0.32	0.27	0.24	0.94	1.02	0.93
Calcium	1.73	1.88	1.79	1.60	1.42	1.89
Cobalt	0.23	0.22	0.19	1.03	1.11	1.16
Copper	4.99	4.83	4.22	1.60	1.61	1.37
Iron	0.35	0.24	0.26	0.36	0.36	0.41
Magnesium	1.79	1.82	1.72	-	-	-
Manganese	0.19	0.19	0.21	1.54	1.38	1.39
Molybdenum	1.03	1.04	0.73	1.32	1.30	1.50
Phosphorus	1.55	1.60	1.44	23.4	20.0	21.9
Sulphur	1.81	1.66	1.68	-	-	-
Selenium	79.5	78.1	78.4	219	229	250
Silicon	1.94	2.01	2.39	-	-	-
Zinc	22.9	22.2	22.5	2.5	2.3	2.8

* All differences were not significant except that between herbage iron concentrations on uniform and gradational LSP ($P < .05$). Concentration units as given in Tables 6.2 and 6.3.

LSP profiles gave very similar "mean effects" for herbage Ca (1.73-1.88mg/kg DM), Mg (1.72-1.82g/kg DM), S (78.1-79.5µg/kg DM), P (1.44-1.60g/kg DM), Mn (0.19-0.21g/kg DM) and Zn (22.2-22.9mg/kg DM). Herbage Si in samples from contrasting profiles (2.39% silica DM) was slightly higher than in those from the uniform profiles (1.94% silica DM).

Examination of the soil data (Table 7.1) showed that although no significant differences were detected, there was a slight tendency for samples from contrasting LSP to contain higher extractable Fe (0.41g/kg) than those from uniform profiles (0.36g/kg). Total Se too tended to be higher in samples from constrasting profiles (250ug/kg) than in those from uniform (219ug/kg) and gradational (229ug/kg) landscapes. By contrast, extractable Cu was slightly lower in samples from contrasting LSP (1.37mg/kg) than in those from uniform (1.60mg/kg) and gradational (1.61mg/kg) profiles. The soil data for most elements gave poor relationships with herbage and in some instances, opposing trends were observed. The most affected elements were Fe, P, Mo and Mn and the statistical findings are consistent with the observations recorded in Chapter Six.

7.3.2 Effects of Farm Management Levels

Table 7.2 gives the statistical appraisal of the effects of farm mangement on herbage and soil mineral composition, and soil

Table 7.2. Effect of Management Level on Herbage and Soil Mineral Composition and Soil pH[+]

Parameter	Herbage under Management rated as:			Corresponding Soils		
	Poor	Fair	Good	Poor	Fair	Good
Soil pH	-	-	-	5.2	5.1	5.2
Aluminium	0.29	0.25	0.29	0.91	1.01	0.96
Calcium	1.70	1.92	1.78	1.65	1.62	1.61
Cobalt	0.21	0.21	0.22	1.00	1.03	1.06
Copper	4.62	4.74	4.63	1.24	2.07	1.37
Iron	0.29	0.26	0.29	0.36	0.37	0.40
Magnesium	1.58	2.01	1.77	-	-	-
Manganese	0.20	0.19	0.19	1.44	1.41	1.45
Molybdenum	0.91	0.01	0.85	1.29	1.49	1.34
Phosphorus	1.48	1.66	1.45	22.9	20.4	21.9
Sulphur	1.60	1.82	1.74	-	-	-
Selenium	92.5	70.1	75.1	195	266	242
Silicon	1.90	2.39	2.06	-	-	-
Zinc	24.7	20.5	22.7	2.5	2.6	2.4

[+] Concentrations units same as those given in Table 7.1. No significant differences were detected by the statistical model for all elements.

pH. The data show uniform mineral concentrations, and suggest that the level of management had little or no effect on soil pH and on all mineral compositions. However, herbage concent-

rations of Ca (1.70-1.92g/kg DM), S (1.60-182g/kg DM), Mg (1.58-2.01g/kg DM) and Cu (4.62-4.72mg/kg DM) showed a slight tendency to increase with farm management quality.

Examination of the soil data (Table 7.2) showed that Cu, Fe, Se and to a lesser extent, Co tended to be higher on farms with better management. The data also gave relationships that showed opposing trends with herbage concentrations; the most affected elements were Se, P and to a lesser extent, Al and Zn.

7.4 DISCUSSION

Results presented in this chapter have indicated that the different LSP and farm management classifications had little or no effect on herbage mineral compositions, and may therefore not contribute much to explaining the variabilities observed in Chapter Four. These findings are contrary to reports of previous assessments (Reuter, 1975a,b; Langlands, 1981b) in other parts of the world and could be attributed to several overriding effects which include plant species characteristics and variations in response to soil compositions and properties (see Chapter Five), altitude and geology and associated differences in soil pH (Chapter Six). According to Plucknett and Sherman (1963) and Clark (1977), for example, a high phosphate application on the well-managed farms may be nullified by high concentrations of exchangeable soil Fe and Al through

fixation at low pH. Alternatively, mineral availability to plants may be reduced depending on the intensity of soil leaching.

Good farm management practice may not necessarily reduce the prevalence of mineral deficiencies. For example, raising the soil pH by liming has complex effects: improving the Ca status of the soils; increasing soil availability of certain useful elements (eg Se and P) but also of the antagonist, Mo; and reducing availability of some useful elements (eg Co) as well as the potentially harmful ones (eg Fe and Al).

It is disturbing to note that the long recognition of a P deficiency problem has not led to effective remedial measures even on well-managed farms.

7.5 CONCLUSION

The incidence of mineral deficiencies and excesses (Chapter 4) in the surveyed area may not be influenced by differences in landscape profiles, and the benefits of good farm management appear to be nullified by overriding soil properties of pH and geology, and the strong effects of plant species and altitude.

CHAPTER EIGHT

MERITS OF SOIL EXTRACTION METHODS FOR THE PREDICTION OF MINERAL AVAILABILITY TO PLANTS

8.1 INTRODUCTION

The mineral analysis data for herbages presented in Chapters 4 and 5 showed wide variations between sites and species in the survey area but the contribution of soil composition to these variations were unclear. Under normal agronomic conditions, plant mineral composition is assumed to increase with soil availability to give in most instances a positive linear relationship (Loneragan, 1975). However, the correlations between concentrations of extractable elements in soil and total herbage levels were poor across different altitude levels and bedrock types (Chapter Six). This calls into question the validity of the chosen soil extraction methods.

In retrospect is is possible that good recovery of an added element as a simple inorganic salt may have been a poor indicator of availability *in vivo*. Extraction methods which gave poor recoveries may have faithfully reflected the adsorptive capacity of the soil ie non-availability (see Table 3.5). It was therefore decided to compare the extraction methods with each other in their capacity to predict concentrations of a particular element in the admittedly small number of samples (twelve) used for the method selection exercise (Table 3.2).

Other factors may have contributed to the poor relationship between the mineral status of soils and herbage. The existence

of species effects which were independent of extractable mineral concentrations suggests that criteria of adequacy in the soil will need to be adjusted for different species. It was therefore decided to assess the importance of species x soil interaction in the prediction of herbage mineral status by examining relationships within species.

8.2 MATERIALS AND METHODS

Two approaches were taken. First, the correlations between herbage and soil mineral status were compared for the full data set using the selected methods for soil analysis within and between species. Secondly, the twelve soil and herbage test samples (Table 3.2), randomly chosen to select soil extraction methods with minimal bias from confounding effects of soil bedrock and plant species, were used to reassess the predictive value of the unselected soil extraction methods.

Plant and soil data were subjected to conventional statistical correlation tests and regression analysis.

8.3 RESULTS

8.3.1 Correlations within Batch Analysis Data

Table 8.1 gives the correlation coefficients of the soil-plant elemental relationships obtained from data in Appendices III and IV before species effects were taken into account.

The data show that poor relationships were obtained, and even for the elements Al ($P < .05$), Co ($P < .01$), and S and P ($P <$

.001) whose relationships were significant, the best prediction (Fig. 8.1) accounted for only 23% of the variation (in herbage P composition).

Table 8.1 Relationships between Soil and Herbage Mineral Composition

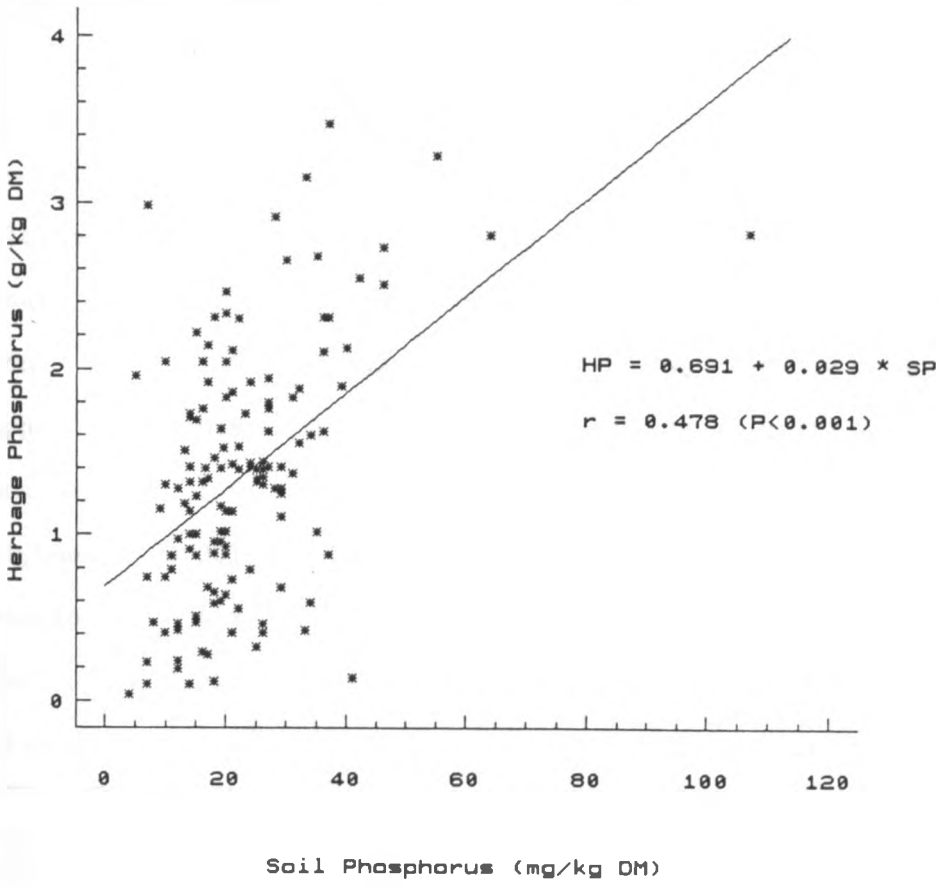
Element	Correlation Coefficient	Significance Level
Molybdenum	-0.040	-
Iron	-0.031	-
Aluminium	0.204	$P < .05$
Manganese	0.032	-
Copper	0.062	-
Zinc	0.027	-
Calcium	-0.048	-
Phosphorus	0.477	$P < .001$
Selenium	0.359	$P < .001$
Cobalt	0.247	$P < .01$

Removal of the high (outlier) value in Figure 8.1 did not improve the relationship for P:Herbage P = 0.559 + 0.0355 soil-P, r = 0.475; $P < .001$). The selected soil extraction methods were least effective in predicting the herbage concentrations of Mo, Fe, Mn, Cu, Zn and Ca.

8.3.2 Within-Species Relationships

Table 8.2 gives the correlation coefficients of the soil-

Fig. 8.1 Relationship between Herbage Phosphorus and Soil Phosphorus



plant elemental relationships for the well represented species (Appendix III v IV).

Table 8.2 Correlation Analysis of the Species' Elemental Relationship with Soil Composition

Element	Correlation Coefficient[+]					
	Napier grass (39)	Nandi setaria (9)	Rhodes grass (45)	Kikuyu grass (9)	Rhodes/ setaria (13)	Natural grass (15)
Aluminium	0.229	0.202	0.061	0.439	0.350	0.270
Calcium	0.141	0.317	-0.084	0.001	0.447	-0.291
Cobalt	0.159	-0.093	0.474***	0.678*	0.177	0.162
Iron	-0.169	0.532	-0.083	0.740*	-0.138	-0.227
Copper	-0.038	0.132	0.159	0.179	0.317	0.456
Manganese	0.145	0.125	0.038	-0.334	-0.205	-0.227
Molybdenum	0.001	0.336	-0.144	-0.429	-0.151	-0.064
Phosphorus	0.488**	0.374	0.386**	0.622	0.411	0.668*
Zinc	-0.221	0.545	0.115	-0.262	0.462	-0.330
Selenium	0.388*	0.484	0.200	0.547	0.415	0.470

[+] Bracketed are the sample frequencies for each species.
Significant level: * $P < .05$; ** $P < .01$; *** $P < .001$.

The data show that concentrations of some elements within a species were better correlated with the soil data giving significant relationships for Fe in Kikuyu grass ($P < .05$); Co in Rhodes ($P < .001$) and Kikuyu grasses ($P < .05$); P in Napier, Rhodes ($P < .01$) and natural grasses ($P < .05$); and Se in

Napier grass ($P < .05$). However, these results suggest that the selected methods remained weak in predicting availability to the species and they were still ineffective for Al, Ca, Cu, Mn, Mo and Zn. The correlation for selenium was notable given that total rather than the extractable element was measured.

8.3.3 Critical Comparisons between Extraction Methods

In order to establish whether the rejected extraction methods might have given a better prediction of soil availability of the elements to the herbages, the narrow data set for the twelve representative samples was subjected to a critical examination as summarized below.

(a) Phosphorus

The results of extractable P obtained with the four different methods and the corresponding composition of the herbages grown on these soils are presented in Table 8.3 below. The extractable soil P concentrations ranged widely irrespective of the method; they also varied from 0.41-2.91g/kg DM in the herbages given some opportunity for obtaining relationships.

The between-method correlations for soil P indicated that 95.3, 95.9, 81.6, 92.7, 90.6 and 88.8% of the variations in one method were predictable from another respectively for methods E/K, F/K, A/E, E/F, A/F and A/K. All correlations were very highly significant ($P < .001$ except for A/E: $P < .01$). However, the good agreement between methods owes much to the uniformity

Table 8.3 Test Results for Extractable Phosphorus Contrasted with Herbage-P

Sample No.	Soil pH	Extractable P in soil (mg/kg)				Herbage P ($g\ kg^{-1}$ DM)
		Method A	Method E	Method F*	Method K	
12	4.5	5	10	28	10	2.91
14	5.2	10	21	18	7	2.31
27	5.0	30	8	18	13	0.89
50	6.2	20	42	37	49	0.88
78	4.6	10	13	20	25	0.88
84	5.8	10	19	14	19	0.91
109	5.6	5	12	7	15	0.74
121	4.7	6	25	15	8	1.00
126	6.5	64	94	107	135	2.82
130	4.8	11	9	15	13	2.22
132	4.4	14	31	26	42	0.41
135	4.1	8	34	20	29	0.93

* Method chosen for batch analysis

with which they agreed on the high extractable P content of sample No. 126. At the levels traditionally associated with soil deficiency (< 20mg/kg) agreement is less close. There is therefore scope for selection of a better method for assessing the soils as sources of available P.

Correlation of the soil extraction results with the corresponding herbage concentrations gave consistently poor relationships ($P < .10$) for all methods and examination of the data in Table 8.3 shows some opposing trends. Sample numbers 12, 14 and 130 gave low extractable soil P and high herbage concentrations. By contrast, sample numbers 50 and 132 had consistently high available P in the soils but the herbages gave very low concentrations. These results suggest that no benefit could have accrued from changing the extraction method.

Although the herbage samples came from different species, it was evident that within Kikuyu and Rhodes there were poor relationships for all methods. Within soil types there was a hint of predictive value for the metamorphosed sedimentary soils but not for igneous or sedimentary soils (cf Table 3.2).

(b) Calcium

The test results for the twelve representative samples (Table 8.4) showed that Ca levels ranged widely from 0.3-16.1 me % in the soils and less so (0.85-2.31g/kg DM) in the herbages. A comparison between the methods of extraction showed that the three methods were highly correlated (ie r^2 = 76.5, 97 and 84% of variation respectively for methods A/E, A/F, E/F). The best relationship given by methods A and F was associated with high recoveries which led to the selection of method A for batch

Table 8.4. Soil Extraction Results for Calcium Compared with Herbage Calcium

Sample No.	Available Ca (me%)			Herbage Ca ($g\ kg^{-1}$ DM)
	Method A*	Method E	Method F	
12	4.0	13.9	8.3	1.13
14	3.6	4.1	3.5	2.00
27	2.5	1.3	1.8	2.31
50	11.4	12.6	16.1	1.65
78	0.6	0.35	1.6	1.28
84	4.7	5.0	7.0	0.85
109	0.7	1.4	0.5	1.47
121	0.9	0.3	1.6	1.49
126	10.9	8.6	14.7	0.92
130	2.7	3.9	5.3	1.36
132	0.3	0.8	2.0	1.93
135	2.5	1.4	4.2	1.23

* Method chosen for batch extraction

analysis (Table 3.5). The data for sample numbers 14, 27, 78 109, 121 and 132 show that the consistently low extractable Ca in soil was associated with high herbage concentrations whereas sample numbers 12 and 126 showed an opposite trend. A method which correlated poorly with the selected method (A) could have

given improved prediction of herbage Ca. However, method E which gave the poorest correlation coefficient did not predict Ca any better than method A or F in the twelve herbage samples, and was particularly inappropriate for sample 12.

(c) Aluminium

As pointed out in Chapter Seven, available Al in soil is of significance in relation to its possible influence on the availability of other elements and also its toxicity to plants. Table 8.5 compares the results of extractable Al obtained with the three test methods in relation to herbage concen- trations. The levels in the representative samples ranged from 0.06-2.42g/kg in the soils and from 0.06-0.65g/kg DM in the herbage. Comparison between the extraction procedures showed that methods B (McClean et al, 1959) and D (Black, 1965) gave the best correlation. Method A tended to extract more Al than methods B and D. However, Table 3.5 showed that the mean recoveries of all the three methods were similar.

Analysis of the data in Table 8.5 showed that Methods A and D picked up maximum available Al in sample number 12 which also had maximum herbage Al. However, Methods A (sample number 84: 0.06g/kg) and B (sample number 121: 1.80g/kg) gave extractable Al which showed opposing trends with the corresponding herbage concentrations (0.43 and 0.06g/kg DM respectively).

Table 8.5 Extraction of Available Aluminium by Various Chemical methods

Sample No.	Available Al ($g\ kg^{-1}$)			Herbage Al ($g\ kg^{-1}$ DM)
	Method A	Method B	Method D*	
12	2.42	1.32	2.10	0.65
14	1.10	0.87	0.98	0.33
27	1.00	0.65	0.71	0.22
50	1.11	0.51	0.72	0.35
78	0.79	0.99	0.66	0.22
84	0.06	0.40	0.11	0.43
109	1.61	1.02	0.98	0.22
121	1.92	1.80	1.51	0.06
126	2.14	1.62	1.13	0.29
130	2.06	0.62	0.53	0.20
132	1.72	0.45	0.57	0.35
135	1.56	0.53	0.42	0.10

* Method selected

(d) Manganese

Concentrations of extractable Mn found in the twelve representative soil samples together with the herbage levels are given in Table 8.6.

Table 8.6. Available Manganese Extracted by Different Chemical Methods in Relation to Herbage Concentration

Sample No.	Extractable Mn, me %			Herbage Mn (g kg^{-1} DM)
	Method A*	Method E	Method F	
12	1.8	2.0	2.6	0.11
14	2.7	1.1	2.2	0.34
27	1.1	0.91	1.6	0.17
50	2.5	2.9	3.1	0.23
78	2.8	3.0	4.2	0.24
84	2.5	3.3	4.6	0.30
109	0.71	1.5	2.2	0.33
121	0.36	0.24	1.9	0.21
126	2.2	3.6	3.1	0.08
130	0.43	0.31	0.54	0.42
132	0.78	0.94	1.7	0.20
135	1.2	0.80	2.4	0.23

*Method chosen for batch extraction

The correlation between the three methods gave similar relationships which accounted for 79, 76 and 86% respectively of the variance for comparisons A/E, A/F and E/F; these were associated with the comparatively uniform recoveries (Table 3.5). Neither of the unselected methods showed a better prediction of herbage concentrations than Method A and all the relationships were poor (Table 8.6). Sample numbers 12 and 126

were found by all the methods to have high extractable Mn associated with low herbage Mn. Method F gave unusually high extractable Mn in sample number 84, which was twice as high as the value given by Method A.

(e) Molybdenum

The twelve samples gave a range of Mo concentrations varying from 0.26 to 5.21mg/kg in the soils, and from 0.27 to 2.87mg/kg DM in the herbages (Table 8.7). Results presented in Table 3.5 indicated that the mean recoveries of added Mo obtained by the four methods were good. However, correlation with the herbage data gave poor relationships suggesting that the four methods were equally inappropriate for predicting availability. The data in Table 3.5 show that although the Lowe and Massey procedure (Method J) gave highest recoveries, correlation with herbage Mo (coefficient = 0.060) was far lower than that given by the ammonium acetate procedure (Method A, coefficient = 0.312) whose better reproducibility had led to its selection for batch determination. The between- method correlations, however, gave similar and highly significant relationships (accounting for 84.9, 71.2, 78.1, 89.4, 84.2 and 74.2% of variation respectively for methods A/F, A/H, F/J, H/J, A/J and F/H). Regression analysis of the data in Table 8.7 showed that sample number 84 was an outlier for all methods having exceptionally high extractable Mo levels when compared to the

Table 8.7. Soil Extractable Molybdenum in Relation to Corresponding Herbage Levels in Test Samples

Sample No.	Available Mo (mg/kg)				Herbage Mo (mg/kg DM)
	Method A*	Method F	Method H	Method J	
12	0.96	0.89	1.38	0.43	2.62
14	0.85	0.73	0.60	0.26	0.92
27	0.64	0.47	0.59	1.18	0.29
50	1.84	1.57	0.96	0.87	2.87
78	2.52	2.96	2.14	3.80	1.28
84	3.81	5.07	4.38	5.21	1.03
109	0.80	1.20	0.56	0.96	1.86
121	0.34	1.11	0.71	0.88	0.56
126	2.53	1.57	2.02	3.11	2.57
130	1.44	1.73	4.29	4.04	0.65
132	1.04	2.55	1.68	0.96	0.93
135	0.48	0.84	1.01	0.57	0.27

* Method selected for batch extraction

herbage concentrations. This discrepancy may have been influenced partly by a high soil pH (Table 8.3) and partly by plant species (eg Kikuyu grass).

(f) Zinc

Table 8.8 gives the extractable soil Zn concentrations obtained in the representative samples using the three test

methods. The available Zn varied from 0.5mg/kg in sample number 130 (Method E) to 12.4mg/kg in sample 126 (Method F).

Table 8.8. Extraction of Available zinc in Test Samples

Sample No.	Available Zn (mg/kg)			Herbage Zn (mg/kg DM)
	Method A*	Method E	Method F	
12	2.6	3.7	5.0	48
14	1.9	4.1	2.8	14
27	1.7	2.2	1.6	15
50	2.8	6.0	3.9	19
78	4.2	6.4	3.3	7
84	4.7	2.1	3.2	33
109	2.0	0.9	1.4	42
121	1.4	1.5	1.1	45
126	8.2	10.6	12.4	41
130	0.8	0.5	1.2	36
132	2.3	1.6	1.2	6
135	3.9	1.9	2.6	39

* Method selected for batch extraction

The corresponding herbage concentrations ranged widely from 6-48mg/kg DM in sample numbers 132 and 12 respectively. Correlations between the three sets of extractable Zn data gave similar relationships that were highly significant ($P < .001$).

However, agreement between sets of data was best for methods A and F. All the three methods gave one unusual observation: regression analysis showed that sample number 126 had high extractable soil Zn which was not reflected in the herbage analysis.

Although high and uniform recoveries were obtained by the three methods each gave very poor relationships with herbage concentrations indicating that the extraction methods did not provide a good index of soil availability to the plants (Table 8.8). No other method was better in predicting herbage Zn than the selected Method A. There was notable inconsistency with which the methods predicted low herbage Zn concentrations in sample numbers 14 and 132.

(g) Copper

The twelve representative soil samples had available Cu concentrations ranging from 0.15-10.02mg/kg by the three test methods (Table 8.9), while corresponding herbage Cu varied between 1.76 and 7.34mg/kg DM. The three methods were highly correlated ($P < .001$), with the ammonium acetate/mixed acid procedures (Methods A and F) giving the best relationship. However, correlation with the herbage concentrations gave poor relationships that indicated poor herbage Cu prediction, despite the good recovery data obtained for added Cu (Table 3.5).

3.5).

The three methods gave high extractable soil values for sample number 14 which were associated with low herbage Cu: this indicated the rejected soil test methods would not have done better than the selected method (A).

Table 8.9 Copper Extraction Test Results in Relation to Herbage Concentrations

Sample No.	Soil pH	Extractable Cu (mg/kg)			Herbage Cu (mg/kg DM)
		Method A	Method F*	Method G	
12	4.5	4.91	6.02	7.40	8.83
14	5.2	6.94	8.98	10.02	4.44
27	5.0	0.65	0.39	3.42	2.46
50	6.2	1.15	2.26	4.35	2.97
78	4.6	2.64	3.36	2.71	1.84
84	5.8	4.68	1.05	3.91	3.58
109	5.6	1.76	0.42	2.48	5.41
121	4.7	0.28	0.42	0.16	3.91
126	6.5	0.21	0.42	0.77	7.89
130	4.8	2.21	1.90	1.52	5.62
132	4.4	1.06	0.65	0.51	2.94
135	4.1	0.15	0.21	0.49	6.21

* Method selected for batch extraction

(h) Iron

The results of available Fe estimated by the three extraction methods showed that the concentrations ranged from 0.13-2.39g/kg (Table 8.10).

Table 8.10 Extractable Iron in Relation to Corresponding Herbage Concentrations

Sample No.	pH of Soil	Extractable Fe ($g\ kg^{-1}$)			Herbage Fe ($g\ kg^{-1}$ DM)
		Method B*	Method C	Method F	
12	4.5	0.13	0.21	0.48	0.35
14	5.2	0.35	0.18	0.72	0.40
27	5.0	0.49	0.61	0.63	0.24
50	6.2	0.34	0.32	0.89	0.45
78	4.6	0.46	0.39	0.79	0.19
84	5.8	0.63	0.38	0.32	0.38
109	5.6	0.56	0.40	0.82	0.20
121	4.7	1.98	1.75	2.39	0.03
126	6.5	0.47	0.33	0.86	0.38
130	4.8	1.79	1.60	1.84	0.24
132	4.4	0.41	0.36	0.59	0.25
135	4.1	2.22	1.38	1.88	0.04

*Method selected

Although variable recoveries were obtained (Table 3.5), the three test procedures were highly correlated ($P < .001$) and gave similar relationships for extractable Fe in the soils. Methods

B and C gave the best correlation; Method F gave high recoveries but poor reproducibilities between duplicate determinations as did Method C.

The soil-plant correlation tests gave strong, negative relationships (coefficients -0.720 to -0.761) with each extraction method, suggesting that they were totally ineffective in predicting herbage Fe; neither Methods C nor F could have predicated better than Method B, the selected procedure.

8.4 DISCUSSION

Tables 8.1 and 8.2 indicated that the selected soil extraction methods gave poor relationships with herbage concentrations between and within herbage species, suggesting that available soil composition cannot provide good prediction of herbage content for any of the elements under the field conditions in W. Kenya. A critical comparison of the data in Tables 8.3-8.10 showed that the rejected soil extraction methods would not have done better, suggesting that recovery and/or reproducibility between replicates of an added element as a simple inorganic salt is a poor criterion for choosing a particular extraction method.

The failure to obtain relationships between mineral status of soil and herbage may be attributed to a number of reasons: (a) influence of differences in soil pH, organic matter content and drainage on the mineral uptake by plants; (b) effects of

microbiological processes on mineral availability in different soil types; (c) soil contamination of the sampled herbages; (d) influence of soil adsorption properties (e) antagonisms between elements both nutritionally in the plant, and at the soil solution-root interface and (f) unbalanced sampling for the method selection exercise.

(a) Differences in Soil pH, Organic Matter and Drainage

The total quantity of any nutrient absorbed by a plant should be primarily determined by its availability in the soil. Availability to the plant is influenced by the pH, organic matter content and drainage characteristics of the soil. In general increasing soil pH strongly increases the rates of absorption of minerals such as Mo (Stout et al, 1951; Loneragan, 1975) but decreases those of certain metals eg Co (Robson and Loneragan, 1970). Wide, pH dependent variations in mineral uptake by plants have been reported (Fried and Broeshart, 1967; Mitchell and Burridge, 1979) and soils low in pH have been indicated to be high in extractable Al, Fe and Mn (Islam and Elahi, 1954; Weir and Miller, 1961).

The data in Appendix IV show that soils collected in this survey were generally strongly to moderately acidic, but gave wide pH differences (mean 5.1, range 4.1-7.0) across the sites. Correlation of the elemental concentrations in soil herbage with soil pH showed that pH affected in vitro and plant availability differently (Table 8.11).

Table 8.11. Correlation of Batch Elemental Concentrations in Soil and Herbage with soil pH.

Element	Correlation Coefficient[+]	
	Soil Availability/ soil pH	Herbage Concentrations/ soil pH
Molybdenum	0.055	0.337***
Iron	-0.210*	0.033
Aluminium	-0.223**	-0.015
Manganese	0.069	-0.315***
Copper	-0.005	0.035
Zinc	0.389***	0.001
Calcium	0.424***	-0.007
Phosphorus	0.391***	0.280**
Selenium	0.077	0.029
Cobalt	-0.103	0.019

[+] Significance Level; * $P < .05$; ** $P < .01$; *** $P < .00$ 1

Increasing soil pH was associated with an increase in available Zn, Ca, and P ($P < .001$) but a decrease in Fe ($P < .05$) Al ($P < .01$) and no effect on Mo or Mn in the soil. By contrast, herbage Fe, Ca, Zn and Al gave poor relationships with soil pH (Table 8.11) but significant relationships were found with herbage Mo and Mn. Molybdenum in the herbages increased,

while Mn decreased with increasing soil pH ($P < .001$); apart from herbage P ($P < .01$) no relationships were found with soil pH for other herbage elements. The poor relationships are contrary to findings in earlier reports (Stout et al 1951; Robson and Loneragan, 1970; Loneragan, 1975) and could be related to confounding factors such as species differences. However, a more likely reason is that the extractant changed the pH of the soil and that the masking effect was enhanced by the extreme (acidic) condition of the soils.

The reactions of mineral elements with soil organic matter and the consequent effect on availability to plants have been reviewed by Schnitzer and Khan (1972). The reactions include ion exchange, chelation and complex formation. For example, chelation and complexation between trace metal ions and organic matter through carboxyl, phenolic hydroxyl and possibly carbonyl and amino functional groups can yield very strong compounds stable up to pH 8 (Al^{3+}), pH 9 (Fe^{3+}) and pH 10 (Cu^{2+}). Fulvic acid present in the soil organic matter also competes strongly for several metals. The strong binding of metals to chelants and complexing agents in soil solution greatly decreases availability *in vivo* and over estimations of availability *in vitro* may arise because extractants break up the unavailable, complexed or chelated forms (Loneragan, 1975). Studies on the influence of drainage conditions (Burridge et al, 1983) on availability of soil minerals have shown that soil drainage can

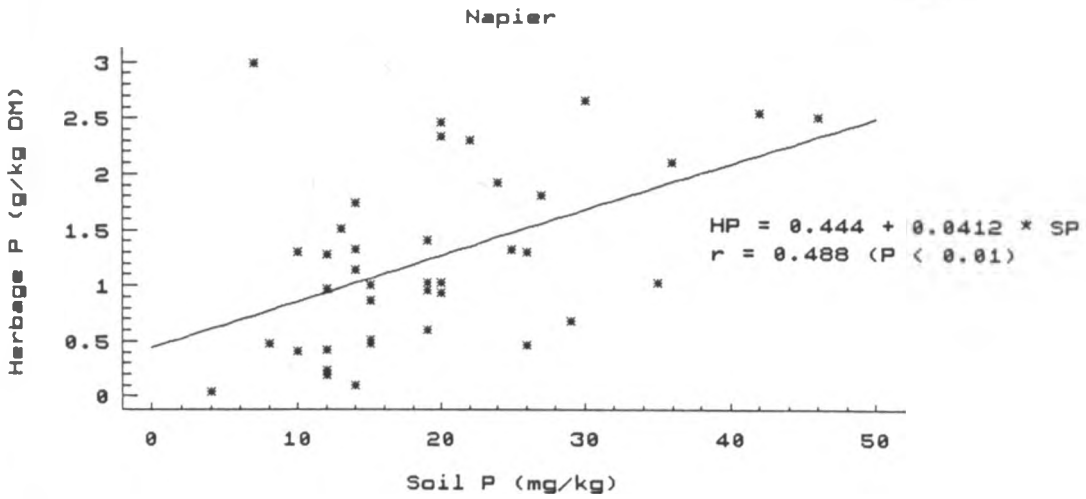
have large effects on the amounts of Co extracted from cultivated soils (Mitchell et al 1957; Berrow and Mitchell, 1980; Berrow et al, 1983). Pedological drainage conditions must be taken into account in the interpretation of extractable soil Co whose uptake by plants is higher in poorly- than freely-drained soils. All soils are completely drained of moisture by the drying process prior to *in vitro* extraction. Poor correlations between extractable soil elements and herbage concentrations may be therefore attributed to differential influences of soil drainage conditions.

(b) Species Differences

Species differences may have weakened the associations between soil and herbage mineral composition in the 'twelve sample exercise'. However, only in the case of P was there substantive evidence (Fig. 8.2).

Table 8.12 summarises the P regression data obtained for the well-represented species using data in Appendix III; it shows that differences lie in the slopes of the relationships and intercepts, with natural grasses having the greatest capacity to utilize "available" soil P and Kikuyu grass the least. The high intercept for Kikuyu grass may reflect the leafy stage at which this species was sampled. The relationship for Nandi setaria is greatly improved by dropping one outlier and if confirmed in a larger population, this plant might be the most reliable species to indicate availability.

Fig. 8.2 Relationship between Herbage and Soil Phosphorus for the Major Species.



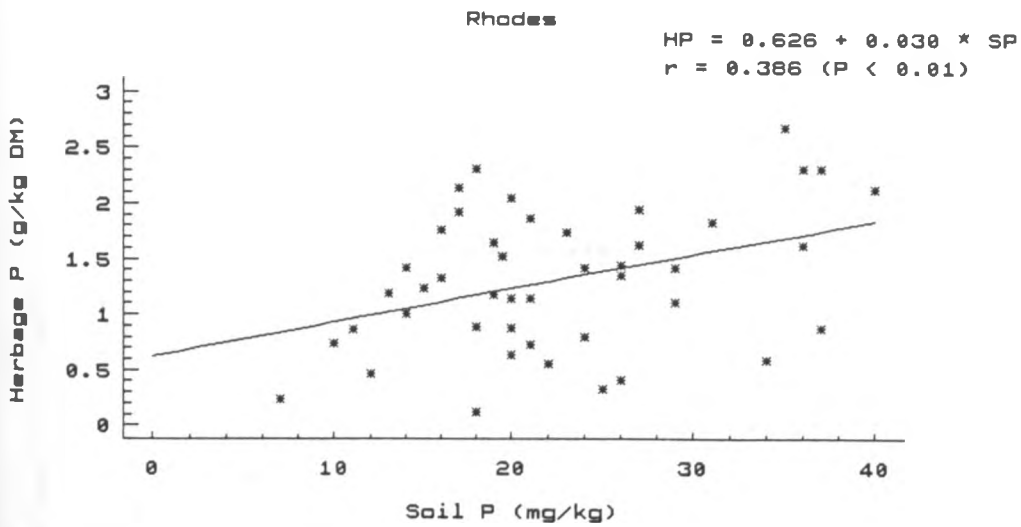
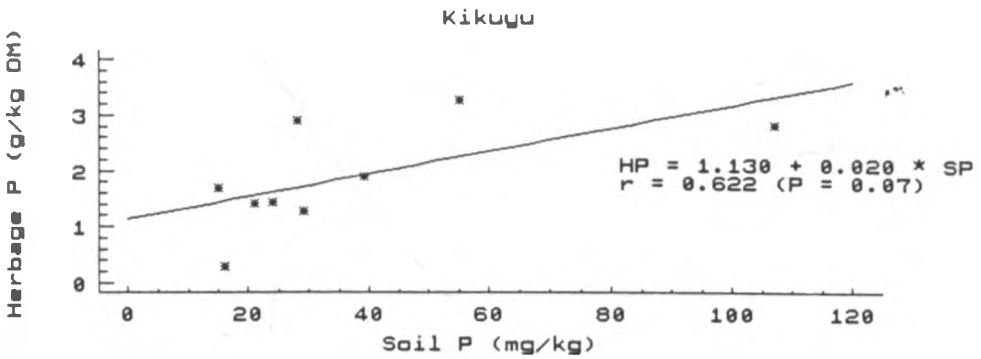
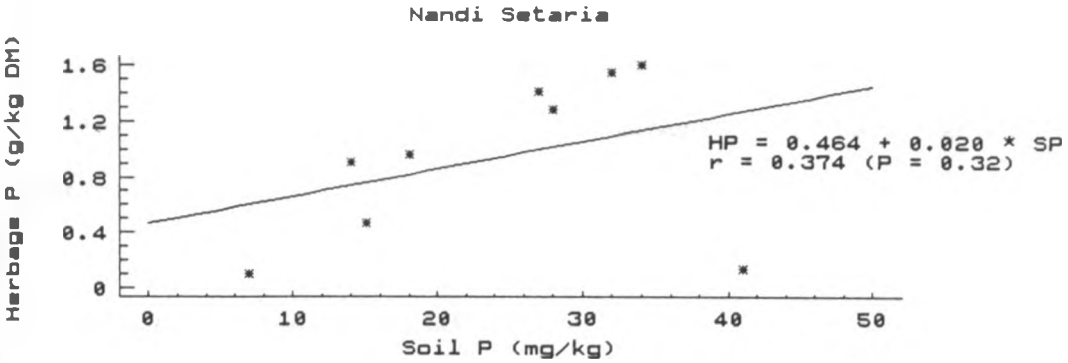


Fig. 8.2 (cont.)


Kikuyu
Herbage P (g/kg DM)
HP = 1.130 + 0.020 * SP
r = 0.622 (P = 0.07)
Soil P (mg/kg)


Nandi Setaria
Herbage P (g/kg DM)
HP = 0.464 + 0.020 * SP
r = 0.374 (P = 0.32)
Soil P (mg/kg)

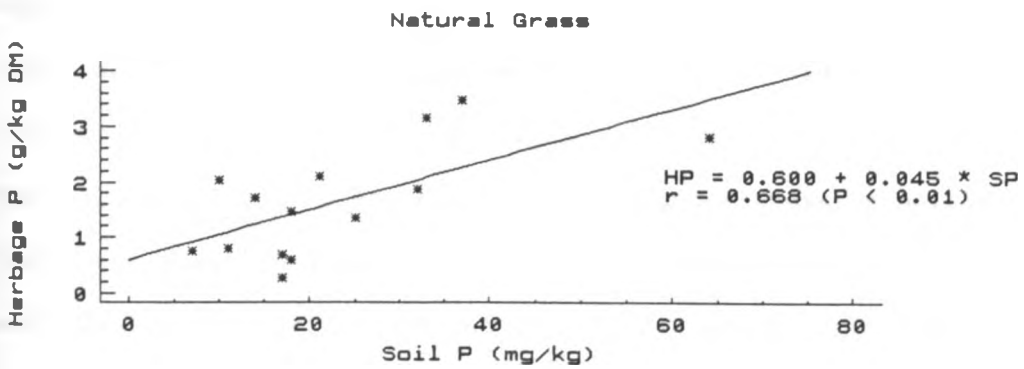

Natural Grass
Herbage P (g/kg DM)
HP = 0.600 + 0.045 * SP
r = 0.668 (P < 0.01)
Soil P (mg/kg)

Table 8.12 Regression Equations Relating Herbage-P to Extractable Soil-P in Different Species.

Species	Constant (Intercept ± SD)	Coefficient (Slope ± SD)	Degrees of Freedom
Napier	0.444 ± 0.259	0.041 ± 0.0121	37
Rhodes	0.626 ± 0.263	0.030 ± 0.0111	43
Natural grass	0.600 ± 0.395	0.045 ± 0.0145	12
Kikuyu	1.133 ± 0.448	0.020 ± 0.0097	7
Nandi setaria	0.464 ± 0.482	0.0197 ± 0.018	7

For other elements, there was little evidence that even within species an alternative extraction method would have improved predictions.

(c) Microbiological Processes

Soils abound in microbes and the surfaces of the soil and of rocks in humid environments are rich in fungi, algae and lichen associations. Microbiological processes beneath the surface of the soil are most intense in the rhizosphere of growing plants, where the exudation of organic species including carbohydrates, amino acids and chelating species by the root provides nutrients for microbial growth (Loneragan, 1975; West, 1981). Metabolic products of bacterial colonies under these conditions have been identified as 2-oxogluconic acid and oxalic acid (Duff and Webley, 1959; Duff et al, 1963) which are powerful

Ca chelants. 2-Oxogluconic acid is capable of attacking and solubilizing many Ca-based minerals and in the process releasing such plant nutrients as phosphate and trace elements. Absorption by the plant may be favoured by microbial activity depending on the pH of the soil and the plant species and this could influence soil:plant elemental relationships. Since air drying would restrict microbial activity, an *in vitro* assessment of a dried soil by chemical extraction methods may not reflect "true" mineral availability under these conditions.

(d) Soil Contamination of Collected Herbage

Under Kenyan conditions, most of the factors that influence soil contamination and ingestion by animals (Chapter Two) prevail and herbages may be contaminated all year round. High temperatures and wind blow during the dry months promote weakening of soil structures making them susceptible to wind erosion and transfer, while during the wet season, rain splash and trampling by animals contribute greatly to contamination of herbage. Techniques for estimating soil contamination of ingested herbage include determination of an acid insoluble residue (AIR) (Mayland et al 1977; McGrath et al 1982) and measurement of titanium and aluminium contents (Millar et al, 1985) in faeces. All these procedures rely on high soil-to-plant concentration ratios for the analyte coupled with non-absorbability in the animal.

Analysis of animal faeces was not considered appropriate in the present study but estimation of soil contamination may be predicted from the Al, Fe and Co data for herbage. Correlation analysis on the data in Appendix III showed that herbage Co increased as both Al and Fe increased ($P < .001$) (Figures 8.3 and 8.4). These results suggest there was possibility of soil contamination of the herbage.

When herbage is analyzed the whole and not just the extractable component of any mineral contamination from soil is attributed to the herbage, potentially distorting the available soil v herbage relationship.

(e) Adsorptive Properties of Soils

The concentration of ionic species in solution is governed by the solubility product principle. However, this process is not always obeyed. In many situations in the soil, pure phases are not formed or the concentrations of the ions are not high enough to reach the solubility product. Under these conditions adsorption equilibria govern the ionic concentrations. Detailed kinetic studies show that this is likely to be the case for elements such as Co, Zn, Mo, Cu, Se and Fe (Quirk and Posner, 1975).

In acid soils, adsorption can affect plant nutrient availability. Nutrient anions such as sulphate, phosphate and molybdate have been found to be adsorbed by mineral surfaces giving rise to poor plant uptakes; measurements of the relative

Fig. 8.3 Relationship between Herbage Cobalt and Herbage Iron

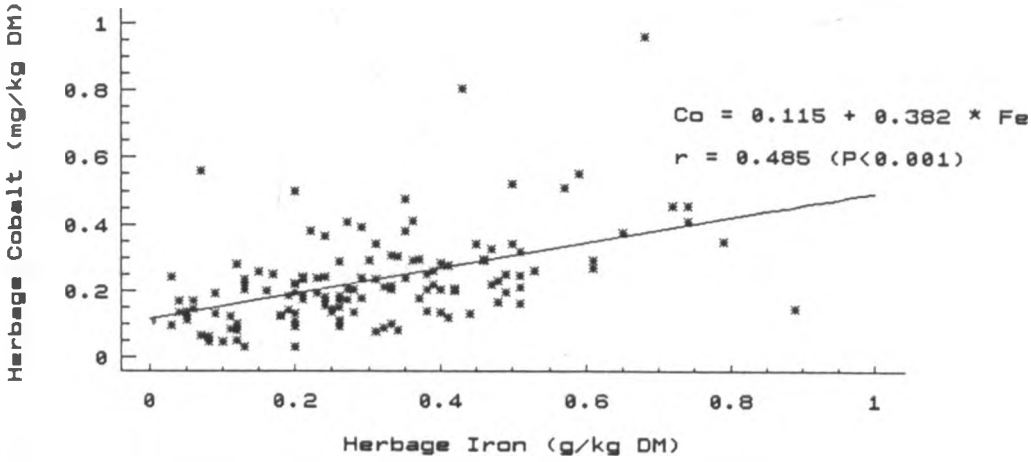
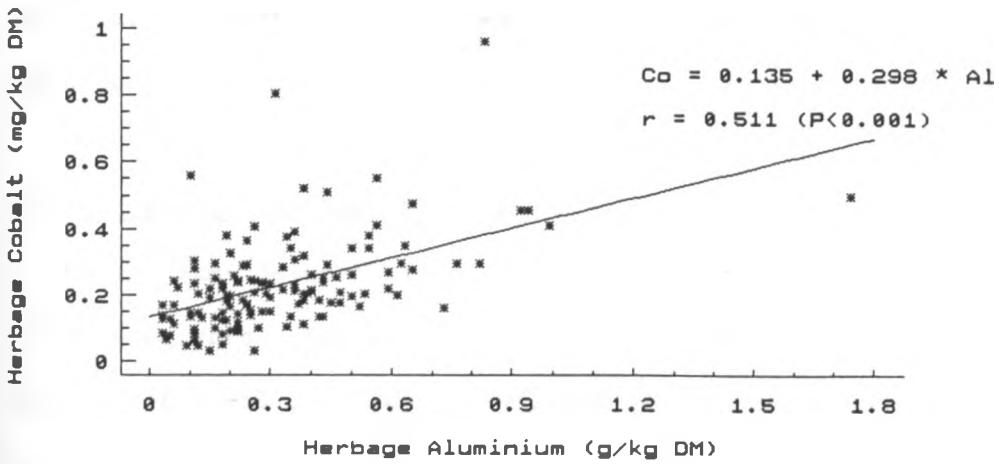


Fig. 8.4 Relationship between Herbage Cobalt and Herbage Aluminium



adsorbing ability of different soils have been of value in predicting anion availability and have contributed to the understanding of the role of soil extractants for predicting plant composition. Differences in the adsorptive capacity of soil colloids can lead to wide variations in the estimated mineral availabilities especially of Mo, Cu and P. The situation may be aggravated by the confounding effects of soil pH. For example, the behaviour of Mo in soil solutions is largely controlled by the reaction of its oxy-anions with Fe oxides. These reactions are very sensitive to pH, being strongest at pH values close to the pKa values for the dissociation of the acid: thus the adsorption of Mo is strongest around pH 4 and decreases with increasing pH (Quirk and Posner, 1975). Hence, changing soil pH frequently induces large changes in the absorption of trace elements. In these cases the dominant processes involved appear to be those of adsorption of trace elements to soil colloids. Poor soil-plant relationships may partly arise where extractants unnaturally disturb adsorption equilibria.

(f) Antagonisms Between Elements

Antagonisms between elements in plants have been reported by several workers. For example, Millikan (1947; 1948), Anderson and Arnot (1953), Mulder (1954) and Geyloff et al (1959) have reported interactions between Fe, Mn and Mo, and have shown that

high concentrations of one can accentuate the deficiency of another in plants. These interactions may have a bearing on the soil-plant relationships. Data in Appendix III showed that herbage Mn decreased as Mo increased ($P < .001$) but increased with Fe and Al ($P < .001$). These data suggest that the high concentrations of Fe and Mn relative to those of Mo may lead to inconsistency in soil-herbage relationships through mutual antagonisms within the plants as well as competition at the site of absorption in the soil solution. The Fe/Mn relationship is contrary to that depicted in Figure 5.1, exacerbating a strong species effect.

(g) Lack of variance

For some elements especially Mo, the data set gave narrow working ranges that prejudiced the detection of significant relationships. The poor performance of the extraction methods could simply be attributed to lack of variation between samples. Samples with wider concentration ranges may have provided a better test of some extraction methods.

8.5 CONCLUSIONS

Results presented in this chapter have shown that soil availability in vitro may not be a good predictor of herbage composition in the survey area. The poor soil-herbage relationships were probably attributable to inconsistencies in the way in which extraction methods simulated soil conditions at

the root surface and results might have been better if the extractants maintained a pH as close as possible to that of the soil.

CHAPTER NINE

INTEGRATING DISCUSSION

Minerals and their functions in the animal become of practical and economical importance only when a deficiency, an excess or an imbalance is reflected in deviating animal behaviour, a lower rate of production, a recognizable disease syndrome and a higher than normal rate of mortality. Having considered the mineral content of pastures as an aid to the diagnosis of deficiencies or excesses which may affect grazing animals and shown that the grazing animal is the focal point in a complex ecological system, it is essential to consider the strategies which might now be used to improve animal production. No reliable statistics are available from which to assess the economic losses caused annually in Kenya or elsewhere by mineral deficiency diseases in farm livestock. However, data such as those provided by annual FAO/WHO Reports on Animal Health clearly indicate that the circumstances leading to the development of mineral deficiency diseases are neither effectively recognized nor anticipated. Although used principally as guides for the formulation of mixed feeds, the ARC (1980) estimates of mineral requirement of farm livestock did provide a basis for the interpretation of data on dietary composition, and depicted areas of suspected nutritional deficiency. McDowell et al (1984) used similar techniques to show the likely prevalence of mineral deficiencies in Latin America.

Results presented and discussed in detail in this thesis show that major problems related to P, Cu, Se, Ca and to a lesser

extent S, Mg, Co and Zn deficiencies are anticipated in the survey area of W. Kenya. Attention was also focussed in the variable but high levels of Al, Fe and Mn found in the herbages which may be toxic by way of antagonism of the already deficient bioessential elements thereby augmenting the size of the mineral imbalance problem.

The danger of relying on unsubstantiated standards of adequacy in the pasture and even in the animal is illustrated by the attempts to define the cause of a wasting disease of Colombian cattle called 'Secadera'. Because of multiple departures from text book standards 'Secadera' has been described as "primarily an induced thiamine deficiency, complicated in some geographic areas by deficiency or imbalance of Zn, Cu and less frequently, Co and Se" (McDowell et al, 1985). Further studies implied that herbage S might be limiting and that the bone indices of Ca and P status were generally low (McDowell et al, 1985). The disease was controlled by feeding a 'complete mineral mixture' with additional amounts of Zn, S, Cu and Se (Miles and McDowell, 1983, cited by McDowell et al, 1984). 'Secadera' is probably a general term for ill-thrift which can arise from various minor nutrient deficiencies, often singly, occasionally in combination but rarely in the abundance indicated by comparison with tables of 'critical' concentrations of nutrients in herbage (Suttle, 1987).

The 'Secadera' example signifies that estimates of requirements such as those of ARC (1980) are basically useful guides and the analysis of soil and pasture samples for mineral

elements is just a starting point or first step in the investigation of mineral imbalances in livestock.

The outstanding questions are first, how can the risks of deficiency be confirmed in animals and second, how can they be controlled.

(a) Assessing the Mineral Status of Grazing Animals

It is suggested that as a follow-up of this work, further information should be obtained from analyses of blood, tissues and organs of a sufficient number of animals, especially cattle, representative of those on farms with anticipated mineral imbalance problems. Each selected farm should be able to provide about 10% (minimum ten animals) of the total herd or flock for the task. Use of whole blood GSH-Px is recommended for Se diagnosis while plasma concentrations of P, Ca, Mg and Cu, and of vitamin B12 for Co (Suttle, 1986a) will provide useful information for assessing the status of these elements in the animals. Additionally, the assay of plasma MMA (Suttle et al, 1986a) will be a useful guide in predicting the prevalence of functional Co deficiency on the selected farms. The determination of liver Cu, Se and vitamin B12 in samples retrieved from abattoir surveys will also be informative.

Results presented in Chapter Eight indicated poor correlations between the extractable soil data and herbage elemental concentrations even within species. Attempts to improve upon the selected procedures were fruitless since none

of the rejected soil extraction methods did any better. Experiments in Scotland (N.F. Suttle, personal communication) have shown plasma vitamin B12 to be better correlated with extractable soil Co than with total herbage Co. It would therefore be worthwhile assessing the direct soil-animal relationship by correlating the soil test results and blood or tissue analysis data. The animal may provide an integrated measure of what the soil-herbage association has delivered over the season whereas a single herbage sample may be unduly influenced by the various factors discussed earlier (eg soil contamination, maturity).

(b) Assessing Responses to Supplementation

Armed with information on the mineral content of animal blood and tissues, diagnostic dosing trials can then be laid down in order to test the response of grazing animals to appropriate mineral supplements. It is suggested that a mineral-supplemented energy and protein concentrate be used for testing responses to Ca, P and Mg; while Cu oxide needles, Se and Co bullets are recommended for assessing Cu, Se and Co deficiency. Supplementation should start prior to pregnancy and records kept of reproductive performance and growth rate of young stock using group sizes of 20 or more. All elements should be given together and if performance is improved, individual elements

should then be removed on the basis of blood or tissue analysis which should indicate the elements most likely to be limiting and lead to specific diagnoses.

(c) Focussing on Problem Areas

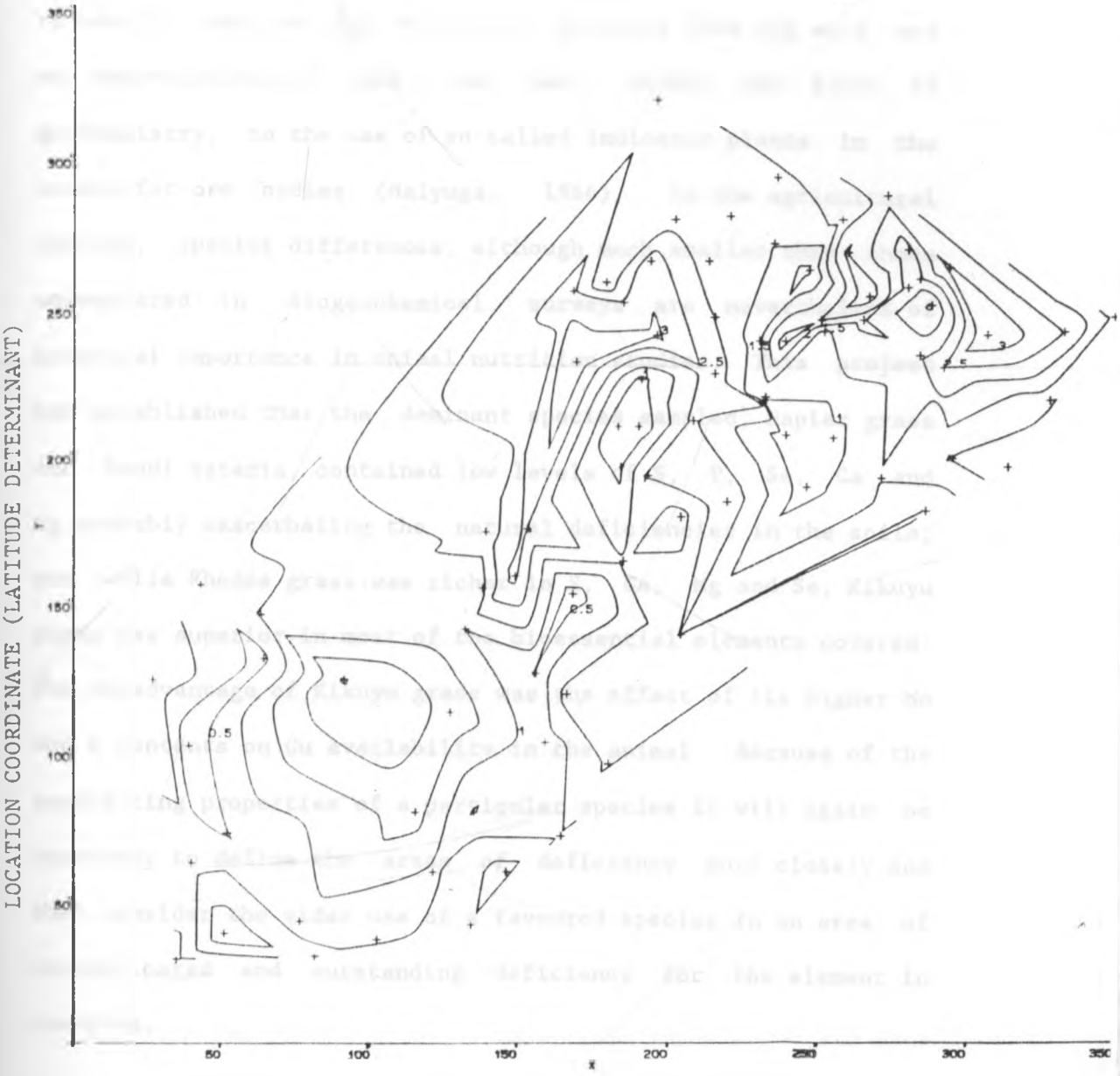
The part of Kenya involved in this study was so large and the potential problems so numerous that there is a need to establish priorities. The results presented in this thesis show that both altitude and geology are important variables affecting mineral composition of herbage, and may influence the extent to which deficiency problems occur in Western Kenya. Variability in the concentrations of the bioessential elements was associated with areas of higher rainfall and high altitudes and sites overlying igneous granite and alluvial deposit bedrocks. Notional deficiencies of Ca, P, Mg, Cu and Se were common in these circumstances. This observation is generally consistent with findings in other parts of the world such as Australia (Gardiner, 1962; Gardiner and Gorman, 1986; Reuter, 1975a,b; Langlands et al, 1981b), and New Zealand (Davies and Watkinson, 1966a). The follow-up assessment of mineral status in animals and response to supplementation should therefore be concentrated in areas situated at the extreme altitudes for Se. Areas below 7000ft ASL should be investigated for Ca, P, S, Mg, Cu and Zn deficiencies, while research on farms overlying igneous granites, alluvial deposits as well as bedrocks whose soils have pH < 5.0 will provide additional information on Se status.

Application of the contour-plot technique (Section 3.10) to map the diversity of pasture mineral status in the survey region was not useful in identifying areas with anticipated mineral imbalance problems. The Mo example given in Figure 9 illustrated this point. The failure may however be attributed to the narrow range of values obtained, both for the herbages and for the soils. Furthermore, the distribution of sample sites was not ideally suited to contour-plotting, which requires even and frequent distribution in two dimensions. The mapping technique may be worth further consideration.

(d) Remedial practices

Once the farms and areas with anticipated problems have been confirmed and the extent of the problem established, appropriate management techniques for providing minerals to grazing animals may then be employed, such as liming to raise soil pH, using mineral-containing fertilizers on pasture fields (Watkinson, 1983), encouraging growth of particular species (eg Rhodes grass), and using 'free choice, complete' mineral supplements (Cunha et al, 1964). Soil pH increases may impose further limitations (Chapter Six) and care should be taken especially with regard to the intensity of liming in relation to herbage molybdenum and cobalt concentrations. However, alterations of soil pH are likely to benefit the farmer from the point of view of increasing available Ca, P, Mg and Se by reducing the interactive effects of Fe and Al.

Fig. 9 Contour plot of herbage molybdenum in Relation to Position of Sampled Sites(+)*



*In this figure, the programme plotter (section 3.10) first assigned each site with the extractable soil Mo concentration (Appendix IV). Sites with the same concentration were then linked by a "contour" to give a zonal picture using identification coordinates described in Table A.1 and listed in Appendix V. Thus the figure depicts soil Mo contours superimposed on the site location map of Fig. 6.1. Similar figures were obtained for all the other elements analyzed but did not yield useful information. However, the technique should be accorded further investigation.

Exploitation of species differences requires particular attention. Species differences in mineral concentrations are related to their ability to obtain minerals from the soil, and an appreciation of this has led, within the field of geochemistry, to the use of so-called indicator plants in the search for ore bodies (Malyuga, 1964). In the agricultural context, species differences, although much smaller than those encountered in biogeochemical surveys are nevertheless of potential importance in animal nutrition studies. This project has established that the dominant species sampled, Napier grass and Nandi setaria, contained low levels of S, P, Se, Ca and Mg probably exacerbating the natural deficiencies in the soils; and while Rhodes grass was richer in S, Ca, Mg and Se, Kikuyu grass was superior in most of the bioessential elements covered. The disadvantage of Kikuyu grass was the effect of its higher Mo and S contents on Cu availability in the animal. Because of the conflicting properties of a particular species it will again be necessary to define the areas of deficiency more closely and then consider the wider use of a favoured species in an area of uncomplicated and outstanding deficiency for the element in question.

(e) Comprehensive Cover

Most of the past studies in Kenya can be criticized for the fact that not all the minerals occurring in traces in plants were studied. In some cases this was due to lack of chemical

methods suitable for the routine screening of large numbers of samples, especially for Cu, Mo, Co and Se determination. These elements present most problems in chemical analysis because they occur at very low concentrations in most plants and soils. Attempts to overcome these problems were made in this thesis, and sensitive and reproducible procedures were standardized (Chapter Three) for future applications and continuity of mineral research in livestock in Kenya.

(f) Conclusion

In summing up the proceedings of the International Symposium on Herbivore Nutrition in the Subtropics and Tropics, Balch and Moir (1983) pointed out that problems of herbivores living in subtropical and tropical climates are certainly increased by the effect of such climates on the mineral content and availability of herbages. This thesis shows that such problems may have increased. Further intensification of farming systems in the survey area may inevitably lead to further subdivisioning. This will make it even more difficult for grazing animals to select a nutritionally adequate diet. An increase in establishment and use of improved pastures as single species stands and the fencing used to restrict stock to the new pastures will continue to result in farmers unwittingly making it difficult for grazing animals to select a diet balanced in respect of minerals. Furthermore, as subdivisions become smaller, opportunities for

diet selection are also reduced, particularly if the subdivisions are made according to soil type and location.

In future investigations a co-ordinated effort to farther the understanding of the soil-plant-animal relationship under the prevailing conditions is necessary. Diagnosis of mineral imbalances in the field is needed at the animal biochemical level, and will require much in common with modern detective work, where a team of specialists in different fields combine their efforts before a particular criminal (or element) can be accused (ISNAR, 1985). In W. Kenya, the task of identifying the minerals which limit animal performance and the extent of their influence has simply been begun by this study.

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APPENDIX I.

LOCATION AND DESCRIPTION OF SAMPLED SITES:

(a) KEY/ABBREVIATIONS

T: Topography of Landscape described as

1. Uniform, gentle slope
2. Gradational
3. Contrasting or Undulating

R: Guide route: c/o Divisional Veterinary Officer:

A. Saboti Division, Trans Nzoia District
B. Kwanza Division, Trans Nzoia District
C. Cherengani Division, Trans Nzoia District
D. Webuye Division, Bungoma District
E. Kanduyi Division, Bungoma District
F. Kimilili Division, Bungoma District
G. Sirisia Division, Bungoma District
H. Tongareni Division, Bungoma District

(b) Location of Farms on Area Maps

Sampled sites can be located as grid points on area maps by extrapolation from the x and y coordinates of each site given in Appendix III. General bearings can be obtained from grid points for five sites at the extremes of the sampled area given in Table A.1. From the x and y coordinates of these sites it can be calculated that 6 units of x or y are equivalent to 1'.

Table A.1. Representative Grid Points for Location of Sites

Farm No.	Sample/ Site No.	Coordinates		Location (Grid point)*	
		x	y	Longitude	Latitude
26	46	196	319	34° 53'E	1° 15'N
49	88	350	246	35°16'E	1° 3'N
63	111	35	29	34° 23'E	0° 43'N
73	124	27	123	34° 22'E	0° 43'N
78	129	168	152	34° 46'E	0° 48'N

* Grid points were accurately determined using area map of scale 1:125,000.

(c) Table A.2 General description of the sampled sites

Farm No.	Location*	R	T	Sample No.	Grass Species
01	Garsen's Farm St. Joseph's, Kitale	A	1	01:	Napier grass
02	Strathyre Farm Endebess	A	1	02:	Rhodes grass
03	Mayer's Farm Kiminini	A	3	03:	Rhodes grass
				04:	Rhodes/star grass mixture
04	N. Mungai's Farm Kiminini	A	2	05:	Rhodes grass + sporobolus isolations
			2	06:	Rhodes grass
			1	07:	Rhodes grass + sporobolus isolations
05	Isaiah's Farm Bukwet, Sikhendu	A	1	08:	Napier Grass
				09:	Rhodes grass + Nandi setaria 50:50
06	Tiot Luget Farm Machewa	A	3	10:	Natural grass dominated by Kikuyu + star grasses
07	Nthenge's Farm Getuamba	A	2	11:	Rhodes grass
				12:	Kikuyu grass
08	Kasoya Estate Farm	A	1	13:	Rhodes grass
			2	14:	Rhodes grass
09	Luri Farm Kitale	A	2	15:	Natural pasture dominated by Kikuyu grass

* Dairy farming was the predominant livestock activity recorded. Area of sampled site varied from 10 to > 6000 acres.

Farm No.	Location*	R	T	Sample No.	Grass Species
10	Manor House Agric. Centre	A	2	16:	Natural grass
				17:	Napier grass
			1	18:	Napier grass
11	Ol Ngatongo ADC Farm	A	1	19:	Rhodes grass
				20:	Rhodes grass + Nandi setaria 50:50
				21:	Nandi setaria
12	Chebelion's Farm Endebess	A	2	22:	Rhodes grass
13	Mill's Farm Kinyoro	A	2	23:	Rhodes grass
				24:	Nandi setaria
14	T. Legemet Farm Saboti	A	3	25:	Natural grass
15	J. Muliro Farm Saboti	A	2	26:	Nandi setaria
				27:	Rhodes grass
				28:	Napier grass
16	Njenga's Farm Wamuini	A	1	29:	Napier grass
				30:	Nandi setaria
17	J. Musundi Farm Tulwet	A	2	31:	Napier grass
				32:	Kikuyu grass
18	Dr. Kimengech's Farm, Mitoni Mitatu	A	1	33:	Rhodes grass
19	Wainaina's Farm Moi's Bridge	A	1	34:	Rhodes grass
20	J. Ngambo Farm Sisal Estate Moi's Bridge	A	1	35:	Napier grass
				36:	Rhodes grass
21	Kimoson Farm Endebess	B	1	37:	Rhodes grass
22	Adamba's Farm Endebess	B	1	38:	Rhodes grass
23	Corner Farm Endebess	B	3	39:	Natural pasture dominated by Kikuyu grass

Farm No.	Location*	R	T	Sample No.	Grass Species
24	Chemeron's Farm Kipsogon	B	3	40:	Rhodes grass
				41:	Nandi setaria
				42:	Natural grass dominated by Kikuyu grass
				43:	Rhodes/Nandi setaria mixture
				44:	Napier grass
25	Zea ADC Farm	B	1	45:	Rhodes grass
26	Japata ADC Farm	B	2	46:	Rhodes grass
27	Kasarate Farm Kapomboi	B	3	47:	Natural grass
28	Katuke ADC Farm Kapomboi	B	2	48:	Napier grass
			1	49:	Sudan grass
			2	50:	Rhodes grass
29	Gidea Farm Kobos	B	2	51:	Rhodes grass
30	Gongwe Farm	B	3	52:	Natural grass
31	Kirita Farm	B	3	53:	Rhodes grass
32	Lunyu Farm	B	2	54:	Rhodes grass
33	Asega's Farm	B	2	55:	Napier grass
				56:	Natural grass dominated by Kikuyu grass
34	Shishi Farm	B	3	57:	Nandi setaria
				58:	Rhodes grass
				59:	Napier grass
35	Tonje Farm Bidii Complex (East)	A	2	60:	Nandi setaria
				61:	Napier grass
				62:	Rhodes grass
36	Bridge Dairy Farm Bidii Complex (North)	A	2	63:	Rhodes grass
			1	64:	Rhodes grass
			1	65:	Napier grass
37	Koitogos Farm (W. Koech)	C	2	66:	Rhodes grass
38	Tongi Farm Kipsaina Scheme	C	2	67:	Napier grass
				68:	Rhodes grass

Farm No.	Location*	R	T	Sample No.	Grass Species
39	Sinyerere Scheme (Otsianda's Farm)	C	2	69: 70: 71:	Nandi setaria Kikuyu grass Napier grass
40	Wambwa's Farm Cherengani	C	2	72: 73:	Rhodes grass Napier grass
41	Karamoja Farm Kapsara, Sinyerere	C	1	74: 75:	Rhodes + Nandi setaria mixture 50:50 Napier grass
42	Mulweye Farm Suwerwa Scheme	C	2	76: 77:	Napier grass Rhodes grass
43	J. Yano Farm Suwerwa Scheme Plot no. 88	C	2	78:	Rhodes grass
44	Sorongai Farm (Dr. Korir's Farm)	C	1	79:	Rhodes grass
45	Mutwot Farm Kachibora	C	3	80: 81: 82:	Natural grass dominated by Kikuyu grass Rhodes grass Napier grass
46	Ngonyeki Farm Cherengani	C	3	83: 84:	Rhodes/Nandi setaria mixture Nandi setaria 50:50
47	T. Malel Farm Sirende	C	2	85:	Rhodes grass
48	Karara Farm	C	3	86: 87:	Rhodes grass Napier grass
49	W. Kibor Farm Top Suwerwa Kapcherop	C	3	88:	Natural grass
50	Jabali ADC Farm	C	3	89: 90:	Rhodes grass Lucerne
51	Jensen's Farm Endebess	A	1	91:	Rhodes/Ndi setaria mixture 50:50

Farm No.	Location*	R	T	Sample No.	Grass Species
52	Bidii Farm Central (C. Odari), Kitale Water Supply Point	A	1	92:	Rhodes grass
53	D. Nasokho Farm Webuye Plains	D	1	93:	Rhodes grass
				94:	Napier grass
54	S. Ngoya Farm Lugulu	D	1	95:	Natural grass
55	G. Muyekhu Plot Misikhu West	D	2	96:	Natural grass
			1	97:	Napier grass
56	W. Sioni Plot Misikhu East	D	1	98:	Natural grass
			1	99:	Sweet potato vines
			2	100:	Napier grass
57	R. Wasike Farm	D	2	101:	Rhodes/Nandi setaria mixture
58	J. Mongou Farm Kapsakwany	F	2	102:	Rhodes/Nandi setaria mixture
				103:	Napier grass
59	Chemoge Farm Kapsakwany	F	2	104:	Kikuyu grass
				105:	Napier grass
60	A. Chemoyiek Farm Kipchiria	F	2	106:	Kikuyu grass
61	Khakina Farm Bungoma	E	1	107:	Rhodes grass
				108:	Napier grass
62	Chrysostin Mini Farm, Kimaeti	E	1	109:	Natural grass
				110:	Napier grass
63	S. Baraza Farm Khasoko	E	1	111:	Napier grass
64	E. Makhanu Kimaeti-Khasoko	E	2	112:	Napier grass
65	Z. Luta, Sibembe Bungoma	E	1	113:	Rhodes grass
			2	114:	Napier grass
66	Victorius Living Ministry & Bible Training Centre Sibembe	E	2	115:	Napier grass

Farm No.	Location*	R	T	Sample No.	Grass Species
67	Bungoma FTC Mabanga	E	2	116: 117:	Rhodes grass Napier grass
68	Nzoia Sugar Nucleus Estate	E	1	118:	Napier grass
69	Sang'alo Institute	E	2	119: 120:	Natural grass Napier grass
70	Sirisia Centre Bulking Plot	G	2	121:	Napier grass
71	Mabonga Farm Kaptana, Masaba Mkt	G	2	122:	Natural grass
72	G. Wachela Cheptais Mkt	G	1	123:	Rhodes grass
73	G. Imo Changala Centre	G	2	124:	Napier grass
74	P. kitui Farm Malakis	G	1	125:	Rhodes grass
75	M. Simiyu Chwele Centre	F	1	126:	Kikuyu grass
76	R. Wanjala Plot Kibingei Mkt	F	2	127:	Rhodes/Nandi setaria mixture
77	Kamusinga Friends School Farm	F	1	128:	Rhodes/Nandi setaria mixture
78	C. Lusenekas Farm Maeni Mkt Kamukuywa	F	2	129:	Rhodes/Nandi setaria mixture
79	S. Kanyonya Farm Webuye Plains	D	1	130:	Rhodes/Nandi setaria mixture
80	D Masibi Makuna School Webuye Plains	D	1	131:	Napier grass
81	Bateta Chembeni Lukusi Market	D	1	132:	Rhodes grass
82	Sifuma's Farm Ndivisi Centre	D	1	133:	Rhodes grass

Farm No.	Location*	R	T	Sample No.	Grass Species
83	Sifuma's Farm Kiminini	H	1	134:	Napier grass
84	Mburu Plot Matisi Market	E	2	135:	Napier grass

* Dairy farming was the predominant livestock activity recorded. Area of sampled site varied from 10 to >6000 acres.

APPENDIX II

HERBAGE RECOVERY RESULTS FOR COPPER AND MOLYBDENUM

(a) Wet-ashing Methods

Method	Digestion Procedure	% Copper Recovered (by AAS)		% Molybdenum recovered	
		Direct Calib.	STD Addition	Direct Calib.	STD Addition
1	$H_2SO_4/HClO_4$	A 77.8	89.9	80.2	89.0
		B 63.1	69.0	74.1	78.4
2	$HNO_3/H_2O_2/H_3PO_4$	A 70.5	88.7	87.7	93.0
		B 68.1	86.8	86.8	95.1
3	HNO_3/H_2O_2	A 77.1	85.6	78.6	88.8
		B 90.0	107.0	93.0	114.0
4	$HNO_3/HCl/H_2O_2$	A 81.2	93.3	94.5	116.7
		B 81.1	107.0	91.8	94.5
5	$HNO_3/HClO_4$	A 98.9	98.9	96.0	97.4
		B 96.4	116.0	92.9	98.0
6	HNO_3/H_2SO_4	A 68.8	107.8	79.1	85.5
		B 71.6	109.1	97.2	92.7
7	$HNO_3/H_2SO_4/HClO_4$	A 98.4	100.0	99.0	97.7
		B 96.0	99.6	97.3	99.1
8	$HNO_3/H_2SO_4/HClO_4$ + Na_2MoO_4	A 97.8	100.3	ND	ND
		B 98.6	99.9	ND	ND

(b) Dry-ashing Methods

Method	Digestion Procedure		% Copper recovered (by AAs)		% Molybdenum Recovered	
			Direct Calib.	STD Addition	Direct Calib.	STD Addition
9	Dry Ashing at 500°C with HNO_3 ashing aid	A	84.5	85.0	94.6	95.0
		B	87.0	91.4	97.8	95.7
10	Dry ashing with $Mg(NO_3)_2$ at 450°C	A	85.1	95.5	92.4	94.0
		B	80.1	79.5	78.1	83.8
11	Dry ashing with Na_2MoO_4 at 450°C	A	85.0	90.1	ND	ND
		B	86.2	90.8	ND	ND
12	Dry ashing with NH_4VO_3 at 450°C	A	84.8	102.0	101.2	95.5
		B	92.2	94.0	89.1	96.2

*Each datum represents a mean of two duplicate digestions

A - Napier grass
B - Nandi setaria
ND - Not determined

APPENDIX III. HERBAGE COMPOSITION (all /kg DM except Si)

Sample No.	Mo (mg)	Fe (g)	Al (g)	Mn (g)	Zn (mg)	Ca (g)	P (g)	Se (ug)	S (g)	Si (%DM)	Cu (mg)	Co (mg)	Mg (g)
1	1.16	0.480	0.52	0.150	7	1.39	1.30	52	0.95	3.83	3.91	0.165	1.67
2	0.57	0.330	0.39	0.300	16	1.52	1.41	56	1.85	5.33	4.65	0.203	1.48
3	0.16	0.400	0.53	0.360	16	1.76	1.44	33	1.52	3.38	3.50	0.203	1.96
4	0.67	0.480	0.36	0.240	17	2.10	1.25	71	1.99	4.19	3.34	0.232	1.28
5	0.22	0.390	0.50	0.320	43	1.51	0.65	73	1.30	4.34	2.43	0.263	0.88
6	1.43	0.290	0.43	0.310	11	1.17	0.32	76	0.86	3.64	1.82	0.239	1.02
7	0.94	0.270	0.37	0.340	19	1.57	0.42	60	1.21	2.78	1.87	0.174	0.64
8	0.76	0.470	0.20	0.200	13	1.43	1.02	58	0.85	2.40	4.98	0.327	1.96
9	2.30	0.270	0.38	0.210	18	1.63	1.39	223	1.08	4.79	3.06	0.203	1.06
10	1.43	0.410	0.65	0.250	37	1.00	3.15	90	1.69	4.82	6.99	0.278	1.85
11	1.56	0.590	0.56	0.240	14	1.48	2.68	247	3.08	5.76	6.46	0.553	1.45
12	2.62	0.350	0.65	0.110	48	1.13	2.91	200	1.74	2.16	8.83	0.475	2.38
13	0.42	0.180	0.19	0.170	20	1.44	1.11	228	2.81	3.40	4.04	0.125	1.24
14	0.92	0.400	0.33	0.340	14	2.00	2.31	220	2.77	5.56	4.44	0.285	1.74
15	1.17	0.480	0.67	0.230	58	1.53	0.29	298	*	*	*	*	*
16	0.66	0.290	0.47	0.200	29	1.91	0.68	94	0.72	4.58	5.49	0.177	1.81
17	0.77	0.120	0.18	0.280	21	1.61	1.73	92	1.00	2.57	*	0.050	*
18	0.64	0.260	0.20	0.240	21	2.39	0.60	131	1.53	2.70	*	0.093	*
19	0.33	0.320	0.40	0.210	42	0.73	1.62	68	2.08	4.02	3.58	0.213	1.83
20	0.73	0.190	0.42	0.310	20	1.67	1.40	55	0.99	5.93	2.40	0.184	0.60
21	0.34	0.500	0.54	0.390	21	0.88	1.55	70	1.27	2.65	3.94	0.341	1.21
22	0.24	0.650	0.34	0.174	27	1.01	2.13	65	1.44	3.25	4.50	0.376	1.43
23	0.58	0.260	0.30	0.240	24	1.86	1.35	87	2.97	6.59	2.94	0.152	1.21
24	1.94	0.350	0.54	0.290	19	0.77	1.60	56	1.26	2.78	2.45	0.378	1.08
25	0.83	0.510	0.38	0.140	29	1.62	1.90	80	1.09	4.99	3.30	0.317	1.19
26	0.60	0.120	0.16	0.190	7	0.86	0.14	55	1.03	0.90	2.69	0.102	1.34
27	0.29	0.240	0.22	0.170	15	2.31	0.89	54	1.24	2.44	2.46	0.242	1.30
28	1.21	0.080	0.09	0.120	22	1.33	1.30	*	*	*	*	*	*
29	0.22	0.390	0.18	0.180	14	0.74	0.04	55	0.97	1.88	3.06	0.218	1.30
30	0.57	2.700	3.00	0.490	22	0.33	0.10	55	1.65	3.12	5.15	0.531	1.02
31	0.66	0.110	0.03	0.160	28	1.02	1.28	59	0.63	4.04	3.78	0.084	0.97
32	2.00	0.720	0.94	0.240	41	1.34	1.69	54	1.50	5.76	3.83	0.455	1.96
33	0.56	0.530	0.40	0.180	38	2.01	1.92	66	1.57	2.76	4.81	0.263	1.65
34	0.23	0.890	0.10	0.230	26	0.99	1.17	30	1.42	5.33	2.27	0.145	1.48
35	1.63	0.040	0.06	0.210	39	2.06	1.92	69	0.87	4.81	3.80	0.168	1.45
36	0.34	0.130	0.40	0.190	28	1.66	1.52	58	2.04	5.37	3.50	0.214	1.21
37	0.23	0.370	0.16	0.280	12	1.33	1.62	93	2.29	5.20	2.71	0.295	1.41
38	1.55	0.340	0.11	0.140	15	1.40	2.31	162	1.34	5.46	3.08	0.304	1.65
39	1.55	0.570	0.44	0.190	18	0.80	1.88	47	1.71	1.54	7.02	0.511	2.23
40	0.69	0.510	0.25	0.260	26	1.41	1.76	62	1.40	2.91	2.81	0.248	1.13
41	0.56	0.510	0.36	0.410	12	1.67	0.96	21	1.12	3.34	4.25	0.211	1.70
42	0.62	0.740	0.92	0.120	43	1.24	1.42	42	1.76	2.70	6.96	0.457	2.20
43	1.12	0.310	0.50	0.410	25	1.56	0.64	59	1.45	3.79	4.06	0.341	2.09
44	0.50	0.230	0.28	0.180	24	1.23	0.10	28	0.93	3.32	2.29	0.237	1.79
45	2.06	0.340	0.18	0.160	22	1.45	2.31	82	1.83	3.96	3.38	0.083	2.36
46	1.25	0.410	0.22	0.210	30	2.39	1.94	16	3.08	2.61	2.98	0.121	2.11
47	2.18	0.310	0.05	0.050	26	2.74	2.81	14	1.65	2.67	2.36	0.079	1.34
48	1.26	0.120	0.11	0.070	34	0.51	2.55	40	0.93	2.70	5.11	0.083	1.43
49	0.94	0.250	0.12	0.300	16	2.10	2.73	350	1.48	4.84	5.17	0.148	1.76
50	2.87	0.450	0.35	0.230	19	1.65	0.88	92	1.85	4.28	2.97	0.341	2.07

APPENDIX III (Cont.)

Sample No.	Mo (mg)	Fe (g)	Al (g)	Mn (g)	Zn (mg)	Ca (g)	P (g)	Se (ug)	S (g)	Si (%DM)	Cu (mg)	Co (mg)	Mg (g)
51	0.37	0.200	0.15	0.210	13	1.80	1.14	58	1.21	4.02	2.52	0.219	2.44
52	0.23	0.330	0.33	0.270	8	0.98	1.34	109	0.78	2.70	2.45	0.214	1.17
53	0.13	0.300	0.24	0.240	19	1.11	1.86	621	1.72	3.19	2.50	0.293	1.57
54	0.86	0.360	0.56	0.190	19	2.57	2.04	427	0.98	3.49	3.81	0.410	1.57
55	0.80	0.150	0.21	0.160	19	2.80	2.30	80	0.83	2.42	3.36	0.257	1.92
56	1.75	0.270	0.26	0.130	20	1.92	2.11	58	0.76	2.50	4.49	0.407	2.07
57	0.36	0.360	0.44	0.260	14	1.41	0.47	56	0.50	3.08	2.21	0.293	0.97
58	0.34	0.120	0.11	0.590	15	0.97	0.12	141	1.71	2.87	2.92	0.281	1.02
59	0.57	0.070	0.10	0.290	18	1.88	0.87	89	0.77	4.11	3.76	0.558	1.52
60	0.25	0.030	0.11	0.300	11	0.86	1.41	9	0.77	1.35	2.46	0.098	2.22
61	0.59	0.380	0.25	0.390	36	1.07	1.80	62	1.14	3.00	2.83	0.139	2.47
62	1.83	0.370	0.45	0.200	13	1.48	0.55	55	1.91	1.93	3.60	0.179	1.17
63	0.26	0.260	0.24	0.250	14	2.00	1.64	92	2.11	3.81	4.09	0.169	1.39
64	0.31	0.230	0.30	0.240	28	1.58	1.32	66	3.01	2.18	3.53	0.191	1.83
65	0.49	0.330	0.36	0.160	20	0.41	1.32	55	0.68	2.76	3.74	0.307	1.26
66	0.27	0.350	0.22	0.330	24	1.72	0.59	131	2.08	3.06	*	0.240	*
67	0.76	0.130	0.18	0.200	24	0.72	1.32	50	0.87	1.31	3.69	0.234	0.86
68	0.56	0.060	0.18	0.190	11	1.92	0.73	78	0.85	1.86	*	0.145	*
69	1.53	0.110	0.18	0.180	27	1.38	1.28	333	1.33	3.08	3.43	0.124	1.45
70	1.65	0.290	0.36	0.140	28	1.76	1.28	200	1.15	5.11	5.77	0.393	2.31
71	2.07	0.260	0.23	0.190	25	2.31	1.40	170	1.17	3.47	3.73	0.287	2.11
72	0.61	0.070	0.04	0.140	5	1.59	0.46	50	1.29	1.13	2.76	0.066	0.57
73	0.84	0.090	0.13	0.310	25	0.79	0.41	267	0.75	3.17	3.20	0.131	1.96
74	1.29	0.740	0.99	0.310	30	1.12	1.37	22	1.24	2.20	4.14	0.412	2.07
75	2.29	0.160	0.19	0.160	24	0.56	0.68	40	0.90	0.81	*	0.201	*
76	1.10	0.200	0.20	0.200	17	1.27	0.51	60	1.00	1.78	5.29	0.196	2.03
77	1.37	0.210	0.28	0.180	20	2.07	1.00	40	1.17	3.55	4.69	0.236	2.05
78	1.28	0.190	0.22	0.240	7	1.28	0.88	77	1.72	2.35	1.84	0.144	0.97
79	2.05	0.280	0.26	0.310	22	1.74	1.19	83	2.20	3.51	3.06	0.206	2.25
80	2.57	0.490	0.50	0.210	14	0.98	0.58	127	0.85	4.28	2.61	0.196	1.57
81	0.57	0.320	0.22	0.250	38	1.70	2.14	67	1.96	5.80	2.51	0.088	1.48
82	2.14	0.080	0.11	0.250	15	1.50	0.47	122	0.81	3.62	3.55	0.064	2.16
83	1.10	0.790	0.63	0.300	36	1.21	0.41	87	0.88	3.02	3.88	0.350	1.48
84	1.03	0.380	0.43	0.300	33	0.85	0.91	67	1.23	2.78	3.58	0.250	1.17
85	1.20	0.330	0.27	0.370	51	1.52	1.23	60	1.73	3.34	2.50	0.102	1.12
86	2.37	0.240	0.25	0.180	27	1.89	1.14	147	1.93	3.83	3.02	0.156	1.35
87	2.60	0.050	0.03	0.060	19	0.85	2.46	160	0.84	4.04	3.78	0.139	1.59
88	1.12	0.610	0.82	0.260	87	1.21	1.46	52	1.30	2.57	*	0.294	*
89	0.50	0.100	0.12	0.180	14	1.31	1.41	54	2.59	3.42	2.14	0.049	1.74
90	0.33	0.260	0.38	0.100	27	3.18	1.76	425	2.10	0.26	7.05	0.114	1.87
91	0.20	0.260	0.28	0.280	38	1.50	1.53	48	1.79	5.76	2.51	0.150	2.12
92	0.29	0.470	0.59	0.290	11	2.01	0.64	64	2.36	2.27	4.21	0.221	1.37
93	0.92	0.460	0.76	0.260	20	1.42	1.41	73	2.21	4.41	4.64	0.294	1.39
94	0.57	0.210	0.24	0.160	17	1.96	0.24	105	1.38	3.75	5.65	0.173	1.54
95	1.71	0.510	0.73	0.090	29	1.55	0.79	120	1.50	3.55	5.40	0.163	2.16
96	1.58	0.460	0.62	0.160	30	1.16	2.04	118	2.10	2.40	7.14	0.296	1.32
97	0.60	0.130	0.15	0.130	17	2.04	2.51	138	1.11	4.69	3.65	0.034	1.56
98	0.44	0.490	0.46	0.370	21	1.93	1.71	96	2.06	4.51	4.61	0.252	1.28
99	0.83	0.680	0.83	0.240	10	3.03	1.16	55	2.61	0.26	9.43	0.960	4.74
100	2.15	0.170	0.16	0.130	12	1.33	1.02	122	1.21	2.70	5.73	0.250	1.63

APPENDIX III (Cont.)

Sample No.	Mo (mg)	Fe (g)	Al (g)	Mn (g)	Zn (mg)	Ca (g)	P (g)	Se (ug)	S (g)	Si (%DM)	Cu (mg)	Co (mg)	Mg (g)
101	0.88	0.610	0.59	0.350	32	1.23	1.83	154	1.35	4.94	3.45	0.268	1.28
102	0.73	0.200	0.16	0.140	14	0.88	1.96	65	0.80	2.91	2.91	0.133	1.56
103	1.06	0.240	0.19	0.200	26	1.40	2.98	105	1.24	2.61	3.48	0.181	1.61
104	1.52	0.200	1.74	0.220	35	1.25	1.43	54	1.10	2.61	5.36	0.500	2.33
105	0.24	0.210	0.26	0.160	23	1.40	0.46	24	0.90	2.25	5.39	0.244	2.25
106	3.11	0.260	0.38	0.070	24	1.15	3.28	31	1.54	3.36	4.93	0.185	2.31
107	2.02	0.260	0.34	0.200	24	1.67	0.23	56	1.17	3.87	2.66	0.104	1.01
108	0.38	0.200	0.26	0.140	34	0.46	0.42	47	0.80	2.05	2.52	0.033	1.01
109	1.86	0.200	0.22	0.330	42	1.47	0.74	82	1.50	3.96	5.41	0.110	1.41
110	3.15	0.060	0.03	0.190	28	1.12	0.47	78	1.10	3.04	4.89	0.171	2.22
111	1.00	0.220	0.19	0.180	15	1.61	1.51	51	1.23	2.87	5.50	0.378	1.89
112	0.95	0.310	0.30	0.160	31	1.30	0.97	40	1.13	3.12	4.64	0.235	1.68
113	0.95	0.400	0.42	0.290	22	2.96	1.73	16	1.46	1.78	3.54	0.137	1.96
114	0.91	0.210	0.23	0.230	14	1.28	2.33	24	1.20	2.48	4.63	0.185	1.87
115	1.82	0.051	0.05	0.130	12	1.31	0.19	22	0.71	3.49	3.10	0.129	1.83
116	0.90	0.420	0.47	0.280	24	1.88	0.87	152	1.59	4.56	3.27	0.209	1.32
117	1.01	0.090	0.15	0.230	20	1.46	1.14	40	1.08	4.19	3.26	0.192	2.56
118	1.21	0.130	0.12	0.090	15	1.24	2.65	32	0.89	3.79	3.35	0.205	1.26
119	0.74	0.280	0.43	0.350	13	1.67	0.28	22	1.28	2.25	4.04	0.136	2.25
120	1.40	0.180	0.03	0.150	27	1.05	0.96	11	1.34	3.92	5.27	0.126	1.08
121	0.56	0.030	0.06	0.210	45	1.49	1.00	20	0.90	3.00	3.91	0.243	1.02
122	2.55	0.430	0.31	0.070	20	1.18	3.47	281	2.60	3.79	*	0.805	*
123	1.90	0.240	0.24	0.160	11	1.65	1.83	303	1.17	2.42	4.09	0.364	1.41
124	1.48	0.080	0.09	0.120	26	1.17	2.10	59	0.85	2.65	3.71	0.048	1.15
125	0.49	0.050	0.06	0.200	5	1.83	0.79	105	1.47	2.29	1.79	0.113	1.08
126	2.57	0.380	0.29	0.080	41	0.92	2.82	40	2.97	1.37	7.89	0.204	1.23
127	4.21	0.420	0.61	0.170	28	1.04	2.04	120	1.64	4.84	3.55	0.202	1.28
128	1.44	0.500	0.38	0.320	47	0.97	1.39	43	2.06	3.81	4.98	0.521	2.22
129	0.28	0.200	0.22	0.260	16	1.69	1.39	140	1.41	3.70	3.44	0.095	1.50
130	0.65	0.240	0.20	0.420	36	1.36	2.22	93	1.96	5.07	5.62	0.167	2.18
131	0.35	0.130	0.11	0.230	31	1.30	1.02	10	1.36	2.50	5.44	0.235	1.72
132	0.93	0.250	0.35	0.200	6	1.93	0.41	12	2.67	1.58	2.94	0.137	2.12
133	0.34	0.200	0.07	0.150	23	2.05	1.34	284	2.25	3.51	6.48	0.223	1.76
134	0.86	0.440	0.16	0.290	15	1.67	0.74	60	1.10	4.15	2.55	0.133	1.92
135	0.27	0.040	0.10	0.230	39	1.23	0.93	52	1.14	2.59	6.21	0.136	1.85

APPENDIX IV. SOIL MINERAL COMPOSITION (all /kg except Ca and Mn)

Sample No.	Mo (mg)	Fe (g)	Al (g)	Mn (me%)	Cu (mg)	Zn (mg)	Ca (me%)	P (mg)	Se (ug)	Co (mg)	pH
1	1.01	1.01	1.17	2.20	1.47	8.4	1.90	26.0	205	0.520	5.2
2	1.26	0.63	1.43	3.30	2.51	2.0	0.50	24.0	227	0.863	5.5
3	2.41	0.52	1.29	1.80	1.48	4.4	2.20	26.0	341	0.605	5.8
4	2.76	0.57	0.87	0.90	0.42	4.2	2.10	29.0	386	0.520	5.6
5	1.30	0.97	1.40	1.00	1.26	1.9	0.54	18.0	341	0.605	4.9
6	1.02	1.10	1.25	0.90	1.10	1.9	0.54	25.0	421	0.807	5.1
7	1.22	0.99	1.18	1.00	1.42	1.9	0.55	33.0	360	0.979	5.0
8	1.55	0.70	1.51	1.60	0.84	3.7	3.10	35.0	227	0.495	6.1
9	1.61	0.69	1.39	1.80	0.86	4.0	3.20	25.0	250	0.410	6.4
10	2.03	0.21	1.10	2.90	10.54	7.4	11.70	33.0	114	0.950	5.3
11	0.88	0.12	2.31	2.00	5.48	4.0	3.60	35.0	295	3.730	4.8
12	0.96	0.21	2.10	1.80	6.02	5.0	4.00	28.0	306	3.163	4.5
13	0.82	0.15	1.40	2.60	9.06	2.9	4.00	29.0	386	0.692	5.0
14	0.85	0.18	0.98	2.70	8.98	2.8	3.60	18.0	391	1.669	5.2
15	2.10	0.57	0.95	1.80	1.69	3.9	1.40	16.0	545	*	6.1
16	2.31	0.61	0.95	2.20	1.48	3.9	1.60	17.0	477	1.292	5.3
17	2.21	0.71	0.94	2.30	1.52	3.0	1.50	14.0	410	1.324	5.5
18	1.98	0.71	0.93	2.20	1.43	4.1	2.10	19.0	426	1.152	5.2
19	2.02	0.31	1.10	2.95	9.70	2.6	2.10	27.0	182	1.464	4.9
20	1.55	0.50	0.76	0.30	3.37	3.5	4.80	16.5	386	1.120	5.3
21	2.03	0.46	1.21	2.25	2.53	5.3	1.70	32.0	291	1.206	4.7
22	3.10	0.08	1.27	3.40	17.29	3.3	7.40	40.0	273	1.378	5.0
23	2.09	0.58	1.55	2.40	3.79	2.9	0.80	26.0	191	1.550	5.1
24	3.11	0.46	0.91	2.60	3.81	3.0	0.70	34.0	184	1.382	5.2
25	3.46	0.04	1.66	3.40	3.58	4.7	10.00	39.0	477	1.120	5.6
26	0.58	0.64	0.72	0.95	0.63	1.5	1.60	41.0	200	1.120	5.8
27	0.64	0.61	0.98	1.10	0.39	1.6	2.50	18.0	219	1.037	5.0
28	0.51	0.65	0.80	0.90	0.87	1.5	1.90	10.0	179	1.037	5.0
29	0.82	0.53	1.25	2.05	2.11	1.8	0.40	4.0	400	1.034	5.4
30	0.81	0.71	1.38	1.80	2.06	1.8	0.50	7.0	386	1.037	5.2
31	0.77	0.63	0.95	1.70	0.84	2.8	1.40	12.0	245	0.930	4.9
32	0.86	0.58	1.01	1.70	0.84	2.8	2.50	15.0	199	1.094	5.1
33	0.81	0.47	1.02	2.50	2.10	3.9	2.30	17.0	405	0.251	5.3
34	1.45	1.01	0.98	1.90	1.48	3.2	1.30	19.0	268	1.463	4.9
35	2.02	0.68	0.71	1.40	1.06	3.9	1.90	24.0	132	1.635	5.3
36	2.15	0.76	0.60	1.40	1.05	2.3	1.80	19.5	138	1.844	5.0
37	2.57	0.11	2.05	4.10	18.60	3.9	0.90	36.0	586	1.565	4.7
38	1.59	0.28	1.21	4.30	11.80	7.6	9.40	37.0	32	1.893	6.4
39	2.68	0.11	1.44	3.50	5.93	8.4	8.30	32.0	86	1.416	5.7
40	2.78	0.67	1.17	1.90	3.37	2.5	1.10	16.0	109	2.319	5.3
41	2.31	0.78	1.10	2.20	2.48	3.4	1.20	18.0	138	3.048	5.4
42	2.93	0.63	0.84	1.80	4.26	2.4	1.00	21.0	117	2.761	5.6
43	3.20	0.50	0.90	2.10	3.21	4.0	1.60	20.0	211	2.588	4.9
44	2.40	0.83	0.72	2.30	3.04	2.5	0.90	14.0	146	3.278	5.0
45	1.77	0.26	1.02	2.70	6.96	3.3	4.40	36.0	359	1.257	5.3
46	1.74	0.14	1.51	3.10	18.13	3.5	3.40	27.0	132	1.910	4.8
47	1.84	0.68	1.51	0.70	0.42	2.9	1.25	64.0	86	1.502	6.4
48	1.74	0.33	0.64	2.30	2.32	3.4	10.80	42.0	314	0.604	5.8
49	1.69	0.30	0.65	2.20	2.40	3.2	10.00	46.0	299	0.692	5.5
50	1.84	0.32	0.71	2.50	2.26	3.9	11.40	37.0	390	0.519	6.2

APPENDIX IV (cont.)

Sample No.	Mo (mg)	Fe (g)	Al (g)	Mn (me%)	Cu (mg)	Zn (mg)	Ca (me%)	P (mg)	Se (ug)	Co (mg)	pH
51	1.55	0.59	0.95	2.10	3.79	4.6	1.35	20.0	291	1.502	5.4
52	1.79	0.56	1.32	2.20	2.31	3.4	0.30	25.0	291	1.421	5.8
53	0.87	0.30	1.25	1.70	2.10	2.5	1.40	21.0	405	1.012	4.9
54	2.09	0.54	0.98	2.10	2.74	6.5	2.10	20.0	182	1.175	5.0
55	3.06	0.28	1.52	2.50	1.48	2.1	0.70	22.0	364	3.298	5.3
56	2.89	0.30	1.51	2.60	1.52	2.0	0.70	21.0	302	3.163	5.2
57	2.68	0.36	1.54	2.10	1.25	1.4	0.25	15.0	477	1.013	4.6
58	2.29	0.41	1.49	2.20	1.18	1.5	1.00	18.0	491	1.956	4.6
59	2.96	0.39	1.59	1.80	1.29	1.8	3.00	15.0	448	1.898	4.8
60	2.09	0.25	1.44	2.30	1.90	1.7	1.00	27.0	432	1.666	5.0
61	2.09	0.29	1.43	2.30	1.89	2.0	1.60	27.0	361	1.324	5.2
62	2.47	0.24	1.46	1.90	1.92	4.1	0.75	22.0	240	1.324	5.0
63	2.47	0.45	1.47	2.20	1.90	1.4	0.60	19.0	106	0.820	4.7
64	2.71	0.50	1.27	2.90	1.74	1.6	1.50	16.0	133	0.749	4.9
65	1.80	0.61	0.99	1.50	2.08	1.5	0.50	14.0	128	0.519	4.8
66	3.38	0.37	1.47	3.30	2.11	2.2	0.52	34.0	410	1.094	4.5
67	1.88	0.43	2.39	0.80	1.27	1.5	0.14	25.0	159	0.931	5.0
68	1.79	0.39	2.19	1.00	1.19	1.3	0.90	21.0	150	1.554	5.1
69	1.74	0.20	1.48	2.40	0.21	7.9	16.40	28.0	523	0.440	4.7
70	2.06	0.21	1.47	2.80	0.56	5.8	10.50	29.0	788	0.368	5.9
71	2.14	0.18	1.30	3.30	0.32	9.2	14.00	19.0	605	0.289	5.6
72	1.84	0.73	1.24	0.80	1.04	2.1	0.70	12.0	136	0.523	4.9
73	1.66	0.84	1.19	1.00	1.08	3.4	0.75	10.0	182	0.807	5.1
74	3.07	0.31	2.34	2.40	0.84	1.3	0.25	31.0	205	1.584	4.6
75	2.99	0.40	2.28	2.80	1.00	1.5	0.35	29.0	154	1.554	4.4
76	0.92	0.52	1.32	1.40	1.26	1.8	0.70	15.0	91	1.421	5.0
77	0.88	0.52	1.32	1.70	1.12	1.8	2.50	14.0	112	0.692	4.9
78	2.52	0.39	1.51	2.80	2.95	2.3	0.60	20.0	341	2.161	4.6
79	3.47	0.96	1.13	1.80	3.79	2.2	0.20	13.0	91	1.508	4.7
80	1.90	0.67	1.13	1.70	3.16	3.8	0.80	18.0	68	1.339	5.0
81	1.86	0.65	1.30	1.90	2.96	4.2	1.00	17.0	394	0.749	4.9
82	1.92	0.71	0.92	1.50	3.24	4.1	3.00	15.0	281	1.037	5.3
83	2.46	0.78	1.13	2.50	3.37	2.9	1.05	21.0	136	0.768	5.7
84	3.81	0.38	1.13	2.50	3.36	3.2	4.70	14.0	112	1.209	5.8
85	0.63	0.65	0.90	2.20	2.31	2.4	1.00	15.0	137	0.849	4.6
86	0.97	0.53	0.61	1.70	1.69	4.6	2.60	21.0	216	0.847	5.2
87	1.18	0.53	0.60	1.40	1.71	6.0	3.50	20.0	136	0.440	5.0
88	1.43	0.72	1.78	1.70	0.84	1.2	0.60	18.0	432	0.462	4.8
89	0.63	1.01	0.83	1.90	0.63	3.4	1.80	29.0	432	0.440	5.0
90	0.56	1.34	0.84	2.40	0.71	3.6	1.30	27.0	690	0.620	5.0
91	2.73	0.56	1.47	2.20	2.11	1.9	1.50	22.0	410	1.669	5.0
92	1.35	0.35	1.85	2.50	1.69	1.7	0.65	20.0	386	1.382	4.4
93	0.95	0.55	1.02	4.00	0.21	1.5	0.40	14.0	432	0.610	4.9
94	0.89	0.50	1.00	0.54	0.30	2.0	0.50	12.0	426	0.610	4.7
95	0.68	0.39	1.44	1.24	1.26	1.4	1.00	11.0	410	1.783	5.2
96	0.49	0.38	0.98	0.99	1.05	1.1	0.60	10.0	386	0.634	4.9
97	1.63	0.45	0.95	1.36	2.11	2.1	1.30	46.0	210	0.749	5.1
98	1.59	0.40	1.01	1.42	1.94	2.1	1.50	14.0	523	0.580	4.8
99	1.71	0.41	0.87	1.36	2.26	2.0	1.50	9.0	295	0.620	5.0
100	1.29	0.50	1.22	1.10	2.31	2.2	1.25	19.0	455	0.577	4.8

APPENDIX IV (cont.)

Sample No.	Mo (mg)	Fe (g)	Al (g)	Mn (me%)	Cu (mg)	Zn (mg)	Ca (me%)	P (mg)	Se (ug)	Co (mg)	pH
101	1.89	0.28	1.36	1.66	5.48	1.5	1.20	20.0	523	0.864	4.9
102	1.64	0.09	1.74	2.01	3.79	4.1	3.70	5.0	682	1.554	5.7
103	1.58	0.10	1.43	1.85	3.78	3.9	3.70	7.0	579	1.382	5.5
104	2.57	0.05	2.16	2.01	7.59	5.8	2.40	24.0	191	1.898	5.5
105	2.66	0.11	2.10	1.38	8.02	6.0	2.50	26.0	98	1.497	6.0
106	2.66	0.26	0.87	2.43	2.53	7.7	13.00	55.0	410	0.577	6.5
107	0.48	0.41	0.11	0.71	0.21	0.5	0.60	7.0	205	0.519	5.8
108	0.61	0.41	0.09	0.62	0.36	0.4	1.00	12.0	241	0.750	5.6
109	0.80	0.40	0.53	0.71	1.05	1.4	0.70	7.0	270	0.580	5.6
110	0.98	0.39	0.40	0.88	1.40	1.4	0.80	8.0	263	0.550	5.8
111	0.39	0.40	0.42	1.27	0.21	1.1	0.65	13.0	182	0.340	5.3
112	1.90	0.36	0.98	1.30	1.69	1.7	0.35	12.0	205	1.497	4.9
113	0.79	0.40	0.45	0.71	0.20	5.1	1.40	23.0	101	0.347	5.6
114	0.95	0.36	0.51	0.73	0.42	5.4	1.60	20.0	126	0.232	5.5
115	1.20	0.50	1.10	1.35	1.48	1.4	0.20	12.0	200	0.922	4.9
116	1.73	0.60	1.14	1.10	1.27	1.0	0.35	11.0	180	0.749	4.9
117	1.84	0.50	1.17	1.27	1.11	1.1	0.20	14.0	105	0.519	4.6
118	0.90	0.52	0.76	0.64	1.07	1.8	3.30	30.0	130	0.289	6.1
119	0.73	1.03	1.21	0.59	0.62	1.4	0.10	17.0	54	0.710	4.8
120	0.68	1.00	1.10	0.55	0.70	2.4	0.10	19.0	81	0.820	4.7
121	0.34	1.75	0.57	0.36	0.42	1.1	0.90	15.0	400	0.330	4.7
122	1.01	0.03	0.38	3.00	1.70	3.3	32.60	37.0	460	1.147	6.1
123	0.73	0.29	1.86	2.70	4.23	3.4	2.30	31.0	280	1.094	5.7
124	1.42	0.57	0.50	0.95	0.64	1.4	4.30	36.0	280	0.749	7.0
125	0.19	0.42	0.08	0.85	0.21	0.6	0.60	24.0	480	0.695	5.4
126	2.53	0.33	0.72	2.20	0.42	12.4	10.90	107.0	460	0.577	6.5
127	1.01	0.71	0.79	0.72	1.05	1.0	2.20	16.0	160	1.090	5.3
128	1.32	0.26	1.36	2.33	2.11	2.4	2.80	22.0	295	1.023	5.4
129	0.24	0.75	1.62	1.63	1.68	1.6	0.60	26.0	340	0.469	4.7
130	1.44	1.60	0.42	0.43	1.90	1.2	2.70	15.0	180	0.864	4.8
131	1.73	0.39	1.10	0.64	0.63	0.5	0.05	20.0	140	0.692	4.2
132	1.04	0.36	0.66	0.78	0.65	1.2	0.30	26.0	120	0.577	4.4
133	1.57	0.27	1.02	2.68	6.75	5.0	7.10	17.0	760	2.128	5.4
134	2.00	0.71	1.02	1.21	1.69	2.7	1.30	10.0	140	0.634	5.1
135	0.48	1.38	0.11	1.20	0.21	2.6	2.50	20.0	40	0.404	4.1

APPENDIX V RELATED FACTORS (key at end of appendix)

Sample No.	Species	Management	Landscape	Area	Xcoord	Ycoord	Altitude
1	1	2	1	1	232	219	6000
2	3	3	1	1	230	238	6030
3	3	3	3	1	219	183	5900
4	3	3	3	1	219	183	5900
5	3	1	2	1	204	178	5780
6	3	1	2	1	204	178	5780
7	3	1	1	1	204	178	5780
8	1	3	1	1	185	163	5380
9	14	3	1	1	185	163	5380
10	15	1	3	1	193	192	6560
11	3	3	2	1	184	195	7340
12	4	3	2	1	184	195	7340
13	3	2	1	1	191	225	5900
14	3	2	2	1	191	225	5900
15	4	1	2	1	231	217	5900
16	15	1	2	1	215	227	5970
17	1	1	2	1	215	227	5970
18	1	1	1	1	215	227	5970
19	3	3	1	1	215	246	6030
20	14	3	1	1	215	246	6030
21	2	3	1	1	215	246	6030
22	3	2	2	1	196	240	6100
23	3	3	2	1	208	211	6065
24	2	3	2	1	208	211	6065
25	4	1	3	1	169	231	6165
26	2	3	2	1	190	209	5900
27	3	3	2	1	190	209	5900
28	1	3	2	1	190	209	5900
29	1	3	1	1	239	206	5970
30	2	3	1	1	239	206	5970
31	1	1	2	1	246	188	6030
32	4	1	2	1	246	188	6030
33	3	3	1	1	255	205	6030
34	3	3	1	1	271	191	6100
35	1	2	1	1	286	180	6000
36	3	2	1	1	286	180	6000
37	3	3	1	1	185	251	6200
38	3	2	1	1	179	258	6300
39	15	1	3	1	168	255	7675
40	3	3	3	1	194	265	6500
41	2	3	3	1	194	265	6500
42	4	3	3	1	194	265	6500
43	14	3	3	1	194	265	6500
44	1	3	3	1	194	265	6500
45	3	3	1	1	213	265	6230
46	3	3	2	1	196	319	7350
47	15	1	3	1	220	280	7220
48	1	3	2	1	202	279	6430
49	10	3	1	1	202	279	6430
50	3	3	2	1	202	279	6430

APPENDIX V (cont.)

Sample No.	Species	Management	Landscape	Area	Xcoord	Ycoord	Altitude
51	3	3	2	1	235	271	6300
52	15	1	3	1	236	293	6170
53	3	1	3	1	247	262	6100
54	3	1	2	1	258	279	6100
55	1	1	2	1	260	268	6070
56	15	1	2	1	260	268	6070
57	2	3	3	1	273	287	6170
58	3	3	3	1	273	287	6170
59	1	3	3	1	273	287	6175
60	2	3	2	1	265	245	6035
61	1	3	2	1	265	245	6040
62	3	3	2	1	265	245	6035
63	3	3	2	1	251	245	5970
64	3	3	1	1	251	245	5970
65	1	3	1	1	251	245	5975
66	3	3	2	1	267	253	6100
67	1	3	2	1	280	256	6330
68	3	3	2	1	280	256	6330
69	2	3	2	1	280	269	6170
70	4	3	2	1	280	269	6170
71	1	3	2	1	280	269	6170
72	3	3	2	1	284	259	6300
73	1	3	2	1	284	259	6300
74	14	1	1	1	294	263	6230
75	1	1	1	1	294	263	6230
76	1	3	2	1	288	250	6590
77	3	3	2	1	288	250	6590
78	3	2	2	1	333	241	6560
79	3	2	1	1	307	240	6070
80	15	1	3	1	284	233	6170
81	3	2	3	1	284	233	6170
82	1	2	3	1	284	233	6170
83	14	3	3	1	292	229	6100
84	2	3	3	1	292	229	6100
85	3	3	2	1	183	209	6150
86	3	3	3	1	295	198	6070
87	1	3	3	1	328	218	6070
88	15	1	3	1	350	246	7610
89	3	3	3	1	314	195	5970
90	5	3	3	1	314	195	5970
91	14	3	1	1	239	238	6035
92	3	3	1	1	252	241	5970
93	3	3	1	2	164	71	4530
94	1	3	1	2	164	71	4530
95	15	2	1	2	159	102	5250
96	15	1	2	2	156	125	5315
97	1	3	1	2	166	118	6310
98	15	1	1	2	166	118	5315
99	11	1	1	2	166	118	5315
100	1	1	2	2	166	118	5320

APPENDIX V (cont.)

Sample No.	Species	Management	Landscape	Area	Xcoord	Ycoord	Altitude
101	14	3	2	2	127	112	5050
102	14	3	2	2	139	173	6300
103	1	3	2	2	139	173	6300
104	4	1	2	2	153	174	6820
105	1	1	2	2	153	174	6820
106	4	3	2	2	149	158	5640
107	3	1	1	2	52	72	4590
108	1	1	1	2	52	72	4590
109	15	1	1	2	40	62	4660
110	1	1	1	2	40	62	4660
111	1	3	1	2	35	29	4400
112	1	3	2	2	51	38	4530
113	3	3	1	2	76	42	4590
114	1	2	2	2	76	42	4590
115	1	2	2	2	81	30	4530
116	3	3	2	2	115	79	4920
117	1	3	2	2	115	79	4920
118	1	1	1	2	121	59	4720
119	15	1	2	2	102	36	4700
120	1	1	2	2	102	36	4700
121	1	3	2	2	64	130	4990
122	15	1	2	2	63	145	5380
123	3	3	1	2	43	155	6625
124	1	1	2	2	27	123	6300
125	3	3	1	2	39	101	4790
126	4	1	1	2	91	123	5250
127	14	3	2	2	132	140	5445
128	14	3	1	2	138	154	5575
129	14	3	2	2	168	152	5315
130	14	2	1	2	134	41	4790
131	1	2	1	2	146	59	4890
132	3	2	1	2	146	59	4900
133	14	2	1	2	180	95	5120
134	3	2	1	2	204	138	5575
135	1	1	2	2	135	79	4790

KEY:

Species

1. Napier
2. Nandi Setaria
3. Rhodes Grass
4. Kikuyu Grass
5. Natural Grasses
10. "
15. "
14. Rhodes + Setaria

Management Rating

1. Poor
2. Fair
3. Good

Area

1. Trans Nzoia District
2. Bungoma District

X and Y Coordinates are arbitrary units (see Table A.1.)

Altitude in Feet ASL

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