Nutrient deficiencies in soils of the Mseleni area, Kwazulu-Natal

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Abstract

This study constitutes a baseline epidemiological investigation into the nutrient status of soils in the Mseleni district of Kwazulu Natal, where nutrient deficiencies have been implicated in the etiology of an endemic disease. Mseleni Joint Disease (MJD) is a crippling, osteo-arthritic condition which afflicts an unusually high proportion of the inhabitants of this district. Home-grown produce and indigenous plants, which form the basis of the local diet, may be nutritionally inadequate due to soil-related nutrient deficiencies. In spite of this, and the fact that epidemiological studies have been successfully utilized to elucidate the etiology of similar diseases elsewhere, studies to date have not included soil chemical investigations.

The main purpose of this study was to assess the nutrient status of soils in the area by means of both chemical analyses and plant growth trials. Water samples were also examined, especially in relation to fluoride levels.

The grey sands of the *Fernwood* soil form which characterize the district were sampled by means of systematic transects through the high incidence area for MJD. Standard laboratory tests were carried out to chemically characterize these soils and to compare them with samples from an adjacent area of red soils. These determinations included pH, clay content, organic carbon by wet oxidation, extractable acidity (in 1 *M* KCl), clay mineralogy (by XRD), bulk elemental composition (XRF) and availability indices for nutrients (KCl, ammonium bicarbonate-EDTA, or, for B only, hot-water extraction). Particular emphasis was placed on the abundance and distribution of potentially important elements (Ca, P, Zn, Mn, Cu, B and Se) in the high incidence area.

Soil test results indicated that the grey sands sampled in the high incidence were sandy, moderately acidic (pH_{H2O} ± 5.8) and highly weathered, possessing low clay (< 4%, predominantly kaolinitic) and organic carbon (< 1.5%) contents and low effective cation exchange capacity (< 2 $cmol_c.kg^{-1}$). The available Ca concentration in the grey sands averaged 250 mg/kg, which is considered low enough to be suboptimal for many plants. Soil P levels (averaging 1.9 mg/kg) were deficient, as were the levels of Zn (averaging 0.4 mg/kg) and Cu (averaging 0.4 mg/kg). Boron (± 0.4 mg/kg) and Mn (averaging 4.5 mg/kg) levels were also very low.

The distribution of available concentrations of the nutrients concerned was extremely variable along the traverses sampled, suggesting a high degree of heterogeneity in the high incidence area and the possibility of the existence of isolated pockets of land where deficiencies might be acute.

Plant growth trials involving maize (*Zea mays*) and Italian ryegrass (*Lolium multiflorum*) were carried out on a composite soil sample from the high incidence area. A subtractive technique was used in the maize pot trial such that each nutrient was omitted in turn from the complete nutrient set, each

treatment being applied to three randomized pots. The lined pots were placed in a phytotron under controlled conditions and watered to field capacity twice daily to ensure optimal growth. Nutrient deficiencies were evaluated by means of deficiency symptoms, dry matter yields and foliar analysis. The ryegrass was fertilized with a Long-Ashton nutrient mixture and grown for 21 days under similar conditions before being analyzed for Se content.

Dry matter yields in the maize experiment indicated that the soil contained suboptimal concentrations of Ca, P, Zn, Cu and B; this was generally supported by various deficiency symptoms in the plants. Critical values from the literature pertaining to foliar nutrient levels in maize plants revealed that Ca (< 0.5%) and Zn (< 20 mg/kg) concentrations in the tissues were suboptimal, whilst the concentrations of P (<0.4%), Cu (<7 mg/kg) and B (<7 mg/kg) were clearly deficient. Three week-old ryegrass contained 0.2-0.4 mg/kg Se in the dried foliage, suggesting that the soil probably contained an adequate level of Se to sustain requirements of grazing animals. The results of the water analysis (Lake Sibaya and a borehole sample) indicated that the F contents of local drinking water are extremely low
(0.02-0.05 mg/L), in agreement with results of previous work.

This study provides the first quantitative chemical data in support of the hypothesis that an environmental factor may be responsible for MJD. It is clear that the plants growing in the soils of the Mseleni district suffer multiple nutrient deficiencies: further research is required to obtain a dietary profile for the local communities and to assess whether these soil-related nutrient deficiencies are in fact translating through the food chain and causing human disorders. Both the ecological and geographical implications of this study require further attention, especially in terms of the possible incidence of MJD in large areas of the Mozambique coastal plain which share similar soils.

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Chapter 1

Introduction

1.1 Overview

Although the intimate relationships between the health of humans, animals and plants and their respective geochemical environments have been recognised for centuries, it has only been in the course of the past three decades that technological advances in the field of analytical chemistry have enabled researchers to begin to understand these interrelationships in any quantitative sense.

One of the most important manifestations of these advances has been the recognition of previously unknown trace element deficiencies in agriculture which had historically led to significant losses of production of both livestock and crops on a global scale. Another field in which analytical breakthroughs have led to significant advances has been that of epidemiology: the study of the distribution and the determinants of disease in human populations. Techniques which allow for the routine measurement of a broad spectrum of elements down to levels previously unthinkable in soil, water, air or a variety of plant and animal tissues have revolutionized researchers' capabilities with regards tackling health-related problems. In a similar fashion geochemists who have traditionally been concerned with the understanding of chemical abundances and processes in natural systems have profited greatly from the new technology. Only relatively recently and in the wake of ever-increasing health problems caused by raised levels of chemical contamination of the biosphere have epidemiologists and geochemists recognized the mutual benefits of interaction in fields which are logically complementary. It is within this context of interaction between the Environmental Geochemistry and Medical professions that this study has been undertaken.

Medical researchers in South Africa have for some twenty five years known of and studied an endemic bone disease known as Mseleni Joint Disease, which affects a rural human population on the eastern seaboard of northern Kwazulu-Natal. Thus far, epidemiological investigations have yielded neither conclusive findings with regard to the causative factors involved in the onset of this disease nor agreement as to which line of evidence is the most plausible. Very little geochemical data have been published to date concerning the natural environment and foods in this area, and those which have been forthcoming appear to be somewhat incomplete. Geochemical insights into the problem have been offered by various medical researchers but have been largely speculative to date due to the paucity of data available, although, as argued below, the nature of the problem is such that more detailed input may potentially be of great interest. The work and the conclusions contained in this dissertation effectively represent an attempt to provide and to interpret some quantitative geochemical data for soils and water from the Mseleni area which have previously remained

unexplored and which may ultimately enhance the overall understanding of this problem. Before considering the geochemical data, however, the nature of this project necessitates a brief introduction to the people, the environment and the endemic disease afflicting the inhabitants of the Mseleni area.

1.2 Background to Mseleni Joint Disease

1.2.1 Introduction

Rural inhabitants of a remote strip of land on the coastal plain of north eastern Kwazulu-Natal (Fig.1A) have long been known to suffer from an endemic bone disease, termed the Mseleni Joint Disease (MJD) after the area in which it was first studied. The sandy, undulating plain is covered variously by forest and grassland and is home to rural people of mixed Tonga and Zulu lineage (Viljoen *et al.*, 1993). Although fairly extensive epidemiological investigations have been carried out in the area, neither the etiology nor the pathogenesis of the disorder are as yet understood (Solomon *et al.*, 1986). A further complication involves dwarfism which is fairly common in the area: it is unclear at present whether this condition is related to a common causal agent (Viljoen *et al.*, 1993). Beighton (1984) has noted that the dwarfs are mostly members of affected families, and that having excluded genetic linkages the logical explanation for this situation is that the Mseleni dwarfs represent the end of the spectrum of severity for MJD. The prevalence of this disease is reported to have major social, economic and health care implications in the area concerned (Yach and Botha, 1985), making further research a priority.

1.2.2 Symptoms and prevalence

The disease seems to be manifested amongst the populace in the form of several separate disorders (Schnitzler *et al.*, 1988). One of these has the features of multiple epiphyseal dysplasia (*epiphysis:* involved in the growth of long bones, *dysplasia:* malformation), another is polyarticular osteoarthritis (arthritis in several joints) and a third is a condition known as protrusio acetabuli (a hip disorder). People with osteoarthritis due to all forms of the disease have been reported to show radiographic osteopenia, or reduction in bone volume (Schnitzler *et al.*, 1988). The disease affects most joints, but the hip joint is most commonly affected (Fellingham and Elphinstone, 1973). Affliction begins with pain and stiffness in the joints and eventually impairs the sufferer's ability to walk to a greater or lesser degree, sometimes resulting in complete crippling and lack of mobility. As mentioned above, a number of individuals with an "unusual dwarfing dysplasia" have been studied; these dwarfs shared some of the symptoms of MJD but were also of short stature (Viljoen *et al.*, 1993). The relationship between the two conditions, if there is one, is not known.

Early work on the disease in the Mseleni area showed that approximately 39% of the female population

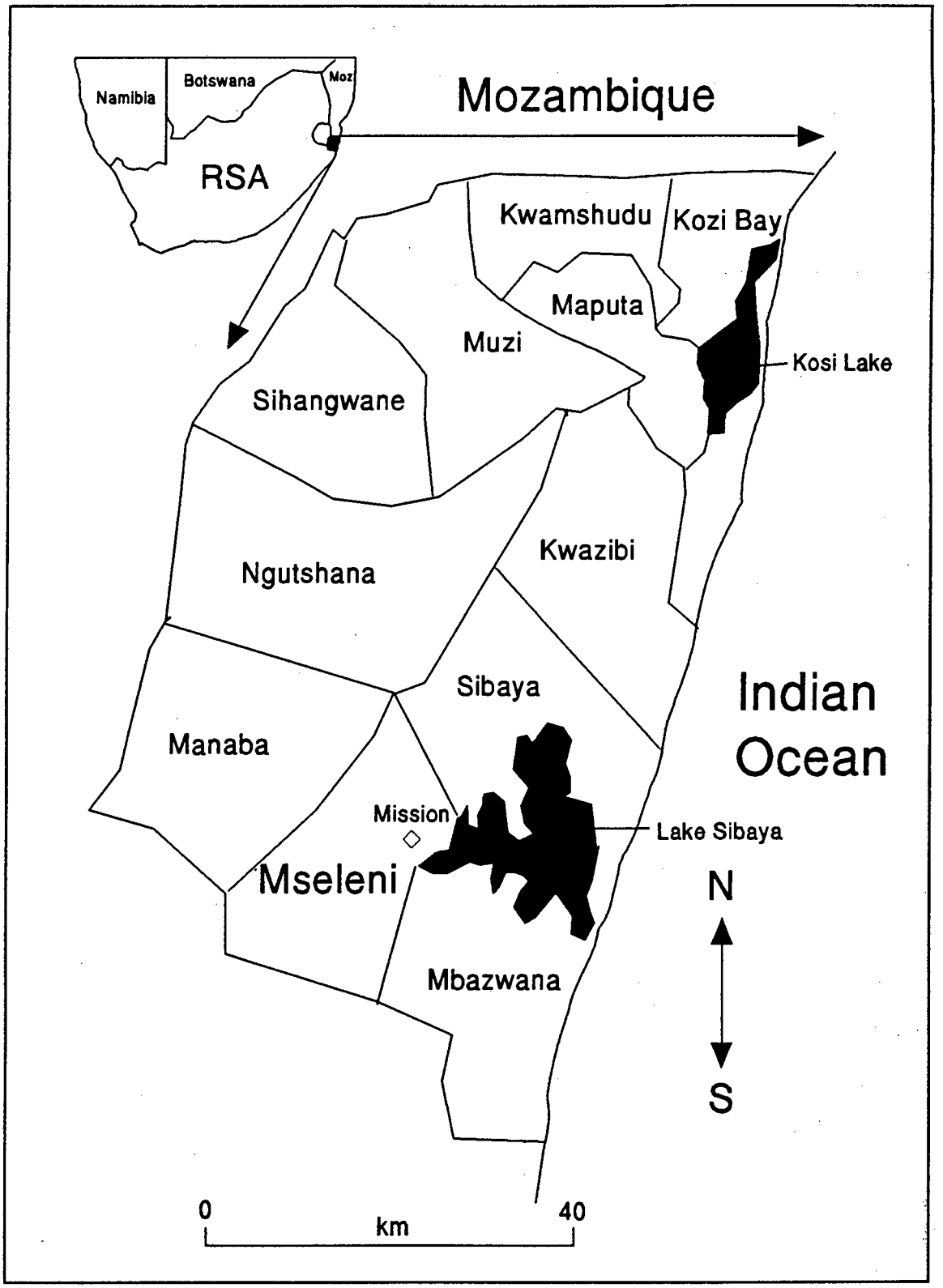


Figure 1A: Areas in which rural dwellers are known to suffer from Mseleni Joint Disease. The disease was originally thought to be localized around the mission hospital, but has since been reported throughout the region.

and 11% of the male population suffered from this disease. It was also discovered, however, that the proportions of affected males and females in the 0-10 age group was equal (Fellingham and Elphinstone, 1973), suggesting an excess of affected females in the older age groups. Persons of either sex and of any age can apparently develop the initial symptoms which result from articular surface irregularity (Fincham *et al.*, 1981). Early studies into prevalence rates (Fellingham and Elphinstone, 1973), however, strongly suggest that women are affected in a much greater proportion than are the men, and that in the majority of cases the disease progresses further with women.

1.2.3 Summary of potential causative factors investigated to date

Two possible types of causal agents for MJD have been investigated, namely i) genetic and ii) environmental factors (the latter group comprising both toxicological and nutritional factors).

i) Genetic factors: Genetic studies (Nurse *et al.*, 1973; Solomon *et al*, 1986; Viljoen *et al.*, 1993) have yielded no persuasive argument for inheritance as a cause of MJD. Population genetic studies indicate that the Mseleni population is not a genetic isolate (Nurse *et al.*, 1973). These workers reasoned that if the disease was inherited, it would have needed a prolonged period of isolation to have reached its present prevalence. In addition to this, the distribution of the disease between the sexes was found to be incompatible with inheritance mechanisms (Nurse *et al.*, 1973).

iia) Toxicological factors: The mycotoxicological studies of Marasas and Van Rensburg (1986) suggested that infection of crops with *Fusarium spp.* was an unlikely cause for the disease. No further work has been carried out in this regard.

iib) Nutritional deficiencies: Nutrient deficiencies have been suggested as a cause for MJD (Fincham *et al.*, 1981, 1985, 1986; van Rensburg and Jaskiewicz, 1984, Schnitzler *et al.*; 1988). Calcium and manganese deficiencies have been mentioned in the literature as possible causes of MJD, although it was noted that the role of the trace elements in bone formation is little known and that any one of several elements or inter-element relationships might in fact be responsible for the condition (Fincham *et al*, 1981).

1.2.4 Climate, soils, water and vegetation

The region as a whole is characterised by a longitudinal (north-south) pattern of landforms parallel to the present coastline. These landforms and their distinctive physical and biotic features form ecological zones (Fig. 1B) differentiated primarily by climatic determinants (Tinley and van Riet, 1981). The study area for MJD has centred on the Mseleni Mission Hospital, Ubombo District, Kwazulu

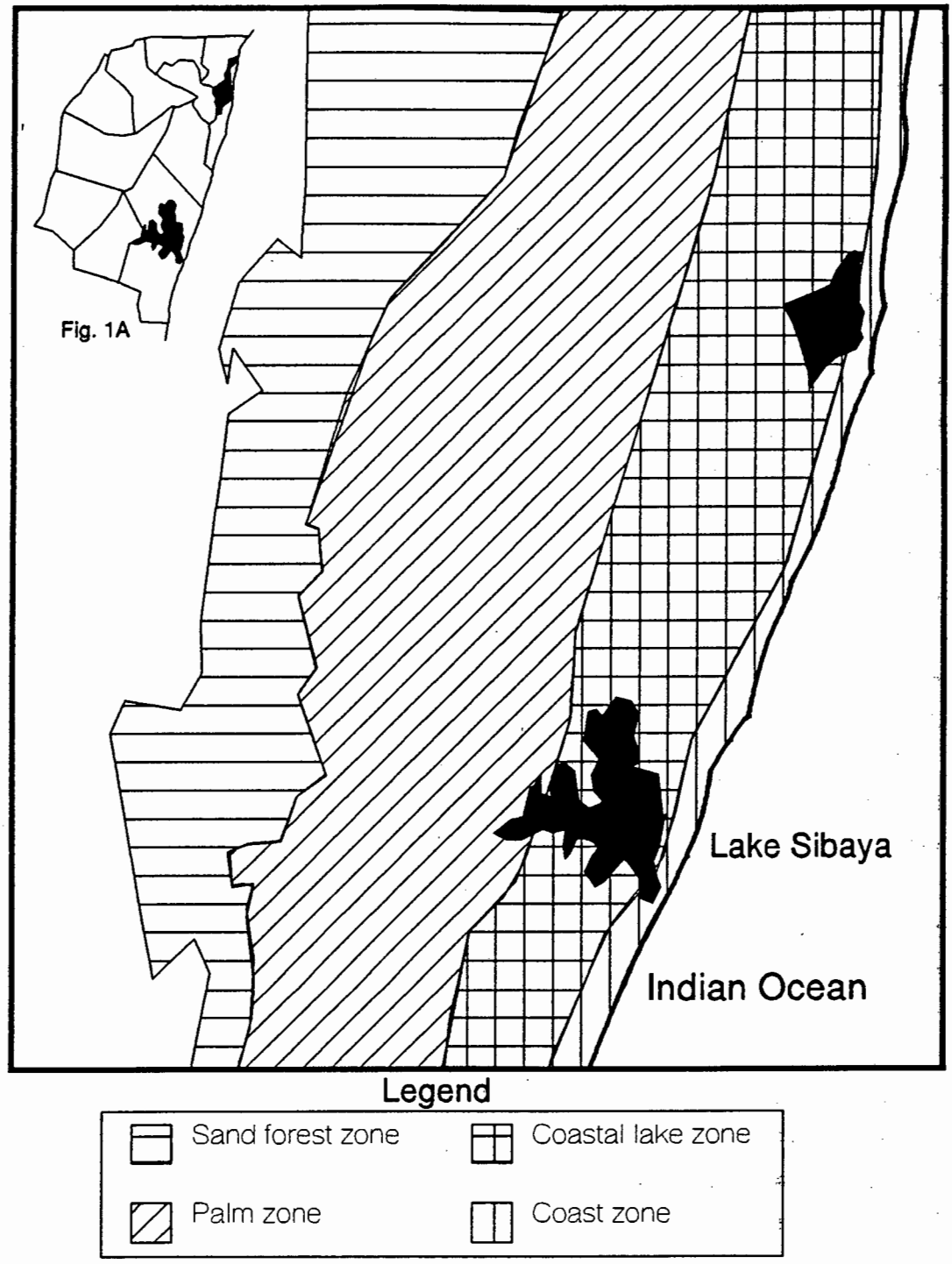


Figure 1B: Bioclimatic zones of the disease affected coastal plain (Tinley and van Riet, 1981); note the longitudinal (north-south) orientation of zones, which corresponds to a climatic gradient (highest rainfall at the coast) across similar soils.

Natal. Mseleni lies within a belt of land which stretches parallel to the coast from just south of Lake Sibaya northwards to beyond the Mozambique border, and almost fifty kilometres inland (Fellingham and Elephinstone, 1973).

This zone, to which the disease appears to be confined, is characterised by a warm, humid tropical climate with most rainfall received in the summer months; i.e approximately 1100 mm per anum (Tinley and van Riet, 1981). The soils concerned are sandy, acidic and leached and have a low moisture holding capacity, having been remobilised by wind in recent geological times (Maud, 1980), although they are now generally stabilised by vegetation cover. These types of soils are usually classified into the *Fernwood* Form in South Africa, being characterised by a deep *E* horizon underlying a relatively poorly developed orthic *A* horizon (Soil Classification Working Group, 1991). The water in the area is generally sourced from either one of the freshwater lakes which characterise the zone, and small rivers or boreholes/pits dug into the sandy aquifer upon which the inhabitants live. The mission hospital also pumps some water from Lake Sibaya into a reservoir and then on to a series of taps in the vicinity.

The indigenous vegetation in the area is predominantly a mixture of treeless grasslands and a savanna-forest mosaic (Tinley and van Riet, 1981): interestingly, although infertile and having a low moisture holding capacity (Maud, 1980), the grey-white sandy soils support a high species diversity, including an inordinate number of endemic species (Moll and White, 1978). *Neoendemism*, which is often related to physiological adaptations to "new" edaphic environs, is seemingly driven both in the Cape Floristic Kingdom (Cowling *et al.*, 1992) and in the Maputaland area by nutrient-poor sandy soils which characterise the geologically young coastal forelands. Another interesting feature of the coastal sands is the preponderance of dwarfed woody species (Moll and White, 1978, Cunningham, 1992), seemingly in accordance with predictions made for physiological adaptation of neoendemics growing on poor soils in the Cape Floristic Kingdom (Stock and Allsopp, 1992).

1.2.5 Lifestyle

The population of the area live in grass or concrete huts grouped into kraals, each kraal consisting of a homestead occupied by a family consisting of a man, his several wives and their children. The people still apparently adhere to a simple traditional way of life to a large degree, each family group still functioning as a self-supporting unit and depending largely upon nature for the provision of their subsistence needs. The area has no municipal electricity or water supplies and few roads; very few of the inhabitants have motorised transport, relying rather on walking through the sandy terrain to reach stores, neighbours or the local mission hospital. The vast majority of the people are peasant farmers living by means of subsistence agriculture carried out on sandy soils characterised by adverse chemical and physical properties which render them "generally infertile and of low agricultural potential" (Maud,

1980). Due to this inherent infertility cultivation is carried out using slash-and-burn methods, with a given patch of sand supporting crop production for two to four growing seasons only (Tinley and van Riet, 1981, Louw, 1984).

Studies into dietary patterns (Lubbe *et al.*, 1973) have shown that the inhabitants of the area rely almost entirely upon home produced foods and wild foods obtained from the veld. Additional foods may be procured through bartering with neighbours or might be bought at the local store, though "on account of the poor economic status of the people, ignorance and traditional food habits", food is normally purchased from a store only when their own supplies have been depleted. Maize, cowpeas, peanuts (home-grown) and a wide variety of indigenous plants form the basis of the local diet. The few animals are only slaughtered for ceremonial or ritual purposes and thus the intake of meat is very restricted: this scarcity of animal protein is exacerbated by the fact the both milk and eggs are hardly ever consumed in the area (Lubbe *et al.*, 1973). Furthermore traditional taboos forbid women and/or children to consume milk, eggs and meat (chicken, monkey, hares or cane rats).

The people rely on water from sundry pits and holes in the sand, the Mseleni river or taps and boreholes which have been established at various points.

1.3 Project rationale and hypotheses

1.3.1 Geochemistry and epidemiology - a global perspective

The usefulness of geochemical surveys in terms of both the explanation and prediction of endemic diseases related to elemental deficiencies and toxicities in human populations has been well established over the last three decades. Conditions such as endemic goitre (I deficiency), the localised incidence of dental caries (F-deficiency) and fluorosis (F-toxicity), dwarfism and poor growth induced by Zn deficiency as well as lesser known diseases such as Keshan Disease (Se deficiency), have all been significantly correlated with trace element anomalies in soils, waters or crops in the respective areas of incidence.

Whereas numerous trace element deficiencies and/or toxicities have been identified and experimentally verified in livestock (Underwood, 1980), ethical and practical limitations have historically limited this experimental approach in human nutrition. Adult volunteers are seldom as susceptible to micronutrient deficiencies as are infants or growing children; furthermore, depletion of any one element to such a degree as to produce clinical signs may take months and lead to irreversible changes (Moynahan, 1980). Hence, epidemiological investigations correlated with geochemical information are of great value to human medicine, albeit fraught with complexities and uncertainties. It must be stressed that the use of

quantitative geochemical data alone in epidemiology never leads to conclusive evidence of a disorder. The data are rather used to evaluate the probability of one or other environmental causal agent, the effects of which must then be tested clinically.

Soil is the pre-eminent source of most biologically important trace elements (Mitchell and Burridge, 1980) which reach humans through plants and animals. Although it is accepted that the total content of a given plant nutrient in a soil may not be a reliable indicator of its capacity to supply that element to the plant, attempts have repeatedly been made to correlate trace element contents of soils or underlying rocks with plant, animal or even human disorders (Mitchell and Burridge, 1980).

1.3.2 Likelihood of elemental deficiencies in the Mseleni area

Mseleni Joint Disease appears to be confined to a unique ecological zone characterized by infertile sandy soils and water which has a "considerably less than optimal concentration" of some essential minerals (van Rensburg and Jaskiewicz, 1984). Farm animals are reportedly few and exceedingly slow growing and therefore unlikely to develop deficiencies, although fish in Lake Sibaya, which is fed largely by groundwater from the affected area, are known to be dwarfed due to poor nutrient supply (van Rensburg and Jaskiewicz, 1984). Dwarfism, as noted above, is also characteristic of plant communities, as is endemism: both may be related to nutrient poor substrates.

Several studies have suggested the possibility of nutritional deficiencies in local humans:

i) biochemical surveys indicated very low fluoride concentrations in the urine of inhabitants from the affected area (Burger *et al.*, 1973);

ii) moderate to severe anaemia attributed to nutritional factors has been found in 42% of males and 53% of females in the Mseleni area (Mayet *et al.*, 1985);

iii) low Ca and Mg levels were found in serum from local women (Fincham *et al.*, 1986);

iv) comprehensive analyses of the dietary staples maize (*Zea mays*) and groundnuts (*Arachis hypogaea*) have indicated the probability of dietary mineral element and protein deficiencies and imbalances (Fincham *et al.*, 1985; Cunningham, 1992); and

v) patients and their unaffected family members seem to be characterised by small stature relative to people living in adjacent areas (van Rensburg and Jaskiewicz, 1984). A gradient of severity in stunting seemingly occurs, with some persons shorter than the arbitrary dwarf standard.

The extremely restricted source of local nutrition and the extensive reliance of the local inhabitants upon plant material grown on the infertile soils renders the population especially vulnerable to trace element and other nutritional deficiencies. Trace element deficiencies tend to manifest themselves in isolated, primitive communities living at subsistence level and whose diets are characteristically unvaried and restricted (Levander, 1987). It is in such communities that the links between the lack of a given element and the human patient are far more direct and less complex than that experienced in communities whose diet is derived from a variety of geographical locations (Moynahan, 1980), enhancing the value and interest in epidemiological and geochemical investigations all the more.

Particularly pertinent to this problem is the fact that bone is often described as the "storehouse" for minerals: following mineral deficiencies or imbalances bones are often "unable to fulfil their function" (Hidiroglou, 1980). Some trace elements found in bone, such as Zn, Cu and Mn, are components of enzyme systems involved in the normal functions of bone and cartilage cells: deficiencies of such elements are thus potentially of great importance in the context of understanding Mseleni Joint Disease.

1.3.3 Existing geochemical data for the area

No soil analyses have been published for the Mseleni area. Preliminary water sampling was carried out by Lubbe *et al.* (1973), who concentrated on comparing data from so called "affected" and "control" areas, concluding that the water was generally of good chemical quality and that there were no outstanding differences between control and affected areas. The integrity of these data and subsequent interpretations may be questioned based on the following:

i) "control" areas used are known to be "affected" areas;

ii) three series of water samples were collected over a period of one year: these were, however, often collected from different sources. Samples from "affected" and "control" areas were not always collected simultaneously, and different containers were used on different occasions. Not all sources were sampled: judging by pH measurements reported the highly important (alkaline) Lake Sibaya water was not studied;

iii) Fluorine concentrations, which must be relevant to the investigation of a disease caused by bone disorders, were reported to be in the range 0.04-0.2 mg/L, whereas optimal concentrations of F over the temperature range experienced at Mseleni are ± 0.7 mg/L (Dept. Water Aff. and For., 1991). No mention was made of this fact.

1.3.4 Methodology

It is clear from the above discussion that the unique coincidence of edaphic and socio-economic conditions found in the Mseleni area exposes the inhabitants to a high risk of nutritionally induced elemental deficiencies. It is furthermore clear that the utilization of quantitative geochemical data to complement epidemiological studies into endemic diseases such as Mseleni Joint Disease has proved to be extremely useful worldwide. Based on these deductions, on the failure of strictly medical investigations in unravelling the aetiology of MJD thus far and on the paucity of available geochemical data, the following *modus operandi* has been adopted in the hope of potentially furthering the understanding of MJD:

1. An investigation into the geochemical properties of grey *Fernwood* soils in the Mseleni area (which is thought to have the highest incidence of MJD) as well as into a very limited number of red (*Hutton*) soils (which form the next soil type inland of the grey sands), in order to understand the likely pattern of element abundances in the soils;

2. The establishment, where possible, of the *actual* soil abundances of those elements thought to be important in the metabolism of bone and joints;

3. An investigation, again where possible, into the *available* fractions of these elements in the soils, i.e. those fractions which are generally biologically available to plants and therefore humans;

4. The potential verification of soil-induced deficiencies by means of soil fertility experiments using both *Zea mays* (maize) and *Lolium multiflorum* (ryegrass) grown in Mseleni soils under experimental conditions;

5. The partial analysis of water samples from two sources (including Lake Sibaya).

Elements selected for investigation were those thought to be involved in the normal growth and metabolism of bone and cartilage: use has been made of both medical literature and professional advice in this regard. The elements selected were as follows: Ca, P, Zn, Mn, Cu, B, Se and F (the last in water samples only). The available concentrations of several plant nutrients not included in this list are also discussed, albeit somewhat superficially, in the following chapters. These data are useful in terms of facilitating a holistic approach to the subject of nutrient supply in the Mseleni area, and may also be useful in terms of further research in the area.

1.3.5 Hypotheses

The above methodology will be used to test the following hypotheses:

1. *The sandy soils of the Mseleni area are generally deficient with respect to one or more elements which are generally considered to be vital to skeletal integrity and/or normal physiological development in animals and/or humans;*

2. *Elemental deficiency(ies) in Mseleni soils will not be spatially homogeneous; rather, a naturally induced variation elemental concentrations will occur, leading to occurrence of pockets of (relatively) low nutrient status;*

3. *One or more elemental deficiencies in the soil will lead to suboptimal growth and nutrient status of plants.*

Having outlined the nature of this project in this chapter, Chapter 2 provides an overview of the origins and possible consequences of soil related nutrient deficiencies. The geochemistry of the Mseleni soil and water samples collected is dealt with in Chapter 3, with special emphasis being placed on the abundance, availability and distribution of selected nutrient elements in the soils of the area. This chemical assessment of nutrient levels in the soils will then be re-examined in Chapter 4, which focuses on the utilization of growth and foliar nutrient concentrations to identify deficiencies in plants grown experimentally on a typical Mseleni soil. The aim of the fifth and final chapter is to synthesize and interrelate the insights and findings reported in the previous chapters, to contextualize the conclusions reached in terms of the existing literature, and finally to highlight some avenues for further research.

Chapter 2

A review of the origins and possible consequences of soil related nutrient deficiencies

Introduction

Soils are the pre-eminent source of nutrient elements for plants, and, through the media of animal feed and food crops, for animals and humans respectively. Nutrient element deficiencies are therefore almost always associated with one or more of the soil properties and processes which affect nutrient availability. The subject of nutrient element deficiency is both broad and extremely well researched, primarily as a consequence of the economic and practical implications of nutrient deficiency in agricultural systems. The aim of this review is to provide the reader with a conceptual framework within which to understand soil related nutrient deficiency, rather than a comprehensive review of the most up-to-date literature regarding individual aspects of such deficiency. The phrase *soil related nutrient deficiencies* has been used with the express purpose of notifying the reader that the many important *plant related* factors involved in nutrient deficiency will not be discussed here. The principal texts used in the construction of this review were as follows: *The Chemistry of Soils* (Sposito, 1989), *Micronutrients in Agriculture* (Mordvedt *et al.*, Eds., 1991), *Soil Testing and Plant Analysis* (Westerman, Ed., 1990), *Russell's Soil Conditions and Plant Growth* (Wild, Ed., 1988a), *Principles of Plant Nutrition* (Mengel and Kirkby, 1978), *Soil Fertility and Fertilizers* (Tisdale *et al.*, 1985), *Environmental Chemistry of Soils* (McBride, 1994) and *Trace Elements in Soil and Plants* (Kabata-Pendias and Pendias, 1985).

2.1 An overview of the principal factors influencing nutrient deficiencies in soils

A useful distinction can be drawn between deficiency resulting from the *absence* of given nutrient elements in soils and deficiency attributable to the *unavailability* of these nutrients in the soil. Geological and climatic factors are arguably the most important considerations in the case of the former, whilst soil chemical factors determine the latter. The activity of soil organisms which are vital to the availability of nutrients in a soil will also be discussed, albeit briefly, in this section.

2.1.1 Factors influencing actual deficiencies of nutrients

2.1.1.1 Parent material

The elemental composition, texture and mineralogy of soils can be related more or less systematically to the nature of the parent material and to the degree to which this material has been weathered (McBride, 1994). Parent material usually dictates the actual abundance of a given nutrient element in

a soil by defining the soil *mineral reserve*, or those weatherable minerals which potentially replenish the stock of nutrients in the soil solution (Sposito, 1989). Soils formed from the weathering of basic rocks (e.g. basalts) are widely recognised as being relatively fertile due to the adequate supply of basic cations (i.e. Ca, Mg, Na and K) arising from the weathering of their primary mineral reserve. Conversely, soils formed from the weathering of acid parent materials (e.g. sandstones and acid volcanics) are often inherently infertile due to the lack of basic cations and other mineral nutrients in their mineral reserve.

Reported deficiencies of many nutrient elements in particular soil types globally may often be simply related to the absence of these elements in the parent material of these soil types. Lucas and Knezek (1972) note that plants grown on regosols, sandy podzols, alluvial and organic soils are prone to develop nutrient disorders due to their low nutrient reserves: this is particularly common in areas of high rainfall (for reasons discussed below).

2.1.1.2 Climate

The impacts of climate on nutrient availability in soils are manifold and intimately related. The intensity and degree of mineral weathering and subsequent solute removal in a given soil environment are dominated by climatic regime. Weathering processes influence the mineralogical make up (e.g. clay mineralogy, sesquioxide content) of a given soil and hence its nutrient retention capacity (McBride, 1994), whilst temperature and soil moisture content are influential in determining the activities of various nutrient elements in the soil solution (Moraghan and Mascagni, 1991) and hence their availability to plants. In this section only those factors contributing to the *absence* of nutrient elements will be discussed: the predominant climatic influences in this context are those controlling the weathering of soil minerals and the subsequent removal of nutrient elements by leaching.

Mineral weathering mechanisms include the displacement of basic cations in mineral structures by solution cations (especially H^+ ions in *hydrolysis*), the addition of water to mineral structures by processes which hydrolyse metal-oxygen bonds (*hydration*) and the removal of electrons from minerals, usually by molecular oxygen, i.e. *oxidation* (McBride, 1994): these processes generally occur wherever water comes into contact with primary mineral particles. Rates of chemical weathering and the subsequent removal of the soluble products depend both on the degree of leaching and temperature. High rainfall and high temperatures tend to result in the both rapid weathering and rapid removal of nutrients from soils, the extreme case of which is observed in the tropics (Kamprath, 1984).

Over three quarters of the soils in humid tropical regimes (rainfall throughout the year) and

approximately two thirds of soils in semi-humid regimes (3-6 month dry season) are highly weathered and leached and are expected to have acidity problems (Sanchez, 1976). The removal of soluble products will be slower if rainfall is interrupted by dry spells and/or if temperature fluctuates seasonally to allow for the recombination of weathering products into clay minerals (Schuffelen and Koenings, 1963). Temperature plays an important part in modifying the effect of rainfall through evaporative effects: *effective rainfall*, or mean rainfall less mean evaporative losses, has been shown to be strongly correlated with measures of exchangeable cations in soils (Donkin and Fey, 1993). In general, the stronger the leaching and the higher the temperatures to which developing soils are subjected, the lower their adsorption capacity and hence the more important the role of the primary mineral reserve. Consequently, the fertility of latosols and of sandy soils depends mainly on the primary mineral reserve (McBride, 1994).

Finally, if sorting occurs after initial weathering, i.e. if remobilization of the soil occurs, the sand fraction will often contain only the very resistant minerals, leading to a significant depletion in the original mineral reserve of the soil. This scenario, an example of which might be the case of wind distributed sands, would be expected to render the newly formed soil more infertile than the initial weathering products of a given parent material (Schuffelen and Koenigs, 1963).

2.1.2 Factors regulating the availability of nutrients

2.1.2.1 Soil solids

Primary minerals

The composition of the primary mineral reserve of a given soil is dictated by the geological parent material and mediated by chemical weathering and solute removal, as discussed above. The importance of primary minerals in terms of the presence or absence of nutrient elements has been discussed above; in this section the role of primary minerals in terms of the availability of the nutrient elements will be discussed, using the element potassium as an example.

The common primary minerals include island, chain, sheet and framework silicates, examples of which are the olivines, pyroxenes, micas and feldspars respectively: a knowledge of the amounts of these minerals in a soil is useful for both chemical and physical reasons. Briefly, the proportion of primary mineral particles in a soil affects the permeability of soils (due to their large size) and hence the rate of throughflow of water, gases and heat which are all important to the rates of weathering, solute removal and sorption processes in soils. Although the large size and correspondingly low surface area of the primary mineral fraction results in this fraction possessing a low chemical reactivity and hence

insignificant adsorption capacity, both the micas and the feldspars play a vital role in the fixation and slow release of the nutrient element K^+ (Mott, 1988).

The main groups of potassium bearing minerals in rocks and soils are the micas muscovite and biotite, and the feldspars orthoclase and microcline: these minerals occur extensively in acid igneous rocks such as granite and less so in mafic rocks. The potassium content of sedimentary rocks varies with their clay mineral content; sandstones generally exhibit low to very low concentrations, whilst significantly higher levels may be observed in shales (Wild, 1988b).

Unlike most other nutrient elements, the exchangeable fraction of potassium in a soil (conventionally measured by extraction with a suitable electrolyte solution) is widely regarded as an incomplete measure of the amount of K which can pass into solution. Structurally bound potassium in micas and feldspars, usually referred to as *matrix* potassium or *non-exchangeable* potassium, is susceptible to both *release* and *fixation* in soils at rates which have a profound influence on potassium availability to plants (Wild, 1988b). In the case of micas, for example, K^+ ions of the mica lattice may diffuse out of the mineral in exchange for other cationic species during weathering (Mott, 1988): depletion of K^+ ions from interlayer sites is favoured by a high H^+ concentration in the surrounding soil solution (i.e. acid conditions) as well as by low levels of K in this medium.

Potassium *fixation* occurs after significant depletion of structural potassium has occurred: interlayer sites become depleted of K^+, although they retain a very high K^+ selectivity relative to divalent ions (Mengel and Kirkby, 1978). Addition of K^+ to such soils results in a strong potassium adsorption to these structural positions causing a contraction of the mineral; K^+ ions become very strongly held and hence unavailable. This phenomenon may be very important in agricultural situations where considerable quantities of K fertilizers may be fixed in this manner after cropping has depleted soil potassium reserves (Mengel and Kirkby, 1978).

Layer silicates

Perhaps the most stable and persistent silicates, which occur as weathering products (*secondary* minerals) in the clay fraction ($< 2\mu m$) of soils, are the layer silicates (McBride, 1994). Layer silicates may contain negligible quantities of nutrient elements as structural components but their small size and correspondingly large surface area result in a high degree of chemical reactivity; this is particularly pertinent to the sorption capacity of a given soil which profoundly influences the availability of nutrient ions.

Most soil colloids, including the layer silicate fraction, carry a negative structural charge caused primarily

by ionic substitution (Kabata-Pendias and Pendias, 1985); this charge may be electroneutralized by cations present in the surrounding solution. In the presence of an excess of cations, the process of cation exchange maintains the electoneutrality of the system, whereby the cations adsorbed onto the solid phase may be replaced by other cations (most often H^+ ions). The ability of a solid to reversibly exchange cations is termed its cation exchange capacity (CEC), and is one of the most important factors controlling nutrient availability in soils (McBride, 1994). The CEC of different soils varies widely both in quantity and quality and can range from 1 to 100 meq/100g soil (Kabata-Pendias and Pendias, 1985). Both the quantity of soil solids possessing significant CEC and the quality of these solids in terms of the relative contribution to the overall CEC of the soil are important: in this context a brief discussion of the major groups of layer silicate minerals is appropriate.

Various structural combinations of tetrahedrally co-ordinated (commonly silica) and octahedrally coordinated (commonly alumina) sheets facilitate the subdivision of the layer silicates into five groups: the kaolinite, smectite, illite, chlorite and vermiculite groups. Kaolinite exhibits a ratio of one tetrahedral sheet to one octahedral sheet (a *1:1 layer silicate*). The ideal structure has no charge, with layers held together by H-bonding (McBride, 1994): for this reason kaolinites do not swell in water, have low surface areas and low CEC. Smectites may be either di- or trioctahedral *2:1 layer silicates*, isomorphous substitution in the tetrahedral and octahedral sheets resulting in individual layers possessing negative charge which may be balanced by exchangeable cations (McBride, 1994). These cations are generally hydrated and easily displaced into solution by other cations, leading to a high CEC for the smectite group. Smectites vary in chemical composition and in the location of structural charge but have in common a relatively low layer charge (McBride, 1994), which allows the individual layers to separate to a large degree in water. Vermiculites are similar in structure to smectites, but possess higher layer charge, leading to a higher CEC and a lower swelling potential. Chlorites are also 2:1 layer silicates, but exhibit zero expansion in water, having relatively low specific surface areas and low CEC.

The affinity of nutrient cations for the surfaces of various clay minerals has been the subject of exhaustive investigations in the literature and treatises on this subject can be found in any standard soil chemistry textbook (Sposito, 1989; McBride, 1994). Important to the consideration of nutrient element deficiency are situations where layer silicate minerals are either absent in significant quantities -often as a result of strong leaching environments which remove the structural components required for secondary mineral formation - or of a variety which is characterised by low CEC (e.g. kaolinite). Kaolinite often dominates the clay fraction of highly weathered soils, for example (McBride, 1994), leading to a low adsorption capacity in these soils and a correspondingly low supply of available nutrient ions. In general, 2:1 layer silicate minerals are more prevalent in temperate than in tropical soils (Mengel and Kirkby, 1978), hence temperate soils often have higher CEC's in relation to their clay contents.

Oxides and hydroxides

Although several oxide (e.g. Ti-, Si-oxides) and hydroxide minerals occur in soils, Fe, Al and Mn oxides and hydroxides are generally the most important in terms of *sorption processes* in soils (McBride, 1994). These oxides and hydroxides commonly occur in soils as coatings on soil particles, as fillings in cracks or as concretions (McBride, 1994). Unlike layer silicate clays, the oxides are not inclined to develop a structural charge as a result of isomorphous substitution which results in their having low CEC in spite of having extensive surface areas (Sposito, 1989).

The surfaces of these soil solids do, however, possess a considerable capacity to chemisorb metal ions as well as inorganic and organic anions. Many trace metals which are important to both plants and animals as nutrients may be chemisorbed by noncrystalline aluminosilicates (allophanes) and oxides and hydroxides of Fe, Al and Mn, which present a similar type of adsorptive site to the soil solution in the form of a valence-unsatisfied OH^- or H_2O ligand bound to a metal ion (McBride, 1994). Soils in the tropics and subtropics often contain significant proportions of these oxides and hydroxides, with the result that these soils often have high adsorption capacities (Mengel and Kirkby, 1978).

Organic matter

Although mineral soils commonly contain only a few percent of organic matter, this organic material may exert an influence on the CEC of soils, often contributing half or more (Jenkinson, 1988). The exchange capacity of humus in well drained, near neutral soils generally lies between 300-550 $cmol_c kg^{-1}$ organic carbon (Jenkinson, 1988); in the case of acidic or poorly drained soils these figures may be slightly lower (50 - 120 $cmol_c kg^{-1}$ organic carbon). Organic matter becomes increasingly important as a source of CEC as the pH of a soil increases: for each unit increase in pH, the change in CEC of humified organic matter is several times higher than that of mineral colloids (Stevenson, 1991). Of the functional groups in humus the most important are carboxylic acid groups, which largely dissociate to the carboxylate ion at pH values found in all but the most acid of soils (McBride, 1994).

As in the case of nutrient-clay surface interactions, a huge body of literature exists which is concerned with the mechanics of the complexation reactions and other processes involved in organic matter-nutrient ion interactions. Organic chelation of micronutrient cations is a particularly important and ubiquitous phenomenon in soils (Stevenson, 1991). Mordvedt *et al.* (1991), Wild (1988), McBride (1994) and Sposito (1989) provide adequate and accessible reviews on this subject.

Of particular interest in the understanding of nutrient deficiencies is the special importance attached to humic substances when these are the only adsorbents present in a given soil: this is pertinent to sandy

soils where humus often might not only be the sole adsorbent present but also an indirect contributor to soil fertility due to the role it plays in soil structure and through its water holding capacity (Schuffelen and Koenigs, 1963). Humus may also constitute an important reserve for certain nutrients (especially N,P,S and B), which are released through biological mineralization (see section 2.2).

Other solids

Carbonates such as calcite are often present in soils, particularly where water loss by evapotranspiration is greater than rainfall. Calcite, which is the most widespread form of Ca carbonate (Ca is the dominant ion in most soil solutions), is often dispersed and may play an important role in regulating the pH of soils (Kabata-Pendias and Pendias, 1985). Variation of soil pH, as noted below, is important to the availability of many nutrient ions; furthermore, a wide range of nutrient ions may either substitute for Ca in calcite or coprecipitate with calcite and may be released upon dissolution of the calcite (Sposito, 1989).

Sulphides, sulphates and chlorides are rare in soils of humid climate regimes, but in arid regions these mineral types may, to a large extent, regulate the behaviour of micronutrients (Kabata-Pendias and Pendias, 1985). Many metallic ions form relatively stable sulphides in flooded soils, whilst others may be coprecipitated with Fe sulphides; this process can be an important one for the regulation of the supply of both S^{2-} and metallic cations in the soil solution. Sulphates, mainly of Fe, Al and Ca, may occur under oxidising soil conditions: these salts are readily soluble and hence important to soil solution chemistry. Chlorides are extremely soluble and only occur in semi-arid to arid environs (Kabata-Pendias and Pendias, 1985).

According to the theory of solid solutions, the solubility of a given ion may be lowered in mixed ionic compound relative to the solubility of the pure compound (McBride, 1994). Consequently, the existence of mixed solid phases in soil may affect the solubility of many of the trace elements in soils.

2.1.2.2 The soil solution

pH

The pH of a given soil solution is recognized as being the main controlling variable affecting soil solution chemistry (Sposito, 1989). The pH controls ion exchange, dissolution/precipitation, redox reactions (discussed below), adsorption and complexation reactions (McBride, 1994), and is hence an extremely important parameter to consider when examining nutrient availability in soil solutions. The scope of pH effects encompasses most soil chemical processes; instead of attempting to outline the multiple roles

of pH in determining soil nutrient availability, the discussion below will be focused on two related aspects, soil acidity and high pH soils, which are frequently associated with nutrient deficiencies throughout the world.

Acid soils (pH < 7) occupy some 30 % of the worlds ice free land area (Baligar and Fageria, 1996). These may be grouped into those soils which have an acidic nature arising from natural weathering processes and those in which acidity has developed anthropogenically (Sumner *et al.*, 1991). The causes of soil acidity will not be discussed here; useful treatises in this regard include reviews by Adams (1984) and Ulrich and Sumner (1991). Soil acidity has a profound effect on the availability of nutrients in soils (Kabata-Pendias and Pendias, 1985; Sumner *et al.*, 1991), two closely related aspects of which involve i) the solubility of nutrient ions, and ii) the influence of pH-dependent charge on the CEC of some soils.

The enhanced solubility of certain metal cations, attibuted variously to pH dependent processes mentioned above, is one of the important consequences of soil acidity (Sumner et al., 1991). Almost the entire suite of nutrient cations shows increased mobility under acidic conditions, preferentially entering the soil solution phase and potentially being removed from the soil given the correct climatic regime (Sumner *et al.*, 1991). This mobility may result in several conditions in the soil: increasing acidity (McBride, 1994), deficiency of nutrient cations (Sumner *et al.*, 1991) or excesses of certain cations leading to elemental toxicity, for example Al, Mn and H (Baligar and Fageria, 1996). The general mobility of cations described often leads to nutrient deficiencies being experienced in acid soils (Clark, 1984), especially when these soils are sandy and poorly buffered. Physiological disorders which often appear in plants grown on acid soils include responses to both macronutrient (i.e. N, P, S, Ca, K, Mg) and micronutrient (e.g. Cu, Zn, Mo, Fe, Mn and B) deficiencies.

Tisdale *et al.* (1985) list the types of soils where deficiencies of the important macro- and micronutrients have been reported, quoting both global and U.S. examples. Acid soils are conspicuous in this list, proving to be potentially deficient in all of the important plant nutrients, especially where leaching conditions are conducive to removal of nutrient cations. A general trend which may be observed in acid, sandy soils in particular is the leaching out of the cations Ca and Mg from the soil, leading to low base status and correspondingly low fertility (Kamprath, 1984).

Another facet of soil nutrient availability affected by soil acidity involves the clay minerals which, as mentioned above, play an important role in ion exchange in soils due to their cation exchange capacities. Ferric or aluminium oxides, either hydrous or anhydrous (as described above under the heading soil solids), are present in nearly all soils, often occurring as coatings on layer silicates (Thomas and Hargrove, 1984). These coatings, which are generally thin, positively charged and bound electrostatically to the aluminosilicate layers (Barnhisel, 1977), introduce a component of pH-dependent

charge to the clays of many acid soils (Thomas and Hargrove, 1984), influencing the CEC properties of these soils. The characteristic cation exchange behaviour of layer silicate-sesquioxide complexes results from the fact that the negative charge on the layer silicate is practically invariant with pH, but the opposite charge on the clay-sesquioxide complexes is not, with the consequence that the net charge varies from positive at low pH to negative at high pH (Sposito, 1989). As a result the effective cation exchange capacities of highly weathered, acid soils (in the tropics, for example) are usually fairly low, and the nutrient status of these soils is often suboptimal (Kamprath, 1984).

The presence of pH dependent charge is not restricted to clay-sesquioxide complexes, and may in fact be associated to a larger degree with the humus fraction in soils (Sposito, 1989). In this case, the pH dependence is generally related to the nature of the humic groups contained in this fraction, the dissociation of which may be strongly influenced by pH (Sposito, 1989).

High pH soils (especially carbonaceous soils) are also conspicuous globally in terms of being potentially deficient in many essential nutrient elements (Tisdale *et al*, 1985). Nutrient deficiencies in such soils are generally caused by the *decreased* mobility of nutrient ions at elevated pH values, in contrast to acid soils where the opposite applies (i.e. *increased* mobility). The solubility of most nutrient cations, for example, decreases with increasing pH values; therefore although a high concentration of a given nutrient may be present in carbonaceous soil, its availability might be extremely low due to soil chemical factors (McBride, 1994).

Redox potential

The importance of pH in controlling soil chemical processes has been stressed above. Soils subject to fluctuations in water content are influenced by another important variable: the reduction-oxidation (or *redox*) potential. Water saturation may lead to a sequence of redox reactions which alter the soil pH and hence the solubility of nutrient ions in the soil solution (McBride, 1994); redox conditions may also exert a profound influence over the chemical form of nutrient ions.

The availability of some elements, notably Fe and Mn, are affected by soil redox conditions (Sposito, 1989). In the case of these elements the bivalent ions which may exist either in the adsorbed or solution form may be oxidised to give products which are insoluble in the common range of soil pH, namely oxides or hydrous oxides, in which the valence of the metal is 3 for Fe and 3 or 4 for Mn. It is well established that alkalinity and aeration favour oxidation, whilst acidity and waterlogging favour reduction (Wild, 1994). Low pE values (pE < 3) destabilize Fe and Mn hydrous oxide adsorbents whilsy stabilizing soil humus adsorbents (Sposito, 1989). The dissolution of the hydrous oxides leads to an increase in the free ion concentrations of anionic nutrients (e.g. B, Mo, inorganic C and P) and of micronutrient

metal cations which were in adsorbed forms. Sposito (1989) notes, however, that the preservation of organic adsorbents generally leads to lower concentrations of metal cations due to surface complexation effects, hence low pE might be expected to indirectly enhance the bioavailability of anionic nutrients but to have little net effect on the bioavailability of micronutrient metals. The relative solubilities of certain nutrient cations, being governed to some extent by the above processes, may result in periodic deficiency being experienced due to waterlogging or drought periods (McBride, 1994).

Apart from the precipitation of hydroxy solids, increases in pE in soils also importantly result in the mineralization of soil organic matter, resulting in an increase in bicarbonate alkalinity and in the concentration of the free ion species of N, P and S in the soil solution (Sposito, 1989).

Ionic strength of soil solutions

The understanding of ion exchange processes at the molecular level involves an appreciation of several fairly complex and highly theoretical models which will not be discussed here: both Sposito (1989) and McBride (1994) provide detailed chapters on this topic. An aspect of ion exchange which does perhaps deserve attention here is the effect of ionic strength of the soil solution on cation adsorption onto soil solid surfaces.

The most accessible example of the effect of ionic strength is that which results in the so called *concentration-charge effect* (McBride, 1994), traditionally referred to as the *ratio-charge law* as announced by Schofield (Wild, 1994). When considering the exchange of a mixture of cations of unequal charge, a shift in favour of the adsorption of higher-charge cations onto soil colloids occurs at lower electrolyte concentrations (McBride, 1994); similarly at high electrolyte concentrations monovalent cations (e.g. Na and K) are preferentially adsorbed onto exchange sites. More generally stated, the activity coefficients of charged inorganic species are dependent on the ionic composition of the solution (Stevenson, 1991). One consequence of this effect is that in humid climates where extensive leaching of salts occurs resulting in dilute soil solutions, the divalent and trivalent cations (e.g. Al^{3+}, Ca^{2+} and Mg^{2+}) tend to dominate exchange sites (McBride, 1994). In soils of arid climatic zones, on the other hand, higher electrolyte concentrations favour Na^{+} and K^{+} adsorption onto exchange sites. In the case of most soils, wide fluctuations in ionic strength occur due to irregular rainfall, drought periods or irrigation, with far reaching consequences for the relative availability of nutrient ions in the soil solution. As soils dry out, for example, some ionic species may reach concentrations higher than their solubility products and precipitate out: calcium, sulphate and phosphates are particularly susceptible to this process (Mengel and Kirkby, 1978). A further important consequence of ionic strength fluctuation is the fact that the CEC and AEC (anion exchange capacity) of variable-charge soils appear to decrease at low ionic strengths, which implies that ion dissociation and resultant losses by leaching are intensified during

soil wetting cycles (Fey, pers. comm., 1996).

Solute interactions

Nutrient deficiencies are often induced in agricultural soils by the use of fertilizers which create imbalances in the nutrient cation composition of the soil solution. Where nutrient ions with similar chemical properties are in competition for absorption by plants the dominance of one ion in the soil solution may well lead to inadequate uptake of another Moraghan and Mascagni, 1991). This concept will be expanded below when individual elements are discussed.

2.1.3 Biological mediation

Soil organisms are present in very large numbers in most soils (e.g. 10^9 bacteria g^{-1} soil) and the resulting levels of biological activity are accepted as being essential to nutrient element cycling within the soil (Harris, 1988a). The microflora (bacteria, actinomycetes, fungi and algae) and microfauna (protozoa and nematode worms) as well as the mesofauna (collembola, mites and others) present in soils are vital to the recycling of nutrients such as carbon, nitrogen and phosphorus among others. A naturally fertile soil may be thought of as one in which soil organisms release inorganic nutrients into the soil from the organic reserves at a rate sufficient to replenish nutrients as they become exhausted by root uptake (Harris, 1988a).

The role of microorganisms in regulating the availability of macronutrients such as N and P in soils is well established, the case of nitrogen providing a useful example in this regard. Microorganisms are known to regulate the release of nitrogen from organic compounds (*ammonification*), the oxidation of ammonia released by this process to both nitrite and nitrate ions (*nitrification*), the reduction of nitrate to nitrogen and its oxides (*denitrification*), and the fixation of atmospheric nitrogen (Harris, 1988b). One of the most important of these functions in the context of nutrient availability is the mineralization of soil organic nitrogen.

Some of the other important roles played by soil organisms include charge alteration of a given element (during redox changes generally), complexing of an element by organic acids or other compounds produced by microorganisms and microbial accumulation or mobilization of an element (Kabata-Pendias and Pendias, 1985). The nature of the soil fauna and flora and the details of soil organism nutrient interactions are discussed in most standard soil chemistry texts and will not be discussed further here: Harris (1988a) has provided a particularly comprehensive and readable account of this fascinating subject. As mentioned at the beginning of this chapter, plant factors such as the mychorrhizal colonization of roots or the role root exudations in nutrient uptake will not be discussed here.

2.2 Causes, evaluation and possible consequences of soil related deficiencies of selected nutrient elements.

Instead of an exhaustive account of all the known plant and animal nutrients, the discussion in this section will focus on a select group of elements which are either known or suspected to be necessary for skeletal integrity in animals and humans; the rationale behind this is to introduce or refresh the reader to concepts and facts related to possible elemental deficiencies which may have a bearing on the etiology of Mseleni Joint Disease. The selected elements, as mentioned in Chapter 1, are as follows: Ca, P, Zn, Mn, Cu, B and Se. Fluorine, although considered necessary to skeletal integrity, has long been known to be derived primarily from drinking water and will not therefore be discussed in this section. In the case of each element a brief description of the geographic occurrence of soil related deficiencies will be followed by a description of the primary causes of such deficiencies, the use of soil tests as a measure for predicting the likelihood of such deficiencies and finally by a discussion of the possible consequences of deficiencies of the particular element in question in relation to both plants and animals/humans. The information presented below is purely factual and is intended to serve partly as a demonstration of the principles discussed in the first half of this chapter, and partly as a a foundation for the discussions involving soil and plant deficiency in Chapters 3 and 4.

2.2.1 Calcium deficiency

2.2.1.1 Distribution and causes

Deficiencies of Ca as a nutrient are uncommon in agricultural situations, due to the fact that neutral and alkaline soils usually contain adequate concentrations of this element and acid soils are generally limed to provide a favourable pH for most crops (Haby *et al.*, 1990). Regions experiencing calcium deficiencies have been fairly comprehensively documented in the United States; affected areas are generally situated on the broad sandy Atlantic coastal plain as well as on the West coast (Adams, 1984; Haby *et al.*, 1990). Many acid soils in the tropics have low Ca contents and low ECEC, which may lead to Ca-deficiency (Kamprath, 1984).

Tisdale et al. (1985) list the most important factors influencing the availability of Ca as the following: total Ca supply, soil pH, CEC, percent Ca saturation of soil colloids, type of soil colloid and the ratio of Ca to other cations in solution. Calcium deficiency is most often associated with coarse textured, acid soils in humid regions which have been formed from parent materials low in weatherable calcium containing minerals and which are subject to removal of Ca by leaching (Adams, 1984; Tisdale *et al.*, 1985): notable amongst such soils are the podzols of the temperate zone and the laterites of the humid tropics (Mengel and Kirkby, 1978). High H^+ activity associated with low pH interferes with Ca uptake, making plants

growing in acid mineral soils vulnerable to Ca deficiency particularly if base saturation is low (Mengel and Kirkby, 1978). CEC is important to Ca availability with respect to the Ca saturation of soil colloids and the type of soil colloids (Haby *et al.*, 1990); many crops respond to Ca applications when the degree of Ca saturation of soil CEC falls below 25 %. Kaolinite clays have been found to provide adequate Ca to plants at saturation values of only 40-50 %, whilst 2:1 layer silicates (e.g. montmorillonite) require a Ca saturation of 70 % or more before an adequate supply is provided to plants (Tisdale *et al.*, 1985).

2.2.1.2 Prediction using soil tests

The total calcium content of soils is generally of no use in determining the likelihood of Ca-deficiency in plants (Tisdale *et al.*, 1985).

Exchangeable and soluble Ca as extracted by 1*N* NH_4OAc at pH 7 are generally regarded as being available to plants (Lanyon and Heald, 1982), although other fractions, e.g. that extracted by 1*N* KCl or 0.025*N* $CaCl_2$, may also be used to predict calcium availability. No critical levels, or soil concentrations below which deficiency is likely to be induced in most plants, can realistically be established for Ca in soils due to the strong dependence of such deficiency states on plant genotype (Adams, 1984), soil factors (discussed above) and growing conditions (Haby *et al.*, 1990). Melsted (1953) observed Ca deficiency symptoms in maize grown on acid soils containing < 400 mg/kg extractable Ca, noting that Ca deficiency was not evident until other nutrient deficiencies were corrected by large applications fertilizers. This value may be pertinent to the Mseleni area, where maize is grown as a staple crop on similar soils.

2.2.1.3 Calcium deficiency in plants and animals

Calcium is a macronutrient essential to all higher plants, with normal concentration ranges in plant tissue ranging from 0.2-1 % (Tisdale *et al.*, 1985). Calcium is required for cell elongation and cell division and is known to have an important role in the structure and permeability of cell membranes (Mengel and Kirkby, 1978).

Calcium deficiency results in the failure of both the terminal buds and apical tips of roots (i.e. growing points) to develop, leading to growth stunting (Tisdale *et al.*, 1985); young leaves may also become deformed and chlorotic and eventually necrotic at the margins (Mengel and Kirkby, 1978). Inadequate calcium nutrition in maize plants prevents the emergence and unfolding of new leaves, the tips of which are generally colourless and covered in a sticky gelatinous material which causes them to adhere to one another (Tisdale *et al*, 1985). Calcium deficiency also impairs membrane permeability, leading to a

gradual disintegration of cellular membranes (Clark, 1984). The growth of fruits and storage tissues (e.g. peanuts, tomatoes) depend largely on soil Ca availability, hence Ca deficiency is often manifested in these parts (Mengel and Kirkby, 1978).

Adequate calcium intake in animals is primarily required for the optimal growth and mineralization of the skeleton, and Ca deficiency is therefore generally manifested in the form of skeletal disorders. Calcium deficiency is most widely known to result in osteomalacia (involving a softening of the bones due to impaired mineralization), rickets (involving limb-bone deformities) and osteopenia. The latter - involving a reduction in bone mass - includes osteoporosis, which involves decreased bone volume due to bone resorption (Nordin, 1976; Heaney, 1981). These symptoms may often coexist: experimentally induced Ca deficiency in rats, for example, has been shown to result in increased bone resorption, decreased matrix apposition, delayed onset of mineralization and a decreased calcification rate (Schnitzler et al., 1988). In rural communities where subsistence agriculture is the predominant source of nutrients, an important complicating factor in dietary Ca deficiency in humans is the apparent inhibitory effect of dietary oxalates on Ca uptake (Pingle and Ramasastri, 1978). These oxalates are most often consumed in the form of green leafy vegetables belonging to *Amaranthus spp.*; India and South Africa are two examples of countries where such a potential problem has been identified (Schnitzler *et al.*; 1988, Pingle and Ramasastri, 1978).

2.2.2 Phosphorus deficiency

2.2.2.1 Distribution and causes

Phosphorus deficiency is both widespread and well characterized in agricultural systems due to the role of P as an essential macroelement in plants. Sandy, podzolic soils in particular are characterized by low P content, although many other soil types induce P deficiency regardless of total P concentrations (Mengel and Kirkby, 1978).

The concentration of P in the soil solution appears to be the most important factor governing the availability of this element to plants (Tisdale *et al.*, 1985); this is influenced by the rate and extent to which P is immobilized by biological factors and by the reaction of P with the mineral fraction of soils. Soils high in soluble Fe and Al, for example, react with ortho- and polyphosphates (some of the many forms of soil phosphorus) to form a variety of insoluble compounds (Tisdale *et al.*, 1985). Soluble phosphates also undergo reactions in soils high in clays (especially 1:1 clays, such as kaolinite) which convert them to relatively insoluble forms. Soil pH has an important influence on the fixation of soluble P in soils (Tisdale *et al*, 1985), adsorption of P by Fe and Al oxides declining with increasing pH: P availability in most soils is at a maximum in the pH range from 6.0-6.5. A full treatment of the many

facets of soil P availability are dealt with adequately in Tisdale *et al.* (1985), and will not be discussed further here.

2.2.2.2 Prediction using soil tests

Soil tests for phosphorus have been designed almost exclusively to assist in fertilization regimes in order to optimize large scale crop production. EDTA- and DTPA based extractants are most commonly used in soil tests for P (Fixen and Grove, 1990). As in the case of calcium, no absolute critical levels for soil P concentrations can be established; sufficiency is instead usually calculated using a combination of soil tests and plant responses (Fixen and Grove, 1990).

2.2.2.3 Phosphorus deficiency in plants and animals

Phosphorus is a macronutrient in plants, commonly constituting between 0.1-0.4 % of plant tissue (Tisdale *et al*, 1985). Phosphorus has many essential functions, the most important of which is probably its role in energy storage and transfer (Mengel and Kirkby, 1978): ATP, or adenosine-triphosphate, and ADP (A-diphosphate) are the most important compounds in this regard. In fact, almost every metabolic reaction of any significance in plants is thought to proceed via P derivatives (Mengel and Kirkby, 1978). In addition to the metabolic role of P, it is an important structural component of a wide variety of biochemicals, including nucleic acids, coenzymes, nucleotides, phosphoproteins, phospholipids and sugar phosphates (Tisdale *et al.*, 1985).

Phosphorus deficiency results in a reduction of RNA synthesis, which in turn reduces protein synthesis in plants (Mengel and Kirkby, 1978); this leads to a reduction in vegetative growth, characterised by thin stems, and limited root system development. Fruit trees show reduced growth rates of new shoots and possible retardation of fruit and seed formation. The stems of many plant species suffering from P deficiency are characterized by a reddish hue originating from an enhanced formation of anthocyanins (Mengel and Kirkby, 1978).

Phosphorus in animals plays a critical role in almost every aspect of life (Lee *et al.*, 1981). Instead of reviewing all aspects of P deficiency in animals, bone related disorders will be focused on in this discussion. The role of P in the regulation of bone formation is well recognized. Phosphorus has been demonstrated to stimulate both bone matrix synthesis and mineral deposition (Lee *et al.*, 1981).

Phosphorus deficiency is known to lead to the development of both osteomalacia and rickets in humans (Nordin, 1976; Lee *et al.*, 1981). Although the metabolic pathways involved in P and Ca deficiencies are very different (Nordin, 1976), the symptoms are similar to those described above for calcium deficiency.

2.2.3 Zinc deficiency

2.2.3.1 Distribution and causes

Zinc deficiencies in plants and animals are widespread in the U.S.A., whilst occurrences have been reported in Canada, western Europe, Great Britain, Israel, New Zealand, Australia, Central and Southern Africa and Brazil (Tisdale *et al.*, 1985). Soil conditions frequently associated with zinc deficiency in soils include: acid, sandy soils low in total zinc; neutral or basic soils, especially when calcareous; soils with a high content of fine clay and silt; soils high in available P; some organic soils; and finally subsoils exposed by land levelling operations or by wind and water erosion (Tisdale *et al.*, 1985).

Aubert and Pinta (1977) concluded that parent material has a much greater effect on soil Zn content than do pedogenic factors. These workers noted that basic eruptive rocks (e.g basalt) generally have a higher Zn content than acid eruptives, metamorphic or sedimentary rocks (e.g. limestone or sandstone). As a result extensively leached, coarse textured soils high in silica (e.g. those resulting from the weathering of sandstone) are frequently deficient in Zn, as are calcareous soils (Moraghan and Mascagni, 1991). Zinc availability varies with pH, decreasing as soil pH increases due to both specific and non-specific processes (Moraghan and Mascagni, 1991). In the case of light textured, acid soils Zn adsorption may be reduced at lower pH values by competing cations, resulting in high mobility and leaching of Zn (Kabata-Pendias and Pendias, 1985).

Organic matter content in soils is strongly correlated with Zn concentrations, resulting in higher levels of extractable Zn often accumulating in topsoils (Kabata-Pendias and Pendias, 1985; Tisdale *et al*, 1985): hence the removal of topsoils can often lead to Zn deficiency. Solubility and availability of Zn are negatively correlated with Ca and P content, with Zn deficiency in plants often associated with soils rich in Ca and P or in soils containing high levels of Ca saturated minerals (Kabata-Pendias and Pendias, 1985). Other element interactions which affect Zn include antagonistic interactions with divalent Cu, Mn and Fe cations which compete for similar sites (Tisdale *et al.*, 1985).

2.2.3.2 Prediction using soil tests

The need to identify soils with inadequate amounts of Zn and the other micronutrients became apparent in the 1930's when deficiencies were confirmed under field conditions (Martens and Lindsay, 1990). It was soon recognized that the plant availabilities of these micronutrients was a function of soil properties rather than of total elemental abundances: the improvement of extraction procedures

the present day, a useful account of which has been provided by Martens and Lindsay (1990).

Although a range of laboratory extraction techniques has been used for Zn and the other micronutrient cations (e.g. Cu, Mn and Fe), those which have found most favour in routine soil analysis have necessarily been designed to: i) solubilize a fraction of the available forms of the given nutrient in soils with variable properties; ii) be amenable to rapid and accurate analysis and iii) produce results which are correlated with crop responses to the given nutrient under differing conditions (Martens and Lindsay, 1990). Extractant solutions most commonly used for soil Zn evaluation have been EDTA-$(NH_4)_2CO_3$, NH_4HCO_3-DTPA, NH_4HCO_3-EDTA and DTPA-TEA (Martens and Lindsay, 1990). Research using these tests and different soil types from around the world has shown that values of about 0.8-1.4 mg/kg Zn in a soil constitute the range of values likely to cause deficiency in plants (Martens and Lindsay, 1990).

A level of uncertainty exists about any *critical level* obtained using micronutrient soil tests, due mainly to factors such as sampling variability, seasonal weather variations and soil chemical parameters, for example P content (Martens and Lindsay, 1990). In the case of zinc, however, such tests have been shown to be particularly successful in separating soils into Zn deficient and sufficient categories (Martens and Lindsay, 1990).

2.2.3.3 Zinc deficiency in plants and animals

Zn is absorbed by higher plants predominantly as a divalent cation which functions either as a metal component of enzymes or as a functional, structural or regulatory cofactor for a large number of enzymes (Kiekens, 1995). Zn is involved in carbohydrate and protein metabolism, and is also required for the synthesis of tryptophan, a precursor for the synthesis of indoleacetic acid (Kabata-Pendias and Pendias, 1985). The most characteristic visible symptoms of Zn deficiency in dicotyledons are short internodes (rosetting) and a decrease in leaf expansion (little leaf); these stunted growth characteristics are often combined with chlorosis of the youngest leaves (Romheld and Marschner, 1991). In monocotyledons, especially maize, chlorotic bands occur along the midribs of leaves combined with red, spot-like discolouration (Bennet, 1993).

Zinc is essential for the function of more than two hundred enzymes or related proteins in animals (Ament, 1991; Miller *et al.*, 1991). This element plays a key role in numerous essential processes including protein synthesis, DNA and RNA metabolism, carbohydrate and lipid metabolism and energy metabolism, mainly through its role as a component of metalloenzymes. Most Zn in mammals is bound in the bones and is not readily available during periods of low Zn intake or increased demand, e.g. during healing (Hidiroglou, 1980).

Zn deficiency has been identified in pigs, poultry, cattle and humans (Ament, 1991). Many functions which are related to protein synthesis are suppressed by Zn deficiency, including growth, cellular immunity and fertility (Smith and Gawthorne, 1975). Common symptoms of Zn deficiency in animals and humans include skin lesions, stunted growth, reproductive failure, problems with wound healing, skeletal defects, egg shell defects and dwarfism (Miller, 1983). Zinc has been found to be the principal limiting factor in the growth of children (Prasad, 1982), especially amongst persons who have inadequate protein intakes. Zinc deficiency is most prevalent in subsistence populations whose diet is dominated by cereal crops: the availability of Zn in such diets is very poor due to high phosphate and phytate content (Prasad, 1982). Nutritional deficiency of Zn in humans is fairly common globally, especially in developing countries: dwarfism attributed to Zn deficiency was first reported from Egypt and Iran and has since been documented in many other countries (Miller, 1983).

2.2.4 Manganese deficiency

2.2.4.1 Distribution and causes

Tisdale *et al.* (1985) report that 13 million acres in thirty states in the United States may be low in Mn: these deficient areas are more widespread in the humid regions of the East (e.g. the Atlantic coastal plain) than in the arid calcareous soils of the West. Manganese deficiency in England is most often associated with peaty soils and soils high in organic matter content (Reuter *et al.*, 1988), in Denmark, China and India deficiency is associated with calcareous soils, whilst in Australia and Scotland podzols are often deficient. Manganese deficiency is often associated with impoverished soils which had parent materials either inherently low in Mn or from which Mn had largely been removed by weathering and leaching, as well in soils characterized by high pH and free carbonates (Reuter *et al.*, 1988). Some soils identified with Mn deficiency are: thin, peaty soils overlying calcareous subsoils; alluvial silt and clay or marsh soils derived from calcareous materials; poorly drained calcareous soils high in organic matter; calcareous soils freshly broken up from old grassland; and very sandy acid mineral soils which are low in native Mn content and where the limited quantities of available Mn may have been leached from the root zone.

Parent materials known to result in soils with low Mn content are crystalline shales, sandstones and acid igneous rocks (Moraghan and Mascagni, 1991). Manganese deficiency commonly occurs in poorly drained sandy soils and in soils which fluctuate between a well drained and a waterlogged condition. The activity of Mn in solution is governed largely by pH and redox conditions: high soil pH and Eh favour the insoluble oxide forms of Mn, whilst low pH levels and redox potentials favour the more soluble divalent species (Kabata-Pendias and Pendias, 1985). Organic matter may limit the availability of Mn in basic soils with a high humus content due to the formation of unavailable chelated Mn^{2+} compounds,

whilst high levels of available Fe may induce Mn deficiency (Tisdale *et al.*, 1985).

2.2.4.2 Prediction using soil tests

Similar soil tests to those described above for the assessment of soil Zn levels are used for the extraction of Mn from soils. Due to the fact that Mn deficiency is very weather dependent in the case of most field crops (Martens and Lindsay, 1990), soil testing procedures may be of limited value in the evaluation of areas of potential Mn deficiency (Tisdale *et* al., 1985). Foliar analysis is a useful complementary technique to soil analysis in order to assess likely areas of deficiency. Critical extractable soil Mn concentrations as determined by various researchers over the last three decades using the extractants mentioned above (under *zinc*) range from 0.2-4.7mg/kg Mn (Martens and Lindsay, 1990). Sims and Johnson (1991) report that field and greenhouse research have consistently demonstrated that the most successful approach to the identification of Mn deficient soils is the use of an availability index incorporating both extractable Mn *and* pH.

2.2.4.3 Manganese deficiency in plants and animals

Mn is an essential element for plants, in which its major function is related to the oxidation-reduction process (Kabata-Pendias and Pendias, 1985). Divalent Mn is known to be a specific component of two enzymes and can also substitute for Mg^{2+} in other enzymes: the element appears to participate in the oxygen evolving system of photosynthesis and also plays a basic role in the photosynthetic electron transfer system (Romheld and Marschner, 1991). Well known symptoms of Mn deficiency in cereals include greenish grey spots, flecks and stripes on the basal leaves, whilst the condition in dicotyledons is characterised by interveinal chlorosis of the younger and middle leaves (Romheld and Marscher, 1991).

Manganese is essential for growth, reproduction and skeletal development in all species of animal which have been investigated to date, including chickens, rats, pigs and cattle (Leach, 1976). Manganese is associated with only a very limited number of metalloenzymes, but may activate numerous metal enzyme complexes as a cofactor (Ament, 1991). This element is also a constituent of bone, having important metabolic functions which include roles in cartilage and bone formation (Hidiroglou, 1980).

Deficiency in animals results in retarded growth, skeletal abnormalities, ataxia (postural defects) of the young and reproductive failure: although species responses differ, skeletal abnormalities and postural defects are common to all (Leach, 1976). The role of Mn in mucopolysaccharide synthesis has been blamed for cartilage and bone abnormalities (Hidiroglou, 1980) as well as for abnormal egg shell formation (Leach, 1976).

Unequivocal Mn deficiency has not as yet been demonstrated in humans (it has not been examined for ethical reasons), although Xilinas (1983) and co-workers linked soil induced Mn deficiency to congenital hip disorders in certain rural populations in Fance, Canada and the U.S.A. in a series of epidemiological studies.

2.2.5 Copper deficiency

2.2.5.1 Distribution and causes

Copper deficiency in plants has been reported from many countries (Davies and Jones, 1988), including large tracts of the U.S.A. and Canada (Tisdale *et al.*, 1985): in the latter case deficiencies are generally associated with either intensive cultivation of peats and mucks or with organic or very sandy soils. Sandy calcareous soils in Canada are often copper deficient (Tisdale *et al.*, 1985).

Organic chelation and complexation are thought to be the most important factors controlling Cu solubility in soils (Kabata-Pendias and Pendias, 1985), with deficiency symptoms often being associated with soils high in organic matter (Moraghan and Mascagni, 1991). Copper deficiencies in crops may occur if the total soil Cu content is low, for example when podzols are brought into agricultural production or in soils with inherently low Cu parent materials such as granites, sandstones or sandy glacial deposits (Davies and Jones, 1988; Moraghan and Mascagni, 1991). Deficiencies are usually caused by soil conditions which reduce the availability of an otherwise marginal soil reserve, an example of which occurs due to organic matter complexation of Cu in peat bogs. High Zn concentrations in soils tend to accentuate Cu deficiencies through the mechanism of competition for similar sites (McBride, 1994).

2.2.5.2 Prediction using soil tests

As in the case of Zn and Mn, the most useful methods in terms of predicting plant responses to Cu have employed EDTA and DTPA extractants (Martens and Lindsay, 1990; Sims and Johnson, 1991). Critical Cu levels range from 0.12-0.25 mg/kg for DTPA and from 0.4-1.0 mg/kg for EDTA solutions (Sims and Johnson, 1991). Complicating factors in the interpretation of soil Cu values include the fact that low amounts of Cu translocation from roots to shoots in various plant species sensitive to Cu deficiency may lead to low correlations between plant Cu concentrations and the Cu supplying power of the soil as tested by soil micronutrient extraction techniques (Martens and Lindsay, 1990). Plants also differ in their sensitivities to Cu (Tisdale *et al.*, 1985), implying that soil tests have to be calibrated for each species or for species with equal sensitivities (Martens and Lindsay, 1990).

2.2.5.3 Copper deficiency in plants and animals

Copper occurs in several vital plant enzymes, fulfilling an essential role in the following processes: photosynthesis; respiration; carbohydrate distribution; N reduction and fixation; protein metabolism; and cell wall metabolism. Copper controls the production of RNA and DNA in plants, and its deficiency inhibits their reproductive abilities as well as their disease resistance (Kabata-Pendias and Pendias, 1985).

In general, Cu deficiency depresses reproductive growth (pollination and the formation of seeds and fruits) more than vegetative growth (Romheld and Marschner, 1991). Other typical symptoms of Cu deficiency are chlorosis (white tip), necrosis, leaf distortion and dieback, as well as wilting (impaired water transport) and shoot bending (Romheld and Marschner, 1991).

Copper in animals and humans is most important in haemoglobin synthesis, neonatal development, skeletal development, pigmentation, fertility and reproduction, co-ordination and nerve function and connective tissue maturation (Harris, 1983): all of these are affected by Cu containing metallo-enzymes. Cu deficiency in livestock was first noted in the form of anaemia: subsequently a host of abnormalities in Cu deficient animals and livestock grazing on Cu depleted pastures have been reported, including abnormal bone formation, reproductive failure, spontaneous bone fracture and "swayback" disease in sheep which is related to demyelination of the spinal cord (Smith and Gawthorne, 1975). Anaemia related to Cu deficiency has been traced to a Cu-protein found in the plasma, which has been shown to catalyze the oxidation of Fe^{2+} by molecular oxygen (Ament, 1991).

Bone disorders related to Cu deficiency have been observed in many species (Smith and Gawthorne, 1975; Harris, 1983): the structural integrity of bones and cartilage are impaired in Cu deficient tissues, leading to fragility, loss of strength and osteoporosis (Hidiroglou, 1980). Fractures, enlarged joints and other signs of deficiency related disorders are also apparent (Hidiroglou, 1980). Copper deficiency may also be responsible for scurvy like changes in long bones: the typical changes include osteoporosis and metaphyseal and soft tissue calcification (Ament, 1991). The Cu containing enzyme *lysine oxidase* appears to control the integrity of collagen and elastin (the body's support proteins), explaining some of the above disorders (Ament, 1991).

2.2.6 Boron deficiency

2.2.6.1 Distribution and causes

Tisdale *et al.* (1985) report soil B deficiencies in 4 regions in the U.S.A.: the Atlantic coastal plain, the

Pacific coastal area, the Pacific Northwest and northern Michigan, Wisconsin and Minnesota. These deficient areas extend northwards into Canada. Kabata-Pendias and Pendias (1985) report B deficiency as being widespread globally, with the lowest values having been determined in Polish and New Zealand soils.

Less than 5 % of soil B is generally available to plants (Tisdale *et al.*, 1985); tourmaline, the main B containing mineral in most soils, is quite insoluble and hence resistant to weathering, thus often supplying inadequate concentrations of B in the soil solution for plant growth. Soils derived from granites and other acid igneous rocks are often poor in B. Since B is usually present in the soil as a neutral molecule, it is very mobile (Moraghan and Mascagni, 1991); in fact it is regarded by some as the most mobile element among the micronutrients (Kabata-Pendias and Pendias, 1985). Due to this high mobility B movement in the soil generally follows the water flux, being leached downwards in humid climates and being concentrated in surface horizons in arid or semi-arid regions (Kabata-Pendias and Pendias, 1985). Boron deficiency is often encountered in soils with pH > 6.5, due to the fact that B is strongly adsorbed by sesquioxides, organic matter and soluble aluminium as pH is raised (Sumner *et al.*, 1991).

Organic matter may be one of the main sources of B in acid soils; the greater availability of B in surface soils compared with subsurface soils is undoubtedly related to the greater quantities of organic matter present in the former (Tisdale *et al.*, 1985). There appears to be a relationship between B and Ca in plants: low Ca supply leads to low B tolerance, whilst high Ca supply leads to greater B requirements (Tisdale *et al.*, 1985).

2.2.6.2 Prediction using soil tests

A hot-water-soluble extraction procedure for determining B availability is used almost exclusively for soil B evaluation (Kabata-Pendias and Pendias, 1985; Sims and Johnson, 1991). The range in critical levels using this procedure is variously reported as 0.1-2.0 mg/kg (Sims and Johnson, 1991) or 0.1-0.3 mg/kg (Kabata-Pendias and Pendias, 1985). Johnson and Fixen (1990) report that the hot water extraction procedure has proved to be consistently reliable for estimating available B concentrations in soils.

2.2.6.3 Boron deficiency in plants and animals

Boron is neither an enzyme constituent nor does it directly affect enzymatic activities (Romheld and Marschner, 1991): instead it forms very stable complexes with certain organic compounds which include sugars and their derivatives as well as constituents of cell walls. The most important roles for B in plants include cell wall formation and stabilization, lignification and xylem differentiation, and the translocation of sugars (especially in plants such as sugar beet).

Boron deficiency in plants commonly leads to abnormal pollen tube growth and pollen germination disorders which are highly important to crop production (Romheld and Marschner, 1991). Boron deficiency symptoms may also appear at the terminal buds and youngest leaves as retarded growth or necrosis. Increases in the drop of buds, flowers and developing fruits are also widespread, as a result of B deficiency, as are failure to set seed and the malformation of fruit (Tisdale *et al.*, 1985).

Boron is not known to be an essential element in animals, although tentative evidence of links between soil related dietary B deficiency and the susceptibility of bone to osteo and rheumatoid arthritis have been reported by Newnham (1981).

2.2.7 Selenium deficiency

2.2.7.1 Distribution and causes

Areas of the world where low Se soils have been identified include large areas of the U.S. and Canada, New Zealand, Australia, Scotland, Finland, Sweden, Austria, Germany, France, Russia, Turkey, Greece and South Africa (Tisdale *et al.*, 1985). Many of the low-Se soils in the U.S.A. are reported to be characterised by either low total Se or by low availability of Se (acid and poorly drained soils).

Selenium availability is governed principally by pH and redox conditions (Tisdale *et al.*, 1985). In alkaline, oxidized soils, selenates (SeO_4^{2-}) are the dominant soil form; these oxyanions bond weakly to oxides and other minerals, resulting in high mobility of Se in neutral to alkaline soils (McBride, 1994). In slightly acid soils which are oxidized, selenites (SeO_3^{2-}) prevail, showing considerably lower mobility than selenates due to a tendency to chemisorb strongly onto oxides and aluminosilicates and to precipitate as the insoluble ferric selenite (McBride, 1994).

Selenium availability has been reported to be higher in the alkaline pH range than in the acidic range, although the lowest availability seems to occur at a neutral or slightly acidic soil pH. In wet, acid or humus-rich soils the insoluble reduced forms of Se predominate, rendering Se bioavailability very low; in addition, the inherent Se levels in most soils are low (Kabata-Pendias and Pendias, 1985), so that crops often contain Se levels which could produce deficiencies in animals/humans (McBride, 1994).

2.2.7.2 Prediction using soil tests

Soil tests for Se are not widely used to assess the availability of this element; plant tissue tests, especially those involving typical forage material for livestock susceptible to Se deficiency/toxicity are

normally utilized instead (Wan *et al.*, 1988).

2.2.7.3 Selenium deficiency in animals

Se is not known to be essential for plants, but is important in forage materials due to the sensitivity of ruminants and other animals to both Se deficiency and toxicity (Davies and Jones, 1988). Of particular concern are the Se accumulator plants (e.g. the genus *Astragalus*) which may contain up to 0.9 % Se.

Glutathione peroxidase is a selenoprotein which detoxifies lipid peroxides and protects cellular and subcellular membranes against peroxide damage in conjunction with vitamin E and other antioxidants (Miller *et al.*, 1991), making Se an essential element in animals. A major symptom of Se deficiency which has been observed in all livestock is *White Muscle Disease*, or nutritional muscular dystrophy, which is associated with excessive peroxidation of lipids. Other symptoms of Se deficiency in animals include pancreatic atrophy (observed in chicks), *hepatosis dietetica* (involving intralobular haemorrhage and hepatic necrosis) and *Mulberry Heart disease* (mottling and dystrophy of the myocardium) in pigs; both the latter conditions result in sudden death (Miller *et al.*, 1991).

Selenium is efficiently transferred up the soil-plant-animal-human food chain so that geographical differences in the availability of the Se in soil for uptake by plants account for most variations in the Se content of foods (Levander, 1987).

Studies in Finland suggested an inverse relationship between Se status and the incidence of cancer in humans (Levander, 1982); this relationship has since been reported from other parts of the globe. *Keshan disease* is an endemic cardiomyopathy which has been recognised in China and Siberia for more than a century, especially in peasant populations consuming a limited and unvaried diet (Zhang, 1986): low Se status (measured in soils, water and foodstuffs) appears to play the primary etiological role in this disease. *Kashin-Beck disease* is an endemic osteoarthropathy (disabling, polyarticular degenerative joint disease) prevalent in parts of northern China, Korea and eastern Siberia. Nutritional Se deficiency is one of the suspected causes of this disease, which is characterised by symptoms such as dwarfism, swollen joints and general stiffness (Levander, 1987).

Although superficially similar in many respects to Mseleni Joint Disease (MJD), the symptoms of Kashin-Beck disease include aches, muscular weakness, cramps, fatigue (Zhang, 1986), symmetrical stiffness, swelling and pain in interphalangeal joints (Levander, 1987), none of which are found in MJD sufferers. Shortened fingers and toes and locking of joints are also typical features of Kashin-Beck disease which are absent in the case persons afflicted by MJD. The radiographic features of the

two diseases (on analysis of bone tissue) also appear to differ (Beighton, pers. comm., 1996).

2.3 Conclusions

An understanding of soil related nutrient element deficiencies in soil-plant-animal systems generally demands a careful consideration of the interactions between many interrelated factors, the most important of which possibly include geological parent material, climatic regime, the nature of both the soil solids and the soil solution and biological mediation of nutrient cycling by soil organisms. Soil parent material and climatic factors dictate to a large extent whether a given nutrient will be present or absent in adequate concentrations within a given soil, whilst the compositions of the soil solids and solution generally govern the availability of nutrient elements within the soils.

Soils are the primary source of nutrient elements for plants, and hence for animals and (either directly or indirectly) humans. Soil related deficiencies of two macronutrients and five micronutrients have been reviewed in order to put into context the results of soil and plant analysis reported in Chapters 3 and 4. It is clear that both soil genesis and soil conditions are important in delineating areas of nutrient deficiency, which is characterised by a range of plant and animal responses.

Of these responses, those affecting the skeletal systems of animals and humans are clearly the more important in any discussion on the etiology of Mseleni Joint Disease. Both calcium and phosporus deficiency in humans may lead to the development of osteomalacia and/or rickets, whilst calcium deficiency can also cause osteopenia (the latter has been described in patients with MJD). Zinc deficiency in humans and animals may lead to stunted growth (including dwarfism) and skeletal defects, both of which are prevalent at Mseleni. Manganese deficiency causes skeletal and cartilage defects in animals: humans have not been experimented upon and it is unclear whether similar effects would be expected, although some workers have suggested that congenital hip disorders in several rural populations worldwide have been caused by soil related Mn deficiencies.

Copper deficiency in animals has been reported to impair the structural integrity of bone and cartilage in animals, leading to abnormal bone formation, premature fracture and osteoporosis, all of which are observed in the human population at Mseleni. Boron defiency has been linked to osteoarthritis in rural black populations in South Africa, whilst selenium deficiency has been suggested as a possible cause of Kashin-Beck disease in China, which manifests as an endemic polyarticular osteoarthropathy.

The reasons for investigating possible deficiencies of the above elements should be self evident at this stage: the following two chapters will focus on this task, referring to some of the basic factual information contained in this chapter as a foundation for further discussion.

Chapter 3

Geochemical characterization of Mseleni soil and water samples

3.1 Introduction

The objective of this chapter is to report and discuss geochemical data pertaining to forty six soil and two water samples collected in the Mseleni area in the relatively cool, dry winter month of July, 1996. Having explained the rationale behind this type of investigation in Chapter 1, and having explored the utility of soil analysis with respect to selected nutrient deficiencies in Chapter 2, this is the first of two chapters dealing specifically with the assessment of nutrient availability in the study area itself. Special emphasis is placed on the abundance, availability and distribution of selected nutrient elements in the characteristic *Fernwood* soils of the Mseleni area, in accordance with the hypotheses presented in Chapter 1.

It should be noted at this juncture that the prediction of nutrient deficiency based on the results of soil tests alone is not an infallible approach. Such results should instead be regarded in the context of this project as providing a basis for understanding deficiencies induced in plants growing on the soils. This subject is broached in Chapter 4.

3.2 Materials and methods

3.2.1 Sampling strategy

Sampling was carried out over a period of two weeks. Given the time and resources available for this project, two options seemed to be available: one was to carry out a regional characterization of soils in which comparisons could be drawn between different areas, especially those with apparently differing incidences of Mseleni Joint Disease (MJD). This approach would have involved collecting a relatively small number of samples from the high incidence area, in order to facilitate a valid statistical comparison with other areas. The other was to focus sampling largely on the high incidence area (Fig. 3A), characterizing soil chemical properties and investigating the spatial variation of these properties through systematic transect sampling. This approach would entail sampling along transects both parallel and perpendicular to variations in topography and vegetation patterns, and, if time permitted, would include a few samples (both soil and water) of interest from elsewhere which might offer additional geochemical insights or provide a basis for recommending further studies.

After some deliberation the latter approach was adopted. Two traverses were sampled through the sandy

Fernwood soils (Fig. 3B) in the vicinity of the Mseleni mission hospital, whilst two red sand and two mudpan samples (Fig. 3B) were collected further inland (Fig. 3A). Two water samples were also collected, one from a tap supplying Lake Sibaya water to rural homesteads and another from a borehole sunk into the sandy aquifer underlying the area.

3.2.2 Sampling localities

The longer of the two soil traverses was oriented west-east (Fig. 3A), roughly bisecting the high incidence area for MJD. This was carried out in order to firstly transect the topographic features in the area (oriented north-south), secondly to investigate any maritime influence on soil chemistry and thirdly to investigate whether the high incidence area is geochemically distinct from surrounding areas. Thirty soil samples were collected along this traverse, including 24 topsoil and 6 subsoil samples. The distance between sampling sites was one kilometre in a direction perpendicular to the coastline.

The second traverse was oriented north-south (Fig. 3A), and positioned such that it fell entirely in the high incidence area. Twelve samples, 9 topsoil and 3 subsoil, were collected, with an intersample spacing of 500 m. The reasoning behind the positioning of this traverse was as follows: the high incidence area would be well characterized; the possibility of spatial inhomogeneities in soil chemical properties within this area of similar relief, edaphic features, rainfall and vegetation type could be investigated; any impact of subsistence farming on soil chemical quality could be accomodated (a high proportion of the land is inhabited); and, finally, the relative removal of nutrients in this elevated ridge area could be investigated.

Two red, sandy soils (*Hutton* form) were collected ± 30 kilometres inland (Fig. 3A), in order to get a rough idea of how similar these are chemically to the *Fernwood* soils. These red sands constitute the next soil type inland of the *Fernwood* soils, which dominate the sandy coastal plain. In addition, two soil samples were collected from dry mud pans situated in topographic lows in an area of grey *Fernwood* soils (Fig. 3A). This was done in order to ascertain whether a significant proportion of clays and nutrient cations were being leached from adjacent ridges of grey sand into topographic lows, as might be expected in this environment.

The nature of the waters sampled has been described above; the sampling localities are depicted in Figure 3A (Lake Sibaya water was sampled from a tap near sample 12 of the WE traverse). Both waters were sampled for the purpose of investigating local fluoride levels, in order to complement the published analyses of Lubbe *et al.* (1973). These workers apparently failed to sample Lake Sibaya, which is an important water source in the area.

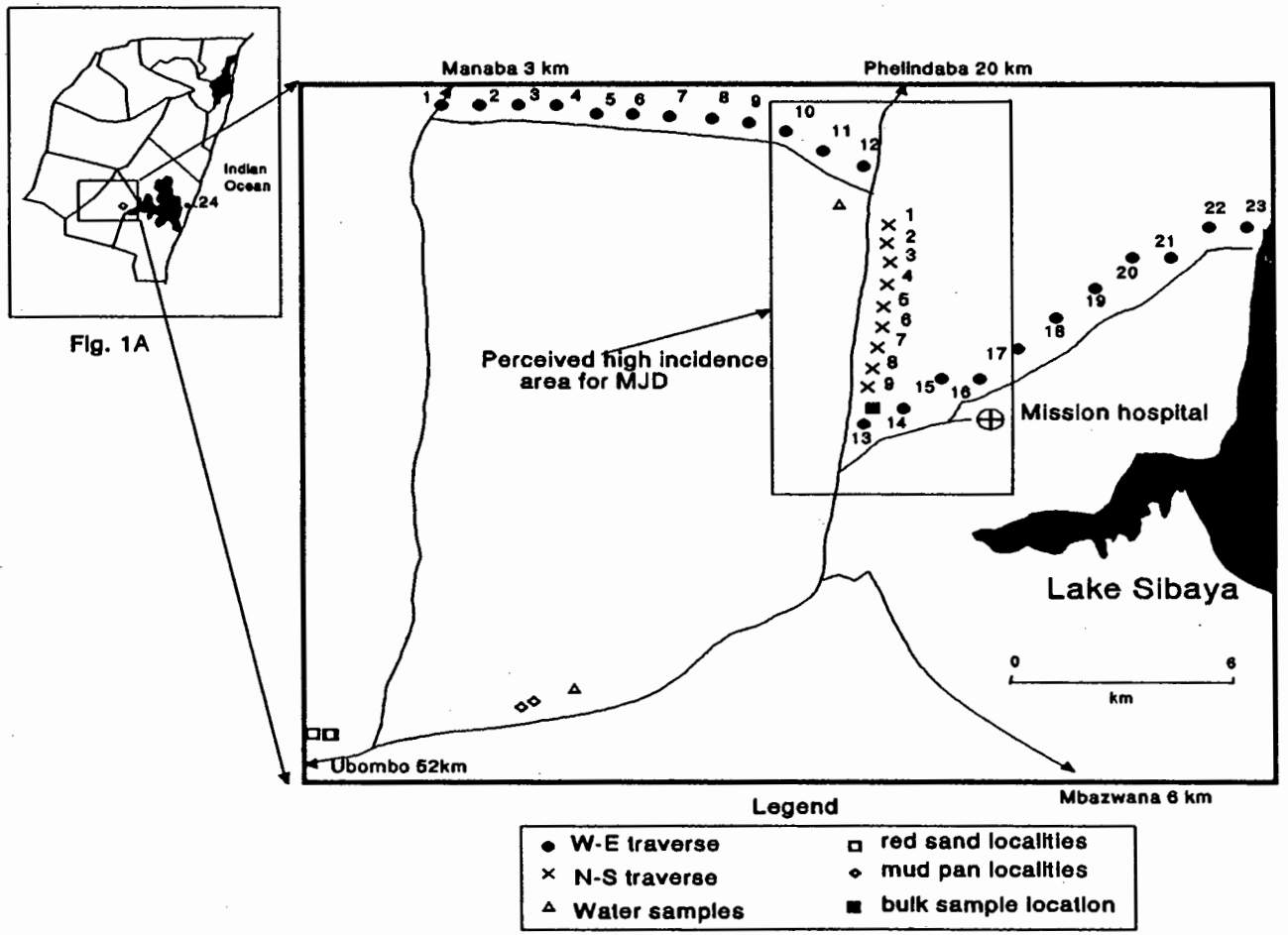


Figure 3A: Sampling localities relative to local roads; note the orientation of sample no. 24 (WE traverse) on Fig.1A in the top left hand corner.

(a)

(b)

(c)

Figure 3B: Field examples of the three soil types investigated: (a) grey sands (*Fernwood Form*); (b) mud pan; and (c) red sands (*Hutton Form*).

3.2.3 Sample Preparation

Soils were air dried for three days then hand crushed to pass a 2mm screen and stored in plastic jars. Water samples were kept refrigerated at about 4°C and filtered through a 0.45 μm filter prior to analysis.

3.2.4 Soil analysis

A detailed account of each method is provided in Appendix A; the following sections summarize the analyses which were carried out.

3.2.4.1 pH

The standard method for pH measurement was used, as described by McLean (1982). Soil and MQ water were mixed in a 1:2.5 soil:water ratio and allowed to equilibrate for thirty minutes before being analyzed on a *Crison Micro pH 2001* meter fitted with a combination glass electrode. The pH ($CaCl_2$) of the samples was obtained using the same procedure but equilibrating the soil with a 0.01 *M* $CaCl_2$ solution instead of water.

3.2.4.2 Extractable acidity and exchangeable basic cations

These parameters were determined by an external laboratory. Extractable acidity, Ca and Mg were determined by the standard *KCl extraction* method (Thomas, 1982). In this method soil was mixed with 1 *M* KCl in a 1:10 ratio, stirred and the resulting solution filtered. The filtrate was then diluted and an aliquot was titrated with 0.01 *M* NaOH to determine extractable acidity, whilst another was analyzed for Ca and Mg by atomic absorption methods. Exchangeable potassium levels were estimated using the *Ambic-2* method (Farina *et al*, 1992). In this method an extracting solution containing 0.25 *M* NH_4HCO_3, 0.01 *M* $(NH_4)_2EDTA$ and 0.01 *M* NH_4F was mixed with the soil for ten minutes (in a 10:1 ratio); the resulting solution was then filtered and an aliquot of the filtrate was diluted and analyzed for K using atomic absorption methods.

3.2.4.3 Organic carbon

Organic carbon was determined by the standard *Walkley Black* method (Nelson and Sommers, 1982). In this method organic matter in soil is oxidised by treatment with a hot mixture of $K_2Cr_2O_7$ and sulphuric acid. After completion of this reaction, the excess dichromate is titrated with iron (II) ammonium sulphate hexahydrate. The reduced dichromate is assumed to be equivalent to the organic C present in the sample, assuming that soil organic matter has an average valence of zero.

3.2.4.4 Clay content

The clay content of selected samples was determined using an abridged version of the standard *Pipette* method (Gee and Bauder, 1986). Soil samples were initially dispersed, using a Calgon (sodium hexametaphosphate + sodium carbonate) dispersing solution, whereupon the resulting solutions were washed through a 53 μm sieve in order to isolate the silt and clay fractions. Sedimentation procedures were then utilized to separate out the clay fraction, a representative portion of which was sampled in solution using a Lowy pipette. This portion was recovered by oven drying, weighed and the result extrapolated in order to assess the clay content of each sample.

3.2.4.5 Clay mineralogy

The clay size fraction (< 2 μm) was separated from the soil samples using sedimentation and simple ion exchange techniques in a non-standard method which is detailed in Appendix A. The clay then was pipetted in a known concentration onto a glass slide and analyzed by X-ray diffractometry (XRD). Scans were carried out over the range 5 - 65° 2θ, using CuKα radiation.

3.2.4.6 Abundances of selected nutrient elements

The total abundances of selected elements (Zn, Cu and Mn) were analyzed by X-ray fluorescence (XRF) techniques. Dried samples were crushed to a fine powder using a swingmill and were fashioned into powder briquettes after the method of Norrish and Hutton (1964) before being analyzed with a Phillips X'Unique PW 1400 spectrometer. Extractable concentrations of P, K, Zn, Cu, Mn and Fe were determined by an external laboratory using the *AMBIC-2* method (Farina *et al., 1992*). The extraction procedure used in this method has been described for the determination of K in section *3.2.4.2* above; P concentrations in an aliquot of the filtrate produced from this extraction were measured potometrically, whilst the concentrations of the other elements were determined using atomic absorption methods.

Extractable concentrations of B were determined by another external laboratory using the standard *Hot-Water Extraction/curcumin* method (Bingham, 1982). In this method B was extracted from the soil using hot water (2:1 soil to water ratio) and 0.05 *M* $CaCl_2$, before being determined photometrically by the curcumin method.

3.2.5 Water analysis

3.2.5.1 pH and electrical conductivity

A *Crison Micro pH 2001* pH meter was used to measure the pH of the water samples, whilst the electrical conductivity of the samples was measured on a *Crison Micro CM 2201* instrument.

3.2.6.3 Major cations and anions

Water samples were filtered using both 0.2 μm and organic filters and diluted such that their electrical conductivities were below 100 μS/cm before being analyzed for major cations and anions by ion chromatography techniques, with a *Dionex 300 Series* High Pressure Ion Chromatography instrument.

3.2.6.4 Fluoride determination

Fluoride concentrations were measured using the standard *electrode* method (Standard Methods for the Examination of Water and Wastewater, 1985). An aliquot of sample was added to an equal volume of a buffer solution containing NaCl + CDTA + 6 *N* NaOH; fluoride concentrations were determined in this solution using an ion selective electrode (a *Corning Ion Analyzer 255*).

3.3 Results and discussion: soils

3.3.1 pH

Soil pH values are presented in Table 3.1. The majority of the pH values for the *Fernwood* soils (i.e. samples from the WE and NS traverses, hereafter termed *grey sands*) fall into the moderately acid pH range (Soil Classification Working Group, 1991), although a few appear to be near neutral or even slightly alkaline and one (NS 9) is moderately alkaline. The *Hutton* soils (hereafter termed *red sand* samples) are best placed in the slightly acid category, whilst the mud pan samples are both strongly alkaline.

These values fall within the expected range for humid region mineral soils (Tan, 1993) as might be expected given the subtropical climate experienced at Mseleni and the sandy nature of the soils there. In humid regions a natural acidification process (as discussed in Chapter 2) is ensured by the dissolution and removal by leaching of alkaline minerals, leaving an acidic residue: this process is probably primarily responsible for the moderately acidic nature of the grey and red sands near Mseleni. The alkaline nature

Table 3.1: Soil pH values: WE indicates samples from the west-east traverse, NS from the north-south traverse, RS indicates red sands and MP the mud-pan samples.

Sample No.	pH (H_2O)	pH ($CaCl_2$)	Δ pH	Sample No.	pH (H_2O)	pH ($CaCl_2$)	Δ pH
WE 1	5.58	4.57	1.01	WE 21	6.09	4.26	1.83
WE 2	5.79	4.78	1.01	*WE 21$_D$*	*5.67*	*4.89*	0.78
WE 3	7.12	6.33	0.79	WE 22	5.47	4.36	1.11
WE 3$_D$	*7.13*	*6.39*	0.56	WE 23	5.95	4.63	1.32
WE 4	5.85	4.60	1.25	WE 24	6.19	5.09	1.10
WE 5	6.26	5.07	1.19				
WE 6	5.46	4.28	1.18	NS 1	5.83	4.59	1.24
WE 6$_D$	*5.64*	*4.23*	1.41	NS 2	6.12	5.11	1.01
WE 7	5.73	4.54	1.19	NS 3	6.63	5.42	1.21
WE 8	6.20	4.94	1.26	*NS 3$_D$*	*6.06*	*4.72*	1.34
WE 9	6.53	5.69	0.84	NS 4	6.10	4.91	1.19
WE 9$_D$	*6.41*	*5.04*	1.37	NS 5	6.47	5.73	0.74
WE 10	6.06	4.79	1.27	NS 6	5.79	4.79	1.00
WE 11	6.22	5.79	0.43	*NS 6$_D$*	*5.59*	*4.37*	1.22
WE 12	6.56	5.85	0.72	NS 7	6.97	6.08	0.89
WE 13	6.39	5.50	0.89	NS 8	7.07	6.15	0.92
WE 14	6.63	5.77	0.86	NS 9	7.77	6.90	0.87
WE 15	6.18	4.95	1.23	*NS 9$_D$*	*6.33*	*5.45*	0.48
WE 15$_D$	*6.02*	*5.01*	1.01				
WE 16	5.98	4.63	1.35	RS 1	6.68	5.57	1.11
WE 17	5.50	4.18	1.32	RS 2	6.43	5.44	0.99
WE 18	5.84	4.34	1.50				
WE 18$_D$	*5.87*	*4.59*	1.28	MP 1	7.71	7.17	0.54
WE 19	6.41	5.36	1.05	MP 2	7.96	7.19	0.77
WE 20	5.71	4.45	1.26				

D = subsoil (values given in italics).
Δ pH = pH (H_2O) - pH ($CaCl_2$)

of the mud pan samples is possibly related to an accumulation of basic cations and any leachable carbonates in topographic lows.

The magnitude of ΔpH (pH measured in H_2O less pH measured in $CaCl_2$) for a given soil is often instructive, as it may be understood in terms of the cation exchange and buffering capacities of the soil (McLean, 1982). Most soils exhibit a ΔpH value of ± 1 pH unit: soils with a high cation exchange capacity (CEC) may have higher values due to the larger number of cations held on exchange sites and hence liberated in the presence of an electrolyte, whilst those with a very low CEC may exhibit lower values than this. In the case of the grey sands ΔpH values are often close to one pH unit, although a degree of variability is exhibited and several samples have ΔpH values of up to 1.8 pH units. This latter characteristic is unusual. One possible explanation for this might be in terms of the very low buffering capacity of these sands: whereas in most soils a degree of acid buffering capacity exists in the form of base cations which might neutralize at least some of the acidity liberated by the action of an electrolyte solution (i.e. $CaCl_2$), these sands probably have very little or no acid buffering capacity, hence the high ΔpH values (Fey, personal communication, 1996).

3.3.2 Extractable acidity, acid saturation and effective cation exchange capacity

3.3.2.1 Extractable acidity

Table 3.2 contains values of extractable acidity (H + Al), acid saturation and total cations. Exchangeable acidity values are fairly arbitrary (Thomas, 1982) but may be related to an existing cation exchange capacity in the soil (discussed below) and are often used in agriculture as a rapid means of determining lime requirement (McLean, 1982). In mineral soils such as those dealt with in this thesis and in the pH range of interest, exchangeable acidity is effectively comprised of H ions obtained from the hydrolysis of exchangeable, trivalent Al (Sposito, 1989).

The values provided in Table 3.2 indicate that levels of exchangeable acidity (where measurable) are negligible in the grey sands and below detection in the red sand and mud pan samples. The use of these values in determining acid saturation for the various soils is described below in section 3.3.2.2. Interesting to note is the good correlation between high exchangeable acidity values and Δ pH values discussed above.

3.3.2.2 Effective cation exchange capacity and percentage acid saturation

The sum of exchangeable (or, more practically, extractable) cations is often termed the *effective cation exchange capacity* (ECEC) of a soil (Sposito, 1989). The ECEC values reported in Table 3.2 (*Cations*)

Table 3.2: *KCl extractable* acidity, exchangeable cations (*KCl* and *AMBIC-2* derived values) and acid saturation values for all soil samples. WE indicates samples from the west-east traverse, NS from the north south traverse, RS indicates red sands and MP the mud-pan samples.

Sample	Al+H	Cations	Acid Saturation	Sample	Al+H	Cations	Acid Saturation
	$cmol_c L^{-1}$		%		$cmol_c L^{-1}$		%
WE 1	0.05	1.79	3	WE 21	0.2	0.71	28
WE 2	0.05	1.59	3	*WE 21$_D$*	0	1.35	0
WE 3	0	5.91	0	WE 22	0	1.47	0
WE 3$_D$	0	5.25	0	WE 23	0.05	1.18	4
WE 4	0.05	2.14	2	WE 24	0	2.01	0
WE 5	0	2.38	0				
WE 6	0.3	0.7	43	NS 1	0	1.91	0
WE 6$_D$	0.25	0.9	28	NS 2	0.05	2.14	2
WE 7	0.05	2.73	2	NS 3	0.05	1.99	3
WE 8	0	3.78	0	*NS 3$_D$*	0	1.57	0
WE 9	0	3.48	0	NS 4	0.05	1.96	3
WE 9$_D$	0	4.58	0	NS 5	0	3.53	0
WE 10	0	1.82	0	NS 6	0.15	2.29	7
WE 11	0	6.7	0	*NS 6$_D$*	0.05	1.48	10
WE 12	0	2.18	0	NS 7	0	4.22	0
WE 13	0	1.39	0	NS 8	0	2.87	0
WE 14	0	2.28	0	NS 9	0	5.82	0
WE 15	0.05	1.78	3	*NS 9$_D$*	0	3.65	0
WE 15$_D$	0	2.3	0				
WE 16	0	1.04	0	RS 1	0	4.64	0
WE 17	0.1	1.41	7	RS 2	0	4.06	0
WE 18	0.15	1.01	15				
WE 18$_D$	0	1.86	0	MP 1	0	9.94	0
WE 19	0	3.08	0	MP 2	0	28.35	0
WE 20	0.1	0.92	11				

D = subsoil (values given in italics).
The *Cations* column includes values determined for Ca + Mg + K + Al + H.

are very low for all samples, with the exception of the mud pan samples which might be expected to contain a higher proportion of clay and organic matter and hence have higher CEC. There appears to be no general trend in terms of ECEC in the grey sand topsoil versus the subsoil samples.

The relative proportions of exchangeable acidic:basic cations occupying exchange sites in a soil is particularly important in terms of the suitability of the soil for plant growth, which has lead to the development of two related measures, termed *acid saturation* and *base saturation* (McBride, 1994). These are expressed in terms of the relative percentage of the exchange sites occupied by either acidic or basic cations, respectively.

The extractable acidity values reported in Table 3.2 are generally very low or below detection. As a result, acid saturation values range from zero (some grey sands, the red sand samples and the mud pan samples) to 43 %, with most (grey sand samples) falling in the range 0-10 %. Base saturation of 90%, i.e corresponding to those samples with acid saturation in the 0-10 % range, is considered sufficient to prevent elemental toxicity in most plants (McBride, 1994). It must be stressed that due to the relatively inert nature of these soils, a figure of 90 % base saturation does not imply that enough basic cations are present to maintain sufficient levels of soil *fertility*. In samples WE 6, WE 6_D and WE 21 the acid saturation values exceed 20 %, indicating a potential hazard for the growth of certain plants.

3.3.3 Organic carbon

Organic carbon values are reported in Table 3.3. It is clear that organic carbon values in all the samples are low, ranging from 0.3 % - 1.5 % in the grey sands, from 0.1 % - 0.5 % in the red sands and from 1 % - 3 % in the mud pan samples. Organic carbon values in the topsoils were found to be consistently higher than those in the subsoils, as might be expected. Organic matter is known to be a major contributor to the cation exchange capacity of most soils (McBride, 1994), due to the negative charge associated with the dissociation of acidic functional groups in organic compounds: low organic carbon values hence almost certainly contribute to the low ECEC of the grey sand and red sand soils reported above.

3.3.4 Clay content (selected samples)

Clay contents for selected soil samples are reported in Table 3.4. The grey sands (*WE* and *NS* prefixed sample names) have very low clay contents, ranging from 1 % - 4 %. The two red sands appear to fall into the same category, whilst the mud pan samples contain considerably more clay (11 and 25 % respectively). In explaining the latter phenomenon it seems likely that i) clays which might have formed or been present in the grey sands have been leached out of these sands and have accumulated in depressions to form mud pans, and ii) that the cations neccessary for clay formation might also have

Table 3.3: Organic carbon values as determined by the Walkley Black method: WE indicates samples from the west-east traverse, NS from the north-south traverse, RS the red sand and MP the mudpan samples.

Sample	Organic carbon	Sample	Organic carbon
	%		%
WE 1	0.66	WE 21	0.20
WE 2	0.49	*WE 21$_D$*	0.43
WE 3	1.15	WE 22	0.45
WE 3$_D$	0.94	WE 23	0.45
WE 4	0.82	WE 24	0.42
WE 5	0.78		
WE 6	0.49	NS 1	0.53
WE 6$_D$	0.43	NS 2	0.48
WE 7	0.92	NS 3	0.38
WE 8	0.81	*NS 3$_D$*	0.22
WE 9	1.43	NS 4	0.45
WE 9$_D$	0.76	NS 5	0.96
WE 10	0.45	NS 6	0.74
WE 11	1.21	*NS 6$_D$*	0.41
WE 12	0.47	NS 7	0.62
WE 13	0.32	NS 8	0.34
WE 14	0.42	NS 9	1.17
WE 15	0.98	*NS 9$_D$*	0.63
WE 15$_D$	0.46		
WE 16	0.45	RS 1	0.47
WE 17	0.41	RS 2	0.35
WE 18	1.12		
WE 18$_D$	0.85	MP 1	1.15
WE 19	1.21	MP 2	2.70
WE 20	0.28		

D = subsoil (values given in italics)

accumulated in such depressions and hence allowed for *neoformation* of clays in these topographic locations. This concept is discussed in more detail below (in section 3.3.5).

Negative charge associated with isomorphous substitution within the structures of layer silicate minerals is generally the major contributor to the exchange capacities of mineral soils (Sposito, 1989). In the case of mineral soils possessing low clay contents, such as those being investigated, the CEC of the soil might as a result be expected to be low, as is indeed observed. The latter statement should be qualified in terms of the nature of the clay minerals in question, however, which is discussed below in section 3.3.5.

Table 3.4: Clay contents determined for selected samples; WE indicates samples from the west-east traverse, NS from the north-south traverse, RS the red sand and MP the mudpan samples.

Sample	Clay content	Sample	Clay content
	%		%
WE 1	1.1	NS 3	2.2
WE 3	3.9	NS 5	3.4
WE 5	2.8	NS 6	2.8
WE 6$_D$	*1.1*	*NS 7*	*3.4*
WE 8	3.9	NS 9	2.8
WE 13	1.6	RS 1	3.4
WE 20	2.2	RS 2	3.9
WE 23	1.1	MP 1	11.7
WE 24	2.2	MP 2	25.7

D = subsoil (values given in italics)

3.3.5 Clay mineralogy

Representative X-ray diffractograms for each soil are illustrated in Figure 3C. Kaolinite is the dominant clay mineral and quartz the subdominant mineral in each. Mica occurs as a subsidiary mineral in all soils, whilst a small amount of an undifferentiated 2:1 layer silicate occurs in the mud pan sample.

The predominance of kaolinite in all three soil types indicates that these soils have been subjected to intensive weathering. Such an advanced stage of weathering of soils is generally brought about in

strongly oxidising environments and/or under conditions of intensive leaching, according to the widely quoted *Jackson-Sherman* weathering scheme (Sposito, 1989). This condition is understandable in the Mseleni area given the relatively high rainfall, high temperatures and free drainage generally prevalent there.

The existence of an undifferentiated 2:1 layer silicate in the mud pan may be understood in terms of

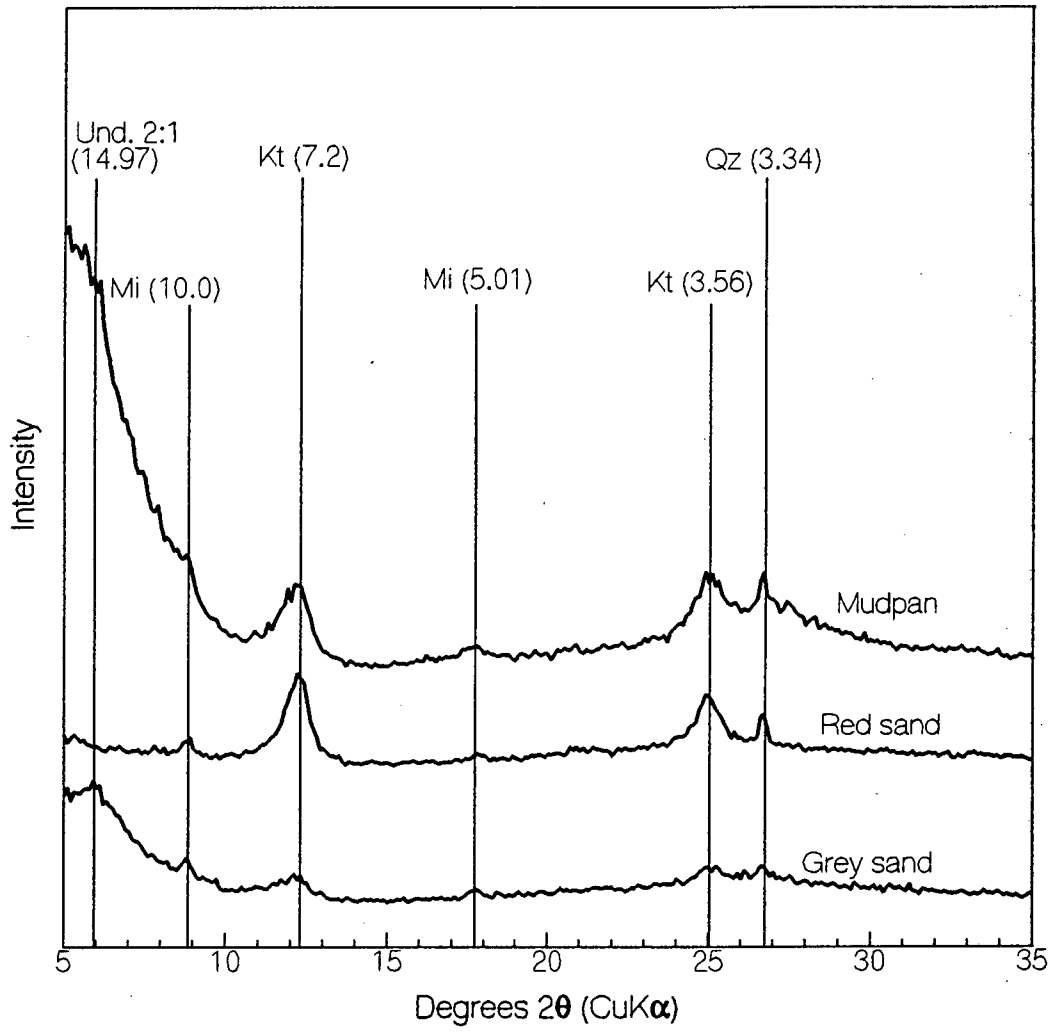


Figure 3C: X-ray diffractograms for the three soil types sampled. Peaks are labelled using the following convention: Mi = mica, *Kt* = kaolinite, *Qz* = quartz, *Und. 2:1* = undifferentiated 2:1 layer silicate. Relevant D spacings are provided in parentheses.

neoformation processes, involving the recombination of silica, aluminium, iron and the various base cations released as a consequence of primary mineral dissolution into new, low temperature, secondary minerals (McBride, 1994). In such scenarios the particular minerals formed are controlled by the leaching intensity of the local environment and to what extent the environment is confining (McBride, 1994), allowing the more soluble leaching products (silica and the base cations) to accumulate. The mud pan environment is indeed confining and is almost certainly host to a proportion of the base cations leached from the surrounding grey sands; as a result it may well represent the only suitable environment in this landscape for complex secondary mineral formation.

The small particle size of the clay fraction ensures that it generally possesses a very high surface area and as a result generally contributes greatly to the chemical reactivity of soils. This contribution clearly depends on the relative chemical reactivity of the individual constituents of the clay fraction, which is usually discussed in terms of the specific area and cation exchange capacity (CEC) of each (McBride, 1994). In the light of the above considerations, it is clear that none of the soils studied are likely to have a significantly reactive clay fractions, being characterised by relatively inert clay minerals which in turn translate into the low cation exchange capacities reported in Section 3.3.2.2.

3.3.6 Elemental abundances

Extractable concentrations of the macronutrients Ca, P, Mg and K are reported in Tables 3.5 (a) and (b), whilst total (where applicable) and extractable micronutrient concentrations (i.e. Zn, Mn, Cu and Fe) are provided in Tables 3.6 (a) and (b). Extractable concentrations of B for selected samples are presented in Table 3.7. The distribution of the elements Ca, P, Zn, Mn and Cu in the grey sands along the two traverses are displayed graphically in Figures 3D and 3E. The discussion in Chapter 2 regarding elemental deficiencies and in particular the prediction of possible deficiencies using soil testing is referred to extensively below. Although the available concentrations of Mg, K and Fe are reported in this chapter, these values are only discussed very briefly. These elements were not considered important in the context of MJD, and are included to complement the geochemical characterization of the grey soils in terms of nutrient levels. Soil test results for extractable selenium have not been reported due to the fact that such values are practically meaningless in terms of the prediction of Se deficiency (Wan *et al.*, 1988). The investigation of selenium availability in the grey sands is deferred until Chapter 4.

3.3.6.1 Calcium

Total calcium concentrations in soils are not useful in determining Ca availability (as noted in Chapter 2), hence total calcium was not determined in the soil samples. *Potassium chloride extractable* calcium

concentrations in the grey sands ranged from 30-630 mg/kg (i.e. mg Ca/kg soil), averaging 250 mg/kg, whilst the red sand samples contained 346 and 393 mg/kg respectively. The mud pan samples contained significantly higher levels of Ca than the other soil types (1100 and 3600 mg/kg soil respectively). Surface samples contained consistently higher concentrations than subsurface samples in the case of the grey sands.

Although no critical limits have been established for extractable calcium in soils, the findings of Melsted (1953) might well be used to assess the likelihood of calcium deficiency in maize plants growing on Mseleni soils. Melsted (1953) found that extractable calcium concentrations < 400 mg/kg in acid, sandy soils caused deficiency in maize. Using this criterion, the majority of the grey sands sampled would be expected to induce Ca deficiency in maize; if not, then these samples could at least be regarded as having very low available calcium concentrations. As noted in Chapter 2, calcium defiency is often associated with coarse grained, acid soils in humid climates with low mineral reserves: hence the low calcium content of the grey sands in particular are not unexpected.

The relatively high calcium concentrations in the mud pan samples are probably a consequence of leaching of calcium out of the surrounding grey sands and subsequent deposition in topographic lows as might be expected in the well drained, high rainfall area where the soils were collected. The higher abundances of calcium in the surface soils relative to the subsoils might be expected due to the weathering interface at the surface (cation release) as well as the presence of higher clay and organic matter contents (i.e. higher CEC) near the surface .

The distribution of extractable calcium in soils along the two grey sand traverses (Figure 3D) is marked by a high degree of heterogeneity, particularly in the WE traverse: this may be due to the interplay between localized differences in drainage conditions and topography and factors such as soil pH and the abundance of adsorption complexes. Interestingly, the NS traverse shows a relatively uniform distribution broken by three higher values towards the south end, where samples were collected from cultivated lands. Although it is highly unlikely that any commercial fertilizer has been used on these cultivated soils, the use of slash-and-burn methods of fertilization is widespread in the area and the higher calcium values may well reflect an anthropogenically induced recycling of plant matter. Alternatively, there may be a general decrease in the concentration of calcium towards the north due to natural causes.

3.3.6.2 Phosphorus

Total phosphorus concentrations are not useful in ascertaining whether plants on a given soil are likely to experience P deficiency (Tisdale *et al.*, 1985); as a result total P was not determined on any of the

Table 3.5 (a): *Ammonium bicarbonate - EDTA* extractable concentrations of the elements P and K and *KCl extractable* Ca and Mg concentrations in soil samples from the west-east traverse.

Sample	P	K	Mg	Ca
		mg/kg		
WE 1	1.9	23	35	162
WE 2	0.5	21	38	133
WE 3	0.6	44	135	594
WE 3_D	*0.5*	*18*	*122*	*588*
WE 4	1.3	22	52	193
WE 5	0.8	32	48	231
WE 6	0.8	13	9	30
WE 6_D	*0.5*	*10*	*31*	*29*
WE 7	1.7	57	51	260
WE 8	6.1	82	45	399
WE 9	1.7	102	68	388
WE 9_D	*0.3*	*71*	*86*	*464*
WE 10	0.8	16	22	204
WE 11	15.6	114	161	628
WE 12	1.6	13	35	227
WE 13	0.4	15	23	136
WE 14	0.7	16	39	232
WE 15_D	*0.3*	*11*	*43*	*161*
WE 15	0.5	8	53	224
WE 16	1.0	10	24	94
WE 17	1.1	3	17	147
WE 18_D	*1.0*	*3*	*26*	*75*
WE 18	1.6	20	41	189
WE 19	1.4	11	82	303
WE 20	0.5	3	20	77
WE 21_D	*0.8*	*24*	*13*	*35*
WE 21	1.3	17	31	131
WE 22	1.5	23	25	141
WE 23	0.6	30	28	97
WE 24	2.5	22	55	182

D = subsoil (values given in italics)

Table 3.5 (b): *Ammonium bicarbonate - EDTA* extractable concentrations of the elements P and K, and *KCl extractable* Ca and Mg concentrations in soil samples from the north-south (NS) traverse, the red sand (RS) area and the mud pans (MP).

Sample	P	K	Mg	Ca
		mg/kg		
NS 1	1.1	29	39	183
NS 2	0.7	21	42	214
NS 3	1.0	47	36	178
NS 3_D	*0.8*	*8*	*34*	*141*
NS 4	1.0	22	32	210
NS 5	2.2	28	53	397
NS 6	0.9	26	32	242
NS 6_D	*1.0*	*13*	*37*	*116*
NS 7	4.1	27	51	483
NS 8	1.0	22	44	318
NS 9	3.7	65	108	629
NS 9_D	*2.2*	*50*	*81*	*356*
RS 1	2.1	69	111	393
RS 2	2.2	60	116	346
MP1	20.8	474	181	1091
MP2	50.8	865	613	3574

D = subsoil (values given in italics)

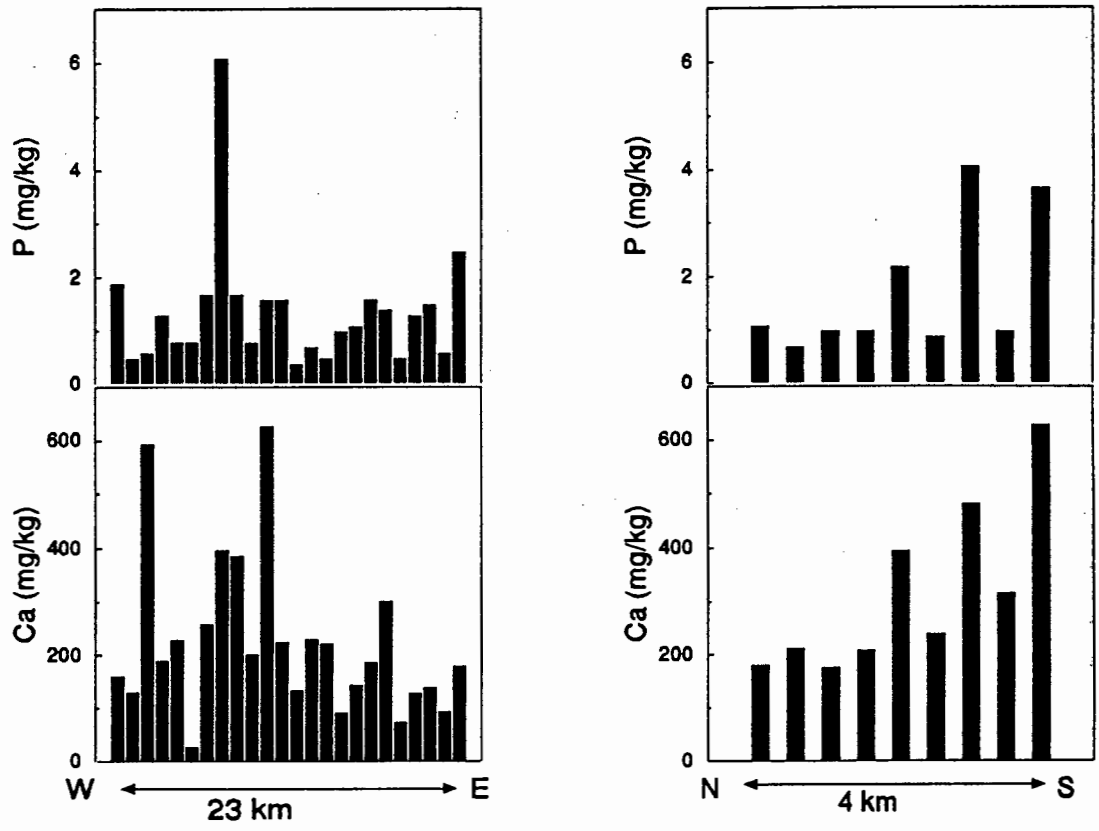


Figure 3D: Distribution of extractable concentrations of Ca and P along the west-east and north-south traverses (note the difference in horizontal scale between the traverses). Sampling distance along the west-east traverse was 1000 m and along the north-south traverse 500 m.

As mentioned in Chapter 2, soil P tests have generally been designed for the prediction of crop response to P addition (i.e. fertilization), are complicated by many variables, and are not amenable to the critical value approach. The levels observed in the grey sands (i.e. 1.9 mg/kg on average), however, would almost certainly be expected to induce P deficiency in most field crops, according to experienced researchers (Fey, personal communication, 1996).

The elevated concentrations of P in the mud pan samples may probably be explained by the same mechanisms as those used above to explain the elevated calcium concentrations in these samples. The distribution of extractable P concentrations along the WE traverse is more homogeneous than observed in the case of calcium (see Figure 3D), although a similar distribution pattern is observed in the NS traverse (as might be expected if, as hypothesized, organic material has indeed been recycled on the cultivated fields at the south end of the traverse).

3.3.6.3 Magnesium and potassium

A brief discussion of these elements is appropriate, although, as mentioned above, they have not been selected for particular attention in this investigation. Exchangeable magnesium concentrations in the grey sands range from 10-160 mg/kg, averaging 49 mg/kg. According to Haby *et al.* (1990), Mg levels between 0-25 mg/kg generally induce deficiency symptoms in most crops, whilst levels between 26-50 mg/kg may induce deficiency in sensitive crops (e.g. sugarbeet, potatos and fruit). It seems likely then that Mg concentrations in the vast majority of grey sands are adequate for the growth of staple crops in the Mseleni area. Magnesium levels in the red sands (± 110 mg/kg) are probably adequate for plant growth, as are those in the two mud pan samples (181 and 613 mg/kg respectively).

Potassium concentrations in the grey sand samples range from 3-114 mg/kg, averaging 29 mg/kg. Values in the two red sand samples average 65 mg/kg, whilst the concentrations in the mud pan samples are much higher (474 and 865 mg/kg respectively). Critical values for K in soils differ widely according to crop type, soil and climate (Haby *et al.*, 1990). Pertinent values have been reported from Alabama, where soil K levels above 40 mg/kg (for noncalcareous soils with CEC values in the range 0-4.6 $cmol_c/kg$) are considered adequate for maize (Haby *et al.*, 1990). Assuming that this value is appropriate to the Mseleni soils (it is lower than most values reported elsewhere), it seems likely that plants grown on the majority of the grey sands will suffer from K deficiency. Potassium levels in the two red sands are probably adequate, whilst those in the two mud pan samples are definitely sufficient.

3.3.6.4 Zinc

The total Zn concentrations of the grey sands fall within the range 1.7-8.5 mg/kg and average 3.4 mg/kg

total Zn (see Tables 3.6 (a) and (b)). Values for the two red sand samples appear to be higher, averaging 10.5 mg/kg, whilst the two mud pan samples have higher values still (13 and 46.5 mg/kg respectively).

Mean total Zn contents in surface soils from a wide variety of countries range from 17 to 125 mg/kg ; the lowest reported values are generally found on podzols and sandy soils and may range from less than 5 mg/kg upwards (Kabata-Pendias and Pendias, 1985). As in the cases of the other micronutrients, total Zn abundances in soils are not particularly useful in assessing Zn availability (Tisdale *et al.*, 1985). However, as discussed in Chapter 2 (section 2.2.1.1), many extensively leached, coarse-textured soils such as those found at Mseleni have proved to be deficient in Zn. This is generally due to a combination of parent material and weathering factors, and clearly appears to be the case with the Mseleni soils, where *total* Zn concentrations fall just above the approximate critical levels for *available* Zn.

Extractable Zn concentrations in the grey sands range from 0.1-1.8 mg/kg, averaging 0.4 mg/kg, whilst those for the two red sand samples fall at the bottom of this range (0.1 mg/kg). The two mud pan samples contain < 1 mg/kg extractable Zn. Zinc concentrations in the subsoil samples (grey sands) appear to be consistently lower than those in the correspondling topsoil samples.

The Cedara Grain Crops Institute in Natal has carried out field calibrations of the ammonium bicarbonate-EDTA extraction technique in order to assess critical values for Zn in soils with regards to plant growth. A value of 1.5 mg/L has been found to be most reliable (Channon, pers. comm., 1996), which translates into ± 1 mg Zn/kg soil if the density of the Mseleni soils is taken into account. This value, incidentally, falls within the range given above (0.8-1.4 mg/kg) for critical values derived using similar methods internationally (Martens and Lindsay, 1990). Only five samples of the entire soil sample suite (including grey sands, red sands and mud pan samples) contain concentrations of extractable Zn in excess of this critical limit, and then only by a very small margin. Zinc deficiency would thus almost certainly be expected in plants growing on Mseleni soils.

The distribution of extractable Zn concentrations (Figure 3E) along the two traverses indicates that this element is not distributed homogeneously in the Mseleni landscape. An example of this feature is the clustering of high values observed in the WE traverse; Zn concentrations may also be observed to vary from 0.1 mg/kg to 1.3 mg/kg within the space of one kilometre (Fig. 3E).

Table 3.6 (a) Total (XRF) and *AMBIC-2* extractable trace element concentrations for the west-east traverse.

Sample	Zn (total)	Zn (AMBIC)	Mn (total)	Mn (AMBIC)	Cu (total)	Cu (AMBIC)	Fe (AMBIC)
			mg/kg				
WE 1	2.6	0.3	253	8.5	16.7	0.5	30.5
WE 2	2.2	0.2	148	7.4	7.4	0.3	19.1
WE 3	4.6	0.4	404	3.3	17.4	1.0	13.9
WE 3_D	*3.1*	*0.1*	*363*	*0.4*	*11.2*	*0.9*	*9.6*
WE 4	2.4	0.1	145	3.1	6.1	0.3	22.1
WE 5	3.1	0.2	289	2.9	16.0	0.4	13.8
WE 6	2.9	0.1	182	14.0	8.1	0.3	10.6
WE 6_D	*2.9*	*0.1*	*144*	*1.3*	*7.4*	*0.4*	*17.3*
WE 7	5.9	1.3	289	15.3	16.7	0.6	36.0
WE 8	7.1	1.8	179	5.4	8.8	0.7	13.5
WE 9	8.5	1.3	215	1.0	9.9	0.6	23.2
WE 9_D	*5.9*	*0.3*	*205*	*2.2*	*9.2*	*0.6*	*23.5*
WE 10	4.7	1.2	269	6.7	16.4	0.7	11.6
WE 11	5.8	1.4	369	9.3	21.2	0.8	11.6
WE 12	4.0	0.2	177	1.5	13.5	0.3	7.6
WE 13	2.2	0.3	177	0.8	12.2	0.3	10.7
WE 14	3.0	0.1	115	0.5	9.2	0.1	10.2
WE 15	3.6	0.3	235	6	7.6	0.3	16.5
WE 15_D	*3.2*	*0.1*	*145*	*0.4*	*14.8*	*0.3*	*10.0*
WE 16	1.9	0.3	210	4.3	14.8	0.5	24.3
WE 17	1.7	0.1	180	7.7	9.0	0.3	19.3
WE 18	2.5	0.1	200	4.1	15.6	0.3	23.5
WE 18_D	*2.3*	*0.1*	*159*	*0.9*	*6.6*	*0.3*	*21.7*
WE 19	2.5	0.3	215	3.2	7.2	0.5	12.8
WE 20	1.9	0.2	168	5.9	6.5	0.3	11.2
WE 21	2.1	0.2	184	4.3	6.2	0.3	22.6
WE 21_D	*1.8*	*0.1*	*175*	*0.9*	*13.0*	*0.1*	*21.8*
WE 22	1.7	0.3	216	9.5	14.8	0.3	17.0
WE 23	2.1	0.1	189	5.7	9.1	0.2	15.7
WE 24	4.8	0.2	291	1.5	10.7	0.3	13.1

D = subsoil (values given in italics)

Table 3.6 (b): Total (XRF) and *AMBIC-2* extractable trace element concentrations for the north-south (NS) traverse, red sand (RS) and mud pan (MP) samples.

Sample	Zn (total)	Zn (AMBIC)	Mn (total)	Mn (AMBIC)	Cu (total)	Cu (AMBIC)	Fe (AMBIC)
			mg/kg				
NS 1	2.9	0.1	156	7.1	6.3	0.3	26.3
NS 2	2.3	0.1	178	6.6	9.1	0.2	10.7
NS 3	2.8	0.1	261	2.3	13.6	0.3	10.0
NS 3_D	*5.8*	*0.1*	*216*	*3.9*	*8.0*	*0.3*	*12.2*
NS 4	3.4	0.4	197	8.3	7.3	0.4	12.7
NS 5	3.4	0.3	231	1.9	13.6	0.4	9.9
NS 6	3.1	0.3	198	4.4	6.7	0.4	10.1
NS 6_D	*2.5*	*0.1*	*172*	*1.7*	*12.4*	*0.1*	*25.7*
NS 7	4.2	0.7	253	2.3	13.0	0.8	8.8
NS 8	3.8	0.4	203	2.2	11.8	0.4	4.1
NS 9	3.8	0.7	232	1.8	10.4	0.7	7.2
NS 9_D	*2.8*	*0.4*	*192*	*2.0*	*8.9*	*0.4*	*15.3*
RS 1	11.4	0.1	543	9.3	14.4	0.3	7.9
RS 2	9.5	0.1	400	1.8	8.9	0.4	19.9
MP 1	13.0	0.6	239	2.1	17.9	0.7	48.9
MP 2	46.5	0.9	384	22.0	24.0	0.8	8.4

D = subsoil (values given in italics)

Zinc concentrations do not appear to follow any obvious trend along the WE traverse (moving seawards). Those along the NS traverse appear to increase towards the south (as observed with Ca and P), in spite of the fact that these samples were collected parallel to the topographic features and isohyets of the region. This may be explained by a consideration of the absolute abundances of Zn in the soils which seems to correlate well with the available concentrations. These higher absolute abundances, in turn, may well be related to the suggested recycling of organic matter as detailed above under the discussions of both calcium and phosphorus distribution. Alternatively, a general decrease may be evident northwards due to various natural processes.

3.3.6.5 Manganese

The total Mn concentrations of the grey sands fall within the range 115-404 mg/kg and average ± 200 mg/kg total Mn. Values for the two red sand samples are higher (404 and 543 mg/kg) whilst the two mud pan samples contained concentrations of 239 and 380 mg/kg respectively.

Total Mn concentrations in soils generally vary greatly: sandy soils and podzols in the USA, for example, show a variation between 7-2000 mg/kg (Kabata-Pendias and Pendias, 1985). Total Mn values for a given soil have very little significance for plant growth, given the strong dependence of available concentrations on pH and redox discussed in Chapter 2. It may be noted, however, that the total Mn contents of the Mseleni soils are far higher than the critical levels reported for available Mn; i.e. the sands are not apparently inherently deficient in Mn by virtue of the *absence* of this element, as was suggested in the case of Zn.

Extractable Mn concentrations in the grey sands range from 0.4-15.3 mg/kg, with a mean value of 4.5 mg/kg in the WE traverse and 3.7 mg/kg in the NS traverse. Concentrations in the two red sand samples differ significantly (9.3 and 1.8 mg/kg respectively), as do those in the mud pan samples (2.1 and 22.0 mg/kg respectively). The extractable Mn contents of topsoil samples are consistently higher than those in the subsoils.

It is widely accepted that soil analysis for Mn is an unreliable predictor of Mn availability: analyis (including the use of EDTA-based extractants) is generally not correlated with plant uptake, possibly due to plant physiological effects which may not be measured by bulk soil techniques (Smith and Paterson, 1995). As mentioned in Chapter 2, the limited value of soil tests is partly a function of the dependence of Mn availability in soils on both climatic and soil drainage characteristics. The range in critical values for soils which have been found to be deficient in Mn elsewhere (0.2-4.7 mg/kg) is hence not particularly useful: it would appear, however, that the grey sands (in particular) might prove to induce Mn deficiency in plants given the correct interplay of rainfall and drainge conditions.

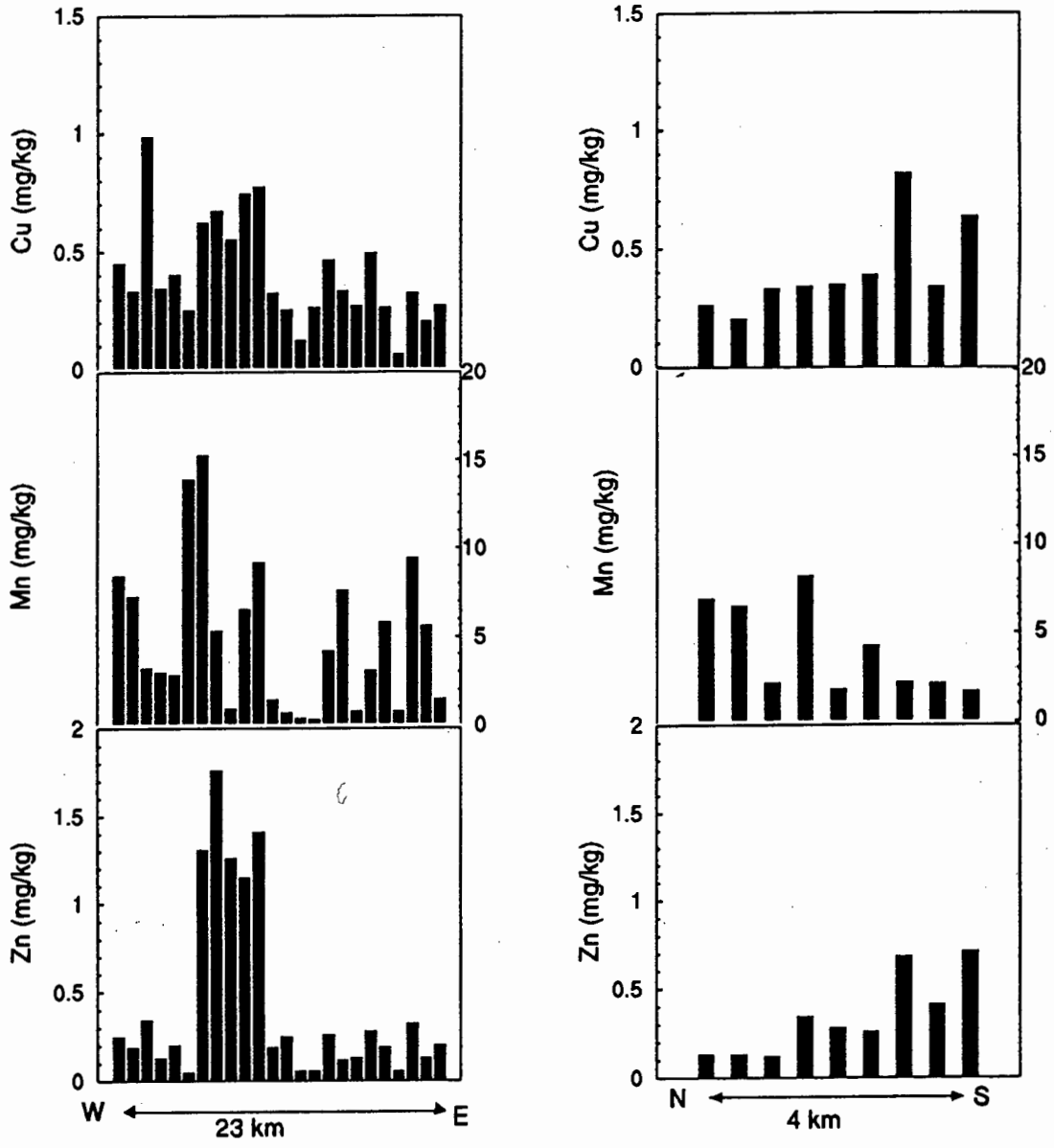


Figure 3E: Distribution of extractable concentrations of Zn, Mn and Cu along the west-east and north-south traverses (note the difference in horizontal scale between the two traverses). Sampling distance along the west-east traverse was 1000 m and that along the north-south traverse was 500 m.

The fact that Mn concentrations in the topsoil samples are higher (i.e. relative to the subsoils) is possibly a function of the higher organic matter content of these samples (organic matter constituting a powerful chelating agent for this element (Tisdale *et al.*, 1985). The distribution of Mn in both traverses (Figure 3E) appears to be highly variable: the distribution of Mn in the WE traverse in particular indicates a clear spatial heterogeneity with regards extractable Mn contents, emphasizing the dependence of measurable Mn concentrations on localized soil conditions. Moraghan and Mascagni (1991) report that oxidation and reduction of soil Mn may occur simultaneously, but independently, at sites in close proximity: the dominant process will dictate the availability of Mn in the soil, and variability of available Mn on a very small scale is widely reported.

An interesting feature of the distribution of Mn across the traverses which is most clearly seen in the case of the NS traverse is the correlation between pH values and Mn content. The last few samples on the south side of the traverse, for example, have the highest pH values (pH > 6) of the traverse and the lowest available Mn concentrations, whilst the highest concentrations (north side) correspond to samples with pH values < 5.1. This is consistent with findings in the literature (Moraghan and Mascagni, 1991) which have shown that rapid oxidation of Mn^{2+} in soils occurs with pH levels > 5.5 (oxidised Mn is generally not bioavailable).

3.3.6.6 Copper

The total copper concentrations for the grey sands fall within the range 6-21 mg/kg; the values determined for the red sand samples are 8.9 and 14.4 mg/kg respectively, whilst those for the mud pan samples are 18 and 24 mg/kg. Mean total Cu values for soils worldwide vary from ± 6-60 mg/kg, with the lowest values being reported for sandy and organic soils (Kabata-Pendias and Pendias, 1985); the Mseleni soils investigated thus fall at the lower end of this range, as might be expected.

Extractable Cu concentrations for the grey sands fall into the range 0.1-1 mg/kg, averaging 0.4 mg/kg. The values determined for the red sand samples are very similar (0.3 and 0.4 mg/kg), as are those determined for the mud pan samples (0.7 and 0.8 mg/kg).

Critical values mentioned in Chapter 2 (i.e. 0.4-1 mg/kg Cu) which are pertinent to EDTA based extractant solutions (such as that used in this study) would appear to indicate that plants growing on all of the soils sampled might be expected to suffer from Cu deficiency. The low Cu values are consistent with the sandy nature of the soils and the resulting marginal Cu reserve (as defined by the low total Cu concentrations in the soils). The distribution of extractable concentrations of Cu (Figure 3E) along the two traverses indicates a similar heterogeneity to that displayed by the other trace metals discussed.

3.3.6.7 Iron

Extracable concentrations of Fe in the grey sand samples ranged from 4-36 mg/kg, averaging 17 mg/kg. Comparative values for the redsand samples were 8 and 20 mg/kg, whilst those for the mudpan samples were 49 and 8.5 mg/kg respectively.

As mentioned in Chapter 2, most soils contain adequate concentrations of Fe and deficiency is usually related to soil properties. Critical values for Fe in soils using the relevant method (4.5-5 mg/kg) indicate that the Mseleni soils probably have adequate Fe for plant growth, although soil tests are conventionally unreliable indicators of Fe availability due to the strong redox and pH dependence of the availability of this element (Martens and Lindsay, 1990).

Table 3.7: Hot-water extractable B concentrations in selected grey sand samples (NS traverse) and in a single redsand (RS) sample.

Sample	Boron
	mg/kg
NS 1	0.35
NS 3	0.36
NS 5	0.91
NS 7	0.78
NS 8	0.48
NS 9	2.09
RS 3	0.25

3.3.6.8 Boron

Total concentrations of B in the soils were not analysed for, due to the fact that less than 5 % of total B is usually available to plants (Kabata-Pendias and Pendias, 1985). Available levels of B were analyzed for in a subset of grey sand samples from the high incidence area, with one red sand sample being analyzed as a means of obtaining a rough comparison of the two soil types.

Extractable concentrations of this element (Table 3.7) in the six grey sands from the NS traverse analysed ranged from 0.25-0.91 mg/kg, whilst the single red sand value was 2.1 mg/kg.

Considering the critical levels for B in soils using the hot water extraction method - i.e. 0.1-0.3 mg/kg - it seems that although the B levels were low in the grey sands, these may supply adequate B for normal plant growth. The slightly higher value obtained for the red sand sample may be related to the well established propensity of B to become sorbed on the sesquioxide fraction of soils (Kabata-Pendias and Pendias, 1985). It is interesting to note that the B concentration in the sample from the southernmost part of the north-south traverse is elevated, as was observed in the case of the distribution of many of the other elements.

3.4 Results and discussion: water

3.4.1 General composition

The pH, electrical conductivities and major cation and anion data for the water samples are contained in Appendix C. These data are not pertinent to the discussion of nutrient deficiency in the study area, but have been included for the benefit of future research in the area.

3.4.2 Fluoride content

The fluoride contents of the two waters were very low (0.02 and 0.05 ppm), as might be expected in an area dominated by leached and relatively inert sands. The main purpose of sampling the water from Lake Sibaya was to compare the fluoride content of this water with data reported by Lubbe *et al.* (1973). As mentioned in Chapter 1, optimal F intake in drinking water in an area with the temperature range experienced at Mseleni is ± 0.7 mg/L. Both the values reported here and many of those reported by Lubbe *et. al.* (1973), which ranged from 0.04-2 mg/L F fall significantly below this optimum level.

The essentiality of F for animals and humans is still open to speculation (Miller *et al.*, 1991). Fluorine is a constituent in bone and teeth and trace amounts have been shown to be beneficial both to the formation of caries-resistant teeth and to the prevention of demineralization of bone in aged individuals (Miller *et al.*, 1991), particularly in areas characterized by low F drinking water (Gron *et al.*, 1966).

3.4 Conclusions

The ubiquitous *Fernwood* soils of the Mseleni area are sandy, moderately acid and highly weathered, possessing low clay and organic matter contents and correspondingly low cation exchange capacities. The poor nature of the parent material and the warm, humid climate experienced in the area are probably the most important factors influencing the character of these soils.

These properties ensure that the abundances of a wide range of important nutrient elements are suboptimal in the grey sands; soil test data indicates that readily available levels of the nutrients Ca, P, Zn and Cu are likely to induce deficiencies in plants growing on these soils, whilst available B concentrations are also very low. Limited sampling of local water sources confirms the data of Lubbe *et al.* (1973), and indicates that fluoride levels in these waters (including the important Lake Sibaya water) are extremely low.

The spatial distribution of nutrient elements appears to be highly variable, and although pockets of land prone to deficiency no doubt occur as a result, the perceived *high incidence* area for MJD does not appear to differ significantly from surrounding areas in terms of soil nutrient supply. A consideration of the data from the west-east traverse indicates that there is no discernable maritime influence on soil chemistry, whilst data from the north-south traverse do suggest a slightly more even distribution of nutrients there which might be expected in soils derived from an area of similar relief, edaphic influences, rainfall and vegetation. There is some indication from the latter traverse that nutrient levels in cultivated soils are elevated relative to virgin soils, which might be attributable to the implementation of slash-and-burn practices by the local people.

The physico chemical character of the *Hutton* (red sand) soils sampled did not appear to differ considerably from that of the *Fernwood* soils, with the possible exception of slightly elevated total concentrations of Zn and Mn observed in the former. Further studies would be required to confirm this assertion, which is based on data from only two soils. The geochemical nature of the two mudpan samples differs significantly from that of the other soil samples, presumably as a consequence of their topographic position, acting as receptor basins for nutrients and dispersed clay displaced by water from surrounding, more elevated parts of the landscape.

It is evident from the above conclusions that plants growing on the soils in the Mseleni area will almost certainly suffer from nutrient deficiencies. As noted in the introductory remarks at the beginning of this chapter, however, such an assertion can only ultimately be confirmed by yield and/or foliar analysis data derived from these plants. This data is investigated in Chapter 4.

Chapter 4

Verification of nutrient deficiencies using plants grown in Mseleni soil.

4.1 Introduction

Chapter 3 dealt with the prediction of nutrient deficiencies using laboratory soil tests, and it was concluded that the grey sands in the Mseleni area, which are of primary interest regarding investigations into the etiology of Mseleni Joint Disease, are likely to be deficient in a variety of nutrient elements. This chapter focuses on the verification of these predictions, by means of a visual, physical and chemical appraisal of the growth responses of plants relying directly upon the grey sands for their nutrient supply. It is important to emphasize that the availability of some elements (and particularly certain trace elements) is better evaluated by plant growth experiments and foliar analyses than by soil tests.

The most reliable method of assessing soil nutrient availability is by means of field trials. Due to the remoteness of the study area, however, this approach was not a feasible one, and the assessment was instead carried out by means of a controlled environment pot trial. The experimental approach adopted was similar to that of Sumner (1970), in which the availability of selected elements was evaluated by a subtractive technique (this work focused on Al toxicity in similar soils). Each element of interest was omitted in turn from a complete nutrient treatment in a randomized set of pots, and plant response was gauged by means of: i) visual recognition of deficiency symptoms; ii) dry matter yield data; and iii) foliar nutrient concentrations. Maize (*Zea mays*, L.), was selected for this experiment, as it is well characterized in the literature, grown ubiquitously in the Mseleni area and forms the basis of the local diet.

A composite sample of grey sand from the high incidence area for MJD was used as the growth medium, in which commercially available maize seed was planted and grown.

In addition to this basic fertility experiment, a rather brief yet informative experiment was carried out in order to assess the selenium status of the same composite grey sand sample. Italian ryegrass (*Lolium multiflorum*) was chosen as a suitable species for this experiment. The reason for this selection was the fact that animals grazing on Se deficient pastures are known to suffer most commonly from deficiencies, and the literature is consequently well supplied with data on Se concentrations in forage grasses.

It should be stressed that both experiments were carried out on a composite soil sample from *one* location in the high incidence area. The spatial inhomogeneity of available concentrations of the various nutrient elements was clearly demonstrated in Chapter 3, and the results and subsequent discussion in this chapter should be considered in this context.

4.2 Materials and methods

Twenty five kilograms of grey sandy (*Fernwood*) topsoil (0-15 cm) was taken from an established (fallow) field, and another twenty five kilograms was collected from a nearby uncultivated area; both were in the high incidence area for Mseleni Joint Disease (see Fig. 3A).

Chemical analyses pertaining to this soil are provided in Table 4.1, together with average concentrations of selected nutrient elements in the grey sands. It is evident that this soil provides a useful yardstick for assessing deficiency, containing slightly higher than average concentrations of the macronutrients and near average levels of the micronutrients.

The bulk samples were air dried and sieved, whereupon subsamples were removed for chemical analysis and for the determination of the moisture holding capacity (MHC) of each soil. The samples were then thoroughly mixed to obtain about fifty kilograms of homogeneous Mseleni soil, and the MHC of the mixture was ascertained. The MHC of the soils was determined using the "sticky point" method. Nutrient stock solutions (see below) were prepared in the laboratory and pipetted onto the soil samples.

Thirty 2.5 kg-capacity pots sealed with 3 kg capacity plastic sampling bags were used in this experiment, the latter being used to eliminate the removal of nutrients from the pots by leaching. Each pot was filled with 1.5 kg Mseleni soil after the nutrients had been added in solution and thoroughly mixed with the soil at the following rates:

N: 125 mg/kg, applied as NH_4NO_3
K: 70 mg/kg, applied as KCl
P: 100 mg/kg, applied as $NaH_2PO_4.2H_2O$
Mg: 50 mg/kg, applied as $MgSO_4.7H_2O$
S: 66 mg/kg, (as above)
Ca: 150 mg/kg, applied as $CaSO_4.2H_2O$: * applied in solid form
B: 1 mg/kg, applied as boric acid
Cu, Mn, Fe, Zn: 3 mg/kg each, applied as nitrates (e.g. $Cu(NO_3)_2$).

As mentioned above, a subtractive technique was used in nutrient additions such that each nutrient was omitted in turn from the complete nutrient set, each treatment being applied to three randomized pots. A basal solution containing N, Mg, S and K was added to eight of the ten sets of pots. Of the two sets of untreated pots, one set contained the virgin and the other the cultivated soil.

Once the pots had been filled with soil they were brought to field capacity by the addition of distilled

Table 4.1: Geochemical information pertaining to the composite grey sand sample used in growth experiments. Extractable concentrations of macronutrients and micronutrients were obtained using the methods described in Chapter 3; average nutrient concentrations for the grey sands are included for comparative purposes.

Soil	P	K	Mg	Ca	Zn	Mn	Cu	Fe	pH ($CaCl_2$)	ECEC	OC	Clay
	mg/kg									$cmol_c L^{-1}$	%	
Comp.	2.3	65	57	309	0.4	3.6	0.3	16	5.45	2.2	0.46	2.4
Avg.	1.9	29	49	250	0.4	4.2	0.4	17				

Comp. = composite soil;
Avg. = average composition of the grey sands.

water, whereupon they were weighed and labelled. Nine maize seeds, which had been soaked in distilled water for approximately 24 hours, were planted in each pot at a uniform depth of 1.5 cm below the surface of the soil (using a marked glass rod to ensure even depth of planting). The pots were then placed in a phytotron and covered until germination, whereupon they were watered to field capacity each day (using distilled water) and rotated around the phytotron to eliminate spatial inhomogeneities in evapotranspiration. The maize plants were thinned to four per pot one week after germination and allowed to grow under conditions detailed below in Table 4.2.

Germination was fairly even, with the result that the 120 seedlings grew at approximately equal rates until such time as nutrient deficiencies began to manifest (about 10 days after germination).

Table 4.2: Growth conditions in the phytotron.

Temperature (night)	21°C
Temperature (day)	23°C
Humidity	50%
Length of day	14 hours
Light intensity	600 W/m^2

Nutrient deficiency symptoms were recorded each week for the various treatments and examples of the more obvious symptoms were photographed prior to harvesting. After five weeks each set of pots representing the various subtractive treatments were photographed alongside the *complete nutrient* set (for comparative purposes) and harvested. The foliar material (stem and leaves) from each pot was removed above a height of 1 cm above the soil, placed in labelled, ventilated brown paper bags and

dried in an oven for 72 hours (at 70°C). The roots were thoroughly washed and oven-dried in similar fashion. The dry matter accumulated in each pot was then weighed to determine yield, whereupon the foliar material was analyzed for Ca, P, Mg, K, Zn, Cu, Mn, Fe and B, after milling and digestion procedures described in Appendix B. Selenium was analyzed for using ICP-MS, B using the azomethine-H method, P photometrically and the other elements by atomic absorption (see App. B).

Two additional pots were prepared in a similar manner to that described above - i.e. 1.5 kg mixed grey sand in each plastic lined pot - for use in the ryegrass experiment. A standard nutrient stock solution - the *Long Ashton* solution (Hewitt, 1966) - was mixed into the soil as described for the maize experiment. This solution contains both the macro- and micronutrients required for optimal plant growth (i.e. excluding Se, which is not an essential plant nutrient). Ryegrass seeds (*Westerwold's ryegrass*) were spread on the surface of the soil after it had been brought to field capacity, and the pots were covered until germination occurred. The grass was allowed to grow for four weeks, under conditions identical to those described for the maize plants (Table 4.2). The ryegrass was then cut, oven dried for 24 hours (70°C) and milled before being digested and analyzed (as described in Appendix B). Selenium analysis was carried out by ICP-mass spectrometry.

4.3 Results and discussion

4.3.1 Maize experiment

4.3.1.1 Yields

Relative dry matter yields for the various treatments are presented in Table 4.3, and illustrations of selected yield responses are presented in Figure 4A. The maize plants grown in pots treated with the *complete nutrient* set produced the highest yield as expected, although those grown using the treatment from which Mn was omitted were statistically inseparable. Although maize has a low Mn requirement (Bennet, 1993), these results suggest that sufficient Mn was present in the grey sand sample for adequate growth and development of the maize plants.

The omission of B, Ca, Cu, Zn and Fe from the nutrient additions resulted in yields which fell short of the maximum by between 10 % and 20 % (Table 4.3). Witholding P had a dramatic effect on yield, which was little more than a third of the maximum even with the addition of other nutrients. It is apparent from these data that all of the elements tested except Mn are deficient in the composite soil sample. The principles underpinning the interpretation of changes in yield in response to nutrient concentrations are summarised by Munson & Nelson (1990). Graphical techniques have been widely

(a)

(b)

(c)

Figure 4A: Comparative maize yields for plants grown on Mseleni soil treated with various nutrient sets: the complete set (which corresponded to maximum yield) is on the right hand side in each photograph, whilst on the left are: (a) *no nutrients added* (cultivated soil); (b) *P omitted*; and (c) *Fe omitted* pots.

used to determine adequacy or deficiency of nutrient elements in plant material: in this approach relative yield is plotted in relation to a specific growth factor. In the process of making generalized statements about plant performance, yield percentages may be assigned to zones on such plots, with 100 % yield representing *adequacy*, 80-100 % falling into a *transition* zone and < 80 % yield representing *deficiency* (Fig. 4B, below).

Table 4.3: The effect of nutrient element treatments on relative yields of *Zea Mays* grown on Mseleni topsoil.

Treatment	Mean yield (3 reps)	S.D.
	%	%
Complete nutrients	100.0	1.7
Mn omitted	99.2	2.0
B omitted	89.5	2.5
Ca omitted	86.1	3.3
Cu omitted	84.7	2.1
Zn omitted	83.5	3.7
Fe omitted	80.4	4.1
P omitted	37.9	3.3
No nutrients added (C)*	29.9	1.0
No nutrients added (V)*	26.4	2.1

* C - cultivated soil, V - virgin soil

According to this scheme, yields in the treatments from which either P or all nutrients were omitted clearly fall into the deficient zone. On the other hand, yields in the absence of added Ca, Zn, Cu, Fe and B fall into the transition zone, corresponding to the condition sometimes referred to as *hidden hunger* (Tisdale *et al.*, 1985). If the *complete* nutrient treatment did not contain optimal nutrient concentrations, then the yields corresponding to several of these treatments (i.e. Ca, Zn, Cu, Fe and B) might well plot in the deficiency zone of such a diagram.

The *no nutrients added* treatments should be viewed cautiously in terms of comparison with the data discussed above. The pots used in these treatments had neither basal solutions (i.e. Mg, K, N and S) nor other nutrients added to them, and as a result have the lowest recorded yields. Assuming the grey sand sample utilized in this experiment was roughly representative of similar sands in the area, these data alone suggest that maize yields in the Mseleni area are likely to be suboptimal due to inadequate levels of one or more nutrient elements. It is likely that the absence of P represented a *masking factor* (Tisdale *et al.*, 1985), in that the absence of P probably resulted in suboptimal concentrations of other nutrients irrespective of whether these were deficient in the soil or not.

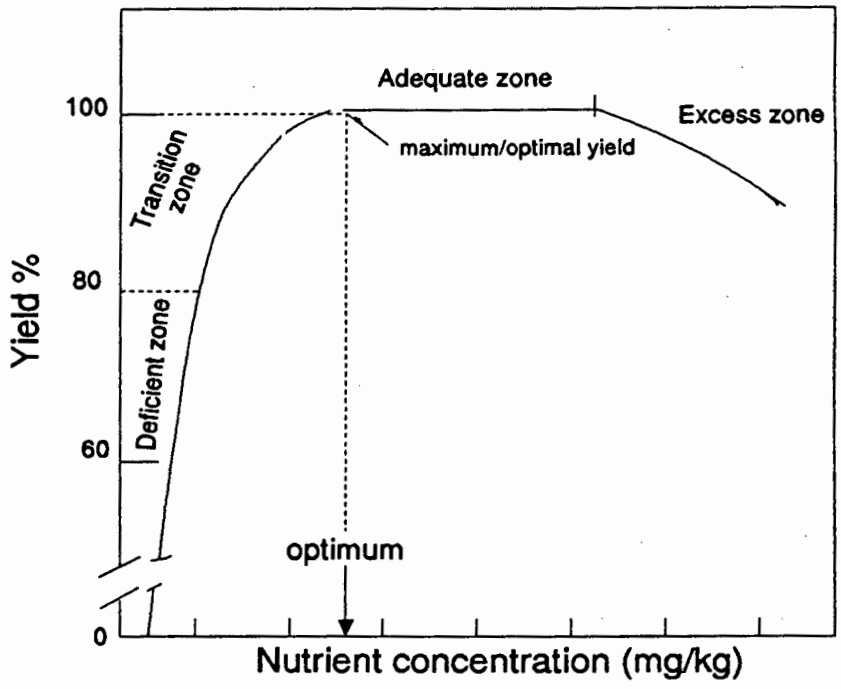


Figure 4B: Schematic illustrating the relationship between percentage of maximum yield and nutrient concentration of a specific plant part sampled at a given stage of development (after Ulrich and Hills, 1967; Dow and Roberts, 1982; *in* Munson and Nelson, 1990, pg. 362).

4.3.1.2 Visual deficiency symptoms

Several deficiency symptoms could be recognized in the maize plants during the course of the experiment. Although the interpretation of such symptoms is both subjective and often equivocal, the fact that deficiency symptoms occurred at all is instructive. The following symptoms were observed (see Fig. 4C):

- a general yellowing of the leaves of maize plants: this is normally attributable to either N and/or P deficiency (Mengel and Kirkby, 1978), and was manifested most obviously in the *P omitted* and *No nutrients added* pots;

- the development of distinct reddish purple midribs, leaf-margins and patches on leaves: this is the most common manifestation of P deficiency (Tisdale *et al.*, 1985), and was also a characteristic feature of the *P omitted* and *No nutrients added* pots (Fig. 4C);

- interveinal chlorosis observed in the leaves of almost all the plants grown in the experiment (Fig. 4C): this is commonly caused by a deficiency of any one of the following elements: Mg, Zn, Cu, Fe or Mn (Tisdale *et al*, 1995);

- necrotic leaf tips: these are often associated with K deficiency, and were observed in all pots, and

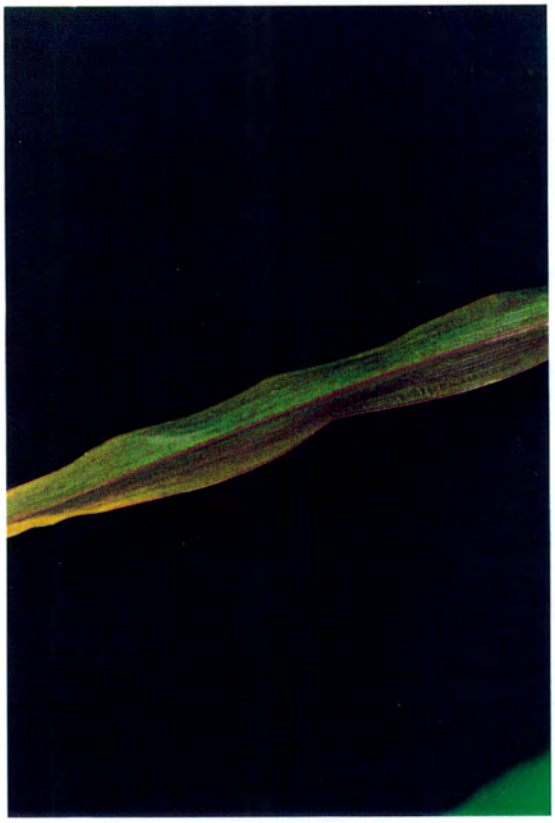
- necrotic patches down the margins leaves in the *Cu omitted* pots (Fig. 4C): this may be interpreted as being a symptom of either Cu or K deficiency (Mengel and Kirkby, 1978; Bennet, 1993). In the light of the fact that these symptoms were only observed in the Cu deficient pots, it seems most likely that deficiency of this nutrient was responsible.

Due to the ambiguous nature of many such symptoms and the qualitative nature of the subsequent interpretations, the use of foliar concentrations of the selected elements (reported below) should be regarded as a more reliable indication of soil nutrient availability. The visual symptoms do indicate, however, that there are clear deficiencies of one or more of the elements which were described in the previous section as possibly falling into the transition zone (on a dry matter yield basis). This suggests that these element are probably better described as falling into the deficiency zone, and the foliar analyses detailed below would be expected to confirm deficiency in all elements besides Mn.

4.3.1.3 Foliar concentrations of selected nutrients

The foliar concentrations of nutrient elements are routinely used to identify nutrient deficiencies in plants and to provide an index of soil nutrient availability. Plant analysis normally requires that a specific part or parts of a given species must be collected at a prescribed time, analyzed, and the test results interpreted (Jones, 1991). The latter stage of this process involves a comparison of the test results with known threshold values, which requires that the first two stages are scrupulously controlled. These normas are commonly reported as: i) *critical values* - those concentrations below which deficiency occurs; ii) *sufficiency ranges* - ranges in foliar concentrations thought to reflect nutrient levels adequate for normal plant growth and metabolism; and iii) *DRIS* (Diagnosis and Recommendation Integrated System) *norms*. In the latter technique, calculated elemental ratio indices are compared to established norms (Jones, 1991) for plants of various ages as a basis for determining the optimum relative concentration or balance between various nutrients.

The most widely used and arguably the most readily applicable approach in the context of this experiment is that employing *critical values*. Various proposed critical values for maize are available in the literature. Of these, it is appropriate to select only those which are best suited to the interpretation of foliar concentrations in whole maize plants 35 days after emergence. Bennet (1995), Clark (1984) and



(a)

(b)

(c)

Figure 4C: Examples of deficiency symptoms observed in maize plants grown in Mseleni soil: (a) reddish patches on leaves, commonly attributed to P deficiency (P omitted treatment); (b) interveinal chlorosis (Zn omitted treatment); and (c) necrotic patches down leaf margins, commonly observed in Cu deficient plants (Cu omitted treatment).

Table 4.4: Foliar concentrations of selected nutrient elements in 35 day old maize plants grown in Mseleni soil, in response to selective fertilizer additions[a].

Treatment	Nutrient concentrations								
	Ca	P	K	Mg	Zn	Mn	Cu	Fe	B
	% (std. dev.)				mg/kg (std. dev.)				
Full nutrients	0.48 (0.04)	0.53 (0.01)	1.79 (0.08)	0.54 (0.02)	74.2 (8.2)	597 (52)	10.2 (1.0)	69.0 (4.6)	25.9 (2.0)
Ca omitted	0.42 (0.02)	0.59 (0.02)	2.03 (0.14)	0.54 (0.01)	68.5 (1.0)	511 (28)	10.2 (0.5)	70.3 (4.2)	22.8 (2.6)
P omitted	0.70 (0.04)	0.15 (0.01)	4.28 (0.48)	0.51 (0.05)	136 (17)	1060 (78)	13.7 (1.8)	92.1 (3.4)	66.2 (14.0)
Zn omitted	0.45 (0.01)	0.61 (0.02)	2.19 (0.15)	0.56 (0.02)	30.8 (0.8)	430 (19)	10.2 (1.1)	81.9 (6.7)	33.4 (3.1)
Mn omitted	0.37 (0.04)	0.56 (0.02)	1.78 (0.11)	0.53 (0.02)	66.3 (4.8)	270 (8)	11.2 (0.1)	72.3 (2.3)	37.0 (0.2)
Cu omitted	0.46 (0.01)	0.64 (0.01)	1.95 (0.13)	0.56 (0.02)	74.6 (6.6)	473 (27)	2.57 (0.34)	87.0 (2.1)	32.4 (0.48)
Fe omitted	0.48 (0.03)	0.60 (0.04)	2.27 (0.28)	0.58 (0.04)	73.0 (4.6)	601 (40)	4.70 (1.37)	64.6 (9.1)	28.2 (1.4)
B omitted	0.40 (0.03)	0.52 (0.03)	1.87 (0.11)	0.53 (0.03)	68.6 (9)	500 (19)	8.5 (0.5)	66.0 (1.1)	4.83 (0.21)
No nutrients*$_C$	0.50 (0.02)	0.13 (0.01)	1.18 (0.05)	0.44 (0.0)	14.7 (1.2)	81.0 (10.2)	1.83 (0.25)	48.3 (12.1)	4.10 (0.0)
No nutrients*$_V$	0.46 (0.04)	0.13 (0.01)	1.55 (0.15)	0.40 (0.04)	14.3 (1.0)	78.0 (9.0)	1.60 (0.1)	44.2 (3.9)	7.70 (0.0)
Critical value[b]	**0.9**	**0.4**	**3.5**	**0.3**	**20**	**50**	**7**	**50**	**7**

*C: cultivated soil; *V: virgin soil

[a] values in parentheses are standard deviations calculated from the values for triplicate treatments (App. D); [b] Critical values from Bennet (1995)

others have synthesized foliar concentration data (i.e. critical values) from the literature pertaining to maize plants which are at the 3- to 4-leaf stage up to 30-45 days after emergence, the span of which includes the plants investigated. Critical values for the elements of interest are reported in Table 4.4, together with mean foliar concentrations of the macronutrients Ca, P, K, Mg and the micronutrients Zn, Mn, Cu, Fe and B in each treatment (values for individual pots are provided in Appendix D. It must be noted in the case of Ca that DRIS norms for analyses of whole young maize plants (Sumner, unpubl. data, 1996) indicated optimal Ca concentrations of 0.53 % for 45 day old plants, which seems to differ significantly from the widely published critical value of 0.9 % mentioned in Table 4.4.

It must be stressed at this point that the focus of the discussion below will be on the fundamental question of whether or not plants grown on the grey sand exhibit foliar concentrations of selected nutrients which represent deficiency. Minor differences in foliar composition may be caused by nutrient interactions (Jones, 1991), and these will not be discussed except where neccessary to assessment of nutrient deficiencies in the soil as a whole.

Macronutrients

Calcium appears to be deficient in foliar material from all treatments if the critical value of 0.9 % is used; interestingly, the *Ca omitted* and *no nutrients* treatments do not have lower Ca concentrations relative to the other treatments (which had Ca added). If, however, the DRIS derived optimal Ca concentration (0.53 %, Sumner, personal communication, 1996) is utilized instead of the critical value (0.9 %), then observed Ca concentrations in the foliar material might be considered generally suboptimal but probably not deficient.

Phosphorus appears to be acutely deficient in the *P omitted* and *no nutrients* treatments. It should be noted that the concentrations of every element except Mg are elevated in the *P omitted* treatment relative to the other treatments. This may have been a result of the small amount of dry matter accumulation in the *P omitted* pots, causing a concentration effect (i.e. the concentrations of these elements actually extracted from the soil in the *P omitted* pots is probably comparable with that withdrawn from pots from other treatments). This kind of effect is well documented, and is considered to be the primary explanation of seasonal changes in the foliar concentrations of certain nutrients in plants grown in the field (Jones, 1991).

Potassium appears to be unequivocally deficient in the maize plants; on the other hand, foliar Mg concentrations appear to be well above the critical value of 0.3 mg/kg, even in the *no nutrients* treatments.

Micronutrients

Zinc appears to be deficient in the *no nutrients* treatments, whilst the *Zn omitted* treatment contains low Zn concentrations. The other treatments (having received Zn fertilization) produced plants with sufficient levels of this micronutrient. These values are difficult to interpret, bearing in mind that the *Zn omitted* pots were fertilized with all the other macro- and micronutrients required to stimulate growth and nutrient uptake (which would not occur in the field area), whilst the *no nutrients* pots were completely unfertilized.

Adequate levels of Mn were observed for all treatments, indicating that the soil conditions resulting from the experimental design were conducive to sufficient concentrations of available Mn in the soil solution. In hindsight it appears that the addition of 3 mg/kg Mn to the pots from all but three treatments (*Mn omitted* and *no nutrients*) was probably excessive, with Mn concentrations reaching near toxic levels in some cases. Copper appears to be deficient in the soil used, as is evidenced by the foliar concentrations for the *Cu omitted*, *Fe omitted* and *no nutrients* treatments. Iron appears to be deficient in the *no nutrients* treatments, although only marginally so. Adequate levels of Fe are evident in the *Fe omitted* treatment, suggesting that available soil concentrations are probably adequate, but that the deficiencies of other elements and/or plant factors have limited Fe uptake in the *no nutrients* pots.

Finally, boron appears also to be deficient: this is suggested by deficient levels in both the *no nutrients* pots as well as in the *B omitted* pots.

4.4.2 Selenium assay using ryegrass

Selenium concentrations in the two ryegrass samples were 0.2 and 0.39 mg/kg respectively. As mentioned in Chapter 2, selenium is an essential element for animals, not plants, hence the foliar concentrations reported above may not, as in the case of the other nutrient elements investigated, be compared with crop derived critical values. Selenium deficiencies in grazing animals generally occur when the Se concentration in forage is less than 0.05 mg/kg (Wan *et al.*, 1988). The ryegrass samples grown on the composite grey sand sample (0.2 and 0.39 mg Se/kg) appear to contain adequate levels of Se, indicating that under optimal growth conditions sufficient Se is available in the soil solution.
The interpretation of these values is purely speculative in terms of the likelihood of selenium deficiency in the field area. Although ryegrass is a convenient forage species in terms of comparison with the literature pertaining to Se deficiency in livestock, it is hardly a species which would be expected to grow at Mseleni. Ryegrass may thus be physiologically different to species prevalent in the area, and these results may consequently only be used as a rough guide to the availability of Se in the soil.

The use of fertilizers to enhance the growth of the ryegrass is also obviously not representative of field conditions. As observed in the case of iron in the preceding section, foliar concentrations of a given nutrient may reflect deficiency in unfertilized soils yet sufficiency where fertilizer has been applied (presumably due to the interaction of soil nutrient availability and plant growth factors). Apart from these factors, there is a host of more general and widely accepted experimental limitations which limit the interpretative value of such an experiment. These may be referenced in most treatises concerned with plant analysis, and will not be discussed in detail here.

4.4 Conclusions

The dry matter yields, visual deficiency symptoms, and foliar concentrations of selected nutrient elements reported here seem to confirm the results of the soil tests discussed in Chapter 3, suggesting that a large proportion of the grey sands in the Mseleni area are deficient in several essential plant nutrients.

Of the macronutrients investigated, Ca concentrations appeared to be suboptimal, whilst P and K were both deficient (Mg adequate). These observations are in agreement with soil test data reported in Chapter 3. The levels of all of the above were slightly elevated in the soil sample used in the experiment relative to the average concentrations in the grey sands, indicating that a fair proportion of these sands would probably induce the same suboptimal growth responses in plants. Of the micronutrient elements, only Mn and Se appeared to be available in adequate concentrations. As mentioned above, however, the interpretation of measured Se concentrations relative to actual (field) concentrations remains highly speculative. As a result, Se "adequacy" cannot be assumed on the basis of these results. Boron and Cu proved to be deficient, whilst Fe and Zn levels suggested that deficiency would almost certainly occur in the field unless a comprehensive fertilization regime was implemented. Soil test data reported in Chapter 3 indicated that Zn and Cu deficiency would in all likelihood occur in these soils, whilst Fe and B appeared to be available in low but sufficient concentrations and Mn concentrations appeared to be adequate. Levels of Zn and Fe in the soil used were identical to the average value for the grey sands, whilst Mn and Cu values differed slightly.

The methods used in this experiment, having been widely calibrated under both greenhouse and field conditions globally, should be regarded as providing a good qualitative estimate of the likelihood of elemental deficiencies in the Mseleni area (with the exception of Se). The extrapolation of the results obtained from experiments on a single composite sample to similar soils in the area of interest is speculative, although the near-average nutrient concentrations in the composite former do suggest that such extrapolation might be valid for a large proportion of the grey sands analyzed. The full implications of the results reported here will be discussed in more general terms in Chapter 5.

Chapter 5

General discussion and conclusions

This study has been directed towards the provision and interpretation of quantitative chemical data for the soils of the Mseleni area in Kwazulu Natal. Medical investigations in the area have increasingly implicated nutritional factors as a possible cause of the endemic joint disease which afflicts local communities. In spite of the fact that the inhabitants of the area rely almost entirely on produce grown in patently poor soils, investigations to date have not included soil analysis. The present study constitutes a baseline epidemiological investigation aimed at quantifying the nutrient status of soils in the high-incidence area and assessing the likelihood of soil-related nutrient deficiencies.

The approach taken involved the testing of three hypotheses: i) that the grey *Fernwood* soils which characterize the area are deficient with respect to one or more elements generally regarded as being vital to skeletal integrity in animals and/or humans; ii) that deficient areas would not be evenly distributed in the landscape, leading to pockets of low nutrient status; and iii) that in the case of those nutrients which are also essential for plant growth, these deficiencies would manifest themselves in the suboptimal growth of crops cultivated on the soil. Standard laboratory techniques were used to chemically characterize both the *Fernwood* soils from the Mseleni area, and a limited number of *Hutton* soils from an adjacent area. Established soil testing procedures were employed to determine nutrient availability, and, finally, in a controlled experiment with young maize plants grown in a typical soil from the area, plant growth and tissue composition responses to nutrient treatments were used to verify nutrient deficiencies. In addition to this, two water samples were analyzed in order to both confirm and supplement earlier geochemical findings pertaining to drinking water in the area.

The results of these various tests have served to confirm all three hypotheses, indicating that the *Fernwood* soils in the area *are* in fact generally deficient in the important bone forming elements P, Zn, Cu and B[1], and contain suboptimal concentrations of Ca (Se availability requires further research); that the spatial distribution of nutrient elements in the area *is* highly variable; and that plants grown in the soils *do* indeed suffer from multiple nutrient deficiencies, involving both these and other nutrient elements (i.e. K and Fe). Results of the water analysis confirmed that fluoride levels in local waters, including Lake Sibaya, are generally very low.

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[1]B is not conventionally regarded as an important bone forming element, but has been linked to osteoarthritis in some rural populations.

Epidemiological studies have tended in general to show that nutrient deficiencies, particularly of trace elements, manifest themselves in isolated, primitive communities who live at subsistence level and whose diets are characteristically unvaried and geographically restricted. The marked poverty of soils in the Mseleni area would almost certainly be expected to extend across large tracts of the broad, sandy coastal plain within which Mseleni Joint Disease (MJD) appears to be confined. The peasant communities living on this plain are especially vulnerable to nutritional deficiencies, eking out a living by means of shifting cultivation on an infertile substrate, in the midst of a large and remote tract of land which has historically been served by a rudimentary infrastructure, minimising opportunities for dietary supplementation with imported foodstuffs. As a consequence, sources of local nutrition have been extremely restricted and dominated by plants grown on what may now be justifiably termed *deficient* soils.

It may well be argued on the basis of these considerations that the Maputaland coastal plain is inherently unsuitable for human settlement based on subsistence agriculture. The nutrient-deficient status of soils in the area has, somewhat ironically, lead to the preponderance of a wealth of indigenous plant species, many of which have probably served to alleviate the difficulties of producing home-grown food and accommodated habitation of an otherwise inhospitable area.

The significance of the present study lies in the fact that it provides the first geochemical data to support suggestions in the literature concerning the possible role of nutritional deficiencies in the etiology of MJD (Fincham *et al.*, 1981, 1985, 1986; van Rensburg and Jaskiewicz, 1984; Schnitzler *et al.*, 1988). Soil and crop plant analysis has formed the cornerstone of epidemiological investigations elsewhere, both into conditions closely related to MJD (Xilinas, 1983; Zhang, 1986) and into other human and animal disorders (Mitchell and Burridge, 1980). Although the need for such analyses was clearly recognized by medical researchers working in the Mseleni district in the mid-1980's (van Rensburg and Jaskiewicz, 1984; Yach and Botha, 1985; Marasas and van Rensburg, 1986), a proposal for multi-disciplinary investigation into these and other etiological factors was rejected by the Medical Research Council (MRC) soon afterwards. It is hoped that the data reported here might provide the stimulus for continued investigations into a possible environmental causal agent in the region.

Motivation for further studies in the area should include a consideration of current social impacts of this disease. These include both extensive human suffering and economic loss due to lack of production, non-attendance at schools, the financing of expensive hip replacement operations and the payment of disability grants by the state. The unique combination of edaphic and climatic factors which prevail in the Mseleni area also provides great academic incentive for further research in that, although nutrient deficiencies may not be experimentally induced in human subjects for ethical reasons, the more isolated parts of this environment constitute a relatively well-constrained natural laboratory. It may thus be

possible to investigate the incidence of a previously undocumented consequences of mineral deficiencies in human subjects there, at least until such time as significant infrastructural and social upliftment exposes the inhabitants to external sources of nutrition.

Additional avenues for geochemical research include the correlation of the incidence of the disease with soil types in the region and analysis of the various crops and other foodstuffs consumed in affected areas to obtain dietary profiles. A further interesting aspect of this problem which undoubtedly deserves attention is the question of whether the disease afflicts neighbouring Mozambique, where rural people subsist in much the same way as on the South African side of the border, on impoverished windblown sands. Ecological issues in the area also warrant consideration: the peculiarity of floral diversity and neo-endemism which have already been alluded to form part of a rich biotic heritage, the management and preservation of which should undoubtedly be linked to a consideration of soil chemical data.

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Appendix A

Analytical methods used in soil and water analysis

1. Soil pH

Method:

The standard method for pH measurement was used, as described by McLean (1982).

Apparatus/Reagents:

2mm sieve; centrifuge tubes; pH meter; 0.01M $CaCl_2$ solution.

Procedure:

Samples were dried and crushed before being sieved through a 2mm sieve. 10g of soil was then mixed with 25ml of MQ water to obtain a 1:2.5 soil : water ratio. After mixing (in centrifuge tubes), the solution was allowed to settle for ± 30 minutes, whereupon pH measurements were made using a Crison pH meter. The procedure was then repeated using a 0.01*M* $CaCl_2$ solution instead of water.

Three pH readings were taken for each sample and for each of the two types of measurement (i.e. pH in water and pH in $CaCl_2$), in order to determine the precision of the data (0.01 pH unit). The pH ($CaCl_2$) data obtained were found to consistently lie within 0.1 pH unit of the corresponding values generated by the Cedara Crop Science Institute for the same soil samples, indicating a satisfactory level of accuracy. The import of pH measurements in this study was not particularly high; it was more important to establish the general range of pH values than to obtain extremely precise values for individual samples.

Calculation:

(no calculation required)

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2. Extractable Acidity, Ca and Mg: Potassium Chloride method

Method:

Extractable acidity, Ca and Mg were determined using the standard *KCl-extraction* method (Thomas, 1982).

Apparatus/reagents:

1 *N* KCl solution; Buchner funnel; 0.01*M* NaOH solution, 1% lanthanum solution; combination diluter-dispenser, phenolphthalein indicator.

Procedure:

i) KCl extraction: 25 ml of 1 *N* KCl solution was added to 2.5 g soil. This was then stirred for ten minutes before being filtered through a Buchner funnel.

ii) Determination of extractable Ca and Mg:
   a) a combination diluter-dispenser was used to take a 1 ml aliquot of filtrate, which was added to 9 ml distilled water and 15 ml of 1% lanthanum solution.
   b) Ca and Mg were determined on the resulting solution using atomic absorption spectroscopy.

iii) Determination of extractable acidity:
   a) A diluter-dispenser was used to take 10 ml of filtrate and add it to 10 ml distilled water containing 2 drops of phenolphthalein indicator.
   b) The resultant solution was titrated with 0.01 *M* NaOH whilst being stirred.

Analyses pertaining to extractable elemental concentrations in the soils (i.e. the *KCl-extraction* and *AMBIC-2* methods) were carried out by external laboratories. This was done because the standard methods used are generally complex, requiring laboratory procedures which enable routine determinations to be made on a regular basis (involving periods of time longer than could be afforded in this project). Prohibitive costs dictated that duplicate samples were not generally submitted to these laboratories; these institutions do, however, subscribe to stringent quality control procedures involving regular audits by independent organizations. The accuracy of the data obtained is thus relatively well assured, which is important in the context of this thesis.

Calculation:

Using the above method for determining extractable acidity, the following formula is applicable:

ml 0.01 M NaOH = meq of acidity per 100 ml of soil

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3. Organic Carbon: Walkley Black Method

Method:

Organic carbon was determined using the standard *Walkley Black* procedure (Nelson and Sommers, 1982).

Apparatus/Reagents:

Balance, Erlenmeyer flasks (500cm^3); burette, pipettes; Potassium dichromate 0.167 mol dm^{-3}; concentrated sulphuric acid; concentrated ortho-phosphoric acid; iron (II) ammonium sulphate (0.5 mol. dm^{-3}); indicator: barium diphenylamine sulphonate.

Procedure:

The soil was ground to pass a 0.35mm sieve, using a porcelain mortar and pestle. Half a gram of air dried soil was transferred to a 500cm^3 Erlenmeyer flask. To this, 10cm^3 $K_2Cr_2O_7$ solution was added (by pipette), and the flask was then swirled to disperse the soil in solution. Concentrated sulphuric acid (20cm^3) was rapidly added to this, and the flask was again swirled until the soil and reagents were mixed. The flask was allowed to cool on a protected surface for ± 30 minutes, following which 150cm^3 de-ionised water and 10cm^3 concentrated ortho-phosphoric acid was added to it. Indicator (1cm^3) was then added and the excess dichromate titrated with iron (II) ammonium sulphate solution. The colour change involved at the end-point was dark violet to green.

Duplicate analyses were carried out on most, if not all samples. The results showed that the organic carbon content of samples was estimated to within 0.1 %, which was satisfactory in the context of this study (only a rough estimate was required). Rapid dichromate methods (including the *Walkley Black* procedure) are sometimes subject to interferences by chlorides, ferrous iron and higher oxides of manganese, leading to spurious results (Nelson and Sommers, 1982). In the soils analyzed here, however, chlorine would not be expected to be present at all (as it is highly mobile), and the redox conditions experienced in the area concerned (i.e. oxidizing conditions) would preclude problems associated with Fe and Mn (such problems are reported to occur under reducing soil conditions, Nelson and Sommers, 1982).

Calculations:

Concentration of $Fe(NH_4)_2(SO_4)_2$ mol dm^{-3} = (10cm^3 $K_2Cr_2O_7$ x 0.167 x 6) / (cm^3$Fe(NH_4)_2(SO_4)_2$

Organic car. % = ([cm^3 $Fe(NH_4)_2(SO_4)_2$ blank - cm^3 $Fe(NH_4)_2(SO_4)_2$ sample] x M x 0.3 x f) / soil mass

where f = 1.3

M = concentration of $Fe(NH_4)_2(SO_4)_2$ in mol dm^{-3}

4. Clay Content: the Pipette Method

Method:

The method used to determine clay content was an abridged version of the standard *pipette* method (Gee and Bauder, 1986).

Apparatus/reagents:

1 litre glass sedimentation cylinders, hand stirrer (metal rod joined to a metal plate roughly the diameter of the sedimentation cylinders), Lowy pipette (22.36cm^3 capacity) and stand, constant temperature room, 53μm sieve with receiving pan, drying oven, various glass beakers;

Calgon dispersing solution: 35.7 g sodium hexametaphosphate $(NaPO_4)_6$ and 7.94 g sodium carbonate (Na_2CO_3) were dissolved into 1 litre of de-ionised water.

Procedure:

Dispersion:

Due to the nature of the soil samples, pretreatment to remove various coatings (e.g. iron oxides/hydroxides or carbonates) which might otherwise prevent dispersion were deemed unnecessary. Calgon dispersing solution (10 cm^3) was added to each 80 g oven-dried soil sample. The suspensions were transferred quantitatively into 250 cm^3 centrifuge bottles and made up to approximately 150 cm^3 volume using de-ionised water. The bottles were then stoppered and shaken overnight on a horizontal reciprocating shaker.

Separation of clay fractions:

The silt and clay were washed through a 0.053 mm sieve into 1L cylinders. The samples were washed until the percolates were clear, whereupon the sand fractions were discarded and the cylinders were made up to 1 L capacity using distilled water. The cylinders were placed in a room with constant temperature (21°C) and stirred thoroughly for thirty seconds (hand stirrer), in a vertical direction. After the appropriate settling time (5 hours 30 minutes) the Lowy pipette was lowered to a depth of 7 cm into each suspension and 22.36 cm^3 (modified pipette) sample was withdrawn from each. These samples were then discharged into tared evaporating dishes. The pipette was then rinsed with de-ionised water each time and the resulting water was added to the sample in the evaporating dish. The water was evaporated and the samples dried in an oven (105 °C, overnight), upon which the dishes containing clay fractions were cooled in a desiccator and re-weighed.

Errors in particle size analysis values using this method are mainly associated with sampling and weighing; clay fractions can generally be determined with a precision of ± 1 % (Gee and Bauder, 1986). Repeat analyses were carried out on several samples, with the precision observed being in agreement

with this published figure (1.1 %).

Calculation:

$$Percent\ clay = [(D-E) \times 1000 \times 100] / (F \times 22.36)$$

where D was the mass (g) of pipetted clay

E was a mass correction for the dispersing agent (0.01 g)

F was the mass (g) of oven-dry total sample (80g)

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5. Clay separation for mineralogical analysis

Method:

A non-standard method was utilized for the separation of clay fractions for subsequent mineralogical analysis.

Apparatus/Reagents:

Plastic bottles, large beakers (1L), plastic buckets, centrifuge tubes, porcelain crucibles, dialysis tubing, silver nitrate solution, 1 *M* NaOH, pH meter, Na_2CO_3 solution, 1 *M* HCl, NaCl.

Method:

Approximately 80 g air-dried soil (<2 mm) was placed in a 250 cm^3 plastic bottle (for each sample). Distilled water was added to this to form a slurry; a few drops of 1 *M* NaOH were added to the slurry and it was shaken. The pH was measured and the procedure repeated until the pH stabilized at ± 9. The bottle was capped and shaken for four hours. The contents were then transferred into a 1L beaker which was then filled with pH 10 Na_2CO_3 solution. The resultant suspension was stirred, covered and allowed to stand for (13) hours. The supernatant suspension was then siphoned off to a depth of 15 cm (values and times worked out using Stokes law). The procedure was repeated with further addition of pH 10 Na_2CO_3 solution; the decantate was accumulated in a large bucket to which 1*M* HCl was added dropwise to restore the pH to $5 < pH < 7$. NaCl was added to promote flocculation. The clear supernatant was siphoned off and discarded. Once this procedure had been repeated four times, the clay concentrate was transferred into a number of centrifuge tubes for centrifugation: the supernatant was again discarded.

The clay was then dialysed by adding a limited volume of water to it, shaking it thoroughly and pouring it into dialysis tubing. The clay was equilibrated with tap water (running) overnight and then with deionised water for two nights. The washing procedure was terminated once it was determined that the chloride (from the salt) had been removed (using silver nitrate). The clay fraction was then stored in a stoppered bottle.

6. Mineralogical analysis of the clay fraction: X-ray diffractometry

Method:

The clay fractions separated by the above method were analyzed using the XRD technique (Whittig and Allardice, 1986).

Apparatus/reagents:

Glass slides, X-ray diffractometer, Pasteur pipette, watch glasses.

Procedure:

The clay fraction obtained in the above method (5) was stored in a plastic bottle. An aliquot (5ml) was pipetted into a tared porcelain crucible for drying and gravimetric determination of the suspension concentration (mg/l). The suspension concentration was adjusted to ± 20 mg/l and a 2 ml aliquot was then withdrawn and put dropwise onto a level glass slide. The slide was covered and put in a cupboard to dry. Slides were analyzed using the XRD technique the following day. Scanning was done over a two theta range of 4-65 degrees, using CuKα radiation. Results were recorded and processed by computer.

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7. X-ray Fluorescence Spectroscopy (XRFS): Zn, Cu, Mn

Method:

Powder briquettes were prepared using the method of Norrish and Hutton (1969), and analyzed using wavelength dispersive x-ray fluorescence techniques (Jones, 1982).

Apparatus/reagents:

Sieb mill; 4% Mowiol solution; lithium tetraborate; wavelength dispersive XRF spectrometer with sample holders.

Procedure:

Sample Preparation:

Soil samples were air dried and then milled for approximately three minutes in a carbon steel Seibtechnik swing mill, reducing grain size to below 50μm diameter. Six grams of the powdered sample was then mixed with 4% mowiol solution using a mortar and pestle, before being mechanically pressed into

briquette form under a pressure of ± 10t. The resulting briquettes were then placed under vacuum for a day in order to desiccate them and to prevent fracture from occurring in the XRF machine (under vacuum conditions).

Analysis:

Trace element concentrations were determined on powder briquettes in an analytical run using a Au x-ray tube. Analytical conditions are listed in Table A1.

Measured intensity data were processed through the computer program TRACE to correct gross peak intensities for background and spectral overlap and to make mass absorption coefficient corrections according to the methods outlined in Duncan *et al.* (1984).

Table A1: Instrumental conditions for determination of trace elements using a Phillips PW1480 WDXRF spectrometer (Willis, 1996).

Element	Collimator	Crystal	Detector	PHS LWL	PHS UPL	Counting time (s)	Concentration range *
ZnKα	F	LiF(220)	FS	20	80	200	0 - 235
CuKα	F	LiF(220)	FS	20	80	200	0 - 227
MnKα	F	LiF(220)	FL	15	75	200	0 - 1700

PHS: pulse height selector
LWL: lower limit; UPL: upper limit
* = all concentrations expressed as part per million (ppm or $mg.kg^{-1}$)

Typical lower limits of detection for the chosen elements were as follows:

Zn - 0.6 mg/kg
Cu - 0.8 mg/kg
Mn - 2.0 mg/kg.

Typical 1 sigma counting errors associated with the determinations were

Zn - 0.2 mg/kg
Cu - 3.0 mg/kg
Mn - 1.4 mg/kg

These were determined from data obtained from two analytical runs, which were averaged out to obtain the reported values.

8. The AMBIC-2 method: P, K, Zn, Cu, Mn and Fe

Method:

The available concentrations of the above elements were determined using the AMBIC-2 method which has been shown to be suitable for the determination of these elements on a wide range of soils (Farina and Channon, 1979).

Apparatus/reagents:

Plastic bottles with silicone rubber stoppers, 100 cm^3 capacity, Whatman number 42 filter paper, funnels and racks, spectrophotometer, atomic absorption spectrometer, combination diluter-dispenser.

i) AMBIC-2 extraction solution: 0.25 $mol.dm^{-3}$ NH_4HCO_3 + 0.01 $mol.dm^{-3}$ $(NH_4)_2EDTA$ + 0.01 $mol.dm^{-3}$ NH_4F + Superfloc (N-127).

Procedure:

1. Extracting procedure: 25 ml extracting solution was added to 2.5 g soil in a bottle; the solution was stirred for ten minutes at low speed (± 400 rpm), before being filtered through Whatman No. 45 filter paper.

2. Analytical procedures:

   i) P determination: a combination diluter-dispenser was used to take a 2 ml aliquot of filtrate, which was added to 8 ml distilled water and 10 ml ammonium molybdate colour reagent B. After 40 minutes the transmission % was measured using a spectrophotometer at 882 *nm*.
   ii) K determination: using the same diluter-dispenser as described above in 2 i), a 2 ml aliquot of filtrate was added to 18 ml distilled water. The K was measured using atomic absorption methods.
   iii) Determination of Cu, Fe, Mn and Zn: these elements were analyzed directly from the filtrate by atomic absorption techniques.

As mentioned previously, precision and accuracy information pertaining to this technique were not established by the author (this work was carried out by the Cedara Crop Science Institute in Natal).

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9. Extractable boron

Method:

Extractable B concentrations were determined by means of the *Hot water extraction/curcumin* method

(Bingham, 1982).

Apparatus/reagents:

Soda glass Erlenmeyer flasks, 50 cm^3, cork stoppers, plastic measuring cylinder, 50 cm^3, plastic funnels, plastic bottles, 50 cm^3 capacity, Whatman no. 542 and no. 41 filter paper, hotplate, oven, pipettes, burette, 50 cm^3, silica crucibles, 30 cm^3, spectrophotometer, balance accurate to 0.1 g
Calcium chloride, 0.05 $mol.dm^{-3}$; curcumin solution: 0.04 g curcumin and 5 g oxalic acid were dissolved in 100 cm^3 ethanol (95%); boron standard solution, 0.5 mg B dm^{-3}; **n.b. Solutions and distilled water were stored in plastic containers to avoid contamination by borosilicate glassware.**

Procedure:

i) Extraction: 20 g air dried soil from each sample was placed in a soda glass Erhenmeyer flask, to which 40 cm^3 distilled water was added. The contents of each flask were mixed manually, whereupon the flasks were stoppered and heated on a hotplate until the temperature reached 80°C. The flasks were then placed in an oven also set at 80°C for five minutes. Three drops of calcium chloride solution was then added to each, before the contents were filtered through Whatman no. 5442 paper (fitted to a plastic funnel) into plastic bottles. A blank was run using 40 cm^3 distilled water and treated in identical fashion.

ii) Determination: 1, 2, 3 and 5 cm^3 standard solution were pipetted into crucibles; 1 cm^3 de-ionised water was used for the blank. 1 cm^3 soil extract for each sample was pipetted into a crucible; to which 4 cm^3 curcumin solution was added. The crucibles were then placed in an oven at 50°C until dry. The salts were washed out (using ethanol) into a 25 cm^3 volumetric flask and made up to volume with ethanol; the resulting solution was then shaken and filtered through Whatman no. 41 filter paper, using plastic funnels. The absorbance of each sample was read using a spectrophotometer, and compared with the standard curve.

These analyses were carried out at the Institute for Soil, Climate and Water in Pretoria, as no suitable equipment was available at UCT. Unfortunately, no duplicate samples were submitted due to the prohibitive cost of the analyses; this laboratory does, however, subscribe to the same stringent quality control program as does the Cedara laboratory. A one percent relative error is expected using this technique (Bingham, 1982).

Calculation:

$$mg\ kg^{-1}\ B\ in\ soil = (b \times 40) / 20$$

if b is the B content of the sample ($\mu g.cm^3$)

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10. Major anions and cations: High pressure ion chromatography (HPIC)

Method:

The concentrations of the major cations and anions in the water samples (except bicarbonate) were

determined using HPIC techniques (Hasset, 1982).

Apparatus:

Electrical conductivity meter, HPIC instrument, 0.2 μm and organic filters.

Procedure:

Samples were filtered through a 0.2 μm filter, diluted using MQ water in order to achieve electrical conductivities of < 100 μS/cm, and then filtered again to remove organic matter and remaining particulates. Analysis was carried out using a Dionex 300 Series suppressed IC system.

Two duplicates were run for each sample on different days to ensure the accuracy of the data and to establish the precision of the technique (see App. C). Charge balance considerations indicate a 30-40 % deficit of negative charge (i.e. anions) which almost certainly represents bicarbonate (not determined).

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11. Fluoride determination

Method:

Fluoride was determined using the standard *electrode method* (Standard methods for the examination of water and wastewater, 1985).

Apparatus/reagents:

Ion-selective meter; magnetic stirrer.
Standard fluoride solutions: 0.5, 1.0 and 2.0 mg F^-/L.
Fluoride buffer: solution containing NaCl+CDTA+ 6*N* NaOH.

Procedure:

The ion-selective electrode was first calibrated using the standard F^- solutions; 10 ml aliquots of sample water were then mixed with 10 ml of fluoride buffer solution, and the resulting mixture was analyzed.

Three duplicate analyses were carried out for each sample. The instrumental read-out produced on analysis was accurate to 0.01 ppm; analysis of the duplicate samples produced identical results, indicating a high degree of precision.

Appendix B

Analytical methods used in plant analysis

1. Plant analysis: selenium

Method:

Organic matter digestion was carried out by a "wet" procedure (Jones and Case, 1990); Se concentrations in the resultant solution were analyzed for by inductively coupled plasma mass spectrometry (ICP-MS) at the Institute for Soil, Climate and Water (in Pretoria).

Apparatus/reagents:

100 ml digestion tubes; heating block; 100 ml volumetric flasks; ICP-MS instrument.
Analytical grade nitric acid: 65 % (Merck GR)
Analytical grade hydrogen peroxide: 30 % (Merck Gr)

Procedure:

Samples were washed to avoid contamination, then dried in an oven at 70°C to minimize chemical and biological changes. Particle size reduction of the dried material was carried out by milling through a 0.84 mm screen. Half a gram of dried, ground plant material was then weighed into a 100 ml digestion tube. Fifteen (15) ml HNO_3 was poured into the tube, which was then heated on a digestion block to 150°C in steps of 50°C. Half a ml of H_2O_2 was then added to the tube twice, with the sample being heated again for five minutes after each addition. Deionised water (5 ml) was then added to the tube, which was heated for another five minutes, removed from the heating block to cool, washed into 100 ml volumetric flasks and made up to volume with deionised water. Aliquots of the resulting solution were then analyzed using an ICP-MS instrument.

The standard range of the ICP-MS instrument used was 5-50 µg/L Se; precision achieved could not be properly estimated due to the small sample number (2). The accuracy of this analysis could not be checked by the author; is should be noted, however, that this laboratory does subscribe to stringent quality control procedures, including audits by independent organizations and that the accuracy of the data might be expected to be satisfactory.

2. Plant analysis: Ca, P, K, Mg, Zn, Cu, Mn and Fe

Method:

After dry ashing and digestion in dilute HCl (Jones and Case, 1990), phosphorus was determined

photometrically and the other elements by atomic absorption spectrophotometry by the Cedara Crop Science Laboratory in Natal.

Apparatus/reagents:

15 ml porcelain crucibles; muffle furnace;
Dilute HCl

Procedure:

Samples were washed to avoid contamination, then dried in an oven at 70°C to minimize chemical and biological changes. Particle size reduction of the dried material was carried out by milling through a 0.84 mm screen. Half a gram of the dried plant tissue was placed into a 15 ml porcelain crucible and ashed in a muffle furnace at 500°C for 6 hours. Once the crucible had cooled, dilute HCl was added to dissolve the ash. Phosphorus was determined photometrically in the resulting solution, whilst the other elements were determined by atomic absorption.

Appendix D contains elemental concentrations for all plants analyzed. Three replicates were available for each treatment; relative standard deviations (giving an indication of analytical precision) for each treatment are reported in Table 4.4.

3. Plant analysis: boron

Method:

The azomethine-H method (Gaines and Mitchell, 1979) was used, after the plant matter had been ashed and dissolved.

Apparatus/reagents:

Porcelain crucibles; muffle furnace; Whatman #1 filter paper; test tubes; test tube shaker; spectrophotometer;
CaO; Azomethine-H reagent; buffer masking solution (ammonium acetate + potassium acetate + tetrasodium salt of EDTA + notrilotriacetic acid); boron stock solution (0.5716 g boric acid diluted to 1L); 1 *N* sulphuric acid

Procedure:

Samples were washed to avoid contamination, then dried in an oven at 70°C to minimize chemical and biological changes. Particle size reduction of the dried material was carried out by milling through a 0.84 mm screen. Half a gram of the dried plant tissue was placed into a 15 ml porcelain crucible;

0.05 g CaO and 4 drops of water was added to this, and it was ashed overnight in a muffle furnace (450°C). After cooling, 10 ml 1*N* H_2SO_4 was added to the crucible, and it was allowed to stand for an hour. The resulting solution was then filtered through Whatman #1 filter paper.

4 ml of the filtrate was pipetted into a test tube, to which 4 ml buffer masking agent and 1 ml Azomethine-H reagent was added. This was mixed thoroughly by hand, and the colour was allowed to develop for 1 hour. Boron concentrations were determined photometrically in this solution.

Appendix C

Results of water analysis

Table C1: Electrical conductivities and pH values for the two water samples collected in the Mseleni area.

Sample name	pH	Electrical conductivity (μS/cm)
Sibaya water	8.10	846
Borehole water	6.70	385

Table C2: Major cations and anions in water sourced from Lake Sibaya and in water from a borehole in a rural area of Mseleni (as measured using the HPIC technique, see Appendix B).

	Sibaya water	Std. dev. (n=3)	Borehole water
Major cations		*(ppm)*	
Na^+	102	8	57
K^+	44	2	33
Ca^{2+}	32	4	10
Mg^{2+}	15	2	8
Major anions		*(ppm)*	
Cl^-	156	10	78
SO_4^{2-}	21	2	17
PO_4^{3-}	1	0	-
NO_3^{2-}	-	3	22

* bicarbonate could not be analyzed for using the HPIC technique; this affects charge balance considerations, as mentioned in Appendix A.

Appendix D

Foliar concentrations of selected nutrients in maize plants grown in Mseleni soil.

Foliar composition data is provided in Table D1 (on pages D2 and D3). Treatments are labelled as follows:

-Ca = Ca omitted
-P = P omitted

etc., as reported in Chapter 4 (Table 4.4).

Table D1: Foliar concentrations of selected elements corresponding to the various fertilization treatments used in the maize growth experiment (C.4).

Treatment	Ca	Mg	K	P	Zn	Mn	Cu	Fe	B
		(%)					(mg/kg)		
Full	0.52	0.57	1.89	0.54	78.3	659	11.6	74.5	28.7
	0.48	0.51	1.78	0.54	81.6	601	10.0	69.3	24.5
	0.43	0.55	1.69	0.51	62.8	531	9.1	63.3	24.6
Nil (c)	0.47	0.44	1.16	0.13	14.4	69	1.5	39.4	4.1
	0.52	0.44	1.24	0.12	13.5	80	2.1	65.3	4.1
	0.51	0.45	1.13	0.15	16.4	90	1.9	40.1	4.1
Nil (v)	0.44	0.43	1.76	0.14	14.1	78	1.6	47.1	7.7
	0.51	0.42	1.46	0.12	15.5	89	1.7	46.9	7.7
	0.43	0.34	1.43	0.13	13.2	67	1.5	38.7	7.7
-Ca	0.43	0.55	1.95	0.58	69.6	497	9.7	65.1	21.4
	0.44	0.54	1.92	0.57	67.2	550	10.1	70.4	26.4
	0.39	0.53	2.23	0.61	68.8	486	10.8	75.4	20.5
-P	0.64	0.46	4.60	0.15	114	1096	11.9	96.1	56.3
	0.73	0.49	4.63	0.16	156	1139	13.1	87.9	56.3
	0.73	0.57	3.60	0.14	137	957	16.1	92.2	86.1

Table D1 (contd.)

Treatment	Ca	Mg	K	P	Zn	Mn	Cu	Fe	B
		(%)					(mg/kg)		
-Zn	0.43	0.54	2.31	0.59	31.8	422	11.7	84.4	35.7
	0.46	0.58	2.28	0.64	30.7	410	9.20	88.6	35.5
	0.46	0.57	1.97	0.59	29.8	456	9.80	72.8	29.1
-Cu	0.47	0.58	2.01	0.64	70.6	478	2.90	88.3	32.0
	0.44	0.53	2.07	0.62	83.9	438	2.70	84.0	32.2
	0.46	0.58	1.76	0.65	69.3	504	2.10	88.7	33.1
-B	0.43	0.54	1.87	0.54	78.4	526	8.00	65.0	4.80
	0.42	0.56	2.01	0.54	70.8	490	9.20	67.6	4.60
	0.36	0.49	1.74	0.48	56.6	483	8.30	65.5	5.10
-Fe	0.44	0.53	1.97	0.57	68.1	545	6.60	61.9	26.8
	0.50	0.59	2.21	0.57	71.8	631	4.10	55.1	30.2
	0.50	0.62	2.64	0.66	79.1	628	3.40	76.8	27.7
-Mn	0.33	0.50	1.72	0.53	60.4	261	11.0	73.7	36.9
	0.42	0.55	1.68	0.59	72.2	280	11.2	74.2	36.9
	0.37	0.55	1.94	0.56	66.4	267	11.3	69.0	37.3