BIOMONITORING AS A MEANS TO DETERMINE THE POLLUTION LEVEL IN STELLENBOSCH

BY

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DATE SUBMITTED: January 1991

"I, Shaun André Davis, declare that Biomonitoring as a means to determine the pollution level in Stellenbosch, is my own work and that all the sources I have used or quoted have been indicated and acknowledged by means of complete references."

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S.A. DAVIS

JANUARY 1991

Dedicated to

My Mother and Father

CONTENTS

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ABSTRACT

A pollution survey was done in Stellenbosch, along two transects. One was south-east and the other north-east of the Corrobrick brickfield - the focal point of this study. Pine and Oak trees, Chasmanthe leaves and lichens were used as bio-indicators for this survey.

The levels of fluoride and sulphur decreased with distance from the main pollution source. Exceptionally high levels were recorded in the Pine and Oak trees within 1.5 km from the brickworks.

The availability of calcium and magnesium in the leaves of Pine and Oak trees appeared to be negatively affected due to their binding with F^- to form insoluble compounds. The brickfield proved to be a major source of especially copper and iron, as their levels in the bark material decreased with increasing distance. Lead levels were found to be correlated with vehicular traffic in the area. Their was some evidence that the iron levels were also linked to the lead concentrations in the bark.

Tip and marginal die-back of Chasmanthe leaves were directly linked to the fluoride concentrations of the leaves.

The chlorophyll degradation ratios of the lichens confirmed that the pollution effect was greatest near the brickfield. The distribution of lichens was closely linked with fluoride and

sulphur dioxide pollution.

OPSOMMING

'n Besoedelingsopname is in Stellenbosch langs twee transekte gedoen, met Corrobrick-baksteenaanleg as die fokuspunt. Die een transek was suidoos en die ander een noordoos vanaf die punt. Denne- en Eikebome, Chasmanthe blare en ligene is as monitor-plante in die opname gebruik.

Besondere hoë waardes van fluoried en sulfaat is in beide die denne en eikebome, binne 1.5 km vanaf die baksteen-aanleg, aangeteken. Dit het hierna afgeneem met toenemende afstand vanaf Corrobrick.

Die beskikbaarheid van kalsium en magnesium blyk negatief beïnvloed te word deur hul moontlike verbinding met fluoried, om onoplosbare komplekse te vorm. Die konsentrasie van koper en yster in die materiaal het afgeneem met toenemende afstand vanaf die aanleg. Die lood konsentrasie in die plantmateriaal blyk gekoppel te wees aan motor vervoer in die gebied. Die resultate toon aan dat daar 'n verband is tussen die yster en lood konsentrasies van die bas van die bome. Die punt- en blaarrandbeskadiging van Chasmanthe blare was direk gekoppel aan die fluoried konsentrasies van die blare.

Die chlorofil-afbrekingsverhouding in die ligene het bevestig dat die besoedeling die ergste was in die onmiddelike omgewing van die baksteen-aanleg. Daar was 'n goeie verband tussen die ligeen-verspreiding en die sulfaat- en fluoriedbesoedeling.

Keywords: Pollution, Oak and Pine trees, Fluoride, Sulphur dioxide

CHAPTER 1

A GENERAL OVERVIEW OF THE RESEARCH DONE ON A NUMBER OF HARMFUL POLLUTANTS, PERTAINING TO ITS EFFECTS ON PLANTS AND HUMANS

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1.1 INTRODUCTION

Air pollution is probably more frightening than water pollution as far as its direct effects on the human body are concerned because we are forced to breathe the polluted air of our cities, while we can usually avoid contact with polluted rivers or streams (Meiring-Naude, 1972).

In a Government report, Air Pollution was defined as follows: Air pollution by the community is the presence in the open air of substances placed there through human agency in concentrations sufficient to interfere directly or indirectly with the convenience, safety or health of members of the community or the full use or enjoyment of their property (Anon, 1971). It is especially during the past few decades that people have become more and more aware of the effect that industrialization has had, not only on their health, but also on their surroundings.

Environmental pollution is usually determined by means of physio-chemical methods, recording concentrations of toxic elements and their compounds in the air. The alternative, is to make use of biological methods, with the help of bio-indicators (Grodzinska, 1982).

A number of European Countries have gone so far as to establish an extensive biological monitoring system to monitor or detect industrial pollutants, for example, metals, fluoride, sulphur dioxide and nitrous oxides (Saunders, 1985).

Using plants has the advantage that they record the cumulative toxic effect of pollution (Reusmann, 1982; Grodzinska, 1982), by characteristic response symptoms. The effects of SO_2 on plant productivity, and also possibly on humans, may occur on time-scales of months or years and the resultant damage is thus due to the accumulation of SO_2 or its derivates during some period of time (Garsed, 1985). This is not reflected when using equipment to take air samples. As opposed to using physio-chemical methods to determine toxic compounds in the air, which are relatively expensive because expensive instrumentation is required, biomonitoring is relatively simple, quick and inexpensive (Grodzinska, 1982).

Of all the pollutants, the effect of O_3, SO_2 and fluoride on vegetation, alone or in combination, is probably attracting the most attention (McLaughlin and Barnes, 1975; MacLean et al., 1982; Mandl et al., 1975; Katainen et al., 1987; Reich et al., 1986). The cuticle is believed to be an almost impermeable barrier to water vapour, carbon dioxide and gaseous air pollutants, except hydrogen fluoride (Carlson, 1985).

1.2 Sulphur Dioxide

Treshow (1970) states that historically, smelters have contributed the greatest amounts of sulphur oxides to the atmosphere. He also refers to work done in 1960, where it was stated that coal burning was recognized as a major contributor to air pollution, hundreds of years before the Industrial Revolution. It presently (1960) accounted for some 60 % of the sulphur dioxide pollution.

Biscoe et al. (1973) found that at low SO_2 concentrations, the stomata tend to stay open. Under the influence of relatively high SO_2 concentrations, the photosynthetic rate was found to have decreased dramatically (Katainen et al., 1987; Soikkeli and Karenlampi, 1984). This would thus influence the growth of the plant negatively.

Numerous authors (Grodzinska, 1982; Johnsen and Sochting, 1973; Staxäng, 1969) reported that a significant correlation between the level of SO_2 in the air and the pH of tree bark existed. An increase in the SO_2 concentration of the atmosphere led to a decrease in pH. Because the tree bark remains in an environment for many years, it records fairly precisely atmospheric changes occuring in this environment and it thus can be an exceptionally sensitive indicator of the environmental acidity. The scarcity of lichens in polluted areas is a well documented phenomenon. Many investigations have shown that

SO_2 is responsible for the disappearance of lichens in and around urban and industrial complexes (Türk, 1982). A wide range of chlorotic and necrotic markings are formed by dicotyledonous plants due to sulphur dioxide accumulation. Acute injury leads to the death of cells, and the area then appears to be water-soaked, dull and grey-green (Halbwachs, 1984). The most pronounced symptoms of sulphur dioxide injury on needle-leaved evergreens, are the reddish-brown discoloration of leaves, shrinkage of tissues, and early defoliation. Toxic concentrations of sulphur dioxide during summer, can lead to necrosis which may appear as tip burn, banding, or basal burn. Injury responses of monocotyledonous plants to sulphur dioxide first appears as a diffuse grey-green discoloration of the leaf-tip. Tip and marginal necrosis is often accompanied by spotted, interveinal flecks or lesions between the midrib and the margins (Treshow, 1970).

As far as the effect of the pollutant on human beings is concerned, Ottaway (1980) reported that SO_2 and its derivates attack sensitive cell layers exposed to air, for example, the conjunctiva of the eye, and more especially the epithelium lining the alveoli of the lungs.

1.3 Fluoride

Heavy contamination with fluorine can occur in the area immediately surrounding an industrial plant, and that is especially true close to brickfield, aluminium smelters, some pottery factories, phosphate factories and glass and steel industries (Allen, 1974; Gilbert, 1973).

Zimmerman and Hitchcock (1956) showed that the tips of Gladiolus leaves may contain 25 to 100 times as much fluoride as the basal section. Oats, onions, pine needles and rushes show the same tendency; margins of fruit tree leaves may contain 2 to 3 times as much fluoride as the basal half, and the margins 2 to 3 times more than the center. It was shown that there was a correlation between the amount of tip and marginal die-back and the concentration of especially fluoride present in the tissue (Compton and Remmert, 1960; Brewer et al., 1957; Coulter et al., 1985). Chang and Thompson (1966) reported that up to 60 % of the fluoride accumulated by naval orange leaves was concentrated in the chloroplasts.

Toxic concentrations of fluorides in the leaves of broad-leaved species can cause characteristic injury symptoms consisting of necrosis, chlorosis, or both. The symptoms are very prominent at the leaf tip and margin. A water-soaked, dull, gray-green discoloration of the tissue is the earliest sign of injury in some sensitive species. A sharply defined narrow, often

reddish-brown band seperated the necrotic tissue from the adjacent healthy tissue. Symptoms of fluoride toxicity on monocotyledonous plants are essentially similar to those on broad-leaved species (Soikkeli and Karenlampi, 1984). Chlorotic spots also develop predominantly along the margins of certain plants, eg. corn and sorghum. Fluoride toxicity symptoms on needles of conifers consist of necrosis beginning at the tip of the current year's needles, and progressing toward the base. The needles are most sensitive when they are elongating and emerging in spring (Treshow, 1970). Taylor and Basabe (1984) reported a reduction in growth in Douglas Fir, due to the influence of relatively high fluoride levels. Although not as common as SO_2, F^- was also shown to be detrimental to the continued existance of lichens in a particular area (Anderson and Treshow, 1984).

Mejstrik (1985) states that it is well-known that fluor (fluoride compounds) affects the activity of enzymes that are of utmost importance in the process of assimilation and respiration, and in the synthesis of carbohydrates, proteins, nucleotides and nucleic acids.

1.4 Sulphur Dioxide and Fluoride

Biscoe et al.(1973) found that at low SO_2 concentrations, the stomata tend to stay open. Mejstrik (1985) and others reported that F^- enters the leaves mainly through the stomata.

It is then logical why the combination of these two pollutants results in more lesions than when they are applied seperately (Mandl et al., 1975).

1.5 Ozone

Significant quantities of ozone present in our immediate environment are formed chemically by the action of ultraviolet light on nitrogen dioxide. Ozone formation is greatest in urban environments, where the necessary chemicals for the reactions are found. A multitude of combustion processes and sources, especially the inefficient internal combustion engines of automobiles, daily emit tons of waste hydrocarbons as well as nitrogen oxides into the atmosphere which can be chemically converted to ozone (Treshow, 1970).

Ozone enters the plant through the open stomata of the leaves (Treshow, 1970; Taylor, 1984). Moist surfaces in the mesophyll tissue provide the medium in which gaseous ozone can dissolve (Malhorta and Khan, 1984). The effects ozone may have on photosynthesis, respiration, and other metabolic processes, are intimately entwined with the initial effects of ozone on the stomatal mechanism and the penetration of ozone into the plant. Any impairment of stomatal opening limits gas exchange and directly affects any subsequent metabolic processes which depends on the availability of carbon dioxide or oxygen (Treshow, 1970). Work surveyed by Malhorta and Khan (1984),

showed that: ozone can cause either an increase or a decrease in amino acid content of plants; an increase or decrease in the level of soluble sugars and carbohydrates in the leaves of exposed plants, and activation of the enzymes involved in phenol metabolism, eg. polyphenol oxidase and peroxidase, which would stimulate oxidation of phenols to quinones and cause accumulation of their polymerization products. These products could be responsible for the necrotic appearance of injured leaves.

1.6 Lead

It was found that exhaust fumes, from automobiles, is the major source of atmospheric lead pollution (Harrison and Laxen, 1981; Grobler et al., 1986; Ormrod, 1984).

The accumulation of lead and its effect on humans has been studied quite extensively. According to Grobler et al. (1986), the storage of lead is cumulative. They also found that blood lead levels were related to vehicular traffic. The best documented effect of lead on blood is its interference with the biosynthesis of haem, which is essential for the production of haemoglobin (the red oxygen-containing pigment in red blood cells)(Harrison and Laxen, 1981). Grobler et al. (1985) found that circumpulpal dentine can concentrate up to 5 times more lead than the total dentine. The most profound effects of lead poisoning are undoubtedly those associated with severe damage to

the central nervous system. At high levels of exposure to lead, neural (brain) damage may result in stupor, convulsions and / or coma and may progress to death (Harrison and Laxen, 1981).

1.7 Work done and Location of study area

A biological pollution survey was done along a NE and SE transect (Fig. 1.1) from a brickfield in Stellenbosch near Cape Town, South Africa. The brickfield was considered to be the focal point in this study.

Bark and leaves of Quercus and Pinus species, as well as Chasmanthe leaves and lichens were used to determine the level of pollution in the area. The decrease or increase of F^- and sulphate and / or sulphur in the material with increasing distance from the pollution source, where applicable, were also studied. The possible effect of the pollutants on calcium, magnesium, sodium, potassium, iron, copper and zinc concentrations was examined. The effect of motor-vehicle exhaust fumes on the lead concentration in the plant samples was investigated. Pollutant effects on the chloroplast pigments were studied. The presence and abundance of lichens along the south east transect was investigated to see if there was any correlation between lichen abundance and pollution. Chlorophyll degradation , as related to pollution will be discussed.

The topography of the area aggravates the effect of the pollutants, because Stellenbosch is in a valley surrounded by three mountainous regions, which cause the emissions to remain in the basin in the absence of air movement.

One of the main reasons for doing this study, was to see if the situation in Stellenbosch had worsened since a previous study (1978 -Raitt, pers.com) was done. If it has, then the pollutants being set free into the atmosphere, could pose a health hazard in the surrounding populated area.

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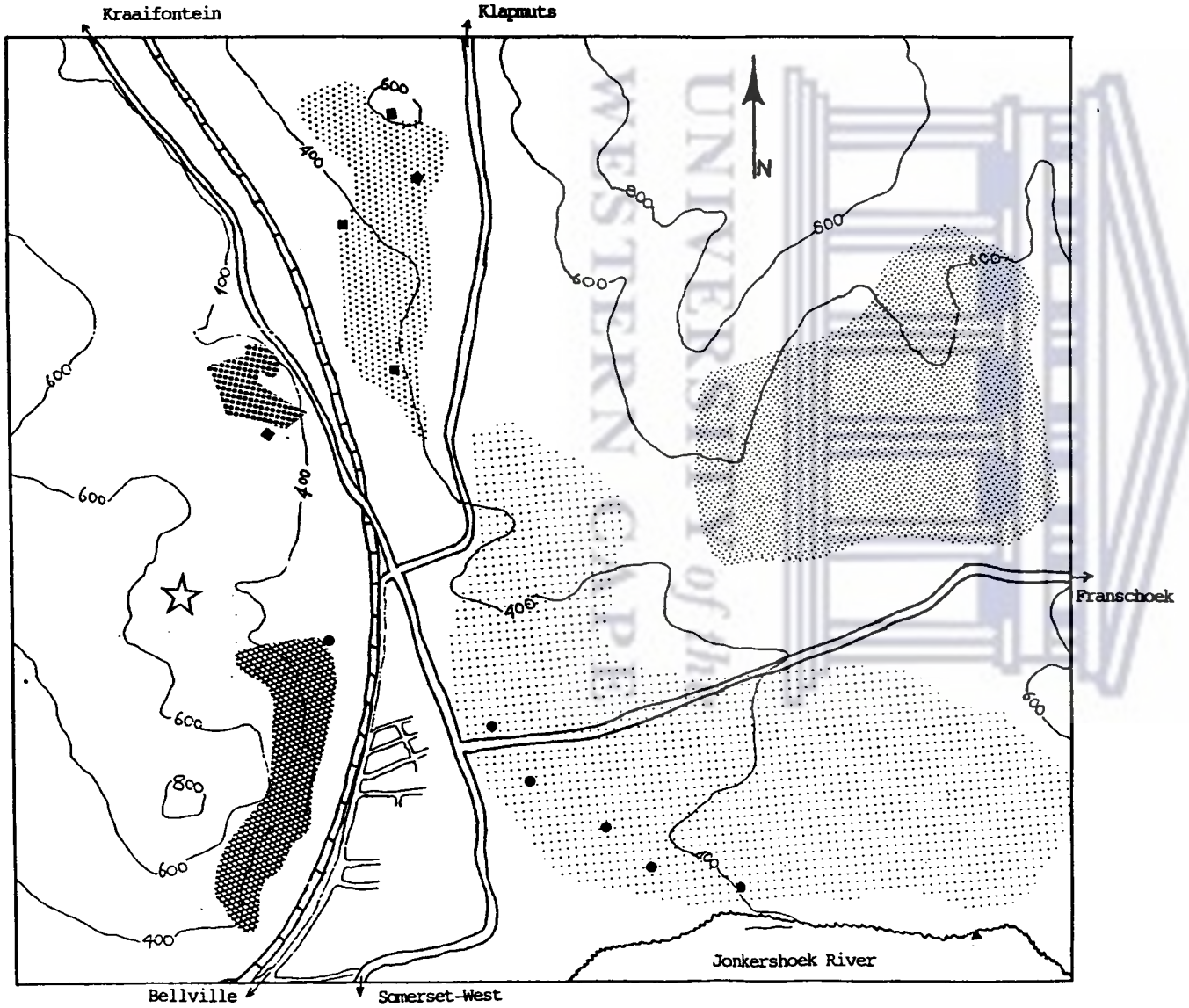
FIG.1.1 THE LOCATION OF THE SAMPLING SITES IN STELLENBOSCH

(0.9X Enlargement of the 1:50000 Topographical map

3318DD Stellenbosch, 1979)



0 1 2 km

KEY

Brickworks

Plankenbrug Industrial Area

RESIDENTIAL AREAS

Cloetesville

Idasvalley

Kayamandi

Central Business District and Residential area

SAMPLING SITES

- SE 1 - SE 6
- NE 1 - NE 5
- CONTROL

Railway Line

Main Access Roads

CHAPTER 2

FLUORIDE AND SULPHATE ACCUMULATION BY OAK TREES ALONG A TRANSECT, AND ITS EFFECT ON THE MINERAL COMPOSITION AND CHLOROPLAST PIGMENTS

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2.1 INTRODUCTION

Characterization of environmental pollution with the aid of bio-indicators can be simple, quick and cheap. A big advantage is that the organisms themselves record the cumulative toxic effect of pollution (Grodzinska, 1982), instead of performing point measurements using machines. A number of European Countries use quite extensive biological monitoring systems to detect industrial pollution, eg. metals, fluoride, sulphur dioxide and nitrous oxides. Germany for instance uses a wide variety of indicator plants (Saunders, 1985). Various authors have found that trees can be used as biological indicators in pollution surveys (Kovacs, et al., 1982).

It is usually reported that pollutants decrease with distance from the source of pollution. This study was done to determine if this is also the case in Stellenbosch.

Sources of pollution in this area include a number of wineries, the Planckenbrug Industrial area and the brickfield. The brickfield was considered to be the focal point of air pollutant emission in the area. Compared to the other industries in the area, the brickfield released large quantities

of visual smoke.

2.2 MATERIALS AND METHODS

The six sampling sites were located at approximately 500 m intervals, on a South-Easterly (SE) transect from the brickfield (Fig.1.1). Each sampling site was a mature oak tree. Samples were collected at monthly intervals, at the same time of the day ensuring that the leaves internal metabolism would be at the same phase (Mansfield and Snaith, 1984).

Bark and leaf material of Quercus species , was collected, put into brown paper bags, and dried at 65°C for elemental analysis. Fresh leaves were also stored in plastic bags, at -12°C, for chlorophyll extraction.

The dried bark was ground with the aid of a Retsch Ball Mill, while the dried leaf material was ground with a Wiley Intermediate Mill to pass through a No.40 mesh sieve, and then stored in screw-top containers.

Student t-tests were used to check for significant differences, in the data obtained.

2.2.1 Determination of bark pH

To determine the pH of the bark, 5 g of ground bark was added to 25 cm^3 of distilled deionised water, and continuously stirred for approximately 2 hours. The pH values were determined by means of a Combination pH Electrode and a Radiometer Ion 85 Analyzer.

2.2.2 Elemental Analysis

2.2.2.1 Fluoride

After reviewing various methods for the analysis of fluoride in plant material (Anon, 1980, Cooke et al., 1976), it was decided to use the selective ion electrode method (B) as described by Anon (1980). For the extraction of F^-, 0.25 g of the material was weighed into 100 cm^3 wide-mouthed plastic containers. To this 20 cm^3 0.05 N HNO_3 was added and the container placed on a magnetic stirrer and stirred for 20 minutes using a Teflon-coated stirring bar. After this period, 20 cm^3 0.1 N KOH was added and the solution further agitated for an additional 20 minutes. To this, 5.0 cm^3 sodium citrate solution, containing 1 $mg.dm^{-3}$ F^-(adjusted pH to 5.5) and 5.0 cm^3 0.2 N HNO_3, was added. A Radiometer Ion 85 Ion Analyzer with a Combination Fluoride Electrode was used for the determination of the fluoride concentration.

2.2.2.2 Sulphate and Sulphur

The ground plant samples were dried at 70°C for 2 hours for the determination of sulphate by means of a Dionex 4000i HPIC. The elution buffer was a mixture of 0.0018 M Na_2CO_3 and 0.0017 M $NaHCO_3$. The 0.1000 g of sample was boiled for 5 minutes, filtered through Whatman No.52 filter paper and made up to 100 cm^3 with deionised distiiled water. The HPIC was calibrated using a sulphate anion standard of 1.50 ppm and the calculation

$$\% \text{ Anion} = \frac{mg.dm^{-3} \times 100}{10 \times 1000 \times \text{mass}}$$

was used to determine the percentage of water soluble anions present in the plant samples. For the sulphur analysis, 1.4 to 4.7 mg of the plant samples from the screw top bottles, was dried prior to weighing. A Carlo Erba Elemental Analyzer, Model 1106, was used for the analysis. The calibration was performed with a certified Phenanthrene standard with 0.848 % Sulphur and was checked after every 10 determinations.

2.2.2.3 Calcium, Magnesium, Potassium, Sodium, Iron, Copper, Zinc and Lead

Of each sample, 0.5 g was folded in cigarette paper, put into a digestion tube and 6 cm^3 of a HNO_3:$HClO_4$ (2:1) mixture was added. A few glass beads were also added to the tubes to curb excessive bumping. The digestion process was considered complete when a colourless solution was obtained. Distilled deionised water was added to the tubes and the solution allowed to cool down. It was then filtered through Schleicher and Schull #595 filter paper, and made up to volume in 100 cm^3 volumetric flasks with deionised distilled water.

A Pye Unicam SP9 Atomic Absorption Spectrophotometer was used to determine the concentrations of the various major cations and trace elements present in the plant material, as described by Allen et al.(1986).

2.2.3 Chlorophyll extraction

The chlorophyll extraction method used in this study is a modification of the one used by Ronen and Galun (1984) for the lichen Ramalina duriaei (De Not.) Jatta. Whole oak leaves, to minimize damage of the chloroplasts, were inserted into 40 cm^3 vials, and submerged in 30 cm^3 of Dimethyl Sulfoxide(DMSO). The vials were then put into a water bath at, 65°C, in the dark, for 45 minutes. The extract was transfered to 100 cm^3 volumetric flasks and

the process repeated twice more and each extract added to the first. The solution was then made up to 100 cm^3 with DMSO. All extractions were done in triplicate. Five cm^3 of the extract was diluted with 5 cm^3 DMSO and this solution was used in the determination of the absorption of the chlorophyll pigments at 435 and 665 nm. All readings were done against a blank of DMSO, using a Varian Techtron Model 635 Double Beam Spectrophotometer. DMSO was used to achieve a uniform method for all chlorophyll extractions, including those of lichens.

2.3 RESULTS AND DISCUSSION

2.3.1 pH of Oak Bark

The pH of the oak bark (Fig.2.1; Table 2.1) was found to occur in a narrow band from pH 3.80 to pH 4.90. The pH at SE 1 was highly significantly lower than that at SE 6 ($t(24)= 7.52$; $p< 0.00005$). The results obtained ten years earlier, by Raitt (personal communication, Appendix 1), show pH of the bark to be similar, but slightly higher. The pH was also in a narrow band and increased with distance from the brickfield. The distribution of lichens correlated highly significantly with bark pH, although the range was so narrow (Fig.2.1; Fig.5.6). Puckett et al.(1973) stated that an indirect effect of SO_2 in fumigated areas, is the

gradual reduction in pH of the surface soils and other substrata on which lichens grow. At the lower pH, dissolved SO_2 is very toxic, so that lichen propagules would stand little chance of surviving in the presence of the pollutant. This could account for the decrease in lichens around the brickfield (see Chapter 5). There was a highly significant positive correlation between bark pH and distance from the brickfield ($r(76)= 0.575$; $p<0.00005$; $y= 3.99504 + (0.138500)x$). Johnsen and Sochting (1973) found in their study, that as they moved closer to the city the bark pH decreased from pH 5 to pH 3 with an increase in ambient sulphur dioxide levels.

2.3.2 Elemental Analysis

2.3.2.1 Fluoride

Mejstrik (1985) states that it is well-known that fluor (fluoride compounds) affects the activity of enzymes which are of utmost importance in processes of assimilation and respiration, in the synthesis of carbohydrates, proteins, nucleotides and nucleic acids.

A highly significant lower F^- concentration was present in the bark at site SE 5 than at site SE 1 (Fig.2.2); $t(24)= 8.857$ and $p< 0.00005$. The samples from SE 3, showed an intermediate F^- concentration as would be expected.

The F^- probably originated from the Brickfield - there being no other factories around that use raw materials which contain F^-. Figure 2.2 indicates that the F^- concentration recorded in the bark at SE 1 was the highest during the summer months, September 1988 to about March 1989. The drop in F^- level for October and November 1988 could probably be linked to a major drop in the production activity, and hence air pollution (Davies, 1986).

A similar pattern of F^- distribution could be expected for the leaves, but probably at a much lower concentration, because the leaves are on the trees for a shorter period of time than the bark. Appendix 2 indicates that the highest F^- concentration was found within the first kilometer from the brickfield in 1978 (Raitt, pers. comm.). This indicated that the brickfield was certainly the main source of F^- pollution. Numerous authors, including Maclean et al.(1982) and McLaughlin et al.(1975), performed HF fumigation experiments on the leaves of various species. Their experiments were performed over relative short periods of time to see the effect of F^- on the photosynthetic apparatus of the plant. This project however was aimed at the cumulative effect of HF on the vegetation. Because the leaves are only on the trees for a limited time, they could not give the cumulative results over a year, as bark may.

2.3.2.2 Sulphate and Sulphur

According to Marschner (1986), the sulphur requirement for optimal growth, is between 0.2 and 0.5 % of the dry weight of plants, but according to Larcher (1980) the requirement is 0.05 to 0.8%. He also states that the sulphate content of plants is a more sensitive indicator of the S-nutritional status than the total S-content. The best indicator being the proportion of sulphate sulphur to total S-content. Figure 2.3(a) indicates a difference between the sulphate concentration obtained at the various sites. Suprisingly the samples collected at SE 3, had the highest sulphate concentration, followed by those of SE 1 which was still significantly higher than SE 5 ($t(24)= 2,558$; $p= 0.0242$). The results of Fig.2.3(b) reflect what was expected in Fig.2.3(a) as the bark at SE 1 had the highest percentage of sulphur present. The difference between bark S contents at SE 1 and SE 5 was highly significant ($t(23)= 3.976$; $p= 0,0006$). In 1978, Raitt (pers. comm.) found that the sulphate concentration in the bark (Appendix 3) and the leaves (Appendix 4) were higher within the first kilometer from the brickfield. This once again indicated that the brickworks is the main source of S in the area, but the current recorded values are much lower than in 1978, possibly indicating that a higher grade of coal could have been used in the drying process.

2.3.2.3 Nutrient status of the trees

2.3.2.3.1 Calcium

Figure 2.4 clearly indicates that the calcium concentration of the bark at SE 1 is the lowest for the duration of the project (also see Table 2.2). Linear regression showed that there was a highly significant positive correlation between the Ca^{2+} concentration of the bark and distance from the pollution source ($r(76)= 0.6856$, $y= 14.2257 + (5.69617)x$; $p<0.00005$). This tendency correlates very well with the increase of pH found in the samples collected (section 2.3.1) at various distances from the brickfield ($t(5)= 8.22$; $p= 0.0004$). This suggests possible leaching of Ca^{2+} at a low pH. A significant positive correlation between the Ca^{2+} leaf concentration (Fig.2.5), and distance from the focal point of this study ($r(51)= 0.3225$, $y= 10.7880 + (1.62259)x$ and $p= 0.0185$), was observed. The Ca^{2+} concentration in the bark is nearly double that in the leaves for SE 1. Since Ca is relatively immobile in the phloem (Raven, 1977 and Clarkson and Hanson, 1980), it can thus continue to accumulate in the leaves in the form of precipitates and crystals. The crystals increase in size and number as the leaves mature and are prominent in many deciduous plants just prior to leaf fall (Baker, 1983). This could explain the marked increase in the Ca^{2+} concentration of the leaves towards the end of the growing

season (May). Allen (1974) states that Ca^{2+} in plants is essential for apical and root development and accumulates in cell walls as calcium pectate. Ca^{2+} reaches the leaves via the transpiration stream and is not relocated as it is a relatively immobile element. Kubowicz et al. (1982) found that Ca^{2+} transport activity rises in the region of cell expansion and, subsequently, declines as the tissue matures. Calcium has a limited role as an enzymatic co-factor (Clarkson and Hanson, 1980), but according to Hanson (1983), there is suggestive evidence that Ca^{2+} has regulatory roles in growth, development and adaptation to environmetal perturbations. The immediate effect of fluorine on the metabolism of the plant is caused by the precipitation of Ca^{2+} in the form of CaF_2, which is insoluble and can result in a Ca^{2+} deficiency (Mejstrik, 1985). He also states that if the Ca^{2+} precipitates, the magnesium present in the chlorophyll molecule might be similarly affected.

2.3.2.3.2 Magnesium

No pattern was found to exist in magnesium concentrations of the bark (Fig.2.6; Table 2.2) over the period of sampling, or with pollution. Marschner (1986) reported that when the level of Mg^{2+} was deficient, or there were excessive levels of K^{+}, the ribosome subunits dissociated and protein synthesis ceased. Figure 2.7 however, indicates a

steady increase in the Mg^{2+} concentration of leaves from when they are formed. Oak trees, being deciduous, loose their leaves during Autumn/Winter and start forming new ones with the onset of Spring. The gradual increase could probably be ascribed to loading via the transpiration stream. Mg^{2+} is an integral part of the chlorophyll molecule (Bidwell, 1979). The importance of Mg^{2+} in the leaves is also reflected by the higher levels in the leaves than in the bark (Fig.2.6; 2.7). Marschner (1986) confirmed the Clarkson and Hanson (1980) statement, that the functions of Mg^{2+} in plants are related to its mobility. Epstein (1972) states that Mg^{2+} is an activator of more enzymes than any other element. Clarkson and Hanson (1980) reported that depending on the relative abundance of K^{+}, Mg^{2+} will also contribute to the neutralization of sugar phosphate, sugar nucleotides, and organic and amino acids. They also state that Mg^{2+} fills the need for a small, strongly electropositve divalent cation which is readily mobile with limited geometric distortion when involved in ionic bonding.

2.3.2.3.3 Potassium

There is no pattern in the distribution of the concentration of K^{+} in the bark amongst the sites, with time or distance (Fig.2.8; Table 2.2). The K^{+} concentration of the leaves (Fig.2.9) is almost three times higher than that of the

bark. According to Salisbury and Ross (1985) potassium is an activator of many enzymes that are essential for photosynthesis and respiration, and that it also activates enzymes needed to form starch and proteins (Evans and Sorger, 1966). Epstein (1972) states that K^+ is the only monovalent cation essential for all higher plants. The element is a major contributor to the osmotic potential of cells and therefore to their turgor pressure, especially in regulating stomatal movement. In addition K^+ is also used in pH stabilization (Marschner, 1986). He also reports on work done in 1968, where it was found that the role of K^+ in protein synthesis is reflected in the accumulation of soluble nitrogen compounds (eg. amino acids, amides and nitrate) in potassium-deficient plants.

2.3.2.3.4 Sodium

The graphs of Figure 2.10 appear to be divided into two sections, the latter half of 1988 showing higher Na^+ concentrations than the early half of 1989. This difference was found to be highly significant ($t(5)= 7.13$; $p= 0.0008$). The readings are relatively constant with no major changes at any of the sites. Figure 2.11 (Oak leaves) however shows no similar pattern. Sodium could assist (together with K^+) in maintaining the solute potential, of the more stressed leaves, at a certain level so as to ensure maximum uptake of CO_2 for photosynthesis. Comparing the values of

SE 1 and SE 6 (leaf part of Table 2.2), it was found that SE 1 had a significantly higher Na^+ concentration than SE 6 ($t(8)= 4.027$; $p= 0.0038$). This is however not the case with the bark. Epstein (1972) concluded that Na^+ is not generally required by green plants, except halophytes and physiologically similar plants. It was found that Na^+ can cause growth stimulation, mainly by its effect on the water balance of plants and therefore, cell expansion. Na^+ can replace K^+ in its contribution to the solute potential in the vacuoles (Marschner, 1986).

Similarities amongst the metal ions

The levels of copper and zinc, are slightly higher in bark than in leaves (Table 2.2) and in the case of iron, the values recorded by the bark, exceeded those of the leaves by far. The iron and copper, but not the Zn concentrations in the bark, increased over the last three months of sampling (Fig.2.12 and 2.14), while the zinc concentration remained the same (Fig.2.16).

2.3.2.3.5 Iron

A lower iron concentration in the bark and leaves was observed in the less polluted areas (Table 2.2). Figure 2.13 indicates a marked increase in the iron concentration from October 1988 onwards. According to Salisbury and Ross (1985) iron plays an important role in certain enzymes and

numerous proteins that carry electrons during photosynthesis and respiration. Jacobson and Oertli (1956) found that where the supply of iron is varied, there is often a good correlation between iron concentration and chlorophyll content. A higher iron concentration in the living leaf material is thus expected, where active photosynthesis and respiration occurs. This is however not the case (Fig.2.12) and alternatively it can be speculated that an external source is responsible for the higher iron concentration in or on the bark. Ho and Tai (1988), found elevated levels of lead, copper, iron, zinc, manganese and cadmium in roadside soil and grass, and this correlated well with the traffic volume. The leaves (Table 2.2) at SE 2 clearly recorded the highest level of iron; this correlates well with the lead values (Table 2.2). The iron value for the bark collected at SE 2 is also high, but still lower than that of SE 1. The bark of SE 1 had a highly significantly higher iron concentration than the bark at SE 6 ($t(24)= 3.499$; $p= 0.0018$) (Table 2.2; Fig.2.12). A highly significant negative correlation, existed between the iron concentration of the bark samples and distance from the pollution source ($r(76)= 0.4860$; $y= 3.27421 - 0.520593)x$; $p<0.00005$).

2.3.2.3.6 Copper

The variation in the copper concentration of the leaves (Fig.2.15) is less erratic than that of the bark (Fig.2.14), which could be an indication that copper does not play as important a role in the bark as it does in the leaves. The high values could merely be a reflection of surface adsorption of the copper being set free in the polluted area. The bark closest to the polluted zone (SE 1) recorded a highly significantly higher copper concentration than that at SE 6 ($t(12)= 3.115$; $p= 0.0089$).

2.3.2.3.7 Zinc

The zinc concentration for the period September to December 1988 appears to be slightly higher than that for January to June 1989. Zinc was present in a higher concentration in the bark (Fig.2.16) than in the leaves (Fig.2.17) (also see Table 2.2). The reverse was actually expected, seeing that in addition to zinc being either a constituent or an activator of several enzymes, it also plays an important role in regulating the level of auxin in the plant (Epstein, 1972). It could thus have an influence on the growth of the plant. This is reflected in Figure 2.17 (leaves). From October to December 1988 there is a markedly higher zinc level in the leaves compared to the period January to May 1989. This could be due to the active growth of the leaves to reach their full photosynthetic capability.

2.3.2.3.8 **Lead**

The concentration of lead in the bark (Fig.2.18) and the leaves (Fig.2.19), is relatively low. The lead concentration of the leaves and bark at SE 2 (Table 2.2) is highly significantly higher than that at the rest of the sites. This could be attributed to the fact that the tree is situated at a four-way-stop where a relatively high amount of exhaust fumes (containing lead) is released from the vehicles. Ormond (1984) refers to work that was done previously, where it was found that internal-combustion engines are one of the main sources of lead pollution. Ho and Tai (1988) also showed that this correlation existed. The bark showed a higher level of lead than the leaves, possibly because it is present on the tree for a longer period than the leaves. Also more lead will be trapped by the relative rough textured bark than by the relatively smooth leaf surfaces (Little and Wiffen, 1977).

2.3.3 Light Absorbance by the Chlorophyll Pigments

Absorbance at 435 nm and 665 nm

Figure 2.20 indicates that the amount of light absorbed by the pigments remained relatively constant for the period, September 1988 to December 1988, after which it decreased. This decrease was found to be significant ($t(3)= 2.499$; $p= 0.0878$). A possible explanation is that the fluoride did

not initially exert any marked effect on the Mg^{2+} ions at SE 1, but this effect was only expressed after December (Fig.2.20) . Because Mg^{2+} is an integral part of the chlorophyll pigment, less pigments will be formed, because less Mg^{2+} will be available for this purpose compared to the leaves at SE 6 growing in a relatively pollution free environment. This means that the chloroplasts remained active for a much longer time than at SE 1. SO_2 could also contribute to the decrease in the photosynthetic ability of the leaves (Black and Unsworth, 1979; Katainen et al., 1987).

What is very obvious when comparing Fig.2.20 with 2.21, is that more absorbance of light took place at 435nm (shorter wavelenght),than at 665nm. Chang and Thompson (1966) found that chloroplasts could contain up to 60 % of the fluoride in the leaves. The decline in the light absorbed, especially by the leaves at SE 1 and SE 2 (Fig.2.20 & 2.21 from Dec 1988 onward), could be a direct result of the F^- pollution.

2.4 CONCLUSION

The results obtained in this study clearly show that fluoride, sulphur, lead, iron, copper and possibly sodium pollution occurs within the area.

Both F^- and SO_2 pollution is very evident within the first 1.5 km from the brickfield, indicating that this is most probably the main source of these pollutants in the area. Raitt (personal comment) also found the distribution of F^- and S to be as reported in this document.

The pH values increased on moving away from the brickfield, and this corresponds well with the increase in Ca values obtained in this study. Previous authors (Staxäng, 1969; Hartel, 1982) have related an increase of SO_2 release, to a decrease in the pH of tree bark. Taking into consideration that coal is used in the baking process of bricks, this can be seen as one of the main sources of SO_2 (H_2SO_4), which in turn can lead to a decrease in the pH of the bark on getting nearer to the source, as was reported elsewhere in this document. It appears as if only Ca^{2+} and Mg^{2+} of the major elements, are adversely affected by especially F^-, as suggested by Mejstrik (1985).

Of the heavy metals, evidence was found for iron, copper and lead pollution within the area. The bark, in all cases, except for Pb, recorded decreasing concentrations of the elements with increasing distance from the pollution source. In the case of iron though, an elevated concentration was found in the bark collected at SE 2. This corresponds well with the elevated level of Pb that was found in the bark at this site. The evidence suggests that it was primarily the motor-vehicles that were the main source of Pb pollution in the area, as suggested by Ho and Tai (1988).

A more detailed leaf pigment extraction could shed more light on the degradation, or not, of the pigments. Subcellular fractionation of the leaves (Chang and Thompson, 1966) would also confirm, or reject, whether the chloroplasts are the main site of F^- accumulation in the Oak leaves.

Anatomical adaptations against pollutants would probably be the plant's first defense mechanism. An anatomical study would surely reveal if such changes had taken place, even if the exposure time is relatively short.

Table 2.1: pH of Oak bark at different sites.(n= 13)

SITE	pH (x±sd)
SE 1	4.15 ± .083
SE 2	4.16 ± .115
SE 3	4.20 ± .256
SE 4	4.40 ± .116
SE 5	4.49 ± .210
SE 6	4.43 ± .101
CONTROL	4.99 ± .124

Table 2.2: The mean concentration of the various elements in Quercus bark and leaves along a pollution gradient (x ± sd) (g/kg Dry Weight).

SITE	ORGAN	ELEMENT									
		Ca	Mg	K	Na	Fe	Cu	Zn	Pb	*F^-	SO_4
SE 1	BARK	16.21/2.12	1.04/0.15	2.30/0.72	0.53/0.72	2.62/0.80	0.006/0.002	0.16/0.14	0.11/0.02	173.9/55.9	0.04/0.0
	LEAVES	10.56/2.33	2.71/0.71	12.99/1.98	1.29/0.31	0.42/0.23	0.005/0.002	0.10/0.40	0.06/0.20		
SE 2	BARK	23.96/4.75	0.77/0.21	2.30/0.77	0.41/0.77	2.58/0.99	0.006/0.002	0.14/0.05	0.39/0.14		
	LEAVES	14.43/4.46	1.93/0.60	12.75/0.78	0.59/0.26	0.46/0.17	0.004/0.002	0.09/0.03	0.09/0.04		
SE 3	BARK	34.37/4.62	0.95/0.62	3.11/0.65	0.70/0.65	2.44/0.79	0.005/0.001	0.15/0.05	0.14/0.03	137.7/35.6	0.06/0.0
	LEAVES	17.03/2.79	2.02/0.34	12.80/0.89	0.81/0.16	0.37/0.13	0.003/0.001	0.10/0.03	0.05/0.02		
SE 4	BARK	24.17/3.34	0.92/0.24	3.37/0.66	0.48/0.66	2.21/0.68	0.004/0.002	0.17/0.17	0.10/0.05		
	LEAVES	13.75/3.60	1.84/0.51	13.54/1.74	0.98/0.30	0.32/0.11	0.003/0.001	0.08/0.02	0.06/0.04		
SE 5	BARK	30.54/4.29	0.95/0.43	2.58/0.73	0.47/0.73	1.25/0.33	0.004/0.001	0.15/0.17	0.08/0.02	31.5/14.0	0.02/0.0
	LEAVES	14.22/4.75	1.66/0.45	12.09/1.17	0.73/0.14	0.25/0.09	0.003/0.001	0.09/0.09	0.07/0.03		
SE 6	BARK	32.70/3.33	1.19/0.26	2.61/0.40	0.57/0.33	1.43/0.88	0.004/0.001	0.17/0.22	0.09/0.04		
	LEAVES	32.70/4.36	2.56/0.54	11.01/1.41	0.98/0.28	0.34/0.15	0.004/0.001	0.09/0.02	0.06/0.01		

* - mg/kg

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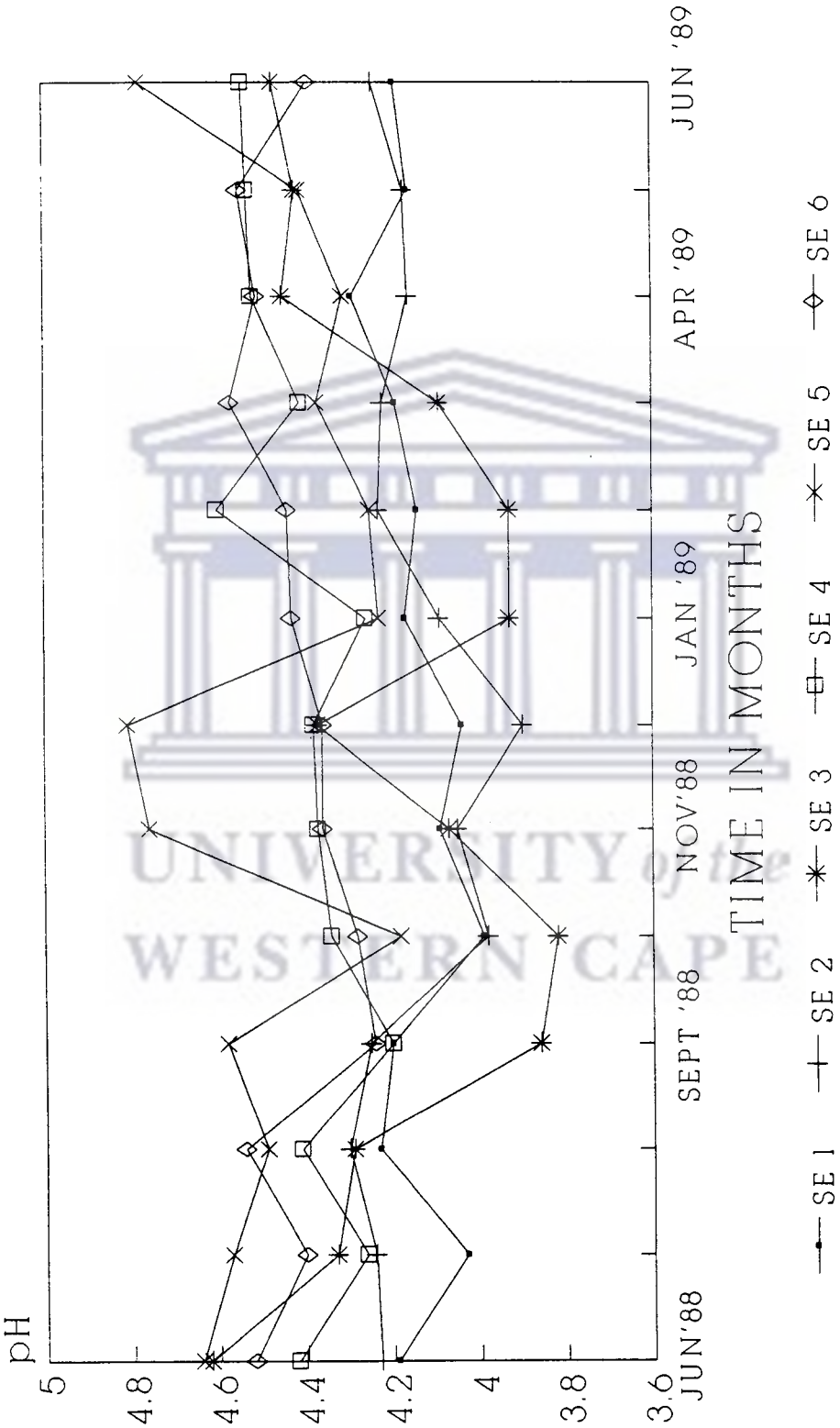


FIG.2.1 ANNUAL VARIATION IN Quercus BARK pH ALONG A POLLUTION GRADIENT

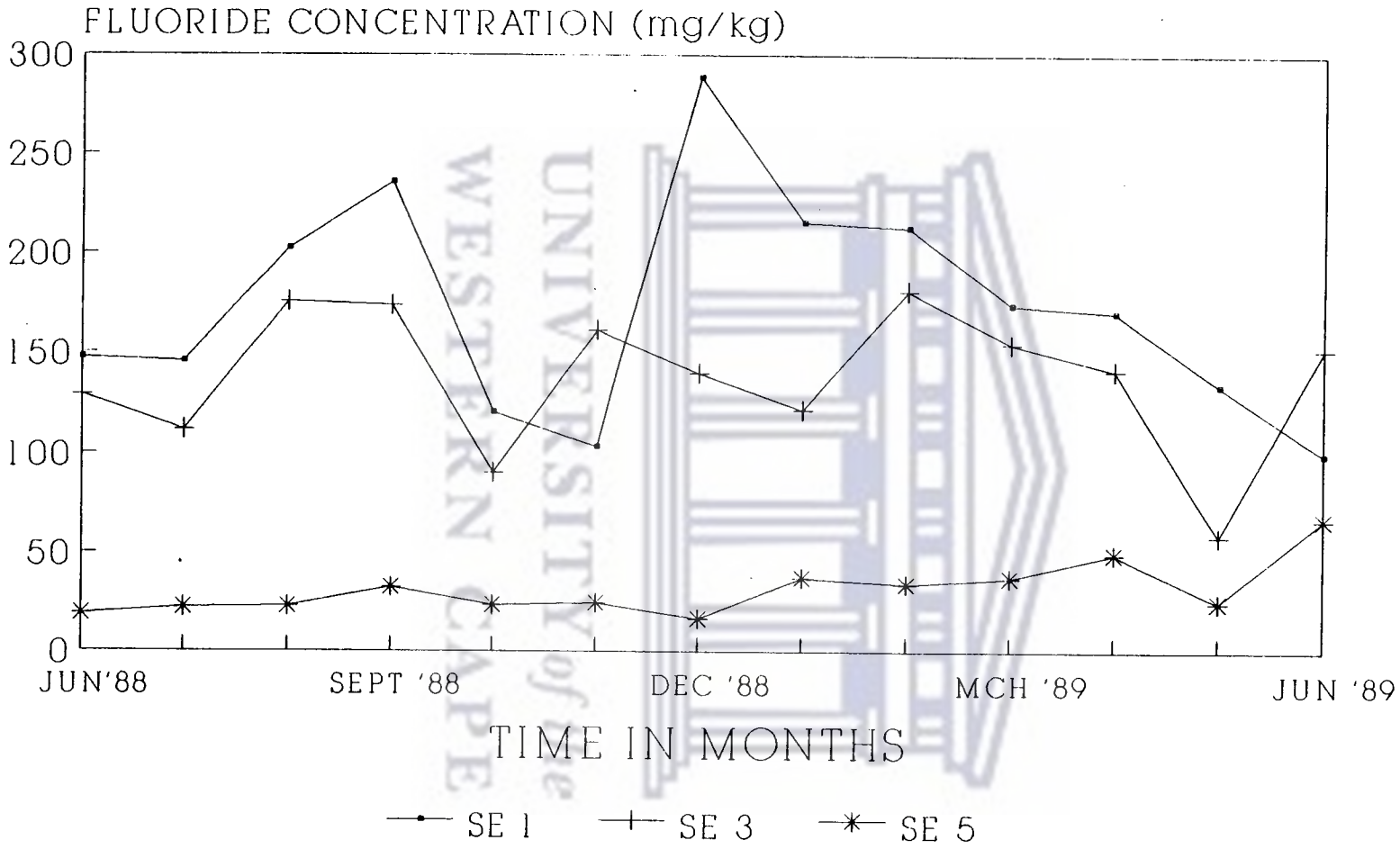


FIG.2.2 ANNUAL VARIATION OF FLUORIDE IN Quercus BARK ALONG A GRADIENT

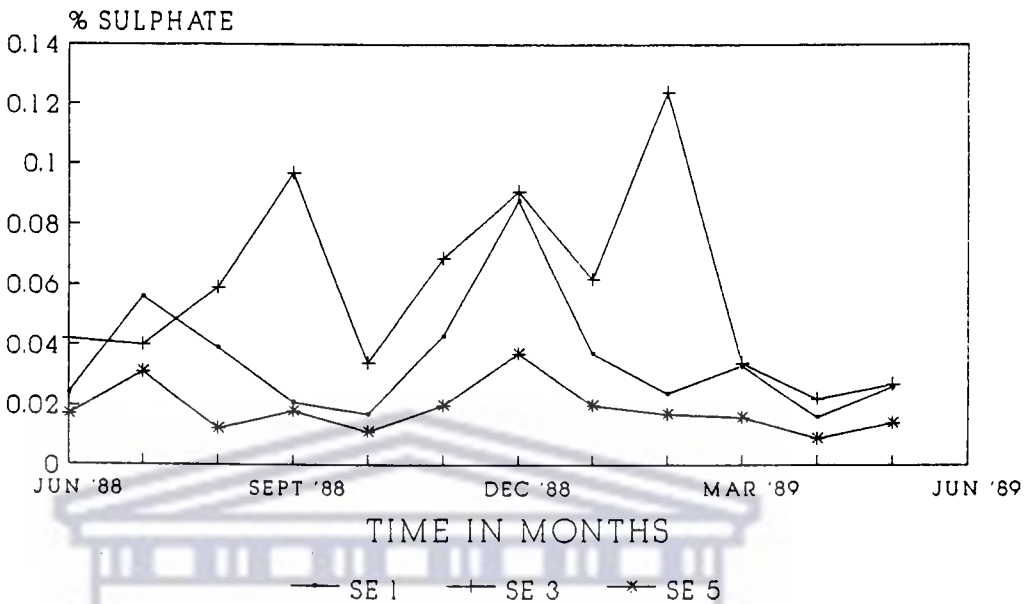


FIG. 2.3a ANNUAL VARIATION OF SULPHATE IN Quercus BARK ALONG A POLLUTION GRADIENT

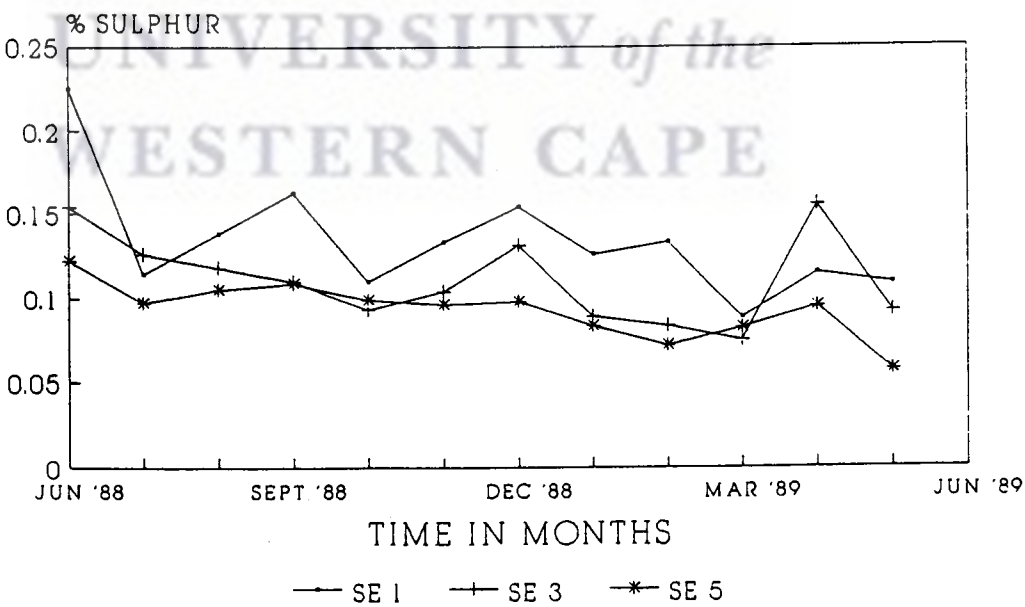


FIG. 2.3b ANNUAL VARIATION OF % SULPHUR IN Quercus BARK ALONG A POLLUTION GRADIENT

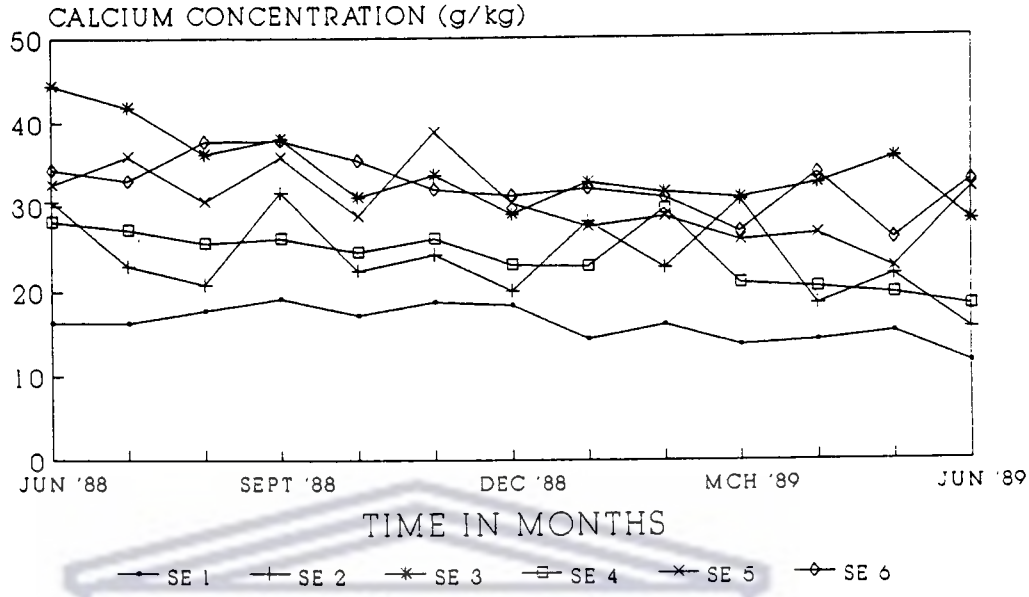


FIG. 2.4 ANNUAL VARIATION OF Ca IN Quercus BARK ALONG A POLLUTION GRADIENT

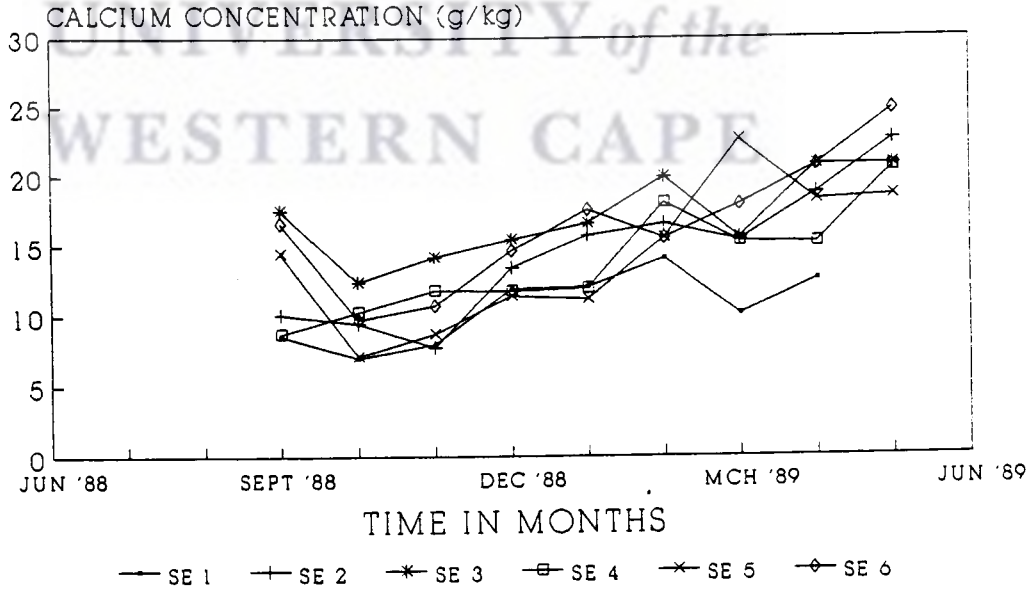


FIG. 2.5 ANNUAL VARIATION OF Ca IN Quercus LEAVES ALONG A POLLUTION GRADIENT

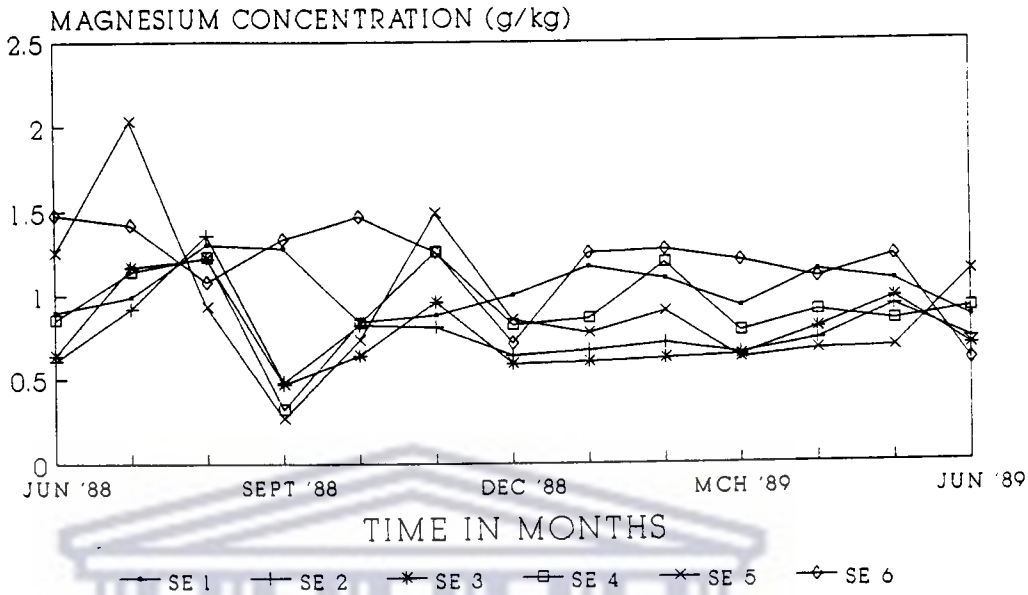


FIG. 2.6 ANNUAL VARIATION OF Mg IN Quercus BARK ALONG A POLLUTION GRADIENT

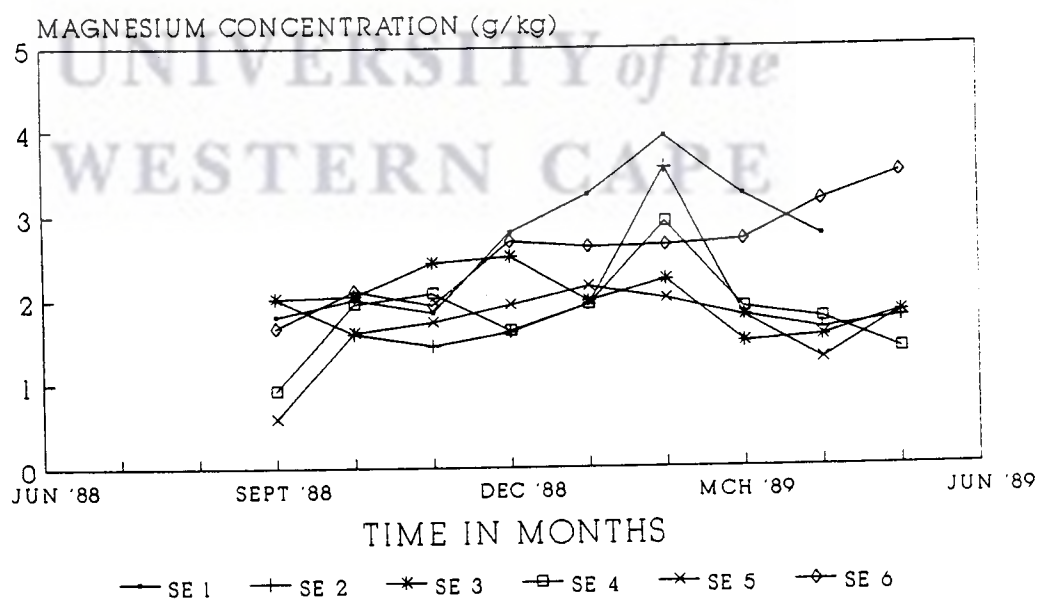


FIG. 2.7 ANNUAL VARIATION OF Mg IN Quercus LEAVES ALONG A POLLUTION GRADIENT

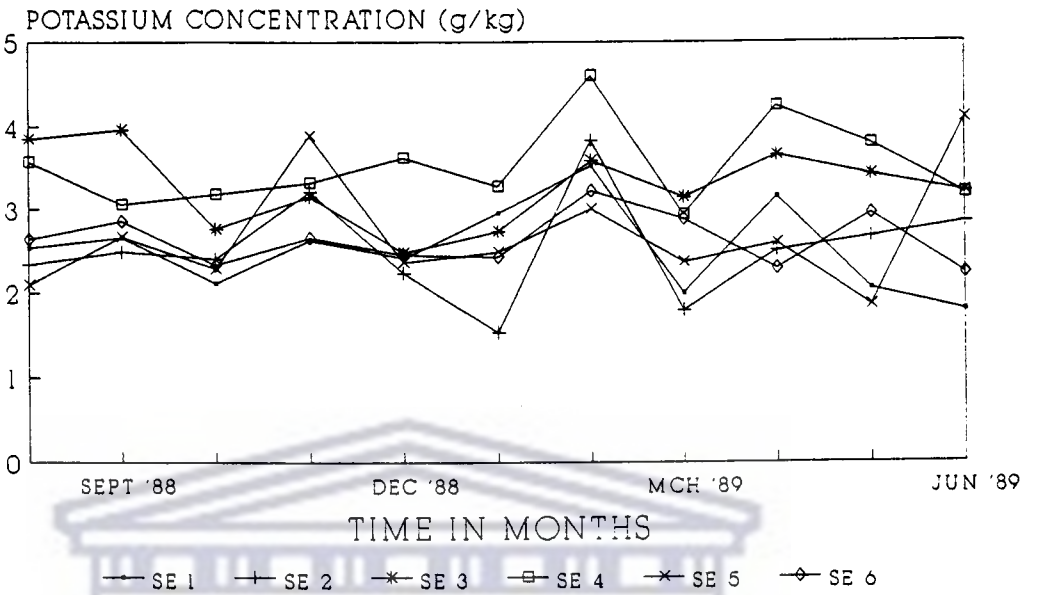


FIG. 2.8 ANNUAL VARIATION OF K IN Quercus BARK ALONG A POLLUTION GRADIENT

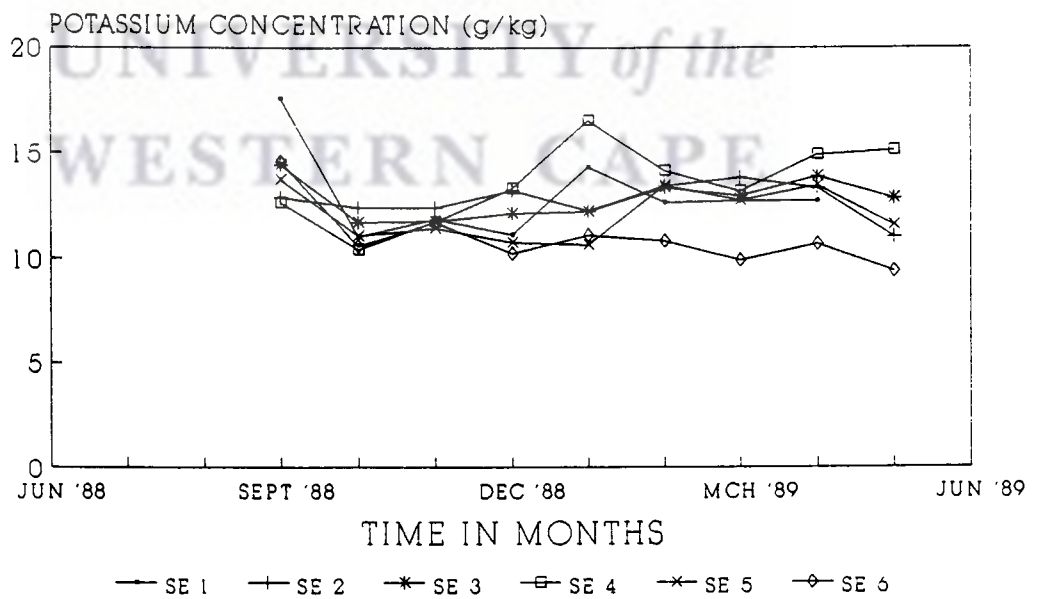


FIG. 2.9 ANNUAL VARIATION OF K IN Quercus LEAVES ALONG A POLLUTION GRADIENT

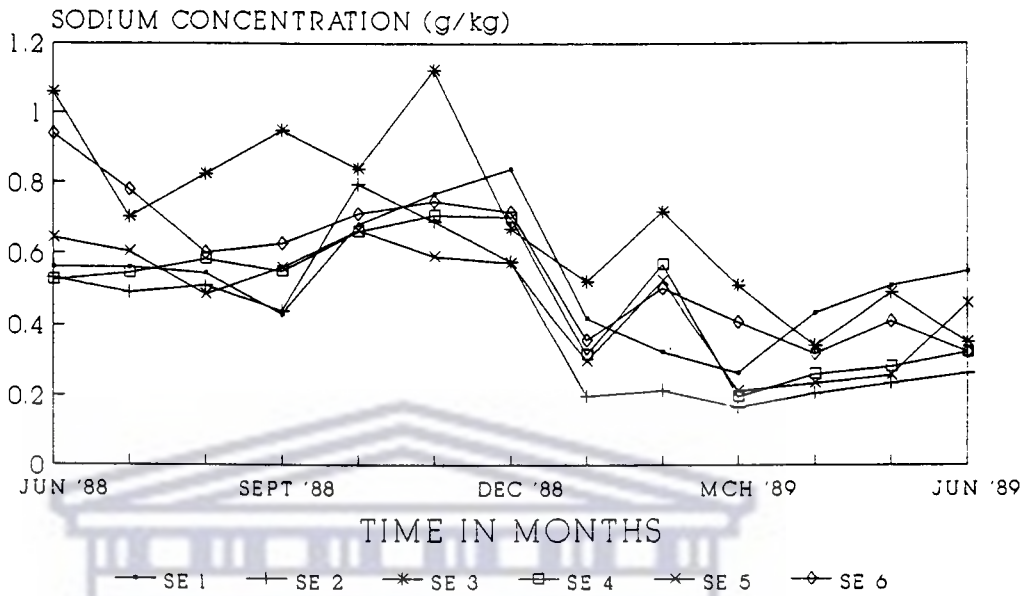


FIG. 2.10 ANNUAL VARIATION OF Na IN Quercus BARK ALONG A POLLUTION GRADIENT

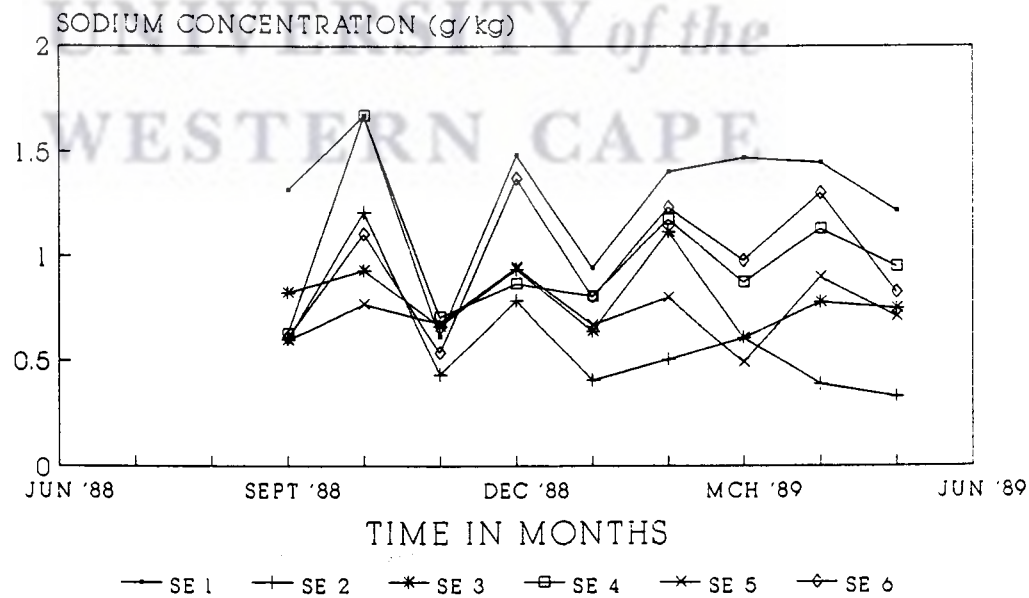


FIG. 2.11 ANNUAL VARIATION OF Na IN Quercus LEAVES ALONG A POLLUTION GRADIENT

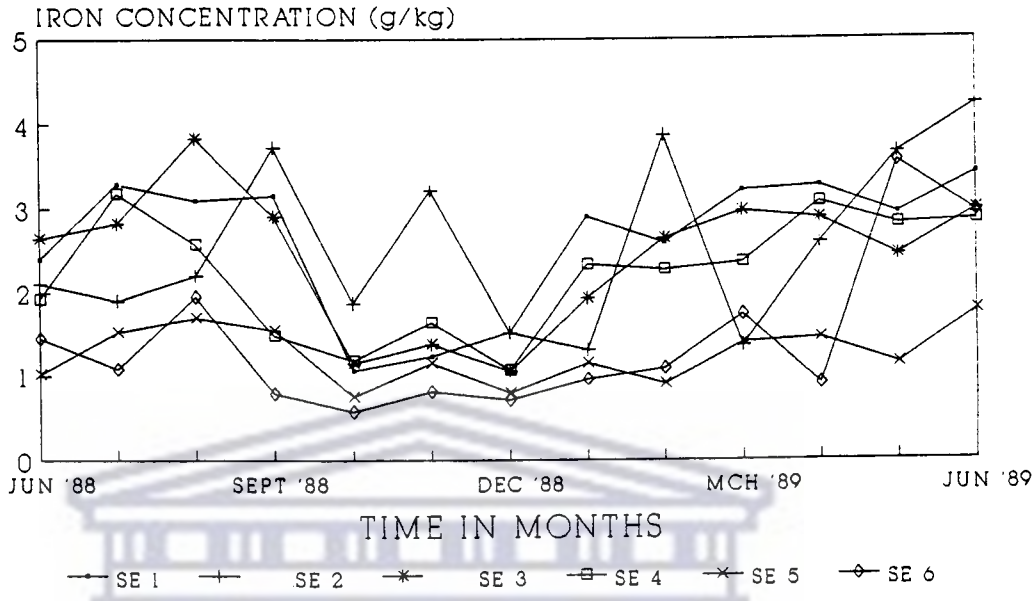


FIG. 2.12 ANNUAL VARIATION OF Fe IN Quercus BARK ALONG A POLLUTION GRADIENT

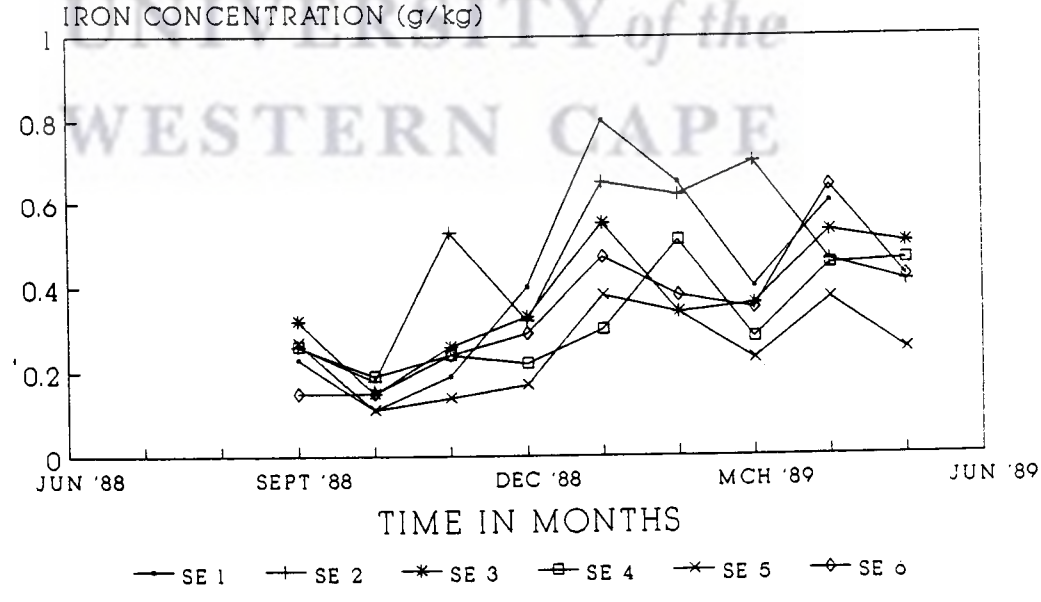


FIG. 2.13 ANNUAL VARIATION OF Fe IN Quercus LEAVES ALONG A POLLUTION GRADIENT

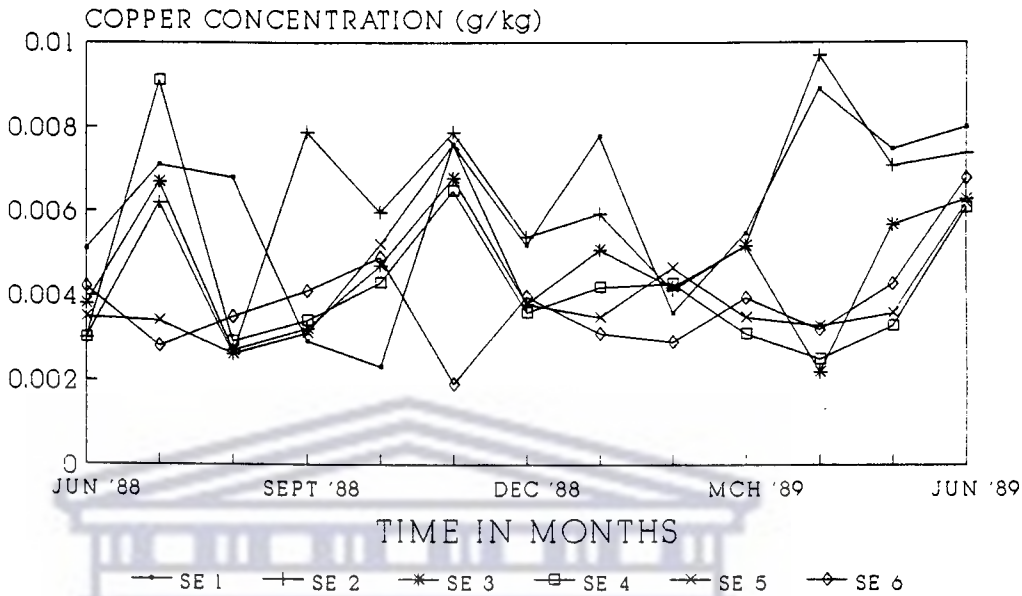


FIG. 2.14 ANNUAL VARIATION OF Cu IN Quercus BARK ALONG A POLLUTION GRADIENT

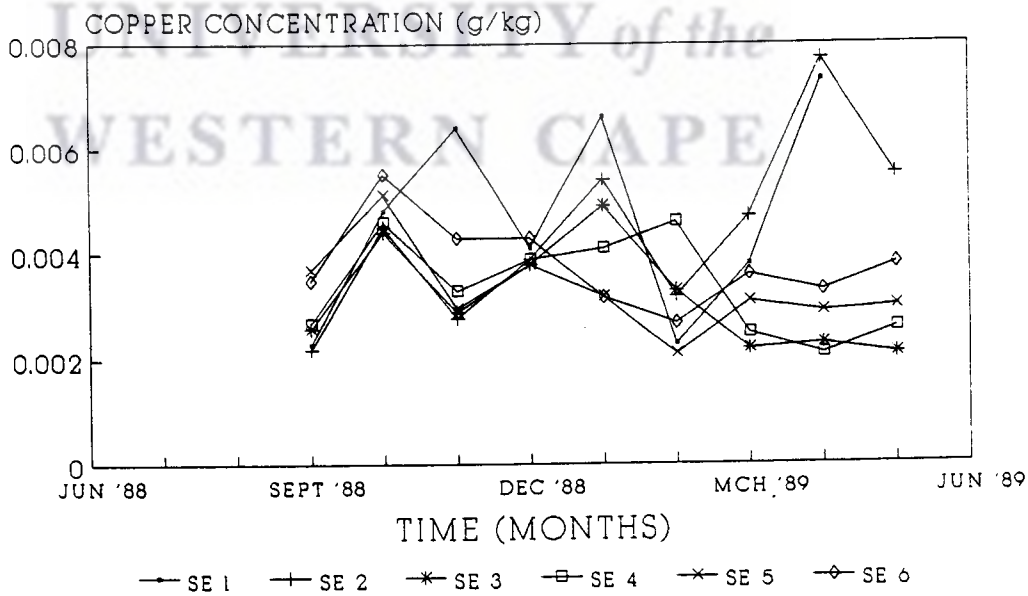


FIG. 2.15 ANNUAL VARIATION OF Cu IN Quercus LEAVES ALONG A POLLUTION GRADIENT

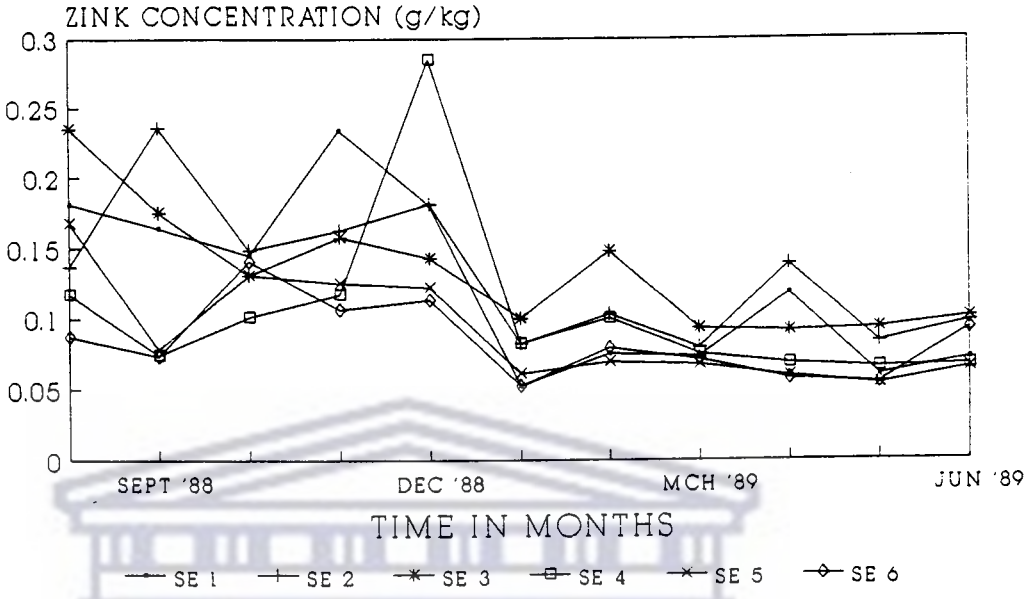


FIG. 2.16 ANNUAL VARIATION OF Zn IN Quercus BARK ALONG A POLLUTION GRADIENT

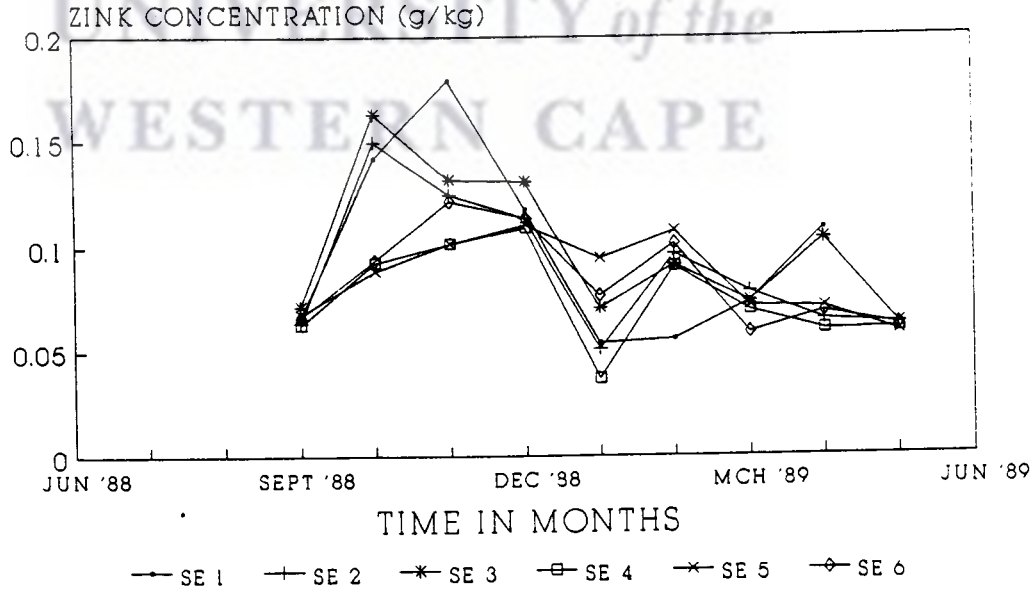


FIG. 2.17 ANNUAL VARIATION OF Zn IN Quercus LEAVES ALONG A POLLUTION GRADIENT

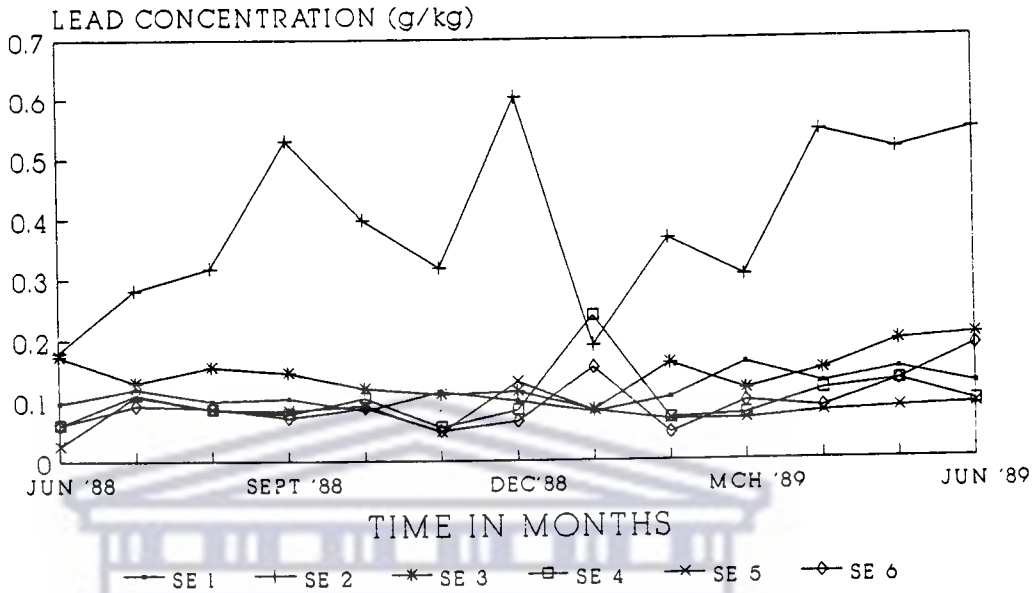


FIG. 2.18 ANNUAL VARIATION OF Pb IN Quercus BARK ALONG A POLLUTION GRADIENT

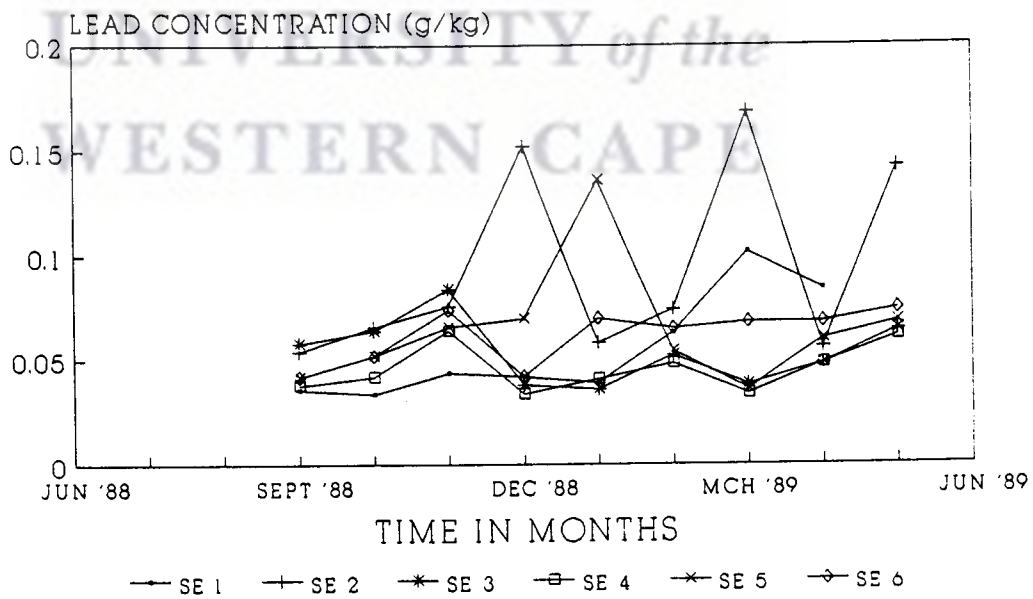


FIG. 2.19 ANNUAL VARIATION OF Pb IN Quercus LEAVES ALONG A POLLUTION GRADIENT

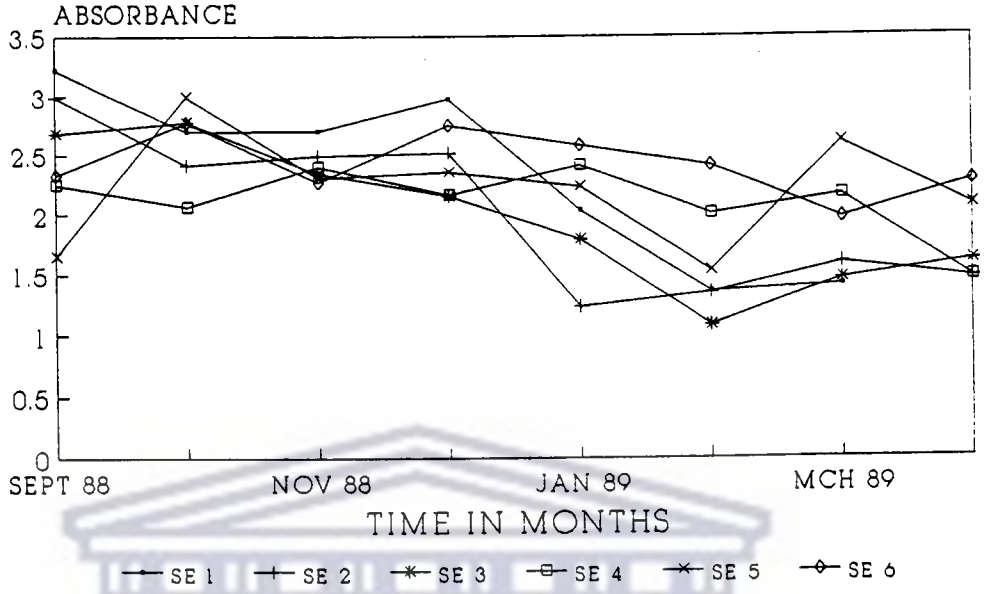


FIG. 2.20 ABSORPTION OF LIGHT BY CHLOROPHYLL PIGMENTS AT 435nm

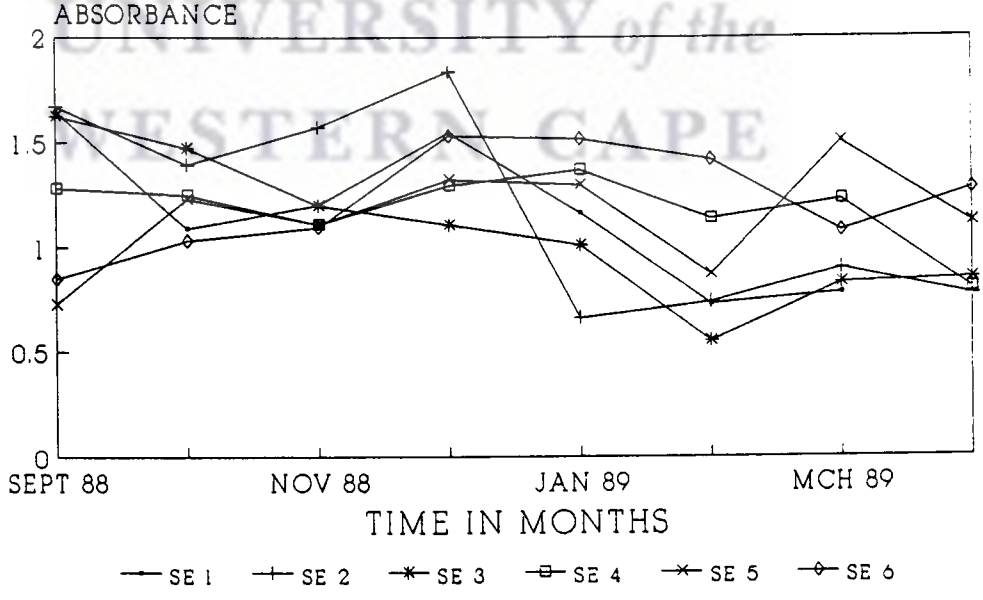
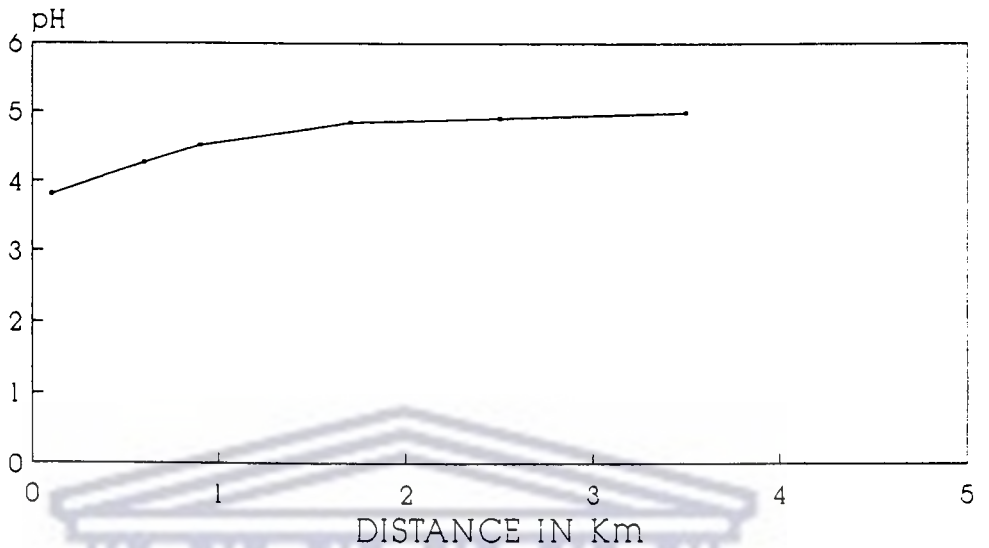
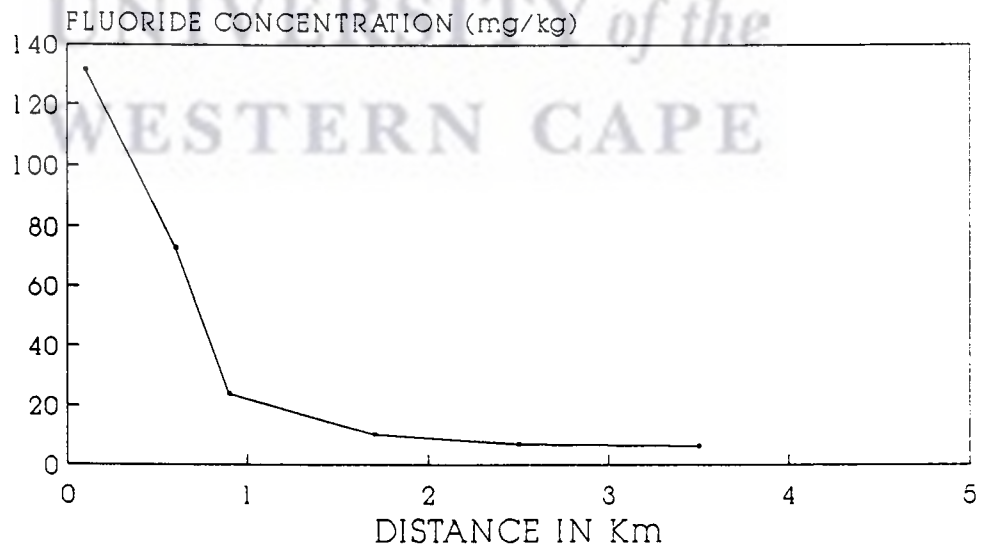


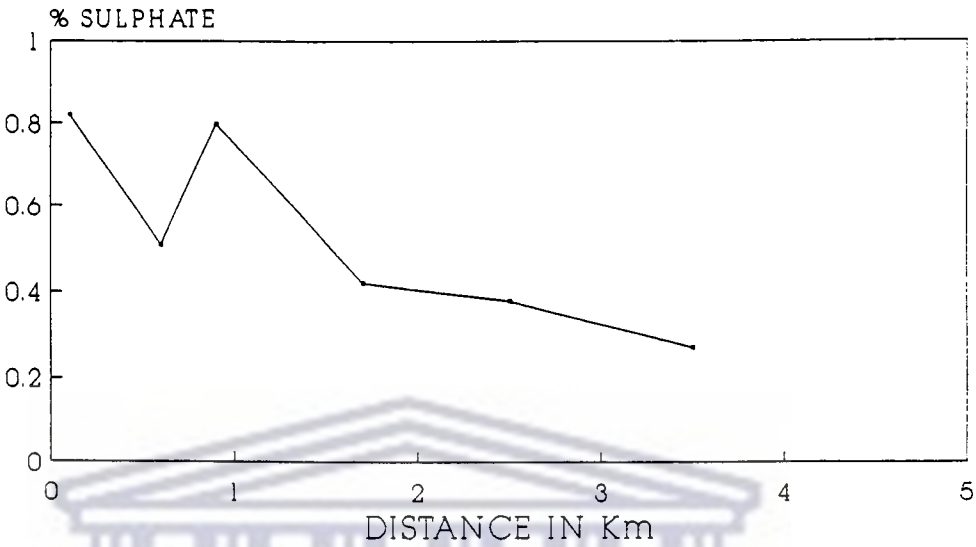
FIG. 2.21 ABSORPTION OF LIGHT BY CHLOROPHYLL PIGMENTS AT 665nm



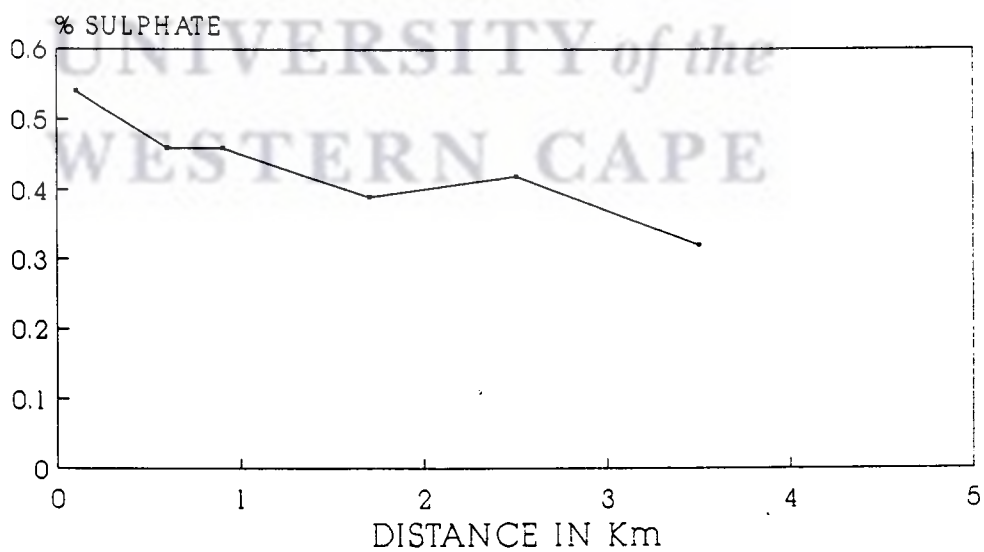
APPENDIX 1. VARIATION IN Quercus BARK ALONG A POLLUTION POLLUTION GRADIENT



APPENDIX 2. VARIATION IN FLUORIDE CONCENTRATION IN Quercus LEAVES ALONG A POLLUTION GRADIENT



APPENDIX 3. VARIATION IN % SULPHATE IN Quercus BARK ALONG A POLLUTION GRADIENT



APPENDIX 4. VARIATION IN % SULPHATE IN Quercus LEAVES ALONG A POLLUTION GRADIENT

pH OF OAK BARK

	JUN'88			SEPT '88		NOV'88		JAN '89			APR '89		JUN '89
SE 1	4.19	4.03	4.23	4.2	3.99	4.09	4.04	4.17	4.14	4.19	4.29	4.16	4.19
SE 2	4.23	4.24	4.3	4.25	3.98	4.05	3.9	4.09	4.23	4.22	4.16	4.17	4.24
SE 3	4.62	4.33	4.29	3.86	3.82	4.07	4.37	3.93	3.93	4.09	4.45	4.42	4.47
SE 4	4.42	4.26	4.41	4.2	4.34	4.37	4.38	4.26	4.6	4.41	4.52	4.53	4.54
SE 5	4.64	4.57	4.49	4.58	4.18	4.76	4.81	4.23	4.25	4.37	4.31	4.41	4.78
SE 6	4.52	4.4	4.54	4.24	4.28	4.36	4.36	4.43	4.44	4.57	4.51	4.55	4.39

FLUORIDE CONTENT OF OAK BARK

	JUN'88			SEPT '88		DEC '88			MCH '89			JUN '89
SE 1	147.6	145.6	202.4	235.8	120.6	103.6	288.8	215.6	212.8	173.8	170	133.8	99.6
SE 3	129	111.6	175.4	173.6	90.4	161.2	140	121.6	181	154.4	141.2	58.4	151.8
SE 5	18.8	22	22.8	32.4	23.2	24.4	16.4	37.4	34	37.2	49.2	24.8	66.8

SULPHATE CONTENT OF OAK BARK

	JUN '88			SEPT '88		DEC '88			MAR '89		
						FIG.2.4a					
SE 1	0.024	0.056	0.039	0.021	0.017	0.043	0.088	0.037	0.024	0.033	0.016	0.026
SE 3	0.042	0.04	0.059	0.097	0.034	0.069	0.091	0.062	0.124	0.034	0.022	0.027
SE 5	0.017	0.031	0.012	0.018	0.011	0.02	0.037	0.02	0.017	0.016	0.009	0.014

SULPHUR CONTENT OFOAK BARK

	JUN '88			SEPT '88		DEC '88			MAR '89		
						FIG.2.4b					
SE 1	0.225	0.114	0.138	0.162	0.11	0.133	0.154	0.126	0.133	0.088	0.115	0.109
SE 3	0.154	0.126	0.118	0.11	0.093	0.104	0.131	0.089	0.083	0.075	0.155	0.092
SE 5	0.122	0.097	0.105	0.109	0.099	0.096	0.098	0.083	0.072	0.082	0.095	0.057

LIGHT ABSORPTION BY OAK LEAF PHOTOSYNTHETIC PIGMENTS AT 435nm

	SEPT '88		NOV '88		JAN '89		MCH '89	
SE 1	3.218	2.701	2.703	2.98	2.04	1.36	1.413	
SE 2	2.99	2.411	2.497	2.521	1.235	1.352	1.599	1.474
SE 3	2.677	2.776	2.346	2.152	1.795	1.079	1.464	1.614
SE 4	2.242	2.066	2.401	2.164	2.416	2.011	2.165	1.475
SE 5	1.651	2.996	2.385	2.355	2.234	1.533	2.61	2.076
SE 6	2.322	2.775	2.272	2.752	2.584	2.412	1.972	2.274

LIGHT ABSORPTION BY OAK LEAF PHOTOSYNTHETIC PIGMENTS AT 665nm

	SEPT '88		NOV '88		JAN '89		MCH '89	
SE 1	1.648	1.093	1.206	1.544	1.167	0.731	0.782	
SE 2	1.668	1.395	1.572	1.832	0.665	0.74	0.982	0.772
SE 3	1.623	1.474	1.205	1.109	1.012	0.553	0.831	0.851
SE 4	1.283	1.252	1.113	1.297	1.369	1.14	1.23	0.882
SE 5	0.728	1.235	1.112	1.325	1.3	0.873	1.503	1.118
SE 6	0.85	1.034	1.098	1.53	1.516	1.419	1.08	1.278

CALCIUM CONTENT OF OAK BARK

	JUN '88			SEPT '88			DEC '88			MCH '89			JUN '89
SE 1	16.34	16.32	17.82	19.28	17.34	18.9	18.52	14.52	16.26	13.88	14.4	15.36	11.72
SE 2	30.44	23.01	20.84	31.7	22.54	24.4	20.22	28.26	22.94	30.74	18.7	21.98	15.74
SE 3	44.48	41.86	36.22	38.08	31.16	33.82	29.12	32.86	31.7	30.98	32.66	35.7	28.2
SE 4	28.12	27.2	25.74	26.28	24.76	26.34	23.18	23.06	29.72	21.12	20.56	19.82	18.36
SE 5	32.48	35.9	30.52	35.88	28.86	38.88	30.36	27.7	28.84	26.1	26.76	22.86	31.88
SE 6	34.32	33.08	37.74	37.88	35.5	32.08	31.28	32.1	31.04	27.08	33.9	26.14	32.92

CALCIUM CONTENT OF OAK LEAVES

SE 1				8.62	7.84	8.84	11.92	12.06	14.08	10.14	12.58		
SE 2				10.14	9.5	7.82	13.42	15.74	16.54	15.4	18.72	22.58	
SE 3				17.54	12.44	14.2	15.46	16.62	19.9	15.58	20.76	20.76	
SE 4				8.76	10.32	11.86	11.78	11.98	18.04	15.28	15.18	20.56	
SE 5				14.46	7.18	8.76	11.42	11.2	15.56	22.6	18.26	18.5	
SE 6				16.64	9.76	10.74	14.64	17.54	15.54	17.9	20.72	24.72	

MAGNESIUM CONTENT OF OAK BARK

	JUN '88			SEPT '88			DEC '88			MCH '89			JUN '89
SE 1	0.896	0.99	1.304	1.28	0.84	0.88	1.002	1.17	1.096	0.93	1.14	1.08	0.86
SE 2	0.61	0.92	1.36	0.48	0.82	0.81	0.64	0.67	0.71	0.65	0.73	0.93	0.73
SE 3	0.64	1.17	1.22	0.47	0.64	0.96	0.59	0.602	0.619	0.64	0.796	0.98	0.69
SE 4	0.85	1.14	1.23	0.32	0.83	1.26	0.82	0.86	1.19	0.78	0.9	0.84	0.91
SE 5	1.25	2.03	0.93	0.27	0.73	1.49	0.85	0.77	0.9	0.62	0.67	0.68	1.13
SE 6	1.48	1.42	1.08	1.34	1.47	1.26	0.71	1.25	1.27	1.202	1.102	1.23	0.602

MAGNESIUM CONTENT OF OAK LEAVES

SE 1				1.81	2.008	1.85	2.802	3.24	3.92	3.23	2.75		
SE 2				2.02	1.602	1.46	1.62	1.94	3.55	1.802	1.63	1.76	
SE 3				2.03	2.06	2.45	2.52	1.99	2.24	1.49	1.55	1.83	
SE 4				0.92	1.96	2.09	1.63	1.94	2.92	1.9	1.76	1.394	
SE 5				0.59	1.62	1.74	1.94	2.16	2.01	1.79	1.28	1.83	
SE 6				1.67	2.12	1.94	2.7	2.63	2.65	2.71	3.15	3.48	

POTASSIUM CONTENT OF OAK BARK

	JUN '88			SEPT '88			DEC '88			MCH '89			JUN '89
SE 1	0.914	1.13	2.53	2.66	2.11	2.63	2.41	2.96	3.52	1.998	3.16	2.05	1.79
SE 2	1.102	0.97	2.33	2.49	2.402	3.22	2.23	1.53	3.83	1.79	2.51	2.68	2.85
SE 3	1.41	3.05	3.85	3.96	2.77	3.16	2.48	2.74	3.58	3.15	3.65	3.42	3.21
SE 4	1.71	3.33	3.57	3.86	3.19	3.33	3.62	3.28	4.602	2.94	4.24	3.796	3.19
SE 5	1.22	2.66	2.89	2.67	2.28	3.89	2.36	2.49	3.01	2.37	2.604	1.85	4.89
SE 6	1.75	3.17	2.64	2.86	2.33	2.66	2.45	2.43	3.23	2.89	2.29	2.96	2.23

POTASSIUM CONTENT OF OAK LEAVES

SE 1				17.57	11.05	11.86	11.14	14.3	12.63	12.696	12.71		
SE 2				12.88	12.41	12.404	13.23	12.26	13.42	13.78	13.31	11.02	
SE 3				14.42	11.696	11.76	12.13	12.21	13.34	12.95	13.84	12.81	
SE 4				12.64	10.43	11.74	13.3	16.5	14.12	13.15	14.87	15.88	
SE 5				13.72	11.06	11.434	10.76	10.66	13.43	12.74	13.42	11.54	
SE 6				14.57	10.59	11.71	10.26	11.09	10.82	9.92	10.68	9.41	

SODIUM CONTENT OF OAK BARK

	JUN '88			SEPT '88			DEC '88			MCH '89			JUN '89
SE 1	0.56	0.56	0.544	0.43	0.68	0.77	0.84	0.42	0.322	0.264	0.434	0.51	0.55
SE 2	0.53	0.49	0.51	0.44	0.796	0.69	0.578	0.198	0.214	0.166	0.206	0.234	0.262
SE 3	1.06	0.702	0.826	0.95	0.94	1.124	0.67	0.52	0.718	0.51	0.34	0.49	0.348
SE 4	0.524	0.544	0.584	0.55	0.66	0.708	0.702	0.314	0.57	0.196	0.26	0.28	0.32
SE 5	0.642	0.604	0.484	0.56	0.664	0.592	0.574	0.296	0.524	0.212	0.234	0.256	0.46
SE 6	0.94	0.78	0.602	0.628	0.712	0.748	0.716	0.356	0.502	0.408	0.316	0.41	0.32

SODIUM CONTENT OF OAK LEAVES

SE 1				1.32	1.67	0.616	1.486	0.948	1.486	1.474	1.45	1.218	
SE 2				0.684	1.214	0.434	0.79	0.486	0.586	0.61	0.388	0.326	
SE 3				0.83	0.934	0.67	0.94	0.644	1.12	0.61	0.78	0.75	
SE 4				0.632	1.67	0.71	0.87	0.81	1.18	0.876	1.13	0.95	
SE 5				0.602	0.77	0.68	0.95	0.676	0.804	0.49	0.9	0.71	
SE 6				0.614	1.188	0.54	1.372	0.888	1.238	0.98	1.304	0.828	

IRON CONTENT OF OAK BARK

	JUN '88			SEPT '88			DEC '88			MCH '89			JUN '89
SE 1	2.4	3.3	3.1	3.16	1.86	1.23	1.52	2.9	2.59	3.22	3.27	2.94	3.42
SE 2	2.1	1.9	2.2	3.73	1.87	3.22	1.51	1.31	3.87	1.36	2.59	3.66	4.23
SE 3	2.65	2.83	3.83	2.91	1.15	1.38	1.85	1.93	2.65	2.97	2.89	2.44	2.98
SE 4	1.92	3.18	2.58	1.48	1.18	1.64	1.87	2.33	2.27	2.36	3.88	2.81	2.86
SE 5	1.82	1.53	1.7	1.55	0.74	1.15	0.79	1.15	0.9	1.4	1.45	1.14	1.78
SE 6	1.45	1.88	1.95	0.78	0.57	0.8	0.784	0.95	1.89	1.74	0.9	3.56	2.96

IRON CONTENT OF OAK LEAVES

SE 1				0.23	0.11	0.19	0.4	0.8	0.65	0.4	0.6		
SE 2				0.26	0.18	0.53	0.32	0.65	0.62	0.7	0.46	0.41	
SE 3				0.32	0.15	0.26	0.33	0.55	0.34	0.36	0.53	0.5	
SE 4				0.26	0.19	0.24	0.22	0.3	0.51	0.28	0.45	0.46	
SE 5				0.27	0.11	0.14	0.17	0.38	0.34	0.23	0.37	0.25	
SE 6				0.15	0.15	0.24	0.29	0.47	0.38	0.35	0.64	0.42	

COPPER CONTENT OF OAK BARK

	JUN '88			SEPT '88			DEC '88			MCH '89			JUN '89
SE 1	0.0051	0.0071	0.0068	0.0029	0.0023	0.0076	0.0052	0.0078	0.0036	0.0055	0.0089	0.0075	0.00802
SE 2	0.003	0.0062	0.0026	0.0079	0.00598	0.0079	0.0054	0.00596	0.00414	0.0052	0.0097	0.0071	0.0074
SE 3	0.0038	0.0067	0.0027	0.0032	0.0047	0.0068	0.0038	0.0051	0.0042	0.0052	0.0022	0.0057	0.0063
SE 4	0.003	0.0091	0.0029	0.0034	0.0043	0.0065	0.0036	0.0042	0.0043	0.0031	0.0025	0.0033	0.0061
SE 5	0.0035	0.0034	0.0026	0.0031	0.0052	0.0076	0.0038	0.0035	0.00468	0.0035	0.0033	0.0036	0.0062
SE 6	0.0042	0.0028	0.0035	0.0041	0.0049	0.0019	0.00398	0.0031	0.0029	0.00396	0.0032	0.0043	0.0068

COPPER CONTENT OF OAK LEAVES

SE 1				0.0023	0.0048	0.0064	0.0041	0.0066	0.0023	0.0038	0.0073		
SE 2				0.0022	0.0045	0.0028	0.0039	0.0054	0.0032	0.0047	0.0077	0.0055	
SE 3				0.0026	0.0044	0.0029	0.0038	0.0049	0.0033	0.0022	0.0023	0.0021	
SE 4				0.0027	0.0046	0.0033	0.0039	0.0041	0.0046	0.0025	0.0021	0.0026	
SE 5				0.0037	0.0051	0.00298	0.0038	0.0032	0.0021	0.0031	0.0029	0.003	
SE 6				0.0035	0.0055	0.0043	0.0043	0.00318	0.0027	0.0036	0.0033	0.0038	

	JUN '88			SEPT '88			DEC '88			MCH '89			JUN '89
ZINK CONTENT OF OAK BARK													
SE 1	0.118	0.588	0.181	0.164	0.145	0.234	0.18	0.052	0.075	0.073	0.118	0.0596	0.071
SE 2	0.087	0.213	0.137	0.236	0.149	0.163	0.181	0.082	0.183	0.0796	0.139	0.083	0.097
SE 3	0.192	0.234	0.235	0.175	0.131	0.158	0.143	0.0996	0.148	0.093	0.091	0.093	0.1084
SE 4	0.517	0.551	0.117	0.074	0.101	0.117	0.285	0.082	0.1082	0.075	0.068	0.065	0.0664
SE 5	0.228	0.727	0.168	0.078	0.131	0.125	0.122	0.061	0.069	0.067	0.059	0.053	0.064
SE 6	0.583	0.358	0.087	0.073	0.141	0.186	0.113	0.052	0.079	0.071	0.057	0.054	0.092
ZINK CONTENT OF OAK LEAVES													
SE 1				0.067	0.142	0.179	0.118	0.054	0.056	0.074	0.189		
SE 2				0.064	0.1496	0.125	0.114	0.051	0.097	0.079	0.065	0.063	
SE 3				0.072	0.163	0.132	0.131	0.071	0.092	0.074	0.104	0.063	
SE 4				0.063	0.093	0.102	0.1096	0.037	0.091	0.07	0.0602	0.0604	
SE 5				0.068	0.089	0.102	0.111	0.095	0.108	0.072	0.071	0.059	
SE 6				0.067	0.094	0.122	0.114	0.077	0.102	0.059	0.069	0.062	

	JUN '88			SEPT '88			DEC '88			MCH '89			JUN '89
LEAD CONTENT OF OAK BARK													
SE 1	0.096	0.12	0.1	0.104	0.084	0.114	0.098	0.081	0.104	0.16	0.126	0.148	0.122
SE 2	0.18	0.292	0.318	0.53	0.398	0.318	0.602	0.19	0.366	0.304	0.544	0.512	0.544
SE 3	0.174	0.13	0.156	0.146	0.12	0.11	0.114	0.084	0.16	0.116	0.148	0.194	0.282
SE 4	0.06	0.11	0.086	0.078	0.104	0.056	0.084	0.238	0.07	0.076	0.114	0.128	0.092
SE 5	0.026	0.108	0.086	0.084	0.092	0.048	0.13	0.083	0.068	0.068	0.078	0.084	0.088
SE 6	0.06	0.092	0.088	0.072	0.088	0.05	0.066	0.134	0.046	0.096	0.086	0.128	0.184
LEAD CONTENT OF BARK LEAVES													
SE 1				0.036	0.034	0.044	0.042	0.039	0.063	0.102	0.084		
SE 2				0.054	0.066	0.076	0.152	0.058	0.074	0.168	0.056	0.142	
SE 3				0.058	0.064	0.084	0.038	0.036	0.052	0.038	0.048	0.064	
SE 4				0.038	0.042	0.064	0.034	0.041	0.048	0.034	0.048	0.061	
SE 5				0.042	0.052	0.066	0.07	0.136	0.054	0.036	0.06	0.068	
SE 6				0.042	0.052	0.074	0.042	0.07	0.065	0.068	0.068	0.074	

CHAPTER 3

THE ACCUMULATION OF FLUORIDE AND SULPHUR BY PINE TREES ALONG A NORTH-EAST TRANSECT AND ITS POSSIBLE INFLUENCE ON THE MINERAL COMPOSITION AND CHLOROPLAST PIGMENTS

INDEX

Page

3.1 INTRODUCTION

Biological monitoring of sulphur dioxide, fluoride, nitrogen oxide compounds and heavy metal pollution has increased over the past few decades. Bark and leaf samples of trees are usually analyzed for the pollutants (Staxäng, 1969; Reusmann, 1982; Johnson and Sochting, 1973) while the effect of the pollutants on the photosynthetic capacity of the leaves has also been studied quite extensively (Soikkeli and Karenlampi, 1984; Black and Unsworth, 1979; Katainen et al., 1987). The advantage of the biological method is that the organisms themselves record the cumulative toxic effect of pollution, and it is simple, quick and inexpensive (Grodzinska, 1982), as opposed to the use of instruments to record point or interval measurements.

Pollution sources in the study area include a number of wineries, the Plankenbrug Industrial area and a brickfield, which in this study was taken as the focal point of pollutant emission. The study area in Stellenbosch comprises a valley surrounded by three mountainous regions causing the emissions to accumulate in the basin during the

absence of wind. This phenomenon increases the effect of pollutants in the valley. The human population in the worst polluted area of Stellenbosch, is likely to be more susceptible to common ailments because of the multiple sources of pollution in the area.

Trees have been widely used as biological indicators, in pollution surveys (Kovacs et al., 1982; Staxäng, 1969). In this paper, distribution and quantities of pollutants absorbed by pine trees are discussed. The effect of pollution on the mineral composition and the chloroplast pigments of these trees are also considered.

3.2 MATERIALS AND METHODS

Five sampling sites on a North-East (NE) transect leading away from the brickworks (Fig.1.1) were selected for this study. Each sampling site was a mature Pine tree, with the distance between trees being approximately 500m. Each tree was sampled at monthly intervals, at the same time of day so as to sample leaves with more or less the same internal metabolism (Mansfield and Snaith, 1984).

Bark and needles were collected, and dried for at least 5 days in a Memmert oven, at 65°C. Fresh needles were also

stored in plastic bags, at -12°C, for chlorophyll extraction. The dried bark was ground with the aid of a Retsch Ball Mill, while the dried needle material was ground to pass through a No.40 mesh using a Wiley Intermediate Mill. Ground material was stored in screw-top containers.

The data for the various elements, at NE 1 and NE 5 were subjected to t-tests, because they represented the sites closest to, and the furthest from the brickworks (polluted and "unpolluted").

3.2.1 Determination of bark pH

For the pH determination of the bark, 5.0 grams of ground bark was mixed in 25 cm^3 of distilled deionised water, and continiously stirred for approximately 2 hours. A single determination was done for each sample. The pH values were determined by means of a Combination pH Electrode and a Radiometer Ion 85 Analyzer (Allen, 1974; Allen et al., 1986).

3.2.2 Elemental Analysis

3.2.2.1 Fluoride

Various methods (Anon, 1980; Cooke et al., 1976) were reviewed for Fluoride analysis. The F^- concentration of the material was determined using a Selective Ion Electrode,

method (B) as described by Anon (1980). For the extraction of F^-, 0.25 g of the material was weighed into a 100 cm^3 wide-mouthed plastic container. To this, 20 cm^3 0.05 N HNO_3 was added and the container placed on a magnetic stirrer and stirred with a Teflon-coated magnetic stirring bar for 20 minutes. After this period, 20 cm^3 0.1 N KOH were added and the solution further agitated for an additional 20 minutes. To this, 5.0 cm^3 sodium citrate solution containing 1 $mg.dm^{-3}$ F^- (adjusted pH to 5.5) and 5.0 cm^3 0.2 N HNO_3 were added. The method was simple, quick and accurate as data was easily reproduced to within 5% of the initial determination. A Radiometer Ion 85 Analyzer with a Combination Fluoride Electrode was used for the determination of the F^- concentration.

3.2.2.2 Sulphate and Sulphur

The plant samples were dried at 70 °C for 2 hours for the sulphate determination, and of each sample 0,1000 g was weighed out accurately and boiled for 5 minutes. The sample was then filtered, using no 52 Whatman filter paper and made up to 100 cm^3 using ultra pure water. The Dionex 4000i HPIC was calibrated using a sulphate anion standard of 1.50 $mg.dm^{-3}$. The elution liquid used, was a mixture of 0.0018 M Na_2CO_3 and 0.0017 M $NaHCO_3$. The calculation used to

determine % anion was as follows:

$$\% \text{ Anion} = \frac{mg.dm^{-3} \times 100}{10 \times 1000 \times mass}$$

The instrument was re-calibrated after every 10 determinations.

For the sulphur analysis, the plant samples were weighed directly from the screw-top containers without further drying. The sample weights taken for analysis varied between 1.4 and 4.7 mg and a Carlo Erba Elemental Analyzer, Model 1106, was used. The instrument was recalibrated after every 10 analysis, using a certified phenanthrene standard containing 0.848 % sulphur.

3.2.2.3 Calcium, Magnesium, Sodium, Potassium, Copper, Iron, Zinc and Lead

Of each sample, 0.5 g was weighed, enfolded in cigarette paper and digested in 6 cm^3 of HNO_3:$HClO_4$ (2:1). Glass beads were used to curb excessive bumping. The digestion process was considered complete when a colourless solution was obtained. Distilled deionised water was added to the tubes, and the solution allowed to cool down. It was then filtered through Schleicher and Schull #595 filter paper, and made up to 100 cm^3. The concentration of calcium, magnesium, sodium, potassium, iron, copper, zinc and lead was determined with a Pye Unicam SP9 Atomic

Absorption Spectrophotometer (Allen et al., 1986).

3.2.3 Chlorophyll extraction

A modification of the method used by Ronen and Galun (1984) was used. Needles were cut in half so as to fit into the vials which were approximately 40 cm^3 in size. The needle tissue was submerged in about 30 cm^3 of dimethyl sulfoxide (DMSO), and the vials heated in a water bath at 65°C in the dark for 45 minutes. The extractant was stored in 100 cm^3 flasks. The process was then repeated twice and each extractant added to the first. The flask content was then made up to 100 cm^3. Five cm^3 from the 100 cm^3 extractant were diluted with 5 cm^3 fresh DMSO and used in the quantitation of the chlorophyll pigments at 435 nm and 665 nm. All readings were done against a blank of DMSO, using a Varian Techtron Model 650 UV-Visible Spectrophotometer. DMSO was used as the extraction medium for all chlorophyll extractions (Oak leaves, Pine needles and Lichens).

3.3 RESULTS AND DISCUSSION

3.3.1 The pH of the Pine Bark

Figure 3.1 indicates that there was not a very large variation in pH amongst the different bark samples. The values obtained are in the range of pH 3.0 to pH 4.2. Generally, bark samples collected at NE 1 were highly significantly less acidic than those from NE 5 ($t(24)= 3.474$ and $p= 0.0020$) (Table 3.1). It appears that the pH of the Pine bark is low (acidic) in general (Hawksworth and Rose, 1976) which could possibly explain why lichens are not common on the bark of pine trees. Puckett et al.(1973) related the increase in SO_2 with a reduction in the pH of the surface of soils and other substrates on which lichens grow. The SO_2 is reflected as SO_4 and sulphur in the plant. They state that at lower pH values dissolved SO_2 is very toxic, to the extent that a lichen propagule would stand little chance of surviving in the presence of the pollutant. No lichens were evident on any of the trees sampled.

3.3.2 Elemental Analysis

3.3.2.1 Fluoride

Evidently fluoride is an essential element for higher animals, but it does not appear to be essential for plants. Heavy F^- contamination can occur in the area immediately surrounding an industrial plant. This is especially true for brickfields, aluminium smelters, some pottery factories, and factories involved in the manufacture of glass and ceramics (Allen, 1974; Purves, 1985). One of the complications associated with F^- damage to vegetation, is the ability of vegetation to accumulate F^- in high concentrations. This may result in F^- toxicity to grazing animals. Since the actual injury to vegetation generally occurs because of gradual F^- accumulation, the duration of exposure, and atmospheric concentrations, will determine the severity of injury (Naegele, 1974). Relatively high F^- concentrations which did not drop below 100 mg/kg were recorded in the bark samples collected at NE 1 (Fig.3.2). Values obtained for samples collected at NE 3 and NE 5 in February and April 1989, could possibly be attributed to productivity of the brickfields (Davies, 1986). The data (Fig.3.2) shows a significantly higher level of F^- near the brickfield ($t(24)= 5.268$; $p= 0.0001$). A drop in the F^- concentration of the bark collected from NE 1 could be

an indication of a drop in the productivity of the brickfield. This correlated well with the drop recorded for Oak Bark (Fig.2.2, Chapter 2). This strongly suggests that there could have been a decrease in production activity at the brickfield (Davies, 1986).

3.3.2.2 Sulphate and Sulphur

Significantly higher sulphate values were recorded at NE 1 than at NE 5 (Fig.3.3a) ($t(12)=2.633$; $p=0.0219$) which strongly suggests that the higher sulphate values (NE 1) could be directly related to the brickfield. The same material was analysed to show the sulphur content, and the results indicated that NE 1 had a highly significantly higher sulphur concentration than NE 5 (Fig.3.3b - ($t(10)= 3.821$; $p= 0.034$). The graph of the sulphate values of NE 1 (Fig.3.3a) strongly resembles that of the F^- values recorded at NE 1 (Fig.3.2), especially the high sulphate and F^- values recorded between Nov. 1988 and Jan. 1989 (Fig.3.2 and 3.3a), indicating that more bricks could have been produced during this period (Davies, 1986). These results compare favourably with those of Figures 2.2 and 2.3 (Chapter 2).

3.3.2.3 Nutrient status of the trees

3.3.2.3.1 Calcium

Hewitt and Smith (1975) refer to the observation made by Florell, that quite small increments of Ca^{2+} resulted in considerable stimulation of the formation of mitochondria in wheat roots as well as an increase in their protein content. They also state that Ca^{2+} is probably multifunctional in that it is required for middle lamellae of cell walls, organelle membranes, nuclear substructure and as a base equivalent for inorganic anions of cells. Ca^{2+} undoubtedly has multiple functions in cellular metabolism. Of these the activation of many enzymes and membrane stability are probably the most important.

A highly significant correlation was found to exist between the Ca^{2+} concentration of the bark, and the distance from the brickfield ($r(59)= -0.6187$; $p< 0.00005$) (Fig.3.4). This was the opposite of what was found in the oak trees (Fig.2.4). This also appeared to correlate well with the decrease in pH when moving away from the brickfield (Fig.3.1). The Ca^{2+} concentration of the needles (Fig.3.5; Table 3.2), however, showed a significant positive correlation with distance ($r(46)= 0.3395$; $p= 0.0182$). This difference could be explained by Mejstrik's (1985) finding that the immediate influence of fluorine on plant nutrition is caused by the precipitation of Ca^{2+} in the form of

insoluble CaF_2, resulting in Ca^{2+} deficiency.

3.3.3.3.2 Magnesium

The bark at NE 1 accumulated a highly significantly higher Mg^{2+} concentration compared to NE 5 (Fig.3.6 - t(24)= 3.050; p= 0.0055). No metabolic significance can actually be attached to it, seeing that the processes in which Mg^{2+} partakes, are in living cells. The concentration of Mg^{2+} was highly significantly higher in the needles collected at NE 5 than at NE 1 (Fig.3.7 -t(24)= 4.47; p=0.0002). This was expected, seeing that the healthier needles were located on the side away from the pollution site. Because Mg^{2+} shares the same chemical characteristics as Ca^{2+}, it is expected that they would behave in the same way. Then, according to Mejstrik (1985), Mg^{2+} can form insoluble complexes with F^-, rendering it unavailable for chlorophyll formation. Mg^{2+} has a critical role in the structure of the ribosomal particles responsible for protein synthesis. The ribosomes consist of 2 or more sub-particles whose association in a functional manner is dependent on the correct Mg^{2+} concentration. It has been calculated that the in vitro concentration of chlorophyll, and therefore of Mg^{2+} in chloroplasts is about 0.2 M. This is some 20 times greater than the average cell concentration and it is therefore not suprising that the first effects of Mg deficiency are often symptoms of chlorosis (loss of chlorophyll). Mg^{2+} is a readily

dissociable ionic activator for many enzymes, but it is probably best known for its role in the chlorophyll molecule (Hewitt and Smith, 1975).

3.3.2.3.3 Potassium

The potassium concentration in the needles (Fig.3.9), is almost three times higher than the K^+ concentration in the bark (Fig.3.8). This justifiably indicates the importance of K^+ in the needles. Potassium is an activator of many enzymes that are essential for respiration. It also activates enzymes needed to form starch and proteins in living material (Salisbury & Ross, 1985), and because bark is dead material, a higher K^+ concentration is expected in the living material. According to Epstein (1972), K^+ is the only monovalent cation essential for all higher plants. K^+ is a major contributor to the osmotic potential of cells and therefore to their water potential. A higher K^+ value would thus be expected for the needles, because they need to regulate their water potential. The bark collected at NE 1, 2 and 3 recorded the higher K^+ concentration, indicating a possible influence by the brickworks.

3.3.2.3.4 Sodium

The Na^+ concentration of the bark (Fig.3.10) is lower than that of the needles (Fig.3.11), especially for the samples collected at NE 2 and NE 3. Table 3.2 indicates that the Na values obtained for the needles collected at NE 1 and NE 5,

are slightly higher than those of the bark. Epstein (1972) surveyed the work of various authors and he concluded that Na^+ is not generally required by green plants. Hewitt and Smith (1975) stated that the beneficial effects of Na^+ in the absence of adequate K^+ lies in the ability of Na^+ to substitute for K^+ in some enzyme systems. According to Brownell and Crossland (1972), higher plants that have the C_4 type of photosynthesis generally have an absolute requirement for Na^+.

3.3.2.3.5 Iron

The higher iron concentrations were recorded at NE 1 and NE 2 (Table 3.2), with NE 2 recording a significantly higher iron concentration than the bark at NE 5 (Fig.3.12 - ($t(24)= 3.754$; $p= 0.001$). This corresponds well with the Pb values obtained for the bark collected at this site (Fig.3.18), suggesting both to be due to traffic. Ho and Tai (1988) found a strong correlation between the logarithmic concentration of the metals (lead, copper, zinc, iron, manganese) and the logarithmic traffic volume, in their study. Iron plays an important role in certain enzymes and numerous proteins that carry electrons during photosynthesis and respiration (Salisbury and Ross, 1985), signifying its relative importance in the needles. Thus a higher iron concentration was expected in the needles, than that recorded by it, in Figure 3.13.

3.3.2.3.5 Copper

The level of copper in the pine needles and bark, does not differ very much (Fig. 3.14 & 3.15). According to Salisbury and Ross (1985), copper is present in several enzymes and proteins involved in oxidation and reduction, especially the respiratory enzymes and chloroplast proteins.

3.3.2.3.7 Zinc

Both Figures 3.16 and 3.17 show the same traits. Until about November 1988 the distribution of zinc in the bark (Fig.3.16) and needles (Fig.3.17), was very erratic, and then from November 1988 onwards the distribution of zinc seems to have stabilized (Fig.3.16 & 3.17). Stabilization of the zinc distribution in the needles occurred (Fig.3.17), with a significant drop in the concentration ($t(4)= 3.67$; $p= 0.0214$). Epstein (1972) reviewed work done by various authors and found that zinc plays an important role in regulating the level of auxin in the plant. It could thus have an influence on the growth of the plant i.e. needle growth. The period from July to December 1988 is probably crucial to the development and growth of the needles, therefore the distribution of zinc as shown in Figure 3.17. Ho and Tai (1988) on the other hand, found that a correlation existed between zinc concentration and the volume of traffic, which was not so evident in this study.

3.3.2.3.8 Lead

In the case of NE 1 the sampling was done on the side of the tree away from the road, but in the direction of the brickworks. This was also the case for NE 2. With NE 5, the road was on the side of the tree from which the samples were collected (in the direction of the brickworks). Ho and Tai (1988) found elevated levels of lead in roadside soil and grass in highly urbanised areas. The data represented in Figure 3.18 and 3.19, suggest that more lead was absorbed/adsorbed by the samples and that a correlation exist between the iron concentration and lead concentration at NE 2. There is a clear difference between the lead concentration in the samples of NE 1 and NE 5, especially towards the latter half of this work. The needles at NE 5 had a highly significantly higher lead concentration than that collected at NE 1 ($t(24)= 3.363$; $p= 0.0026$). The high lead (Fig.3.18) levels recorded in the bark at NE 2 correlated well with the high iron values recorded at this site (Fig.3.12).

3.3.3 Light absorbance by chlorophyll pigments

Absorbance at 435 nm and 665 nm

From October 1988 to April 1989 there was a continuous decrease in the amount of light (435 nm) absorbed by the chloroplast pigments (Fig.3.20). This could definitely not be as a result of the pollution, because the level of

absorbance of the needles at NE 1 and NE 5 were more or less the same. It could however have happened that the needles collected at NE 1 were older than those collected at NE 5, which could explain the difference between the absorbance values. The pattern of absorption for Fig.3.20 differs from that of Fig.3.21. There was an increase in the amount of light absorbed by the chloroplast pigments extracted from samples collected between October and February 1989 (Fig.3.21). The level of absorption is however much lower than that of Fig.3.21. This indicates that more light is absorbed at the lower end of the spectrum for photosynthesis. When comparing Fig.3.20 with Fig.3.21, there is an increase in the amount of light absorbed at 665nm with time, whereas the light absorbed at the shorter wavelength (435nm), is much higher than that at the longer wavelength (665nm).

3.4 CONCLUSION

Fluoride and sulphur dioxide are certainly the dominant pollutants in the area that was studied. There were also definite signs of iron and lead pollution.

With the distribution of F^- and sulphur along the transect, it became apparent that the brickfield area was definitely the main source of these two pollutants. Relatively high levels of F^- were recorded in the pine bark within the first kilometer from the brickfield (Fig.3.2). A similar distribution pattern was also observed for sulphur (Fig.3.3a).

According to Table 3.1, pine trees generally have a low bark pH. The highest pH value was obtained in the vicinity of the brickworks (NE 1) and the lowest at NE 5. Staxäng (1969) correlated a decrease in bark pH with an increase in SO_2 in the atmosphere, and the decrease in pH experienced in this study was contradictory to her findings.

There was a comparative similarity between a decrease in Ca^{2+} concentration and a decrease in the bark pH with distance. The needles on the other hand showed a steady increase with distance from the brickfield. This could probably be due to less F^- being available to form the insoluble CaF_2 in the needles of the trees further away.

The importance of Mg^{2+} in the needles was reflected in the higher recorded concentration in the needles compared to the bark. This was also further substantiated by the higher recorded concentrations further away from the pollution zone.

A higher concentration of K^+ was recorded by the bark in the polluted zone, indicating that the main pollution source could have been the cause of this.

Iron pollution definitely occurred along the transect, with the recorded bark iron concentration at NE 1 and NE 2 being the highest. Comparing Fig.3.12 with 3.18, the level of iron recorded at NE 2 corresponds with that of the lead accumulated at this site, while Figures 3.13 and 3.19 indicate that NE 5 accumulated the most iron and lead respectively. This evidence suggests that internal-combustion engines are responsible for a large percentage of iron and lead pollution in the area. The evidence confirmed what Ho and Tai (1988) found in their study.

An anatomical study, of the needles, could reveal if any structural changes occured in response to the pollution threat.

Fluoride and sulphur pollution is especially significant within the first 1.5 km from the brickworks, with Fe and Pb pollution reflecting traffic rather than industrial sources.

Table 3.1: pH of Pine bark at different sites.(n= 13)

SITE	pH (x±sd)
NE 1	3.77 ± .186
NE 2	3.74 ± .145
NE 3	3.70 ± .180
NE 4	3.70 ± .182
NE 5	3.50 ± .193

Table 3.2. The mean concentration of the various elements in Pine bark and needles along a pollution gradient (x ± sd) (g/kg Dry Weight).

SITEORGAN	ELEMENT									
	Ca	Mg	K	Na	Fe	Cu	Zn	Pb	*F^-	SO_4
NE 1BARK	8.88/1.11	0.81/0.28	1.21/0.47	0.71/3.01	1.13/0.3	0.004/0.001	0.18/0.21	0.067/0.02	198.5/71.7	0.386/0.2
NEEDLES	5.94/0.99	1.19/0.24	9.98/3.01	1.40/0.47	0.73/1.1	0.004/0.001	0.08/0.03	0.050/0.02		
NE 2BARK	8.26/1.78	1.06/0.35	1.94/0.55	1.38/0.24	2.17/0.95	0.006/0.004	0.34/0.26	0.091/0.03		
NEEDLES	7.75/1.74	1.71/0.46	9.64.2.33	5.14/1.34	0.49/0.2	0.004/0.001	0.13/0.05	0.054/0.02		
NE 3BARK	7.37/1.03	0.73/0.45	1.44/0.34	0.82/1.76	0.96/0.28	0.005/0.001	0.20/0.17	0.069/0.02	67.8/32.9	0.226/0.1
NEEDLES	6.47/0.77	1.79/0.4	10.07/1.8	4.07/0.34	0.54/0.18	0.005/0.003	0.1/0.061	0.062/0.02		
NE 4BARK	7.35/1.26	0.58/0.1	0.84/0.2	0.58/0.19	0.76/0.19	0.004/0.001	0.22/0.21	0.070/0.02		
NEEDLES										
NE 5BARK	5.87/0.62	0.57/1.21	1.01/0.3	0.62/1.26	0.7/0.26	0.004/0.001	0.16/0.18	0.074/0.02	56.12/28.50	0.172/0.1
NEEDLES	7.52/1.21	1.68/0.3	10.55/1.3	1.25/0.33	0.49/0.22	0.005/0.001	0.12/0.05	0.075/0.02		

* - mg/kg

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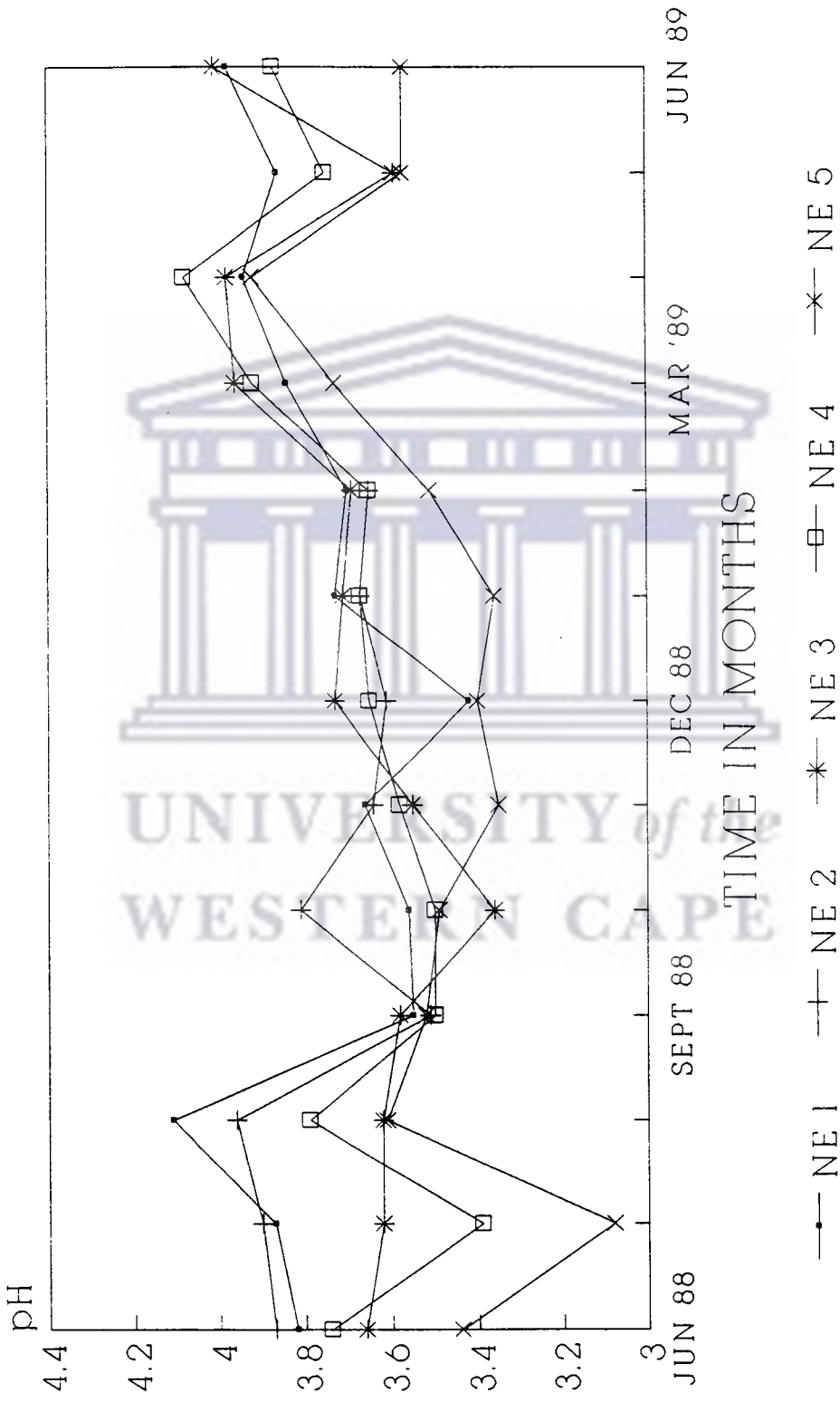

FIG.3.1 ANNUAL VARIATION IN BARK pH ALONG A POLLUTION GRADIENT

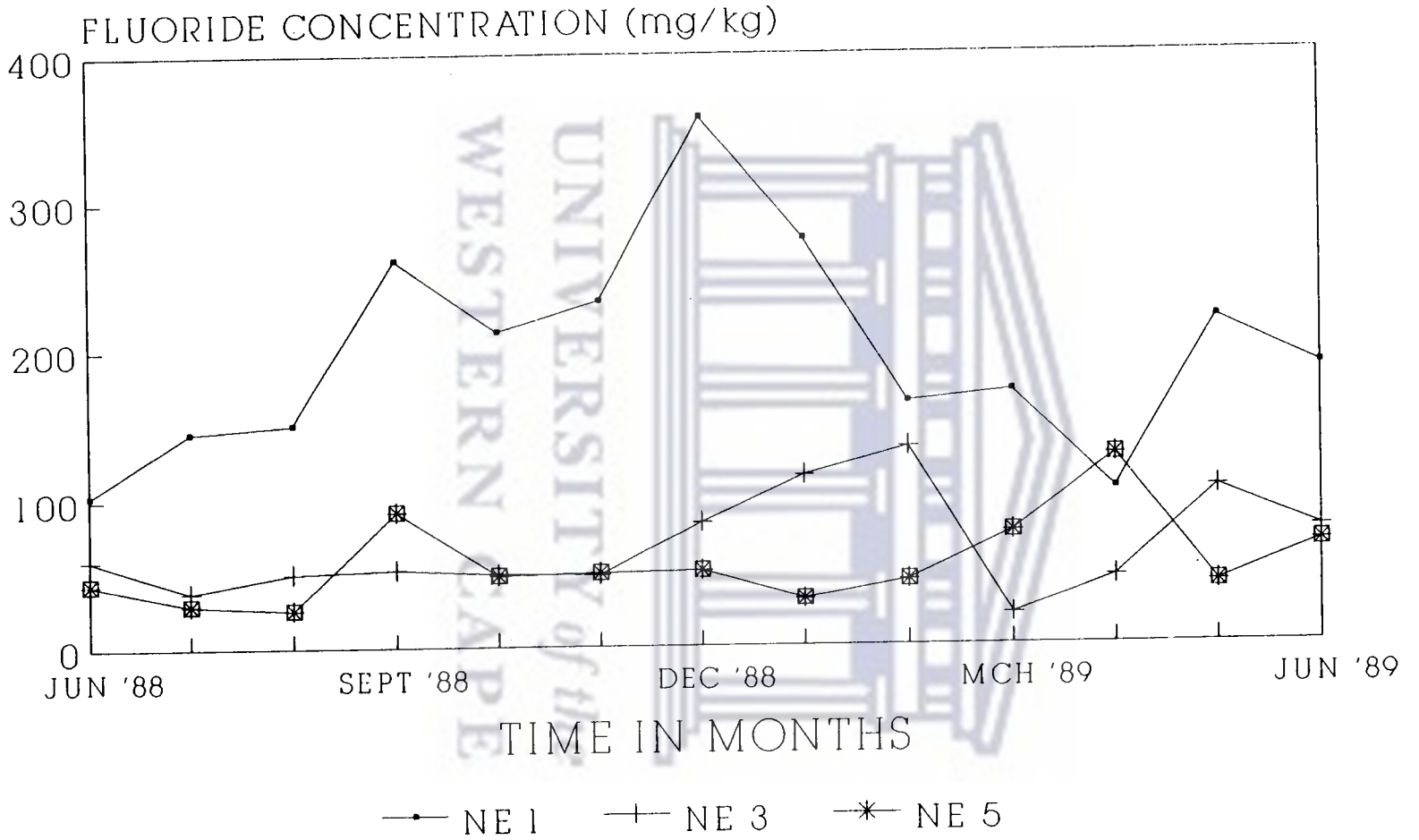

FIG.3.2 ANNUAL VARIATION IN THE FLUORIDE CONTENT OF PINE BARK

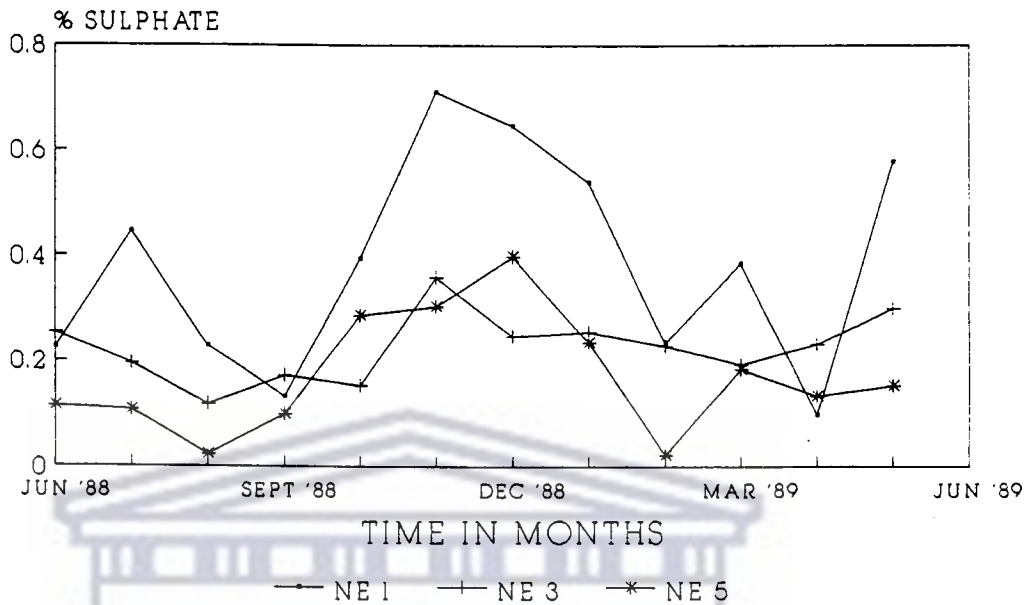

FIG. 3.3a ANNUAL VARIATION OF SULPHATE IN PINE BARK ALONG A POLLUTION GRADIENT

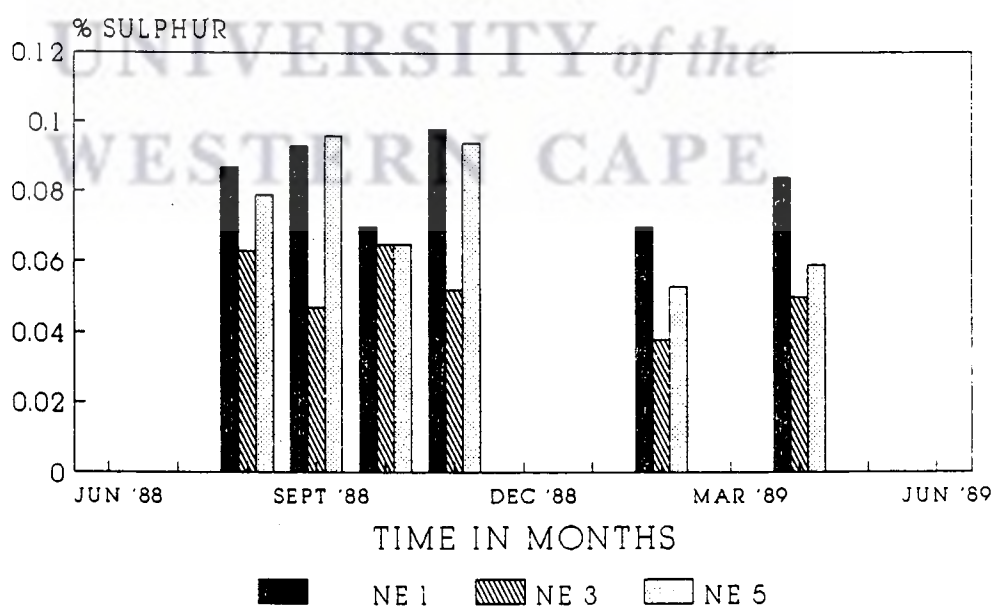

FIG. 3.3b VARIATION OF SULPHUR CONTENT IN PINE BARK ALONG A POLLUTION GRADIENT

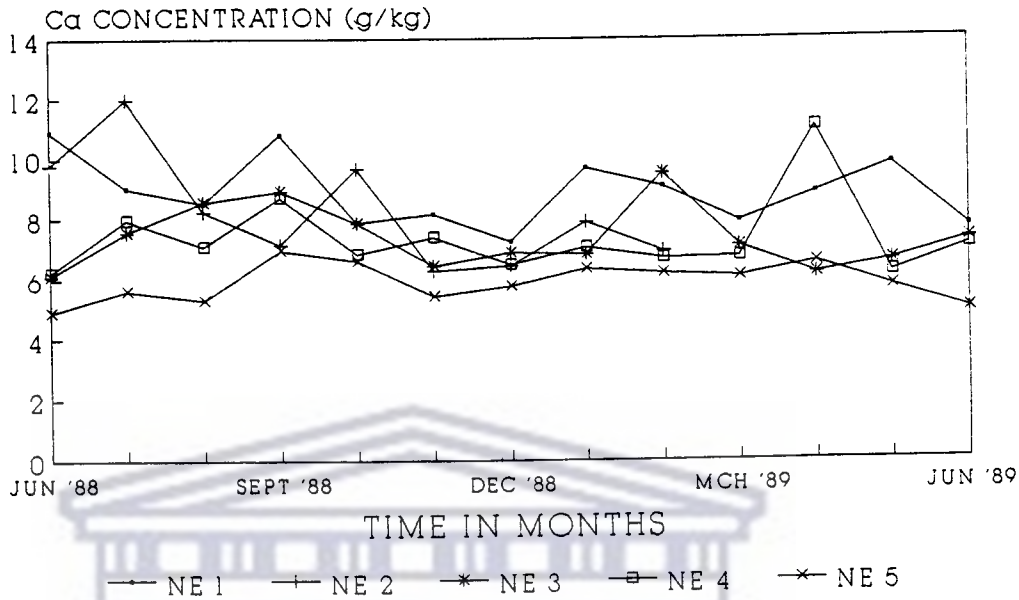

FIG. 3.4 ANNUAL VARIATION OF Ca IN PINE BARK ALONG A POLLUTION GRADIENT

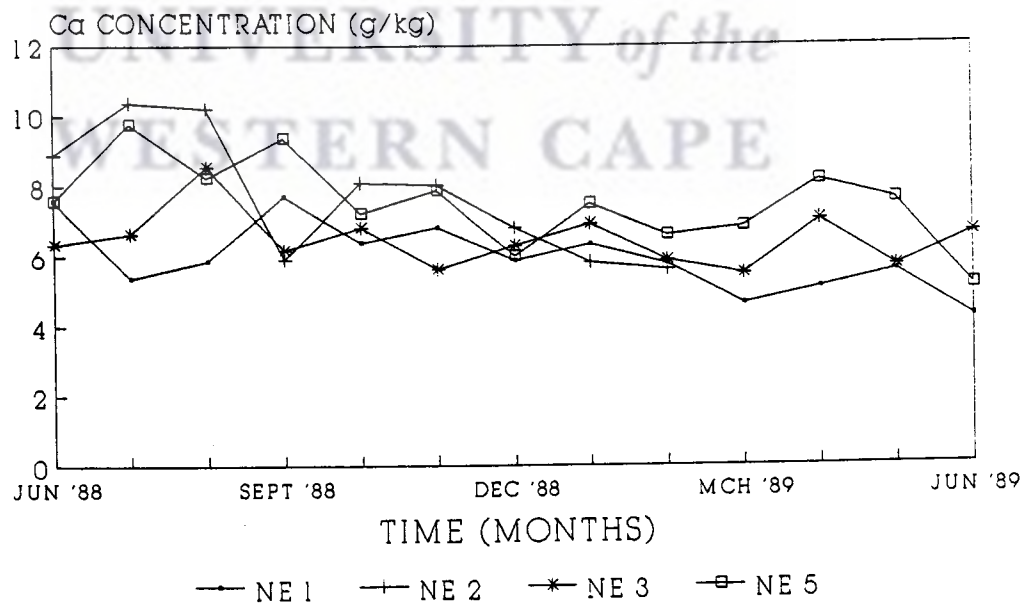

FIG. 3.5 ANNUAL VARIATION OF Ca IN PINE NEEDLES ALONG A POLLUTION GRADIENT

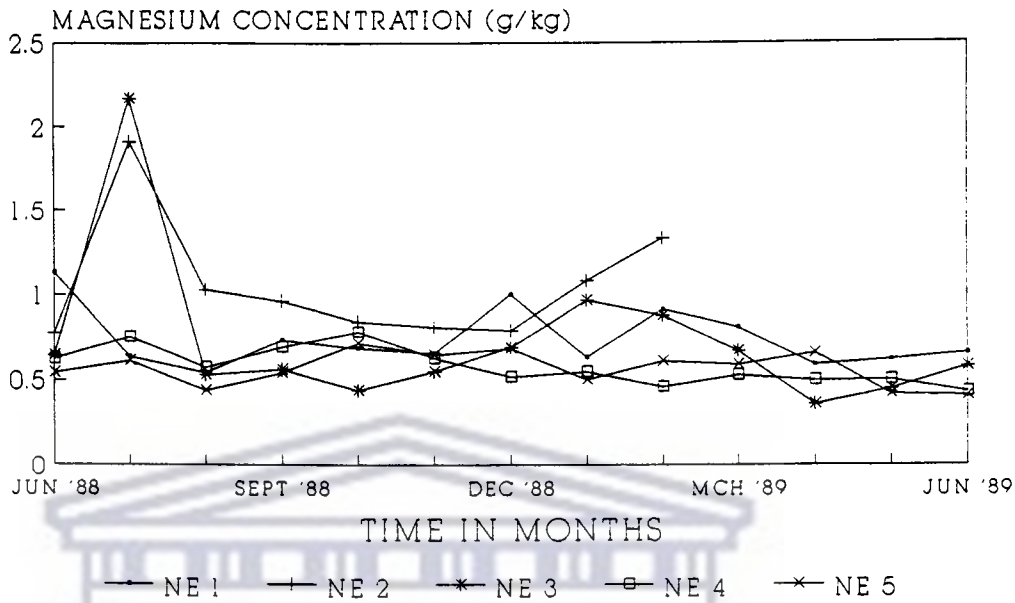

FIG. 3.6 ANNUAL VARIATION OF Mg IN PINE BARK ALONG A POLLUTION GRADIENT

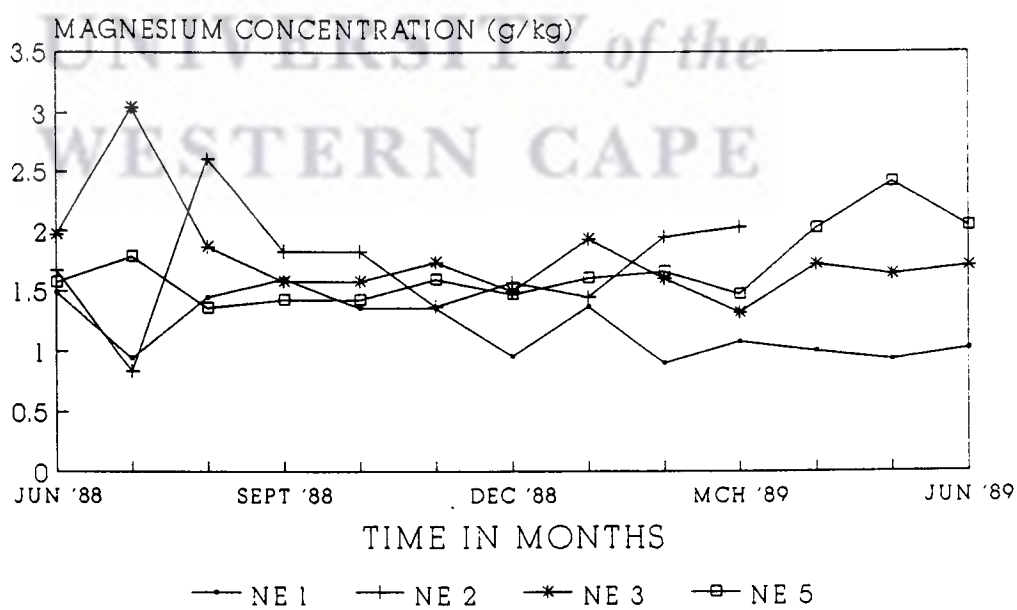

FIG. 3.7 ANNUAL VARIATION OF Mg IN PINE NEEDLES ALONG A POLLUTION GRADIENT

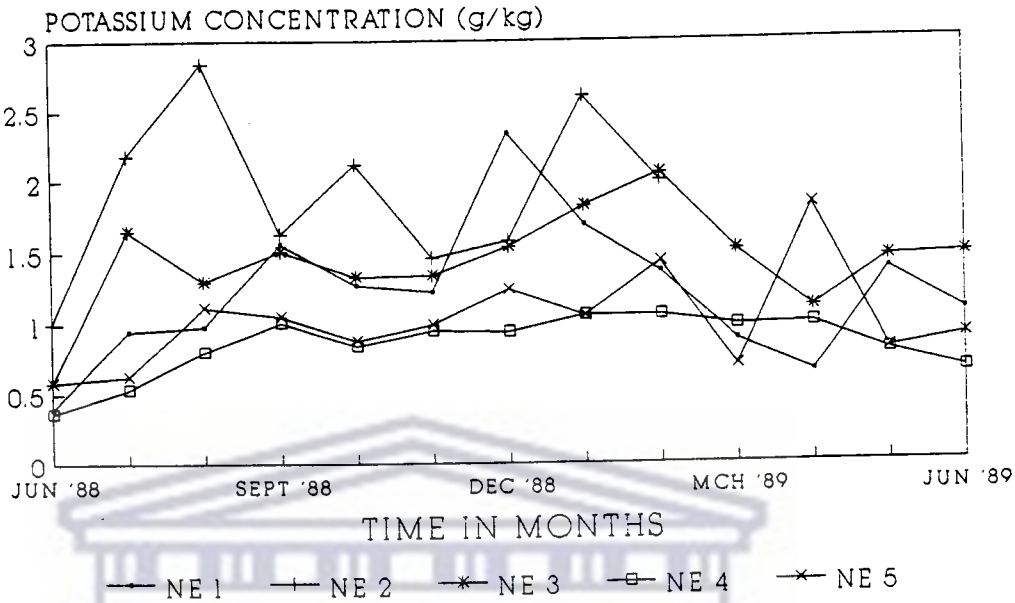

FIG. 3.8 ANNUAL VARIATION OF K IN PINE BARK ALONG A POLLUTION GRADIENT

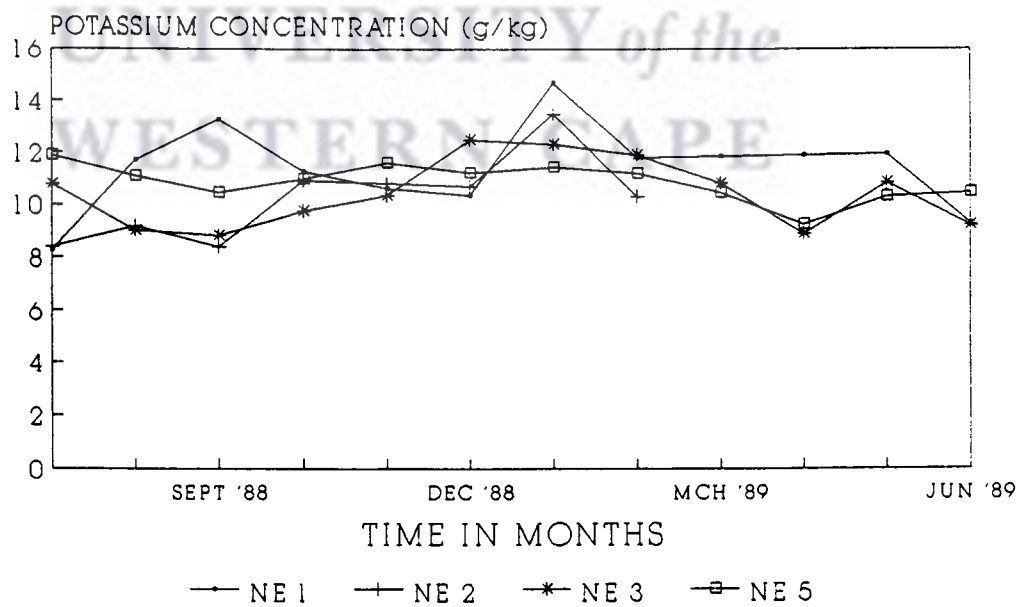

FIG. 3.9 ANNUAL VARIATION OF K IN PINE NEEDLES ALONG A POLLUTION GRADIENT

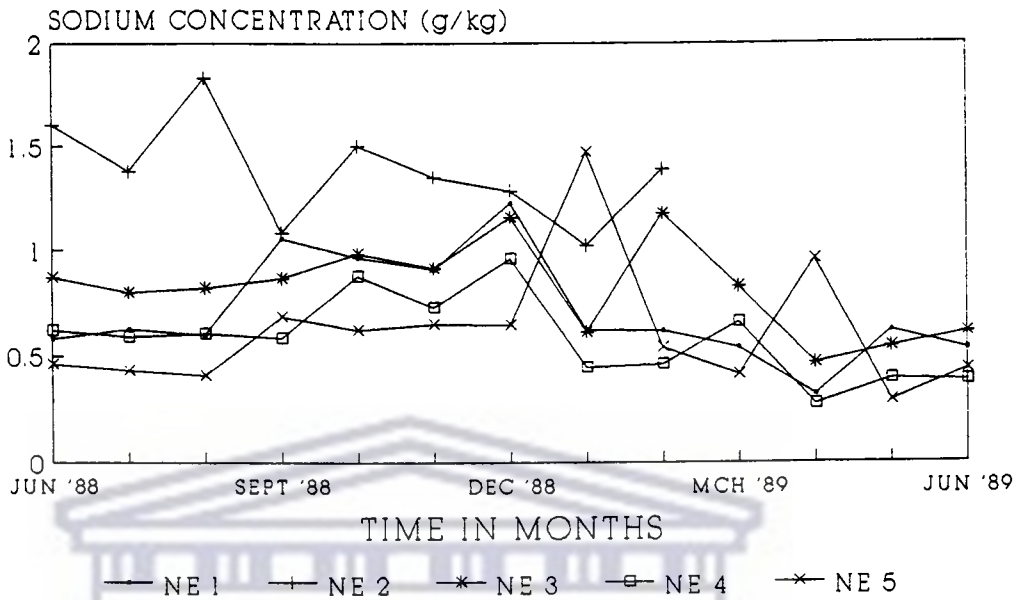

FIG. 3.10 ANNUAL VARIATION OF Na IN PINE BARK ALONG A POLLUTION GRADIENT

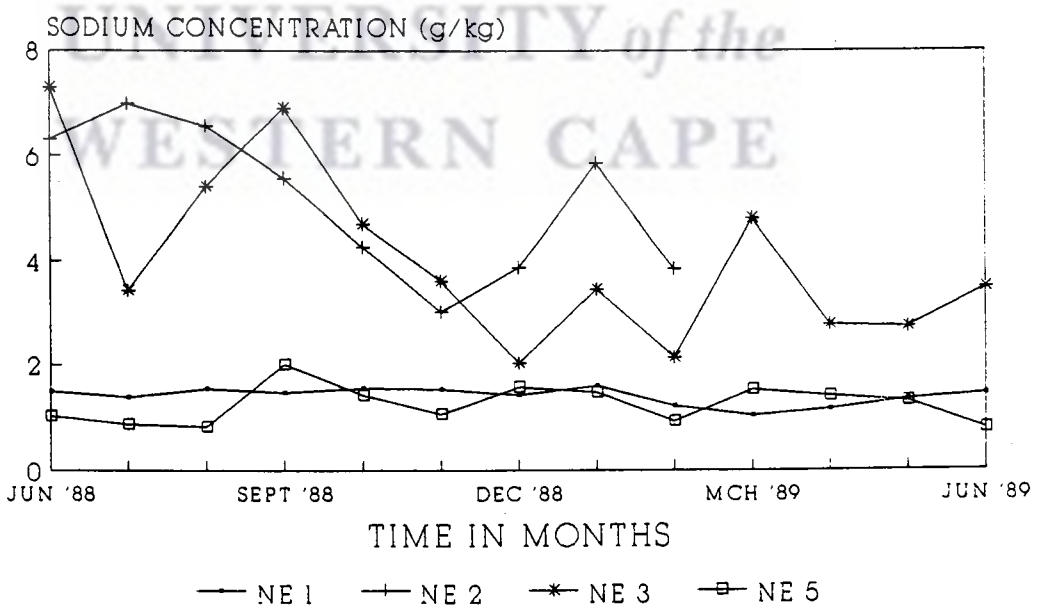

FIG. 3.11 ANNUAL VARIATION OF Na IN PINE NEEDLES ALONG A POLLUTION GRADIENT

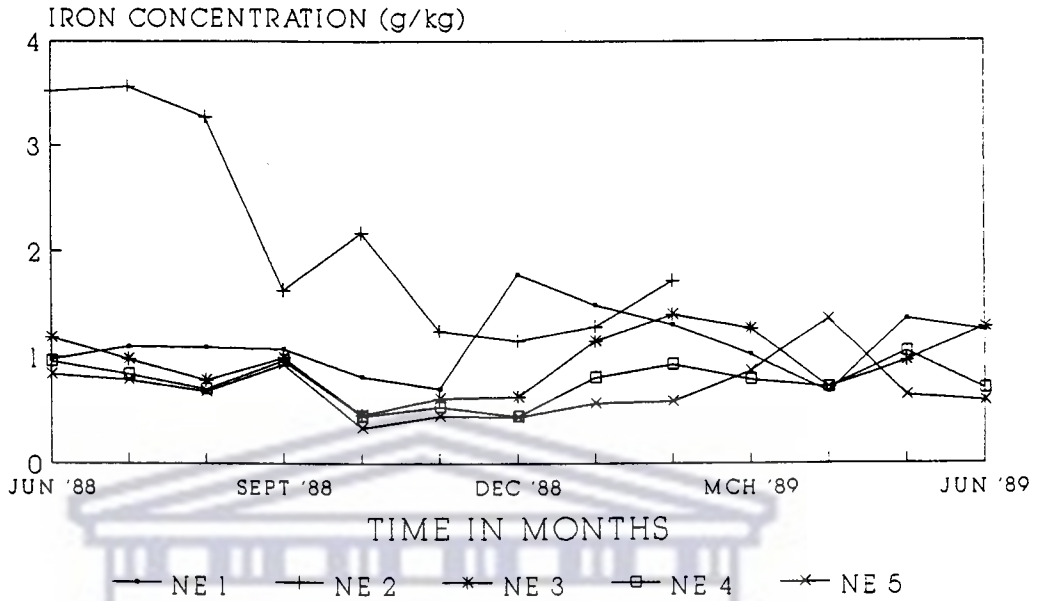

FIG. 3.12 ANNUAL VARIATION OF Fe IN PINE BARK ALONG A POLLUTION GRADIENT

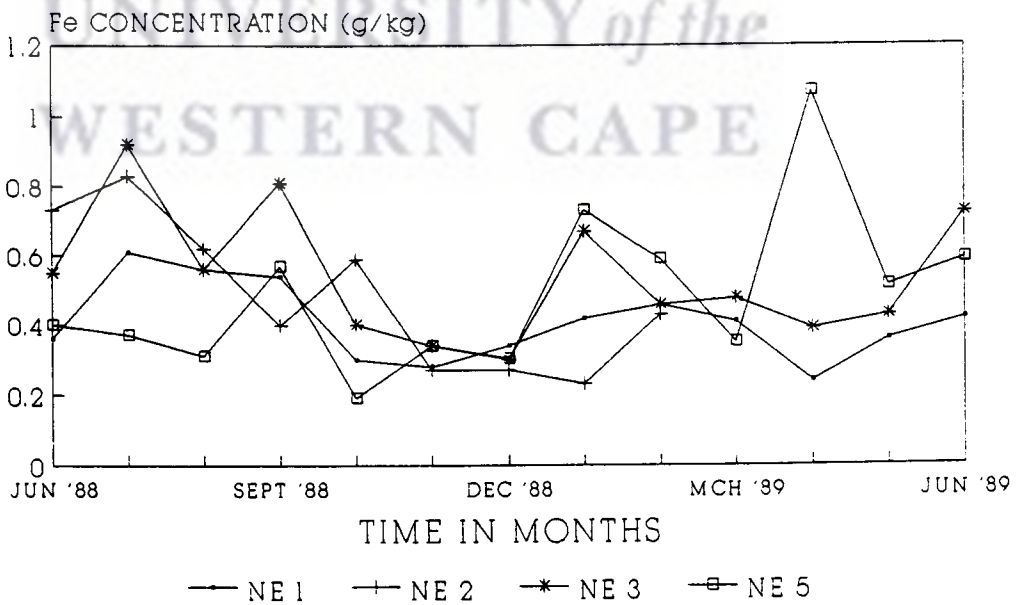

FIG. 3.13 ANNUAL VARIATION OF Fe IN PINE NEEDLES ALONG A POLLUTION GRADIENT

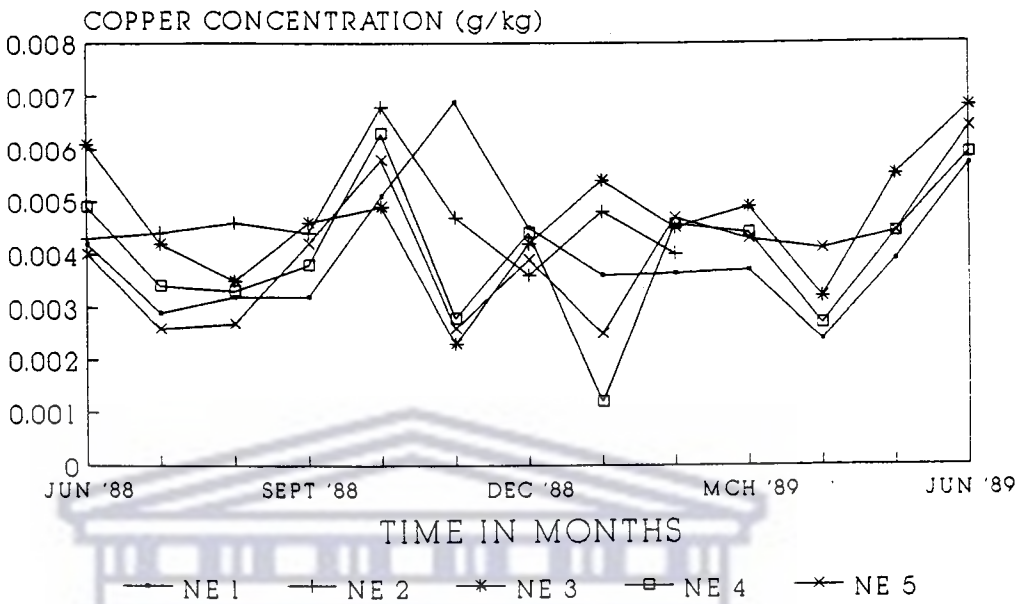

FIG. 3.14 ANNUAL VARIATION OF Cu IN PINE BARK ALONG A POLLUTION GRADIENT

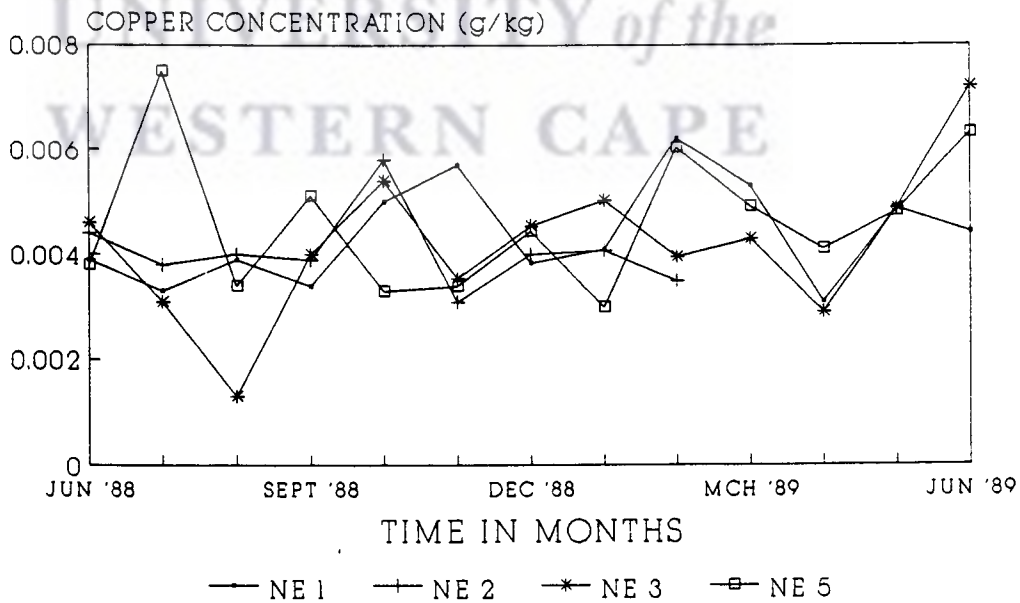

FIG. 3.15 ANNUAL VARIATION OF Cu IN PINE NEEDLES ALONG A POLLUTION GRADIENT

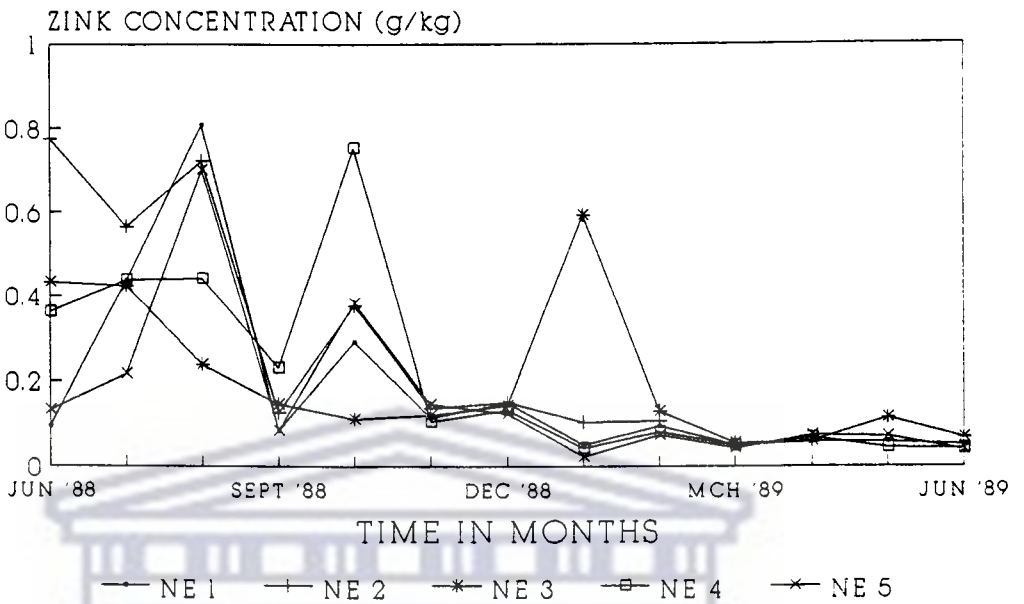

FIG. 3.16 ANNUAL VARIATION OF Zn IN PINE BARK ALONG A POLLUTION GRADIENT

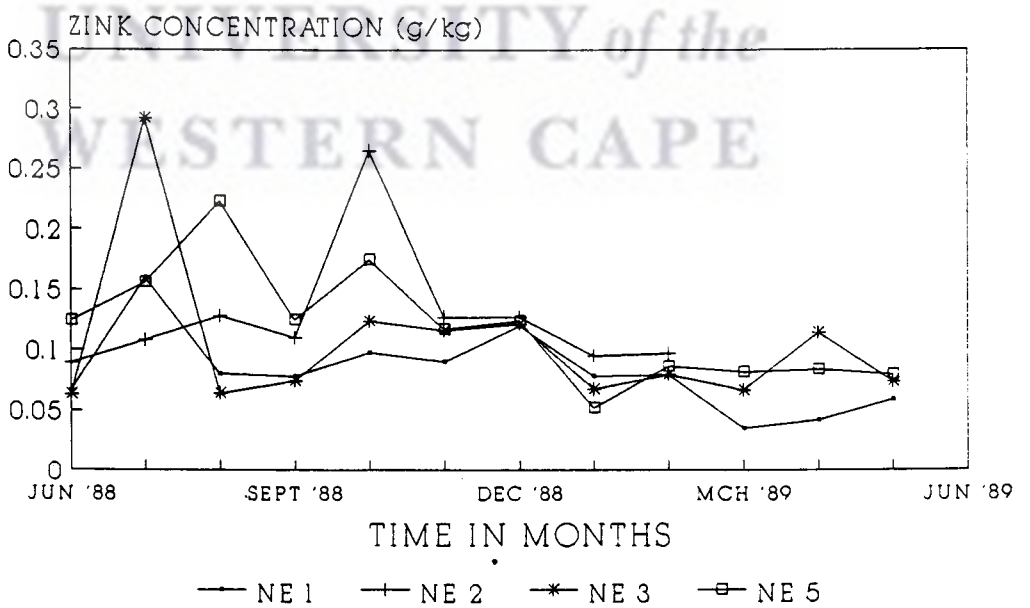

FIG. 3.17 ANNUAL VARIATION OF Zn IN PINE NEEDLES ALONG A POLLUTION GRADIENT

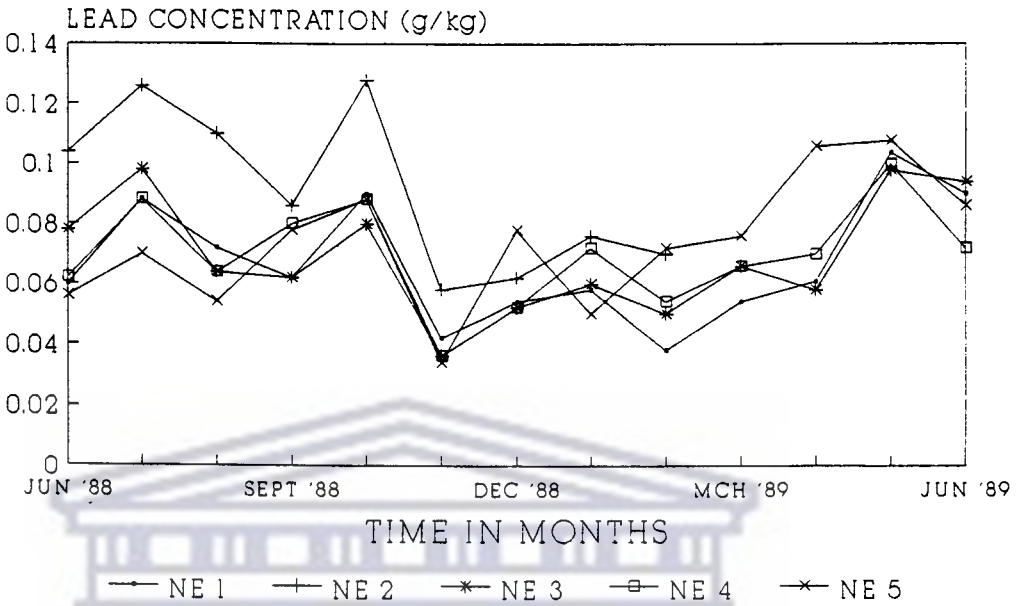

FIG. 3.18 ANNUAL VARIATION OF Pb IN PINE BARK ALONG A POLLUTION GRADIENT

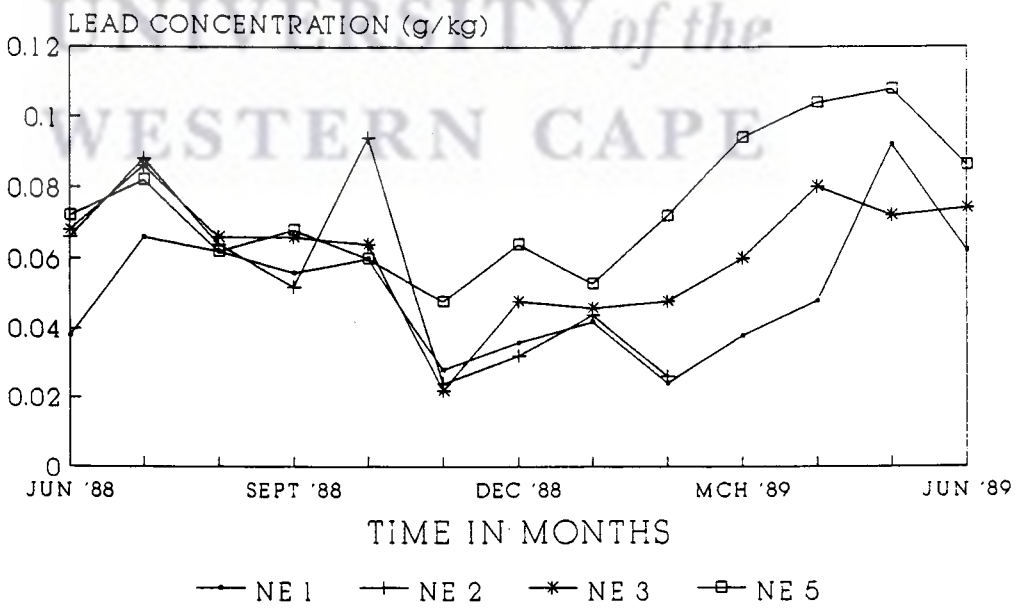

FIG. 3.19 ANNUAL VARIATION OF Pb IN PINE NEEDLES ALONG A POLLUTION GRADIENT

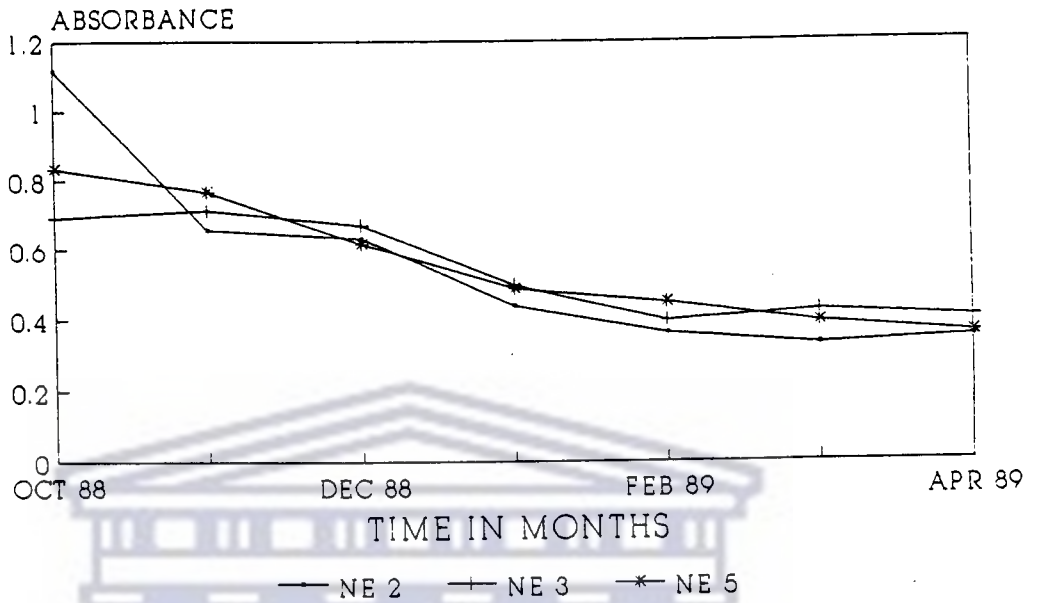

FIG. 3.20 ABSORPTION OF LIGHT BY CHLOROPHYLL PIGMENTS AT 435 nm

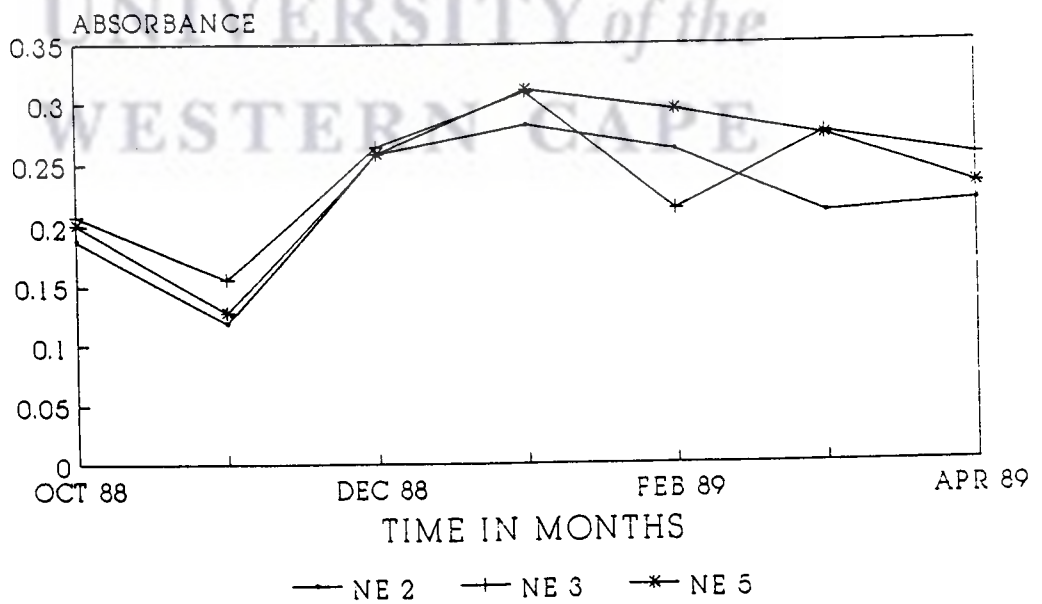

FIG. 3.21 ABSORPTION OF LIGHT BY CHLOROPHYLL PIGMENTS AT 665 nm

pH OF PINE BARK

	JUN 88		SEPT 88		DEC 88				MAR '89			JUN 89
NE 1	3.82	3.87	4.11	3.55	3.56	3.66	3.42	3.73	3.7	3.84	3.94	3.86	3.98
NE 2	3.87	3.9	3.96	3.51	3.81	3.64	3.61	3.67	3.65				
NE 3	3.66	3.62	3.62	3.58	3.36	3.55	3.73	3.71	3.69	3.96	3.98	3.59	4.01
NE 4	3.74	3.39	3.79	3.5	3.5	3.58	3.65	3.67	3.65	3.92	4.08	3.75	3.87
NE 5	3.44	3.08	3.61	3.52	3.49	3.35	3.4	3.36	3.51	3.73	3.92	3.57	3.57

FLUORIDE CONTENT OF PINE BARK

	JUN '88		SEPT '88		DEC '88				MCH '89			JUN '89
NE 1	103	145.2	150.2	261.2	212.2	233	356.8	274.4	163.6	170.5	105	219.4	186.4
NE 3	59.8	38.4	50.4	52.8	49.8	49	83.2	114.6	133.2	21	45.8	105.3	77.4
NE 5	43.9	29.4	25.8	91.6	48.4	50	51.2	31.8	44.2	76.2	127.4	41.6	68

SULPHATE CONTENT OF PINE BARK

	JUN '88		SEPT '88		DEC '88				MAR '89			
					FIG.3.4a							
NE 1	0.226	0.446	0.23	0.132	0.396	0.711	0.647	0.539	0.235	0.387	0.097	0.58
NE 3	0.255	0.195	0.118	0.172	0.152	0.361	0.248	0.256	0.229	0.193	0.232	0.381
NE 5	0.114	0.107	0.823	0.098	0.288	0.305	0.399	0.236	0.0219	0.184	0.133	0.152

SULPHUR CONTENT OF PINE BARK

	JUN '88		SEPT '88		DEC '88				MAR '89			
					FIG.3.4a							
NE 1			0.087	0.093	0.07	0.098			0.07		0.084	
NE 3			0.063	0.047	0.065	0.052			0.038		0.05	
NE 5			0.079	0.096	0.065	0.094			0.053		0.059	

LIGHT ABSORPTION BY PINE NEEDLE PHOTOSYNTHETIC PIGMENTS AT 435nm

	OCT '88		DEC '88		FEB '89		APR '89
NE 2	1.117	0.659	0.634	0.439	0.361	0.329	0.347
NE 3	0.693	0.715	0.672	0.497	0.397	0.426	0.404
NE 5	0.833	0.768	0.617	0.489	0.45	0.392	0.358

LIGHT ABSORPTION BY PINE NEEDLE PHOTOSYNTHETIC PIGMENTS AT 665nm

	OCT '88		DEC '88		FEB '89		APR '89
NE 2	0.188	0.119	0.258	0.282	0.261	0.289	0.217
NE 3	0.208	0.156	0.264	0.309	0.213	0.274	0.254
NE 5	0.201	0.128	0.258	0.311	0.294	0.272	0.231

	JUN '88			SEPT '88			DEC '88			MCH '89			JUN '89
CALCIUM CONTENT OF PINE BARK													
NE 1	10.9	9.02	8.52	10.82	7.88	8.16	7.24	9.66	9.06	7.9	8.34	9.78	7.68
NE 2	9.78	11.98	8.24	7.16	9.68	6.26	6.44	7.88	6.92				
NE 3	6.12	7.56	8.56	8.94	7.86	6.44	6.88	6.82	9.5	7.1	6.16	6.58	7.28
NE 4	6.22	7.96	7.1	8.7	6.84	7.4	6.48	7.04	6.7	6.74	11.04	6.18	7.88
NE 5	4.9	5.62	5.3	6.96	6.6	5.42	5.76	6.34	6.18	6.08	6.54	5.72	4.94
CALCIUM CONTENT OF PINE NEEDLES													
NE 1	7.58	5.38	5.88	7.72	6.4	6.84	5.88	6.36	5.78	4.62	5.08	5.56	4.22
NE 2	8.9	10.38	10.22	5.92	8.1	8.02	6.82	5.82	5.6				
NE 3	6.36	6.66	8.54	6.18	6.84	5.62	6.32	6.94	5.88	5.48	7.02	5.68	6.62
NE 4													
NE 5	7.58	9.76	8.24	9.36	7.24	7.88	6.01	7.5	6.6	6.84	8.12	7.58	5.1

	JUN '88			SEPT '88			DEC '88			MCH '89			JUN '89
MAGNESIUM CONTENT OF PINE BARK													
NE 1	1.13	0.63	0.54	0.73	0.68	0.65	1.006	0.63	0.92	0.814	0.59	0.62	0.66
NE 2	0.77	1.91	1.03	0.96	0.84	0.81	0.79	1.09	1.34				
NE 3	0.64	2.17	0.53	0.56	0.44	0.55	0.69	0.97	0.88	0.67	0.36	0.45	0.58
NE 4	0.62	0.75	0.57	0.69	0.78	0.624	0.52	0.55	0.46	0.53	0.5	0.582	0.43
NE 5	0.54	0.61	0.44	0.54	0.71	0.642	0.68	0.584	0.61	0.59	0.66	0.42	0.41
MAGNESIUM CONTENT OF PINE NEEDLES													
NE 1	1.49	0.94	1.45	1.602	1.36	1.36	0.96	1.37	0.9	1.08	0.998	0.93	1.02
NE 2	1.67	0.83	2.61	1.83	1.83	1.37	1.57	1.45	1.95	2.03			
NE 3	1.97	3.05	1.87	1.58	1.58	1.74	1.5	1.93	1.684	1.32	1.72	1.64	1.702
NE 4													
NE 5	1.57	1.79	1.36	1.43	1.43	1.6	1.47	1.61	1.66	1.47	2.02	2.41	2.04

	JUN '88			SEPT '88			DEC '88			MCH '89			JUN '89
POTASSIUM CONTENT OF PINE BARK													
NE 1	0.39	0.95	0.98	1.56	1.27	1.22	2.34	1.688	1.36	0.88	0.64	1.37	1.07
NE 2	1.002	2.18	2.84	1.63	2.12	1.46	1.58	2.61	1.998				
NE 3	0.58	1.65	1.296	1.51	1.33	1.34	1.54	1.82	2.05	1.51	1.104	1.45	1.47
NE 4	0.36	0.53	0.798	1.008	0.84	0.95	0.94	1.054	1.054	0.98	0.99	0.79	0.65
NE 5	0.58	0.62	1.11	1.05	0.88	0.99	1.24	1.05	1.43	0.69	1.82	0.802	0.896
POTASSIUM CONTENT OF PINE NEEDLES													
NE 1		8.27	11.72	13.25	11.27	10.61	10.35	14.69	11.796	11.85	11.9	11.95	9.29
NE 2		8.39	9.196	8.39	10.92	10.82	10.69	13.48	10.31				
NE 3		10.77	9.02	8.82	9.77	10.35	12.48	12.31	11.9	10.81	8.896	10.85	9.22
NE 4													
NE 5		11.89	11.07	10.44	10.99	11.61	11.22	11.44	11.19	10.44	9.26	10.3	10.44

	JUN '88			SEPT '88			DEC '88			MCH '89			JUN '89
SODIUM CONTENT OF PINE BARK													
NE 1	0.582	0.626	0.598	1.056	0.966	0.908	1.226	0.62	0.618	0.538	0.314	0.616	0.53
NE 2	1.602	1.38	1.832	1.082	1.504	1.352	1.286	1.022	1.39				
NE 3	0.87	0.802	0.824	0.868	0.982	0.914	1.156	0.61	1.178	0.83	0.466	0.542	0.608
NE 4	0.62	0.59	0.606	0.584	0.878	0.728	0.958	0.444	0.46	0.66	0.27	0.368	0.378
NE 5	0.46	0.43	0.486	0.684	0.62	0.646	0.644	1.472	0.54	0.412	0.958	0.284	0.434
SODIUM CONTENT OF PINE NEEDLES													
NE 1	1.498	1.396	1.544	1.478	1.56	1.534	1.43	1.602	1.224	1.04	1.16	1.35	1.436
NE 2	6.308	6.996	6.558	5.574	4.278	3.02	3.868	5.85	3.848				
NE 3	7.298	3.442	5.424	6.984	4.72	3.616	2.03	3.456	2.148	4.82	2.77	2.738	3.486
NE 4													
NE 5	1.826	0.87	0.828	2.01	1.426	1.066	1.574	1.472	0.932	1.53	1.402	1.304	0.79

	JUN '88			SEPT '88			DEC '88			MCH '89			JUN '89
IRON CONTENT OF PINE BARK													
NE 1	0.98	1.096	1.09	1.07	0.82	0.7	1.78	1.494	1.31	1.024	0.68	1.36	1.25
NE 2	3.52	3.56	3.28	1.63	2.17	1.24	1.15	1.29	1.72				
NE 3	1.18	0.98	0.79	0.99	0.46	0.61	0.63	1.15	1.402	1.27	0.72	0.96	1.28
NE 4	0.96	0.84	0.782	0.97	0.45	0.53	0.45	0.81	0.93	0.794	0.72	1.044	0.782
NE 5	0.84	0.79	0.68	0.93	0.34	0.45	0.44	0.57	0.59	0.87	1.36	0.64	0.59
IRON CONTENT OF PINE NEEDLES													
NE 1	0.36	0.61	0.56	0.54	0.3	0.28	0.34	0.42	0.46	0.41	0.24	0.36	0.42
NE 2	0.73	0.83	0.62	0.4	0.59	0.27	0.27	0.23	0.43				
NE 3	0.55	0.92	0.56	0.81	0.402	0.34	0.3	0.67	0.46	0.48	0.39	0.43	0.72
NE 4													
NE 5	0.4	0.37	0.31	0.57	0.19	0.34	0.304	0.73	0.59	0.35	1.07	0.514	0.59

	JUN '88			SEPT '88			DEC '88			MCH '89			JUN '89
COPPER CONTENT OF PINE BARK													
NE 1	0.0042	0.0029	0.0032	0.0032	0.0051	0.0069	0.0045	0.0036	0.00364	0.0037	0.0024	0.0039	0.0057
NE 2	0.0043	0.0044	0.0046	0.0044	0.0068	0.0047	0.0036	0.0048	0.004				
NE 3	0.0061	0.0042	0.0035	0.0046	0.0049	0.0023	0.0042	0.0054	0.0045	0.0049	0.0032	0.0055	0.0068
NE 4	0.0049	0.0034	0.0033	0.0038	0.0063	0.0028	0.0044	0.0012	0.00456	0.0044	0.0027	0.0044	0.0059
NE 5	0.004	0.0026	0.0027	0.0042	0.0058	0.0026	0.0039	0.0025	0.00468	0.00428	0.0041	0.00442	0.0064
COPPER CONTENT OF PINE LEAVES													
NE 1	0.0039	0.0033	0.0039	0.0034	0.005	0.0057	0.00384	0.0041	0.0062	0.0053	0.0031	0.00486	0.0044
NE 2	0.0044	0.0038	0.004	0.0039	0.0058	0.0031	0.004	0.00408	0.0035				
NE 3	0.0046	0.0031	0.0013	0.004	0.0054	0.00354	0.00454	0.00502	0.00396	0.0043	0.0029	0.00486	0.0072
NE 4													
NE 5	0.0038	0.0075	0.0034	0.0051	0.0033	0.0034	0.00444	0.003	0.00602	0.0049	0.0041	0.0048	0.0063

	JUN '88		SEPT '88		DEC '88			MCH '89			JUN '89	
ZINK CONTENT OF PINE BARK												
NE 1	0.094	0.437	0.089	0.0826	0.291	0.113	0.149	0.0498	0.094	0.05	0.058	0.057	0.0504
NE 2	0.774	0.566	0.723	0.125	0.376	0.136	0.15	0.103	0.106				
NE 3	0.432	0.424	0.237	0.146	0.11	0.12	0.143	0.596	0.128	0.055	0.059	0.114	0.066
NE 4	0.362	0.438	0.441	0.23	0.755	0.105	0.132	0.042	0.081	0.049	0.067	0.044	0.041
NE 5	0.131	0.216	0.702	0.084	0.383	0.145	0.123	0.0224	0.073	0.042	0.073	0.069	0.038
ZINK CONTENT OF PINE LEAVES													
NE 1	0.066	0.16	0.0798	0.078	0.098	0.0898	0.1198	0.078	0.079	0.035	0.042	0.059	
NE 2	0.069	0.108	0.128	0.11	0.265	0.127	0.127	0.095	0.097				
NE 3	0.063	0.292	0.064	0.074	0.124	0.116	0.121	0.067	0.079	0.066	0.114	0.073	
NE 4													
NE 5	0.124	0.156	0.223	0.125	0.175	0.117	0.124	0.052	0.086	0.081	0.083	0.079	

	JUN '88		SEPT '88		DEC '88			MCH '89			JUN '89	
LEAD CONTENT OF PINE BARK												
NE 1	0.06	0.088	0.072	0.062	0.09	0.042	0.054	0.058	0.038	0.054	0.061	0.104	0.09
NE 2	0.104	0.126	0.11	0.086	0.128	0.058	0.062	0.076	0.07				
NE 3	0.078	0.093	0.064	0.062	0.08	0.036	0.052	0.06	0.05	0.066	0.058	0.098	0.094
NE 4	0.062	0.088	0.064	0.08	0.088	0.036	0.052	0.072	0.054	0.066	0.07	0.1	0.072
NE 5	0.056	0.07	0.054	0.078	0.088	0.034	0.078	0.05	0.072	0.076	0.106	0.108	0.086
LEAD CONTENT OF PINE NEEDLES													
NE 1	0.038	0.066	0.062	0.056	0.06	0.028	0.036	0.042	0.024	0.038	0.048	0.092	0.062
NE 2	0.066	0.088	0.064	0.052	0.094	0.024	0.032	0.044	0.026				
NE 3	0.068	0.086	0.066	0.066	0.064	0.022	0.048	0.046	0.048	0.06	0.08	0.072	0.074
NE 4													
NE 5	0.072	0.092	0.062	0.068	0.06	0.048	0.064	0.053	0.072	0.094	0.104	0.108	0.086

CHAPTER 4

THE ACCUMULATION OF FLUORIDE AND SULPHATE BY *Chasmanthe* LEAVES ALONG A POLLUTION GRADIENT, AND ITS EFFECT ON THE MINERAL COMPOSITION

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4.1 INTRODUCTION

It is well established that air pollution does occur in the Stellenbosch area (Chapters 2 and 3), especially fluoride and sulphur dioxide pollution. Gladiolus and Chasmanthe are both members of the family Iridaceae (Dyer, 1976). It is thus possible that they could respond similarly to different pollutants by exhibiting characteristic symptoms, to especially F^- pollution. Because F^- is very soluble, it moves fairly easily in the transpiration stream. With the evaporation of water from the leaves, the fluoride tends to accumulate at the leaf tip and margins (Brewer et al., 1957). As a result of the toxic effect of the excess F^-, regions of die-back appear along the margins of the leaves.

The purpose of this study was to make use of plant species already growing in the Stellenbosch area, that are known to be sensitive to pollution (especially to F^-), and to use them as indicator plants. Numerous papers have been published, on the effect that F^- has on Gladiolus plants (Coulter et al., 1985, Brewer et al. 1966 and Hendrix et al. 1958). The correlation between leaf tip die-back and fluoride concentration will be discussed, as well as the influence of the major pollutants, F^- and SO_2, on the mineral composition of the Chasmanthe leaves.

4.2 MATERIALS AND METHODS

Due to the lack of Chasmanthe at all the sites, only the following four sites were used: South-east(SE) 2 was situated next to a road, that was not very much used; SE 4 was in the Stellenbosch University Botanical Garden; SE 6 was beside the driveway of a house and the Control Site was at the back of Coetzenburg Stadium (Fig.1.1). At each site there were between 5 and 10 plants, and a sample consisted of a mixture of leaves cut during a particular sampling period, meaning that only one sample per site was collected. The die-back (Fig.4.1) of 10 leaves was measured before it was dried.

For 1988, sampling was done during July and August. During 1989 however, sampling was done in May and June.

The material collected during 1988 formed the basis for this study, while the material collected during 1989 was used only to see if there was any difference in the F^- concentration of different sections of the leaves. Two sets of leaf material were collected, the first 7 cm from the tip of the leaf blade (t), and the next 7 cm below it (b).

4.2.1 Die-Back of the leaf tips and margins

Leaves were randomly chosen to measure the tip die-back. The leaves were initially cut of just below the region of die-back, or at a length of 10 cm during the 1988 sampling.

Sampling for 1988 was done during July and August. The region of die-back was then measured in the laboratory from the tip of the leaf to the end of the die-back region.

4.2.2 Elemental Analysis

The leaf material collected, was dried at 65°C for at least 5 days, before it was ground with a Wiley Intermediate Mill, to pass through a no.40 mesh sieve.

4.2.2.1 Fluoride

The fluoride concentration of the leaves was determined with a Selective Ion Electrode (Method B), as described by Anon (1980). This method was preferred to the ones reviewed by Cooke et al. (1976). For the extraction of F^-, 0.25 g of the material was weighed into a 100 cm^3 wide-mouthed plastic container. To this, 20 cm^3 0.05 M HNO_3 was added and the container placed on a magnetic stirrer with a Teflon-coated magnetic stirrer bar for 20 minutes. After this period, 20 cm^3 0.1 M KOH was added and the solution

further agitated for an additional 20 minutes. To this, 5.0 cm^3 sodium citrate solution containing 1 $mg.dm^{-3}$ F^- (adjusted pH 5.5) and 5.0 cm^3 0.2 N HNO_3 was added. The analysis performed on the material collected in 1989 was done to see if there was any difference in F^- concentration between the tip and basal sectionss.

4.2.2.2 Sulphate, Nitrate and Chloride

For the methods used in determining the above-mentioned substances in the leaves, see Chapter 2. The sample preparations for nitrate and chloride were the same as for sulphate, but the standards used to calibrate the HPIC were, 1.00 $mg.dm^{-3}$ and 0.30 $mg.dm^{-3}$ respectively.

4.2.2.3 Calcium, Magnesium, Potassium, Sodium, Iron, Copper, Zinc and Lead

Of the dried leaf material, 0.5 g was then weighed out accurately onto cigarette paper, enfolded within and then placed in a digestion tube, and 6 cm^3 of a $HCl:HNO_3$ (2:1) mixture added. Three glass beads were added to curb excessive bumping during heating. The digestion process was considered completed when a colourless liquid was obtained. Distilled-deionised water was then added, and the tubes left to cool. The resulting solution was then filtered through Schleicher and Schull #595 filter paper into 100 cm^3

volumetric flasks, and made up to volume. This material was then stored in brown plastic bottles till further use. The different elements, calcium, magnesium, potassium, sodium, iron, copper, zinc and lead, were then determined using a Pye Unicam SP 9 Atomic Absorption Spectrophotometer(Allen et al., 1986).

4.3 RESULTS AND DISCUSSION

4.3.1 Die-Back of the leaf Tips and Margins

As was expected (Fig.4.1), the lenght of the marginal die-back decreased highly significantly with increasing distance from the brickfield ($r(6)= -0.8437$, $p= 0.0085$). A general increase of the die-back region, over the sampling period was expected, seeing that the leaves were exposed for a longer period of time. The results obtained did imply this. A difference in the age of the leaves could explain the variable results that were obtained for SE 6. Work done by Hendrix et al. (1958), indicated that there was a difference in the sensitivity, amongst leaves on the same plant. To complete an experiment of this nature more accurately, one would need sufficient leaf material to be able to select leaves of the same age.

4.3.2 Elemental Analysis

4.3.2.1 Fluoride concentration of the leaves

Compton and Remmert (1960) referred to work done in 1952, where it was reported that only small amounts of fluoride are usually absorbed from the soil, and that an even smaller amount is transported to the leaves. The F^- concentration of the leaves collected at SE 2, for July and August 1988, is much greater than in the leaves collected from the other sites (Fig.4.2a). This was expected, seeing that the site is situated closest to the brickfield, a major source of F^- pollution (Davies, 1980). A significant negative correlation was found to exist between the F^- concentration in the leaves with distance ($r(6)= 0.8363$; $p= 0.097$). A highly significant positive correlation existed between the marginal die-back and the F^- concentration of the leaves ($r(6)= 0.8552$, $p= 0.0068$), as was found in Gladiolus by Compton and Remmert (1960). A higher F^- value was expected for the samples collected during August (SE 2) than the one recorded during July 1988 (Brewer et al, 1957), but the decrease in F^- concentration could be attributed to the age of the leaves sampled (Hendrix et al., 1958). Another possible explanation, is that the fluoride has bound to the Ca of the leaves, rendering it insoluble, thus recording a lower value on extraction (Mejstrik, 1985).

Leaves collected during May and June 1989, and analysed for F^-, indicated that the top 7 cm(t) of the blade had a higher F^- concentration than the following 7 cm (b) (Fig.4.3b) as was found by other researchers (Compton and Remmert, 1960). More F^- accumulated during June 1989 than during May 1989.

4.3.2.2 Sulphate

Coal is burnt, at the brickfield to bake the bricks. From the burning process, sulphur dioxide gas is set free, which is taken up by plants. In the plant, SO_2 can be converted to sulphate (Mellanby, 1980). The sulphate values reflected in Figure 4.3, show the expected pattern of distribution. The samples collected at SE 2 shows the highest SO_4^{2-} value for both months, with a gradual decline towards the control site (Figure 4.3). The decrease was found to be significant ($t(6)= 0.7154$; $p= 0.046$). This was expected, seeing that the brickfield was the main source of SO_2.

4.3.2.3 Chloride

The chloride content of the Chasmanthe leaves at SE 2 was significantly lower than that of the control site ($t(6)= 3.645$; $p= 0.0356$) (Fig.4.4). Gauch (1972) found that chloride is closely linked to the chloroplasts as chlorine was the most recently confirmed essential element for higher

plants. From the work surveyed by Marschner (1986), it is apparent that chloride is preferentially accumulated in the chloroplasts. Bidwell (1979) refers to the work of Arnon, who demonstrated an absolute requirement for chloride ions in the photosynthesis of isolated chloroplasts. There was a significant correlation between the decreasing F^- concentration and the increasing chloride concentration with distance from the pollution ($t(14)= 2.760$; $p= 0.0281$). Keeping in mind that Chang and Thompson (1966) found that F^- also has the tendency to accumulate in the chloroplasts, one would expect a higher chloride content in the healthier leaves (further away from the brickworks) than was found.

4.3.2.4 Nitrate

Fig.4.5 indicates what appears to be an increase in the nitrate content of the leaves when moving away from the brickfield. The higher nitrate content of the leaves at SE 2 for July and August, and SE 4 for August could be attributed to a difference in age of the leaves. When looking at the various sites of collection, the plants at the control site grew in the shade whilst the other sites were relatively exposed. Work surveyed by Marschner (1986), revealed that nitrate reductase activity is lower under low light intensity. Could this perhaps have been responsible for the relatively high nitrate level at the control site?

A greater number of replicates could have shed more light on the results that were obtained.

4.3.2.3.1 Calcium

Calcium is an important structural component of cell walls, and can affect the permeability of the cell membranes (Bidwell, 1979, Raven et al., 1986 and Salisbury and Ross, 1985). It was thus expected that Ca^{2+} would be present in quite high amounts seeing that new cell formation and expansion actively takes place in the top part of the leaves. A lower Ca^{2+} concentration was expected in the leaves at SE 2, compared to that of the control site (Fig.4.6), due to the possibility of Ca^{2+} being less freely available in the polluted zone because of its bonding with F^- (Mejstrik, 1985). The trend of Ca^{2+} increasing in the leaf material on moving away from the brickfield (Chapter 2 and 3), was not clearly observed in the Chasmanthe leaves. The difference in age could probably have played a role in the results obtained.

4.3.2.3.2 Magnesium

Mg^{2+} is not as immobile in the phloem as Ca^{2+} (Salisbury and Ross, 1985). The results recorded in section 4.3.2.3.1 (Ca^{2+}) were an indication of what could be expected for July 1988 (Fig.4.7). The values recorded for August 1988, however were more what was expected due to the influence of F^-. A significant positive correlation was found to exist

between the Mg^{2+} concentration data with distance from the brickworks (r(6)= 0.7038; p= 0.0514). Chang and Thompson (1966) found that up to 60% of the F^- absorbed by Naval orange leaves, accumulated in the chloroplasts, thus impairing its proper functioning. This could be related to the fact that Mg^{2+} has similar ionic properties to Ca^{2+}, and would thus be affected similarly by F^- (Mejstrik, 1985). Less active chlorophyll molecules would be available for photosynthesis. A possible explanation for the high Mg^{2+} concentration at SE 2 for July, could be that the F^- had not yet reacted with the Mg^{2+}, or that the leaves sampled were more mature than that at the other sites (Compton and Remmert, 1960).

4.3.2.3.3 Potassium

The work reviewed by Gauch (1972), indicated that plants required K^+ in relatively large amounts as was found in this study (Fig.4.8). Work done by Hartt (1969), on sugarcane, revealed that a deficiency of K^+ decreased translocation of labelled photosynthate from the leaves to other portions of the plant. Taking into consideration that Chasmanthe is a geophyte, the plant will need to translocate its photosynthate to the corm. According to Fig.4.8, there were no major changes in the K^+ concentration of the leaves at the various sites.

4.3.2.3.4 Sodium

In the literature surveyed, it was found that Na^+ may partially substitute for K^+ (Gauch, 1972), and that it is mostly beneficial to halophytes (Bidwell, 1979 and Marschner, 1986). Figure 4.9 indicates that there is an increase in the Na^+ concentration as one moves away from the brickfield, with the leaves at the control site recording a significantly higher Na^+ value ($r(6)= 0.6628$; $p= 0.0732$). It was found that in the leaves of plants, in which a high proportion of K^+ was replaced by Na^+, the starch content is lower, but the content of soluble carbohydrates, particularly sucrose, is much higher. This will then favour both cell expansion and phloem transport to sinks such as storage roots of sugar beet (Hawker et al., 1974). This can perhaps also be the case with Chasmanthe.

4.3.2.3.5 Iron

The iron values of the leaves, collected at the different sites, show a decrease as one moves away from the brickfield (Fig.4.10). There was a significant drop in iron concentration at the various sites with increasing distance ($t(6)= -0.6395$; $p= 0.0877$), indicating that the brickfield probably was a major supplier of this metal in the area. If this was not the case, one would have expected a higher iron concentration in the more healthier leaves further away from the pollution source. Iron is essential in the formation of

chlorophyll, although it is not part of the molecule (Bidwell, 1979) and it is thus expected that the iron concentration might be high in the more healthy leaves.

4.3.2.3.6 Copper

The copper values recorded (Fig.4.11) seem to be constant throughout the sampling sites, and with time. The increase in copper for August, over July, could probably be attributed to the growing process of the leaves. Copper is present in the chloroplasts (Bidwell, 1979; Salisbury and Ross, 1985). A higher copper concentration in the healthier leaves, whose chloroplasts are not affected by the F^-, can be expected.

4.3.2.3.7 Zinc

Figure 4.12 indicates that there is an increase in zinc during August compared to July. There is also a gradual decrease in zinc concentration from SE 2 to the control site. Leaves of different ages could possibly be the reason for this pattern. According to Bidwell (1979), a Zn deficiency may result in a substantial increase in soluble nitrogen compounds. Comparing the values of SE 6 and the control site of Figures 4.4 and 4.12, there seems to be a correlation between the relatively low zinc concentration and the relatively high percentage nitrate present in the leaf material.

4.3.2.3.8 Lead

Due to the position of SE 2, next to a road, and SE 6, a relatively higher lead concentration in the leaves, at these sites, compared to the leaves from the other sites were expected (Fytianos et al., 1985). Purves (1985) states that lead contamination of soil from atmospheric pollution is undoubtedly the result of exhaust fumes. The results obtained (Fig.4.13) do not seem to indicate this. It can be that the plants are subjected to exhaust fumes for to short period of time. A larger number of replicates would certainly have given a clearer picture.

CONCLUSION

The results obtained suggest that atmospheric pollutants are harmful to the metabolism of Chasmanthe.

Coulter et al. (1985) related HF concentration to leaf necrosis, so one can link the amount of marginal die-back directly with the amount of F^- absorbed by the plant (Fig. 4.1 and 4.2). The results confirm this relationship. Chasmanthe leaves also showed the existance of SO_2 pollution. From the results obtained by Raitt in 1978 (pers.comm.), it appears that the amount of F^- accumulated by the leaves did not increase dramatically.

The leaves collected at SE 2 recorded a higher SO_4^{2-} level than the rest of the sites.

Because Cl^- and F^- are so similar in their activity, and the results suggest that where F^- dominates, Cl^- decreases, and vice versa, it can be speculated that F^- replaces the Cl^- close to the main source of F^- pollution.

Although Ca^{2+} showed no response to the F^- pollution, the Mg^{2+} concentration in the leaves increased with distance from the polluted zone. This correlated well with the data obtained in Chapter's 2 and 3.

The brickfield apparently was the main source of iron in the area, with the concentrations recorded by the Chasmanthe leaves decreasing with distance from the brickfield.

In general the pollution did not adversely affect the mineral status of Chasmanthe that much. This could perhaps be related to the fact that new leaves are formed every year, while the old ones die off. Due to the lack of plant material, the results for the mineral status of the plants could not be verified, statistically. To do a detailed study of this nature, one has to grow your own plants in a pollutant free environment, and see to it that the plants

are of more or less the same physiological age. Enough plants should be grown to be subjected to pollution at various sites, so that leaves of the same level (position from base) could be used, and thus limiting the number of variables that could influence the results.

The indications are there that fluoride pollution occurs in the area and that the marginal necrosis is a direct result of it.

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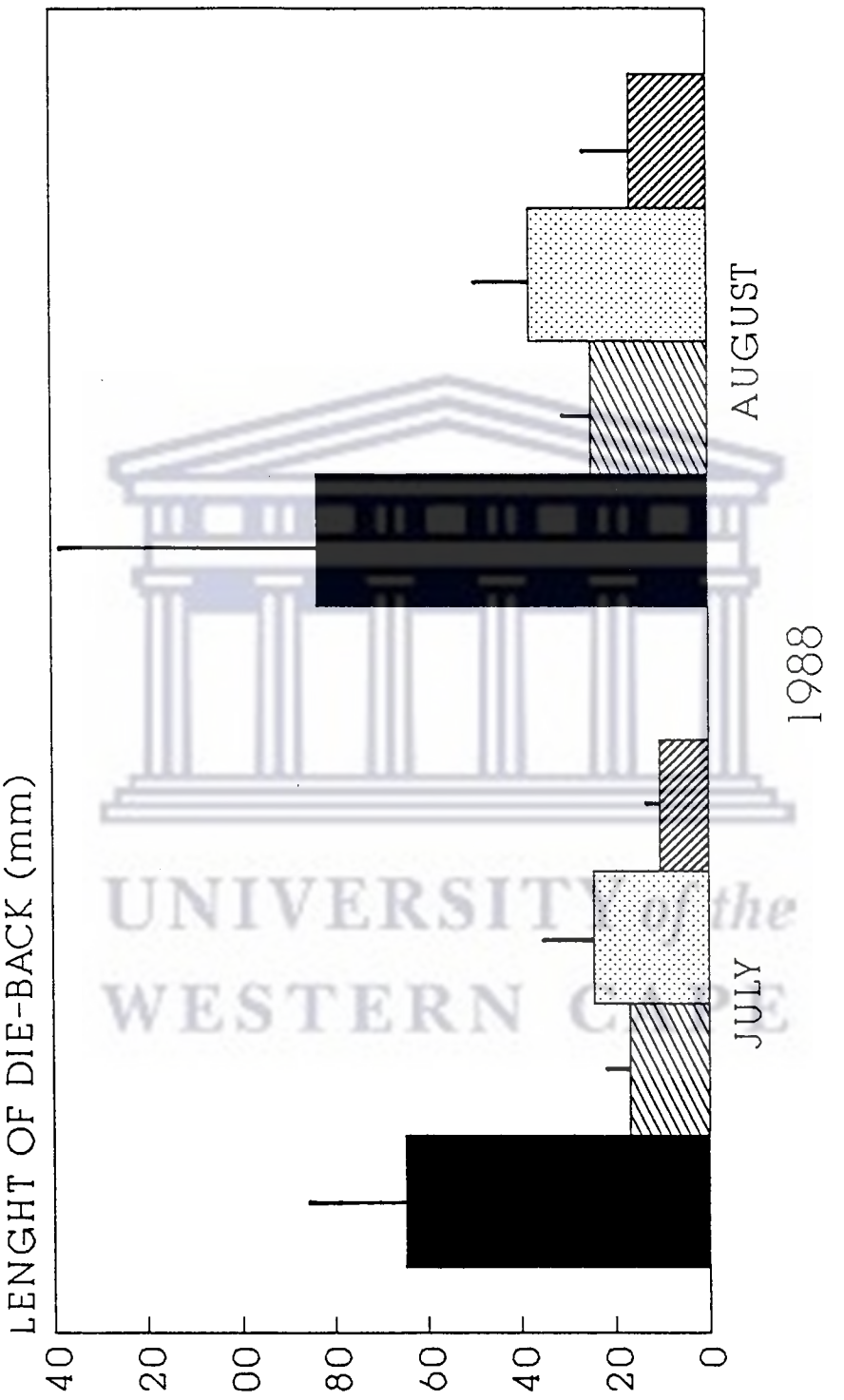


FIG.4.1 DIE-BACK OF Chasmanthe LEAVES ALONG A POLLUTION GRADIENT

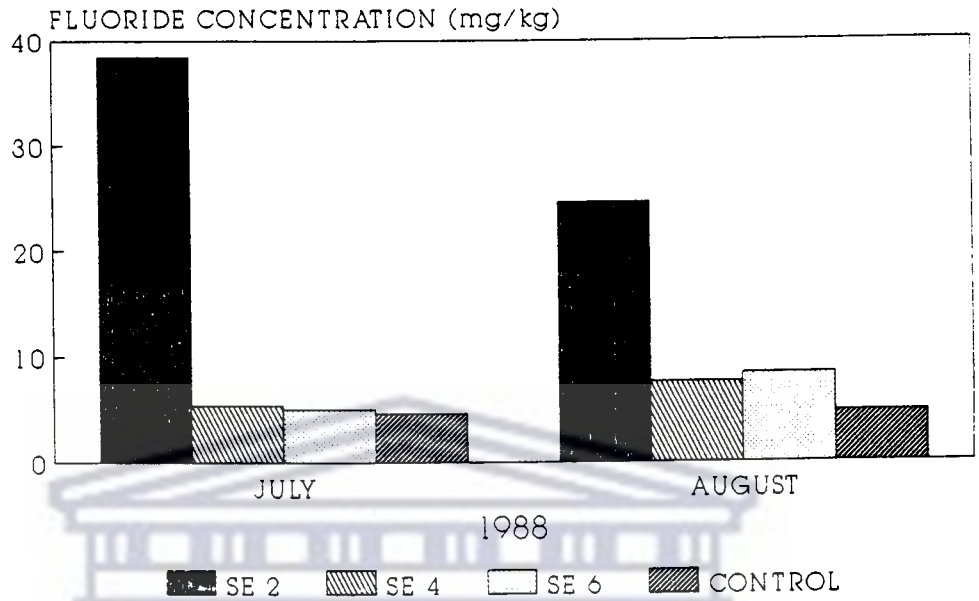
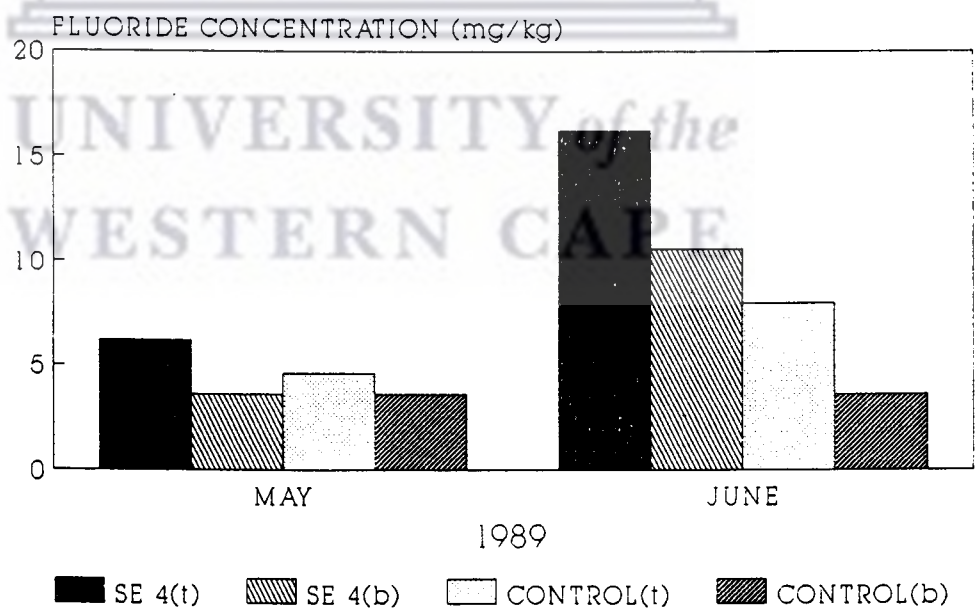


FIG. 4.2a FLUORIDE CONTENT OF Chasmanthe LEAVES ALONG A POLLUTION GRADIENT



t= tip 7cm; b= next 7cm

FIG. 4.2b FLUORIDE CONTENT OF Chasmanthe LEAF SEGMENTS ALONG A POLLUTION GRADIENT

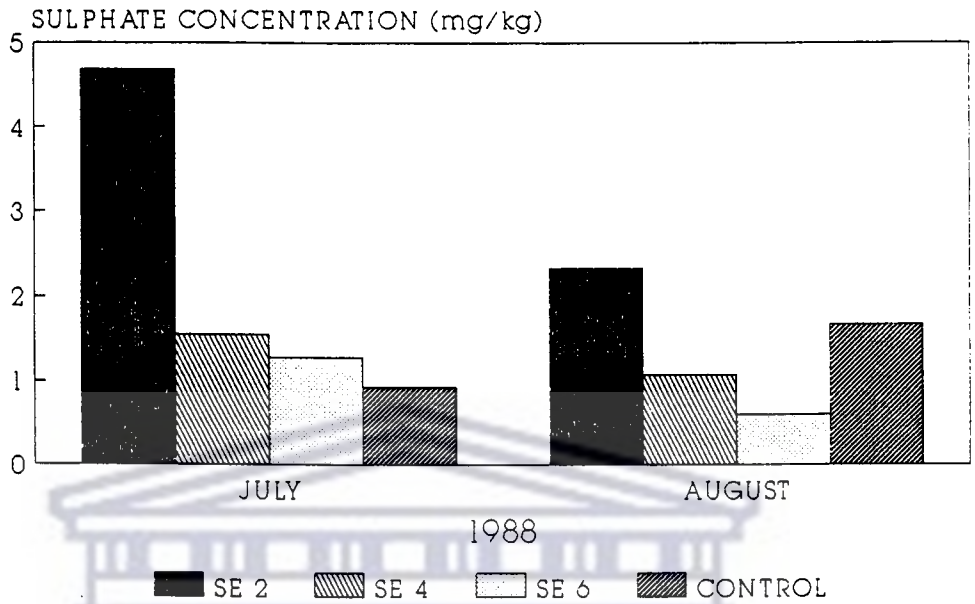


FIG. 4.3 SULPHATE CONTENT OF Chasmanthe LEAVES ALONG A POLLUTION GRADIENT

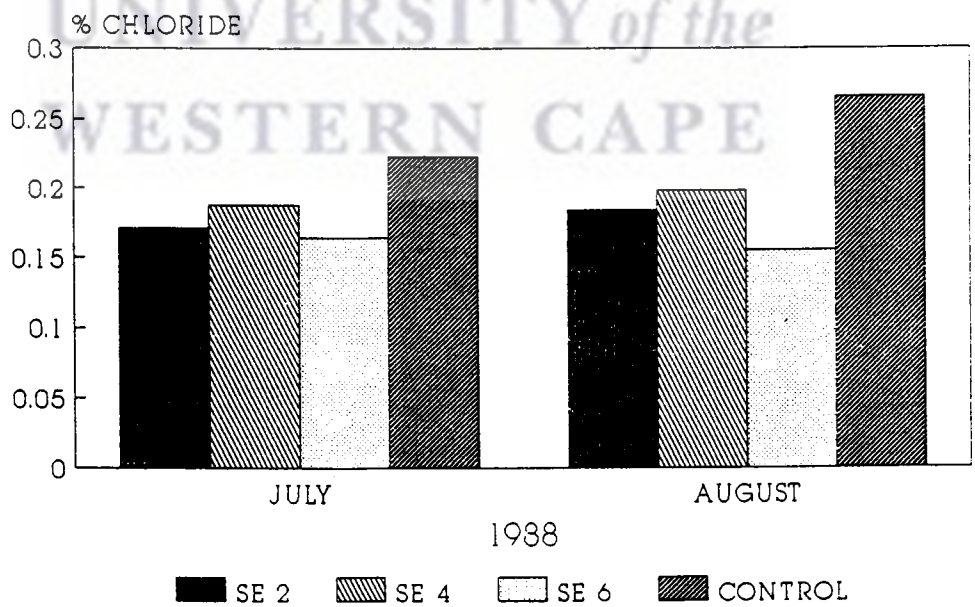


FIG. 4.4 CHLORIDE CONTENT OF Chasmanthe LEAVES ALONG A POLLUTION GRADIENT

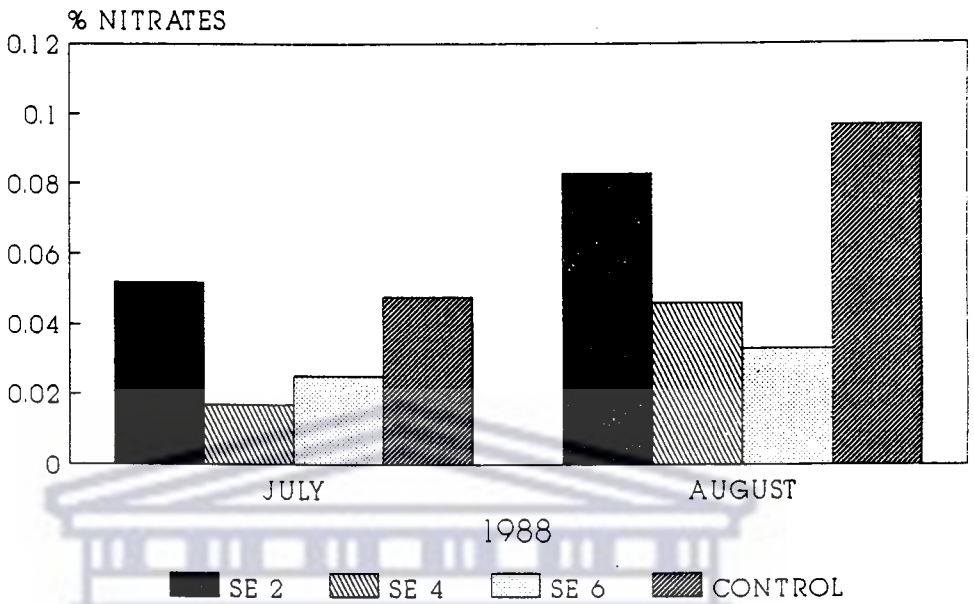


FIG. 4.5 NITRATE CONTENT OF Chasmanthe LEAVES ALONG A POLLUTION GRADIENT

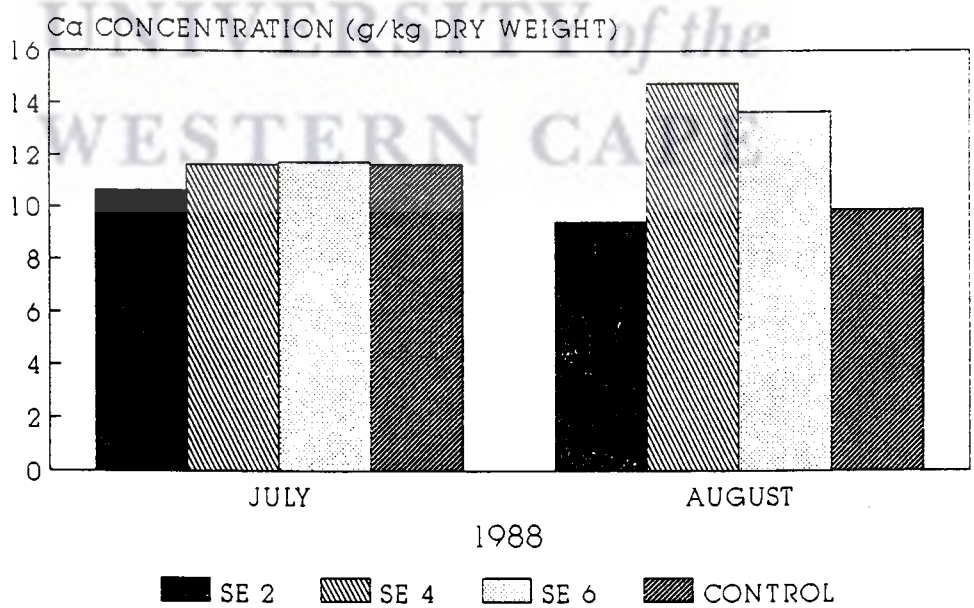


FIG. 4.6 CALCIUM CONTENT OF Chasmanthe LEAVES ALONG A POLLUTION GRADIENT

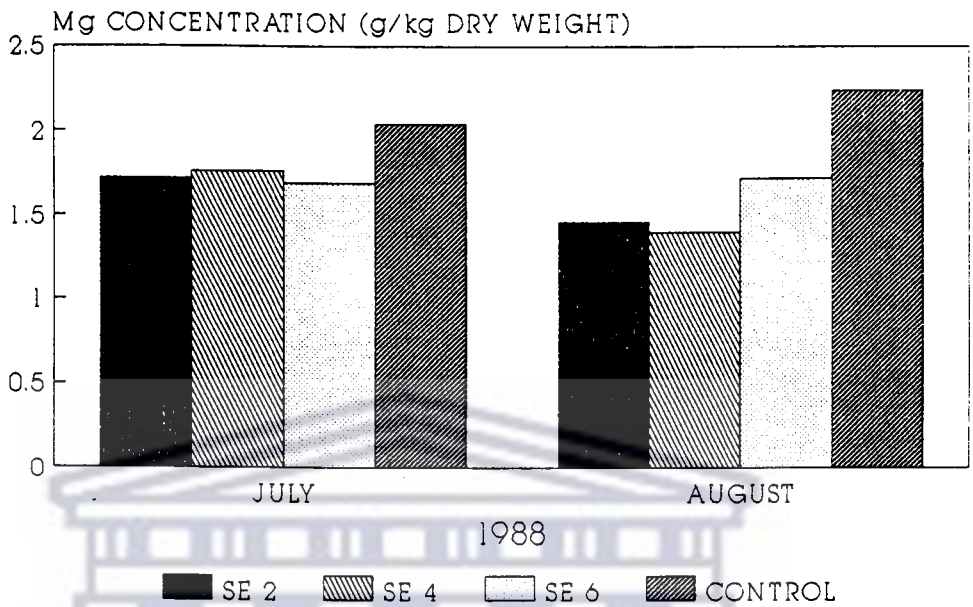


FIG. 4.7 MAGNESIUM CONTENT OF Chasmanthe LEAVES ALONG A POLLUTION GRADIENT

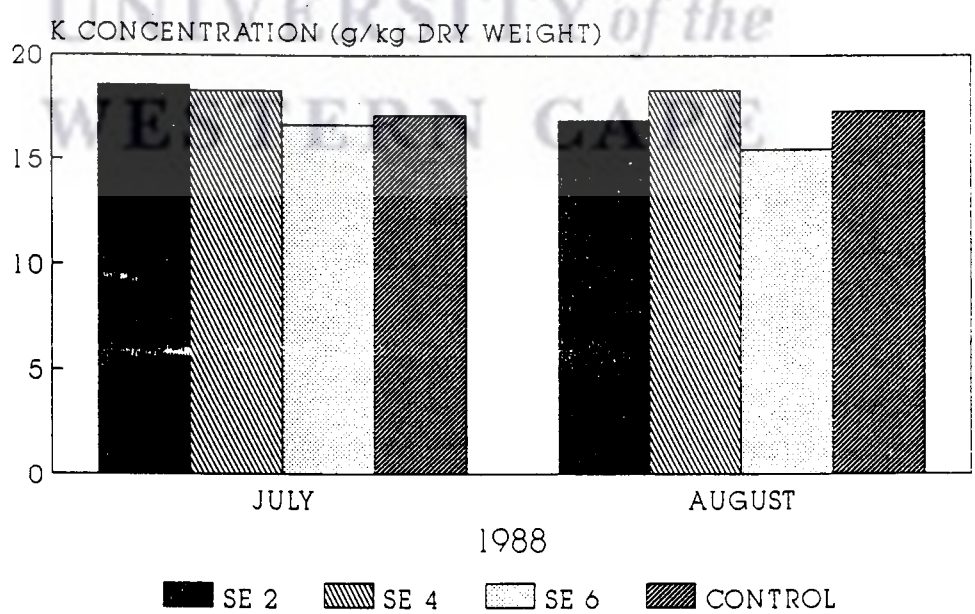


FIG. 4.8 POTASSIUM CONTENT OF Chasmanthe LEAVES ALONG A POLLUTION GRADIENT

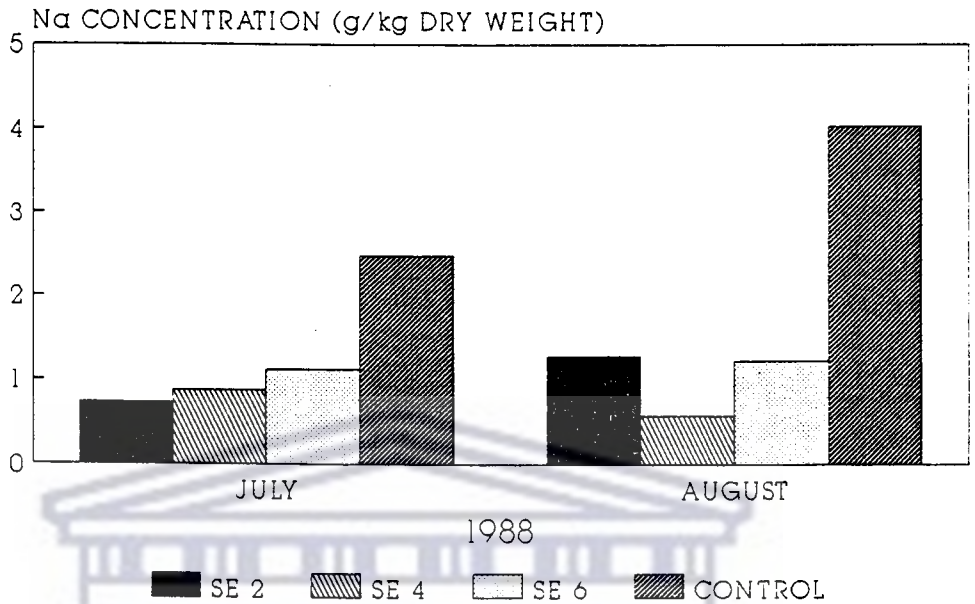


FIG. 4.9 SODIUM CONTENT OF Chasmanthe LEAVES ALONG A POLLUTION GRADIENT

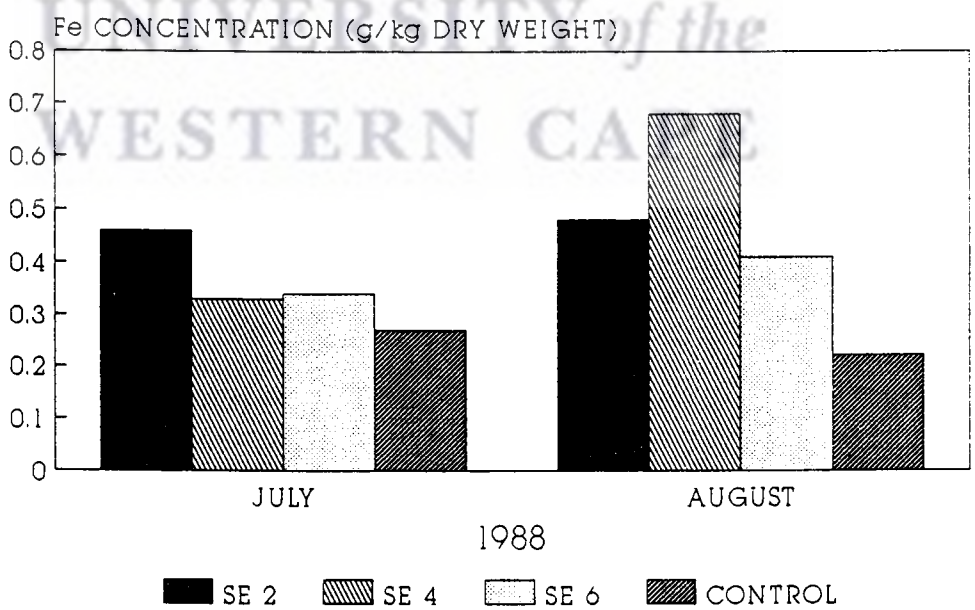


FIG. 4.10 IRON CONTENT OF Chasmanthe LEAVES ALONG A POLLUTION GRADIENT

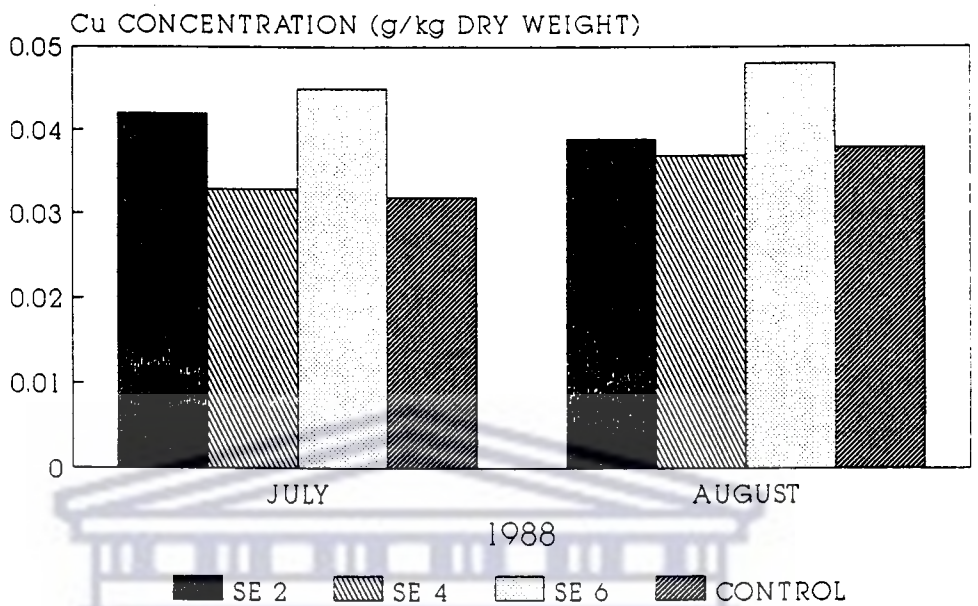


FIG. 4.11 COPPER CONTENT OF Chasmanthe LEAVES ALONG A POLLUTION GRADIENT

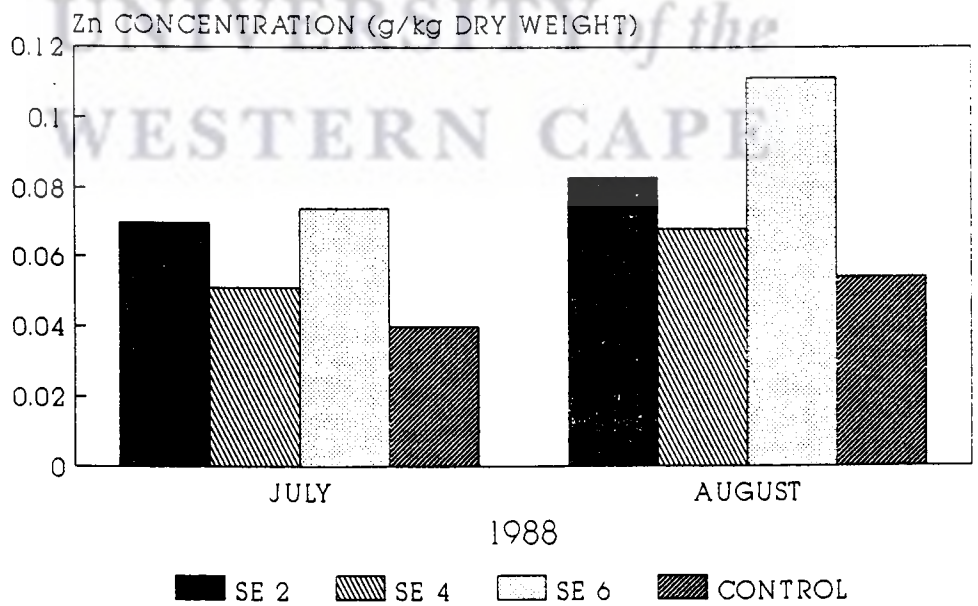


FIG. 4.12 ZINK CONTENT OF Chasmanthe LEAVES ALONG A POLLUTION GRADIENT

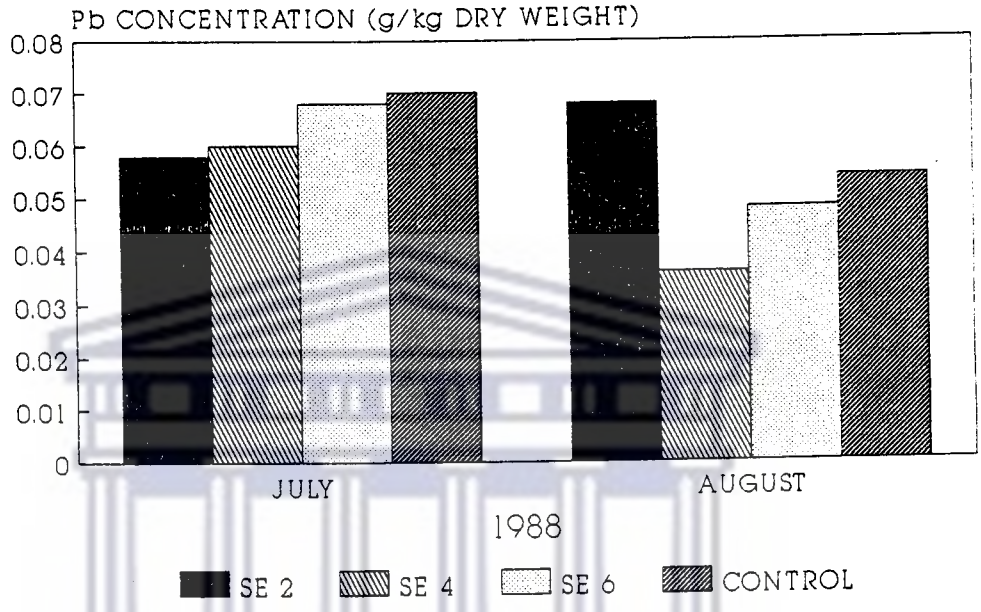


FIG. 4.13 LEAD CONTENT OF Chasmanthe LEAVES ALONG A POLLUTION GRADIENT

DIE-BACK OF CHASMANTHE LEAVES

	JUL '88	AUG '88
SE 2	64.7	83.4
SE 4	16.9	24.85
SE 6	24.7	37.65
CONTROL	10.4	16.5

FLUORIDE CONCENTRATION OF CHASMANTHE LEAF SEGMENTS ---- 1989

t= tip segment ; b= next segment

	MAY	JUNE
SE 4(t)	6.2	16.2
SE 4(b)	3.6	10.6
CONTR(t)	4.6	8
CONTR(b)	3.6	3.6

ELEMENTAL CONTENT OF CHASMANTHE LEAVES FOR 1988

	FLUORIDE		SULHATE		CHLORIDES		NITRATE		CALCIUM		MAGNESIUM	
	JULY	AUGUST	JULY	AUGUST	JULY	AUGUST	JULY	AUGUST	JULY	AUGUST	JULY	AUGUST
SE 2	38.4	24.6	4.686	2.329	0.172	0.184	0.852	0.883	10.61	9.42	1.72	1.46
SE 4	5.4	7.6	1.549	1.878	0.188	0.198	0.817	0.846	11.64	14.77	1.76	1.4
SE 6	5	8.4	1.277	0.602	0.164	0.155	0.825	0.833	11.74	13.66	1.69	1.72
CONTROL	4.6	4.8	0.928	1.673	0.223	0.266	0.848	0.897	11.63	9.86	2.84	2.24

	POTASSIUM		SODIUM		IRON		COPPER		ZINK		LEAD	
	JULY	AUGUST	JULY	AUGUST	JULY	AUGUST	JULY	AUGUST	JULY	AUGUST	JULY	AUGUST
SE 2	18.56	16.85	0.73	1.28	0.46	0.48	0.842	0.839	0.87	0.883	0.858	0.868
SE 4	18.3	18.33	0.88	0.57	0.33	0.68	0.833	0.837	0.851	0.868	0.86	0.836
SE 6	16.64	15.45	1.12	1.22	0.34	0.41	0.845	0.848	0.874	0.111	0.868	0.848
CONTROL	17.09	17.28	2.49	4.84	0.27	0.22	0.832	0.838	0.84	0.854	0.87	0.854

CHAPTER 5

THE RESPONSE OF Ramalina AND Parmelia TO FLUORIDE AND SULPHUR DIOXIDE POLLUTION ALONG A POLLUTION GRADIENT

INDEX

Page

5.1 INTRODUCTION

In areas where correlations with pollutant levels have been established, lichen patterns can be taken as estimates of the level of particular pollutants (Hawksworth and Rose, 1976). The lichen's poikilohydrous nature facilitates the accumulation of high levels of dissolved sulphur dioxide and therefore it is not surprising that they are among the most sensitive plants (Richardson, 1981). Therefore they have been widely used as biological indicators of SO_2 pollution (Hällgren and Huss, 1975; Hawksworth, 1971). In addition to SO_2, lichens may also be used as indicator plants for Hydrogen Fluoride (HF) and fluorine pollution. Davies (1982) concluded that the damage exhibited by Xanthoria parientina could only be attributed to too high fluoride concentrations, seeing that it was growing close to a brickworks. The variety of biological parameters used to assess pollution damages to lichens, include photosynthesis (Puckett et al. 1973) and chlorophyll degradation (Ronen and Galun, 1984).

Lichens occupy a special place in the plant world, due to the fact that each lichen is a double organism comprising a fungus and one or sometimes two algae. The fungus and the

algae are generally assumed to live symbiotically although there is some debate about possible parasitism on the part of the fungus. Since the fungi have no chlorophyll, the algae alone are responsible for photosynthesis (Skye, 1979).

In the Stellenbosch study area, lichens are mainly found on the bark of the Oak trees that grow there. The trees studied occurred in a south-easterly direction from the brickworks.

In this paper the effect of the pollutants on the lichens, in relation to the distance from the main source of pollution, is discussed. The influence of the pollutants on chlorophyll degradation as well as the cover abundance of the lichens on the tree-trunks, is discussed.

5.2 MATERIALS AND METHODS

Bark and branch segments, containing mainly the lichens * Parmelia and + Ramalina, were collected. This material was then divided and put out in six bags of nylon mesh-type material so that each bag had a representative sample of the above-mentioned lichens. This method was preferred to the one used by Le Blanc et al. (1971), who cut circular, lichen-containing disks from trees. During early July 1988 each bag was hung at a height of between 3.5 and 4.5 meters on the trees representing the sampling sites (Fig.1.1), on the side facing the brickworks (Gilbert, 1985), during early July 1988.

* - Thallus of foliose form, prostrate and more or less closely creeping, generally attached to the substratum by rhizinae, but sometimes by a single central 'holdfast' or attachment point, or by most of lower surface;

\+ - Thallus fruticose, erect or pendent, attached only at the base and consisting of branching lobes, solid round or flattened in cross-section or like stems with coralloid laterals (Alvin, 1977).

5.2.1 Photographic Study

The effect of the pollutants was recorded photographically. The bark and branch segments were arranged next to each other and then photographed. The photographic study was not intended to show the lichens lost due to pollution, but only to show the effect of pollution on the lichen colour and structure during its time of exposure. This was done once every three months when material was removed for chlorophyll extraction.

5.2.2 Chlorophyll Extraction

The method of extraction was the same as the one used by Ronen and Galun (1984). This method determines especially the degradation of chlorophyll a molecules. The degradation ratio OD435/OD415 refers to the absorption of light by chlorophyll a and phaeophytin a molecules at wavelenghts 435nm and 415nm. It was shown that no other pigments interfered with light absorption of chlorophyll a at these wavelenghts. As the chlorophyll a is subjected to more pollution, more chlorophyll molecules become inactivated and thus less able to absorb light for photosynthesis.

5.2.3 Lichen Survey

A lichen survey was also done along the south-easterly transect, to study the distribution of the lichens. The different species were identified, and their percentage cover abundance on the tree bark was determined by means of the Braun-Blanquet method as described by Geldenhuys et al.(1988).

5.3 RESULTS AND DISCUSSION

5.3.1 Photographic Evidence

Compared to the grey-green Ramalina, Parmelia was initially greyish in colour, and both had soft textures. At the end of the sampling period, the upright Ramalina was hard, while the Parmelia became leathery. There was little noticeable change in the colour of both Parmelia and Ramalina after the first six months of exposure to the pollutants (Fig.5.1 a & b). During the March sampling, a definite change in the colour of these two lichens was evident. Ramalina had a yellowish colour, and Parmelia was off-white. This was accentuated by June 1989. The colour change of Ramalina correlated well with the degradation of the chlorophyll molecules (Fig.5.2). The correlation between chlorophyll degradation and the change in colour was not so apparent in the Parmelias (Fig.5.3). This correlated well with the

different sensitivities of these two lichens to pollution (Perkins and Millar, 1987 a & b). Chang and Thompson (1966) showed that a correlation existed between chloroplast and fluoride accumulation in Naval Orange leaves. Because chlorophyll degradation decreased rapidly with distance from the brickfield, it can safely be assumed that F^- was mainly responsible for the change in colour as it was a very localized effect (Roberts and Thompson, 1980). Davies (1986) found that there was a correlation between the fluoride concentration in Xanthoria and the distance from the source of pollutant emission.

5.3.2 Chlorophyll Degradation

The bags containing the lichens were put out during early July 1988. The results obtained in September 1988 thus show the effects of lichens that has been subjected to approximately 3 months pollution. The chlorophyll degradation ratio of Ramalina, at SE 1 (Fig.5.2), decreased with exposure time. Comparing the results of the various sites (Fig.5.2), it seems as if the downward trend was followed in most cases. Where this trend did not occur, one can probably ascribe it to the exposure of the lichens to the pollutants. The difference in the degradation ratios between SE 1 and the control site, was higly significant ($t = 6.708$; $p = 0.0005$; Table 5.1) (Ronen and Galun, 1984; Garty et al., 1985). A significant difference was found to

exist between the degradation ratios of the Parmelias of SE 1 and the control site (t= 3.607; p= 0.0113; Fig.5.3; Table 5.2). The data confirms that more pollution occurred at SE 1 than at the control site (Garty et al., 1985). It is the chlorophyll a pigment that gets degraded (Ronen and Galun, 1984; Garty et al., 1985). The differences in degradation ratios were more profound in Ramalina (Fig.5.2) than in Parmelia (Fig.5.3). Previous authors have found that fruticose lichens were more susceptable to pollutants, than foliose lichens (Perkins and Millar, 1987 a and b; Silberstein and Galun, 1988). This is confirmed by the fact that there was a more significant difference between SE 1 and the control site degradation values in the case of the Ramalinas, than the Parmelias. The control site values for both Ramalina and Parmelia (Fig.5.2 and 5.3), showed no apparent interference with the chlorophyll a molecules with time. The results obtained indicate that there was more chlorophyll present in Ramalina than in Parmelia. Kardish et al. (1987) found that a correlation existed between the concentration of ATP and the chlorophyll degradation ratio (OD435/OD415). They found that ATP concentration of the lichen Ramalina duriaei was a more sensitive parameter for the monitoring of pollution in transplanted lichens, than chlorophyll degradation.

5.3.3 Lichen distribution along the South-east transect

The results obtained clearly indicate that there was an increase in the number of lichen species (Fig.5.4) as well as their percentage cover (Fig.5.7) with an increase in distance from the pollution source. Eight lichen species were collected at the control site, while only two species were recorded at SE 1. The correlation between the number of species with distance, was found to be highly significant (Fig.5.4) ($r(5)= 0.8847$; $p= 0.0081$; $y= 0.0589589 + (1.57193)x$).

The resultant percentage cover, of the lichens, parallels the decrease in species number. There was a marked increase in the percentage cover on the trees beyond the 1.5 km distance from the brickfield (Fig.5.5). This increase between SE 1 and the control site was significantly higher ($t(6)= 2.978$; $p= 0.0247$).

Determinations of the pH of the bark, showed a highly significant correlation between the increase in bark pH and distance, (Fig.5.6; $r(4)= 0.9638$; $p= 0.0005$; $y= 3.92660 + 0.253209x$). The results obtained in Chapter 2 of this document, indicate a decrease in the pH of the bark in the polluted zone. Johnsen and Sochting (1973) also found that there was an inverse relationship between the number of lichen species and SO_2 pollution. Hawksworth and Hill (1984) stated that at the lower pH values (below 4), SO_2 is very toxic, but at pH values above 5, it is much less so.

A highly significant correlation between the bark pH and the number of lichen species (Fig.5.7) present was found ($r(5)= 0.8849$; $p= 0.0081$; $x= 4.03810 + (0.130833)y$, $y= -23.2980 + 5.98453x$).

5.4 CONCLUSION

The change in colour, of the lichens, over the time period of this study, could only be attributed to air pollution that occurs in the area.

This change in colour was well supported by the chlorophyll degradation results that were obtained (fig.5.1 & 5.2 and table 5.1). The results also confirm that the fruticose lichen, Ramalina, was more susceptable to pollution than the foliose lichen, Parmelia, as suggested by Perkins and Millar (1987 a and b). The correlation between the number of species, their percentage cover abundance, the bark pH and distance, was highly significant. It would have been interesting to see, if there was a correlation between ATP concentration and OD435/OD415 as Kardish et al. (1987) reported.

Since the study was completed, the brickfield has ceased active operation, and it will be important to keep on monitoring for lichen re-vegetation, if any.

The lichens once again proved to be a very sensitive guide to the degree of pollution that occurs in an area. The absence should clearly be an alarm signal as to the quality of the air we breathe.

Table 5.1 The chlorophyll degradation ratio in Ramalina along a pollution gradient (SE 1 nearest pollution source, n = 13).

SITE	AVERAGE (x ± sd)
SE 1	0.830 ± 0.136
SE 2	1.116 ± 0.199
SE 3	1.135 ± 0.122
SE 4	1.140 ± 0.238
SE 5	1.100 ± 0.050
SE 6	1.392 ± 0.032
CONTROL	1.371 ± 0.032

Table 5.2 The chlorophyll degradation ratio in Parmelia along a pollution gradient.(Site SE 1 nearest pollution source, n= 13)

SITE	AVERAGE (x ± sd)
SE 1	0.752 ± 0.123
SE 2	1.052 ± 0.037
SE 3	0.836 ± 0.242
SE 4	0.904 ± 0.169
SE 5	0.991 ± 0.095
SE 6	1.075 ± 0.053
CONTROL	1.088 ± 0.070

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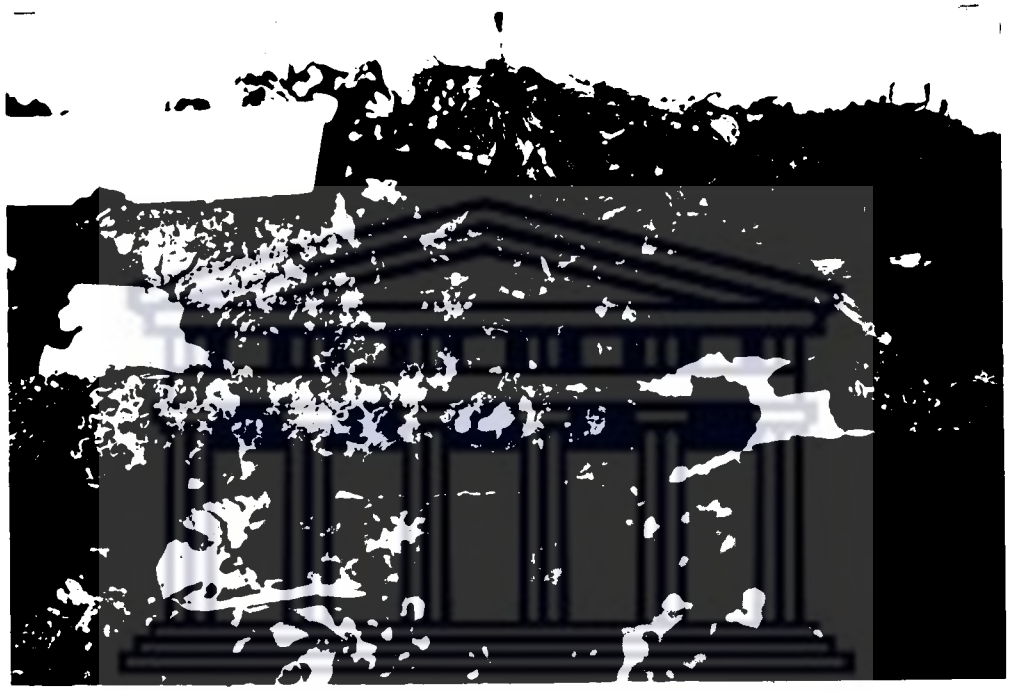
FIG. 5.1 The effect of pollution on the lichens Ramalina and Parmelia.

(a) SEPTEMBER 1988



(b) DECEMBER 1988

(c) MARCH 1989

(d) JUNE 1989

FIG. 5.4 VARIATION IN THE NUMBER OF LICHEN SPECIES WITH DISTANCE

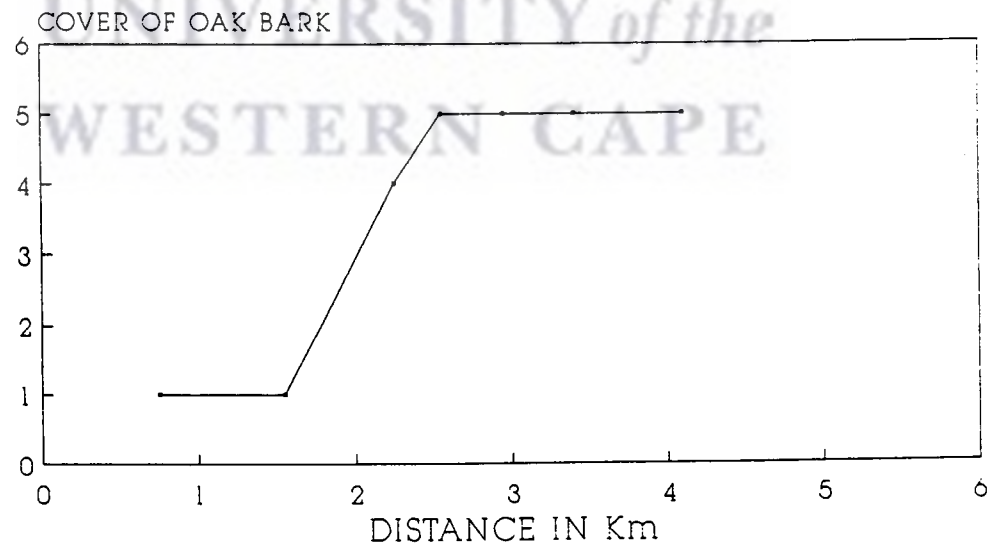


FIG. 5.5 VARIATION IN PERCENTAGE BARK COVER WITH DISTANCE

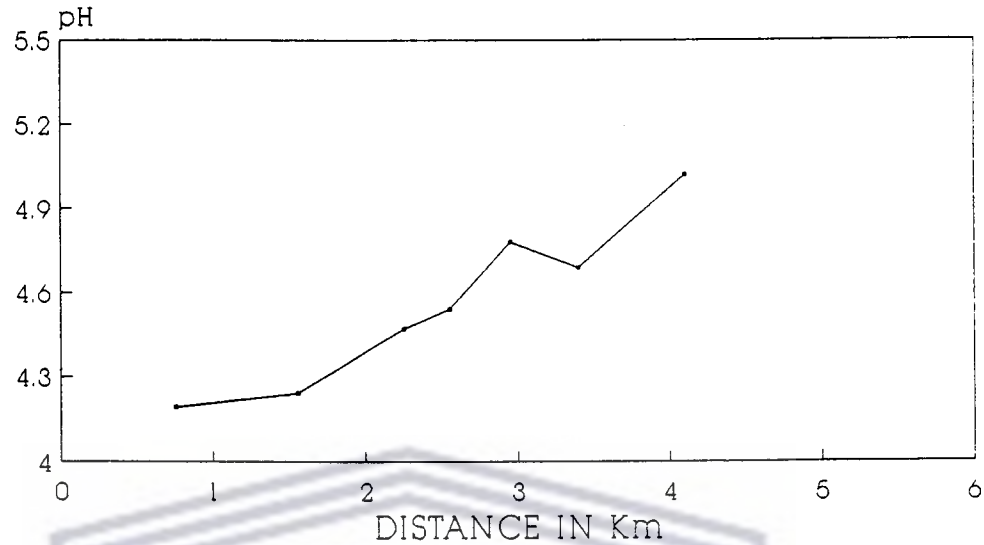


FIG. 5.6 VARIATION IN BARK pH WITH DISTANCE

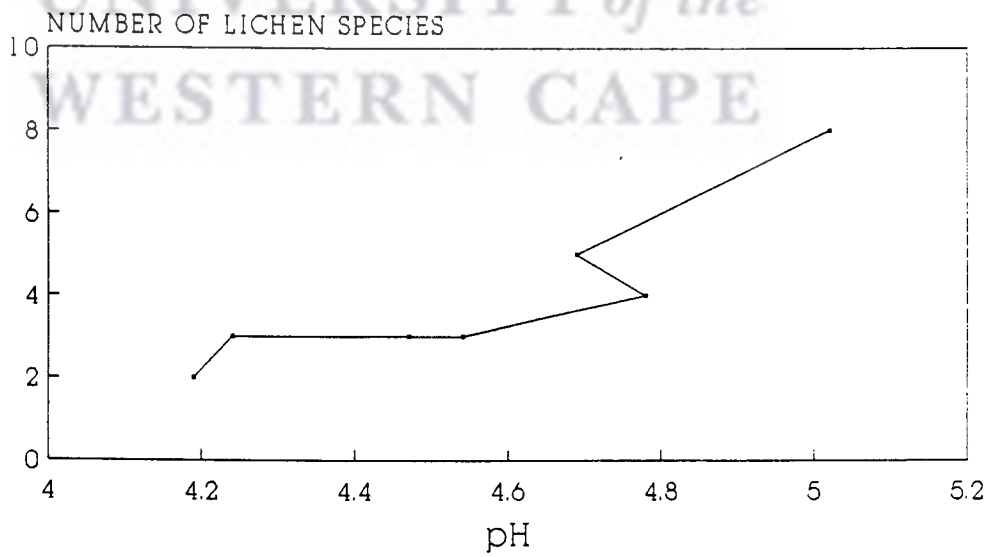


FIG. 5.7 VARIATION IN LICHEN SPECIES WITH pH

CHLOROPHYLL DEGRADATION RATIOS OF Ramalina

	SEPT '88	DEC '88	MCH '89	JUN '89
SE 1	1.047	0.794	0.672	0.806
SE 2	1.284	1.258	1.136	0.784
SE 3				
SE 4	1.398	1.331	1.021	0.809
SE 5	1.142	1.128	1.029	
SE 6	1.424	1.359		
CONTROL	1.332	1.365	1.422	1.365

CHLOROPHYLL DEGRADATION RATIOS OF Parmelia

	SEPT '88	DEC '88	MCH '89	JUN '89
SE 1	0.789	0.936	0.621	0.663
SE 2	0.992	1.06	1.06	1.095
SE 3	1.185	0.929	0.557	0.674
SE 4	0.808	0.687	1.127	0.994
SE 5	1.09	1.081	0.887	0.905
SE 6	1.128	1.021		
CONTROL	1.026	0.945	1.134	1.081

DISTANCE	LICHEN #	BARK pH	% COVER
0.75	2	4.19	1
1.55	3	4.24	1
2.25	3	4.47	4
2.55	3	4.54	5
2.95	4	4.78	5
3.4	5	4.69	5
4.1	8	5.02	5

CHAPTER 6

GENERAL CONCLUSIONS AND RECOMMENDATIONS

are especially alarmingly high along the two transects within 1.5km from the brickworks.

From the results obtained, it is safe to assume that the availability of both Ca^{2+} and Mg^{2+} are negatively affected due to the binding with F^- to form insoluble complexes.

High iron and copper concentrations were also recorded in the immediate vicinity of the brickworks, after which their concentrations decreased. Lead pollution in the area was proven to be related to motor-vehicles. Iron also showed to be closely linked with the lead pollution, as was suggested by Ho and Tai (1988).

Both Figure 6.1 and 6.2 indicate that SO_2 effectively pollutes over a larger area than is the case with F^-. This conclusion is drawn when studying the relatively sudden drop in the F^- concentration in both Figure 6.1 and 6.2 compared to the relatively gradual drop in the sulphate and sulphur concentrations.

The results obtained in this study indicate that the sulphur dioxide pollution had evidently decreased. This can possibly be due to the use of a higher grade coal which does not release as much sulphur when burnt. The evidence also

intervention of concerned residents of the area. It would thus be ideal to have a follow-up study in the future to see the changes that might occur, in the concentration of fluoride and sulphur/sulphate in the vegetation, and the re-colonization of lichens in the area.

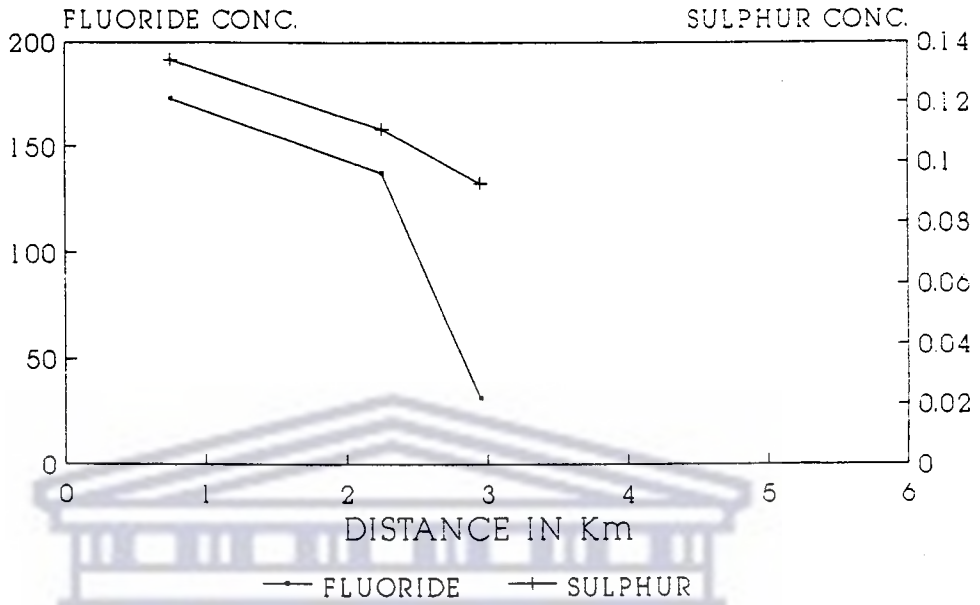


FIG. 6.1 FLUORIDE CONCENTRATION vs SULPHUR CONCENTRATION IN OAK BARK

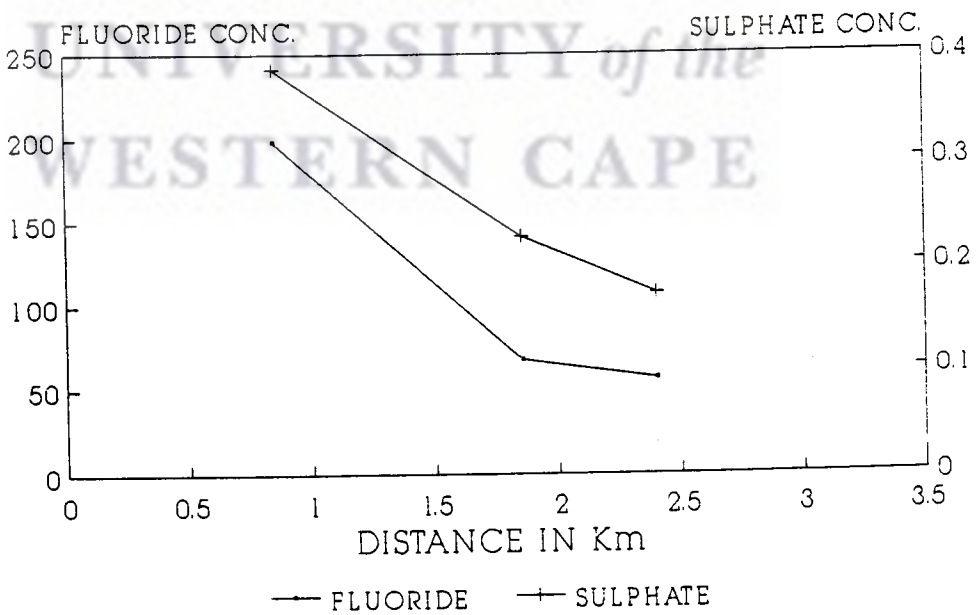


FIG. 6.2 FLUORIDE CONCENTRATION vs SULPHATE CONCENTRATION IN PINE BARK

SULPHUR vs FLUORIDE CONC.
IN OAK BARK

DISTANCE	FLUORIDE	SULPHUR
0.75	173.88	0.134
1.55		
2.25	137.66	0.111
2.55		
2.95	31.49	0.093
3.4		

SULPHATE vs FLUORIDE CONC.
IN PINE BARK

DISTANCE	FLUORIDE	SULPHATE
0.85	198.53	0.386
1.45		
1.85	67.75	0.226
2.15		
2.4	56.12	0.172