ACCUMULATION AND TOXICITY OF LEAD IN SOIL ALONG THE ROAD VERGES IN THE CITY OF CAPE TOWN

By

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DECLARATION

I, Anne-liese Krüger, declare that the contents of this thesis represent my own unaided work, and that the thesis has not previously been submitted for academic examination towards any qualification. Furthermore, it represents my own opinions and not necessarily those of the Cape Peninsula University of Technology.

Signed: 

Date: 23/11/07

ABSTRACT

The widespread use of lead (Pb) and especially Pb from vehicular emissions arising from lead additives in petrol has resulted in high levels of this metal found in various soil samples taken along the road verges of Cape Town CBD. The accumulation of lead was investigated in roadside surface soil by collecting soil samples at various sites along the three major highways (N7, N2, N1), approximately three metres from the road verges and at a depth of approximately 5 cm for a period of 12 months. After digestion with 55% nitric acid the Pb concentrations were determined by using an Inductively Coupled Plasma Atomic Emission Spectrophotometer (ICP–AES).

Results have shown the Pb concentrations in the soil at busy intersections to be higher than at other areas along the roadsides. Lead concentrations found in the roadside soils of the N1 ranged between 200 and 2000 mg/kg and these were of the highest concentrations found compared to the other two highways and were even higher than found in other studies. The roadside soils on the N1 highway, with the most traffic, according to car count data obtained, seemed more contaminated than the other two highways.

It was also investigated whether earthworms *(Eisenia fetida)* accumulated Pb after being exposed to the contaminated soil from the sampling sites. The earthworms in the highly contaminated soil accumulated on average much higher concentrations of Pb than the earthworms in the lower Pb contaminated soil.

A potential biomarker (cell membrane integrity) was applied to determine whether the earthworms experienced toxic stress as a result of the exposure to lead contaminated soil. Behavioural and morphological changes in the earthworms were also observed. The Trypan blue exclusion assay was used to measure the effect of lead exposure on the membrane stability of the coelomocytes in the coelomic fluid of earthworms. In the

highest exposure groups per highway, a significant decrease in percentage viable cells were seen (N7, 36 ± 0.07%; N2 48 ± 0.09%; N1, 34 ± 0.08%). The fact that clear statistically significant responses were seen after the five week exposure period in the highest, as well as lowest exposure groups indicate that these responses could serve as an early warning system of lead exposure. The percentage cell viability (biomarker) used in this study have been useful in identifying toxic stress in earthworms caused by lead in roadside soils. The additional information obtained by using biomarkers could not be obtained by chemical analysis of soil and earthworms alone.

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Men love to wonder and that is the seed of our science –
Ralph Waldo Emerson

GLOSSARY

1) *Lead*: Pb is a trace element in rocks and soils and therefore occurs naturally in the environment (Nriagu, 1978).

2) *Tetra ethyl lead:* (TEL) is an organic compound added to petrol because of its antiknocking properties, which improves fuel efficiency and increases octane ratings (Rosner and Markowitz, 1985).

3) *Biomarkers*: Any biological response to a chemical at the below-individual level, measured inside an organism or its products (urine, faeces, hair, etc), indicating a departure from the normal status, which cannot be detected in the intact organism (Van Gestel & Van Brummelen 1996).

4) *Ecotoxicology:* The study of the toxic effects of various agents on living organisms, especially on the population and communities within ecosystems (Connel *et al.* 1999).

5) *Bio-accumulation:* Some substances increase in concentration, or bioaccumulate, in living organisms as they breathe contaminated air, drink contaminated water, or eat contaminated food. These chemicals can become concentrated in the tissues and internal organs of animals and humans (http://www.familyjeweler.com/fortweb/ bioaccumulation.htm).

6) *Bioaccessible:* It is the fraction of a substance that is available for absorption by an organism (http://www.grc.cf.ac.uk/1rn/resources/bioavailability/difference.php).

7) *Bioavailable:* It is the fraction of the chemical that can be absorbed by the body through the gastrointestinal system, the pulmonary system and the skin (http://www.grc.cf.ac.uk/1rn/resources/bioavailability/difference.php).

8) *Eisenia fetida*: The compost earthworm and also the organism used in this study. It was recommended by Edwards and Coulson (1992) as a standard test organism in ecotoxicological evaluations because of the fact that they are present in the topsoil layers.

LIST OF FIGURES

LIST OF TABLES

TABLE OF CONTENTS

CHAPTER 1: INTRODUCTION

Lead is a trace element found in rocks and soils and thus occurs naturally in the environment (Nriagu, 1978). It is therefore difficult to determine whether or not a soil is contaminated with lead even if the concentrations are low (Ahrens, 1954). Wixson and Davies (1993) defined a lead contaminated soil as being contaminated with lead when this metal's content is higher than that expected from the pedological nature of the soil.

Elevated soil lead levels are generally caused by emissions to the environment due to the increase in human use of the metal and its derivatives (Duffus, 1980; Tong and Che Lam, 2000). Industrial sources of lead include the pigment and dye industry (Olivio *et al.,* 1974), car battery manufacturing (Crandall *et al.*, 1974), municipal waste incinerators and the manufacturing of tetraalkyl additives for petrol (Nozaki *et al.*, 1967). Sewage sludge used as a fertilizer, as well as mining and smelting, are also recognized as major sources of lead contamination. Soil is also a major collection point for this contaminant (Frank *et al.*, 1976; Merry *et al.*, 1983). Major industrial parks are situated relatively close to the major highways in the City of Cape Town and may contribute to lead in soil via the wind. According to Davis *et al.* (2001) such aerial depositions are also recognized as an important source of lead in soil. Industrial emissions (Ren *et al.,* 2006) are a point source of lead and elevated lead levels have been found in the vicinity of many industries that produce, use, recycle or dispose of products containing lead. It is also likely that land and vegetation close to these major lead sources are severely contaminated by lead. Furthermore, lead released into the atmosphere can travel over thousands of kilometers before settling on land or sea (Grandjean, 1975; Tong and Che Lam, 2000). Spillages and chemical leakages, as well as sites that have been contaminated from centuries before, particularly mines, add to further contamination (Hankard and Svendsen, 2004). Urban stormwater run-off containing lead has proven to be toxic to aquatic organisms (Heaney *et al.*, 1999) and toxic effects have been found in urban creeks and rivers (Bay *et al.*, 1997; Jirik *et al.*, 1998; Riveles and Gersberg, 1999; Schiff *et al.*, 2002). However, the greater concern since the

recent past is with the vehicular emissions arising from lead additives in petrol (EPA, 1978; Okonkwo and Maribe, 2004). Tetra ethyl lead (TEL) is an organic compound added to petrol because of its antiknocking properties, which improves fuel efficiency and increases octane ratings (Rosner and Markowitz, 1985). The major causes of environmental pollution arise from vehicular emissions containing unoxidized traces of TEL and its oxidized form of lead (CDC, 1981; Madany *et al.*, 1994; Ren, 2006). In a study done by Swaileh *et al.* (2004) it was found that cars are a source of several pollutants to the environment, particularly lead, (Sutherland and Tack, 2000). Harper *et al.* (2003) found that older vehicles are associated with higher levels of pollutant emissions and in this study, done in South Africa it was found that vehicles are on average older than in developed countries.

The City of Cape Town (CCT) is a relatively densely populated metropolitan area, with 37% of the population living in the Cape Town CBD, Northern and Southern suburbs. Eighty percent of all the job opportunities are localized in the aforementioned areas, resulting in a huge daily movement of people to and from work with high vehicular traffic on the major roads, especially during peak hours (CMC, 1997/8; CCT, 2002). Over the past twenty years the number of vehicles in the CCT has increased by 80%, which is more than in most developing countries. Also, industrial activities in the CCT expanded to such an extent in the past years (CMA, 1998) that there is a realistic chance that the environment in the CCT has been put under increased toxic stress due to increased emission of metals, such as lead.

Due to this widespread use of lead in petrol, vast amounts of lead are introduced into the atmosphere (Rodrigues, 1982), which may cause an accumulation of lead in the upper 0 to 5 cm of the soil (Harrison and Laxen, 1984; Wild, 1993). Accumulation is related to the type of vehicle, traffic volume, topography, prevailing vegetation and winds (Smith, 1976), as well as rainfall and run-off (Zartman *et al.*, 2001). In the latter study it was found that the concentration of metals in run-off varied by season and that rainfall played an important role in the concentration of metals in run-off (Sansalone *et al.*, 1996).

Warren and Delavault (1960) were the first to point out an accumulation of lead in the roadside ecosystem and soon after Cannon and Bowles (1962) demonstrated that grass within, 150 meters downwind of highways were contaminated by lead. Since then many authors (*e.g.* Shy, 1990; Sutherland and Tack, 2000; Nabulo *et al.,* 2006) in many countries have reported contamination of the roadside environment as a result of the use of leaded petrol. Previous studies done by Reinecke *et al.* (1997; 2000) have shown lead to accumulate in South Africa's urban soils. There are several farmlands along the Cape Town highways where crops are grown and livestock are seen grazing. The concern is that these crops that are growing close to these busy roads could contain lead levels that are dangerous to human health (Williamson and Evans, 1972; Nabulu *et al.*, 2006). Low levels of lead are present in food and drink, because of its natural occurrence in the environment, but the levels may be increased due to lead deposition from the atmosphere (EPA, 1978).

Lead exposure is a health hazard, recognized worldwide, forcing the invention of catalytic converters reducing or eliminating the release of lead additives from petrol (Rosner and Markowitz, 1985). Although unleaded petrol has been available in South Africa since 1996 the conversion from leaded to unleaded petrol has been slow. According to von Schirnding *et al.* (2001) only about 25% of the petrol market share in South Africa was constituted by unleaded petrol. Leaded petrol has been phased out since January 2006. However, there is still a real danger of lead contamination at least in the area close to busy roads (CMA, 1998) and also due to lead's long half-life (Pirkle *et al.*, 1985).

There is no knowledge of lead being beneficial to human health. In fact, exposure to lead adversely affects hemoglobin synthesis (causing anemia), the kidneys, the neurological system, as well as the cardiovascular system (EPA, 1998; ILO, 1998). In a study done in Adana in 1997, blood lead levels of traffic policeman, working in busy crossroads were above normal (Kara *et al.*, 1999). The risk of having even higher blood lead levels are people that are in actual contact with petrol, *e.g.* petrol station employees, motor mechanics cleaning their hands with petrol and refinery workers (Kapaki *et al.*, 1998; Duydu and Vural, 1998). According to data from the 1970's, lead in petrol caused an

increase in average blood lead levels in the general population (Shy, 1990). A MRC study done in 1991 revealed that more than 90% of the inner city children of Cape Town had blood lead levels exceeding or equaling the national action level of 10 µg/dl. A recent MRC study showed that although there is an improvement in blood lead levels of children in Cape Town since the introduction of unleaded petrol, some children (up to 29%) attending inner-city schools continued to have abnormally high blood lead levels. High blood lead levels like in these children are associated with hyperactivity, effects on brain development and inability to concentrate, resulting in poor performance in school (MRC, 2002; Ren *et al.*, 2006). Fetal health is also highly sensitive to lead exposure during developmental stages (Mathee *et al.*, 1996) and has been a global public health concern for many years (Tong *et al.*, 2000).

This metal does not only accumulate in soil, vegetation and humans but also in animals (*e.g.* invertebrates) and are like the rest of the ecosystem also adversely affected. Lead is inhaled or ingested by animals and might initially concentrate in the liver and kidneys and thereafter be redistributed to the bones, teeth and brain. Although it may not cause immediate toxicity, it may potentially be mobilized during feverish illness (Duffus, 1980). Data on lead contamination in animals show inhibited enzymes and alterations in biological membranes, which is also related to the formation of lipid peroxides (Lawton and Donaldson, 1991; IPCS, 1989).

Terrestrial invertebrates accumulate metals from soils and vegetation mostly in the gut epithelia or digestive glands, which are their digestive tissues (Hopkin, 1989). The function of these animals in the soil ecosystem is to enhance the soil structure and to decompose organic material (Kammenga *et al.*, 2000). The accumulation of lead has an important implication for their nutritional physiology (Hopkin, 1989) and could therefore cause a retardation of the heterotrophic breakdown of organic matter in the soil (Duffus, 1980). Reinecke *et al.* (2000) have shown that earthworms accumulate lead. In fact, many studies done on earthworms confirm a higher accumulation of metals in earthworms that were collected at road verges than in those earthworms found in non-contaminated

sites (Anderson, 1979; Wright and Stringer, 1980). The concentrations of accumulated metals in the earthworms were found to be less when found further from the road verges (Gish and Christensen, 1973) and also depended on the traffic density (Gish and Christensen, 1973; Czarnowska and Jopkiewicz, 1978; Ash and Lee, 1980). Earthworms are a food source to birds, amphibians, reptiles, mammals and many other vertebrates and the importance of the accumulation of metals in earthworms lie in the potential for it to move into higher tropic levels in food chains (Terhivuo *et al.*, 1994). Because of earthworms' role in soil fertility their protection in the ecosystem is essential (Morgan and Morgan, 1998; Weeks and Svendsen, 1996). Their growth rates may also be affected by lead, as well as their behaviour and reproduction (Helmke *et al.*, 1979).

The earthworm *Eisenia fetida* used in the present study was recommended by Edwards and Coulson (1992) as a standard test organism in ecotoxicological evaluations because of the fact that they are present in the topsoil layers (Bouché, 1992) where lead concentrations are generally higher. *Eisenia fetida* have been used successfully in other studies (Labrot *et al.*, 1996) to determine sublethal effects of lead on some biochemical responses. Davies *et al.* (2003) subjected *E. fetida* to a 28-day exposure period in an artificial soil to assess acceptable levels of lead in soils (Davies *et al.*, 2003).

Earthworms have been reported to be outstanding bio-indicators of the health of soil ecosystems (Kühle, 1983). Since they accumulate metals, analysis of earthworms may provide helpful information on the bioavailibility of metals in soils (Helmke *et al.*, 1979). They are numerous, large, widespread and easy to identify and their relative immobility makes it easy to sample them. Earthworms consume large quantities of the substrate that they live in and their bodies are in full contact with the substrate (Morgan *et al.*, 1986).

Internationally, there is abundant information on lead pollution but to determine the effects of this pollutant on the environment can be very difficult (Depledge *et al.*, 1994). Although the information derived from biological indicators can provide useful information about the status of a site, the proper choice of sensitive and ecologically relevant assays would be the determining factor in the success of these biological techniques (Cortet *et al.*, 1999; Kammenga *et al.*, 2000). Biomarkers originated from human toxicology and have proven to be successful in measuring the effects of chemical exposure to humans or in indicating syndromes or diseases at an early stage (Timbrell, 1998). Sampling of tissues and body fluids is mostly non-destructive and provide accurate estimates of individual susceptibility to disease and internal or effective dose (Forbes *et al.*, 2005).

The use of biomarkers in terrestrial invertebrates to assess potential damage of toxins in the soil ecosystem is also becoming increasingly popular, essentially because they are in direct contact with the soil and food and because they represent a large component of species in soils. Also, being generally densely populated, sampling for analysis rule out the fear of affecting population dynamics (Kammenga *et al.*, 2000).

Biomarkers has been used successfully in ecological risk assessment, although many of these techniques still need validation for monitoring purposes in field situations (Kammenga *et al.*, 2000). Van Gestel and Van Brummelen (1996) defined biomarkers as any biological response to a chemical at the below-individual level, measured inside an organism or its products (urine, faeces, hair, etc), indicating a departure from the normal status, which cannot be detected in the intact organism. Biomarkers supply additional information needed to measure possible harmful effects in the environment (Depledge and Fossi, 1994). These techniques serve as an early warning of pollution-induced stress. Its significance lies in the early detection of the effects of pollution and a response at lower concentrations, before pollution can cause an irreversible effect (Van Gestel and Van Brummelen, 1996).

The most significant characteristics of biomarkers thus, lie in identifying interactions that have taken place between the organism and the contaminant. Equally important is that they measure sublethal effects. Therefore, the known and unknown presence of contaminants can be detected, which allows for preventative and remedial action to be taken. Chemical analysis alone would not supply any information on the adverse effects of the contaminant to organisms. It would basically measure a fraction of the contaminants that are present (Bayne *et al.*, 1985; Haux and Förlin, 1988; McCarthy and Shuggart, 1990; Stegeman *et al.*, 1992).

Environmental contamination and conservation of the environment have become a cause for concern especially in terms of the impact of contaminants on sustainable land use. Regulations and procedures to protect soils are in place in certain countries but no system that monitors the direct effects is in place. Biomarkers could prove to have great value within such a monitoring system (Svendsen *et al.*, 2002).

In ecotoxicology, the biomarker concept is focused on the detection of molecular, biochemical, physiological, or cellular changes after exposure to pollutants to these organisms (Peakall and Shuggart, 1992; Depledge and Fossi, 1994). The NRRT assay (neutral red retention time assay) is used to measure the effect of a toxic substance on membrane stability of lysosomes. This technique has been used successfully by many researchers (Koenig, 1962; Allison and Young, 1969; Nemes *et al.*, 1979; Snyman *et al.*, 2000, 2002; Reinecke and Reinecke, 2003; Svendsen *et al.*, 2004; Hankerd *et al.*, 2004).

Being an established technique it is used in ecotoxicology on earthworms to indicate a response to pollutants in metals such as copper (Svendsen and Weeks, 1997a, b), zinc (Spurgeon *et al.*, 2000), lead, cadmium (Reinecke *et al.*, 1999) and nickel (Scott-Fordsmand *et al.*, 1998). Moore (1985) used NRRT to provide a fast indication of a response to changes in an ecosystem when lysosomal membranes were exposed to toxic substances. As a consequence of the exposure to the toxic substance, the integrity of the membranes was

affected and the accumulated red dye in the lysosomal vesicle diffused out and stained the cytosol. The rate of staining generally correlates with the degree of damage and NRRT assay functioned sufficiently as an early-warning system. The NRRT assay assesses the lysosomal membrane stability of the coelomocytes, obtained from the coelomic fluid in the earthworm. This technique is both fast and simple (Weeks and Svendsen, 1996; Svendsen *et al.*, 2004).

A different assay, the trypan blue exclusion assay, can be used to determine if contaminant accumulation causes alterations in the permeability of the cell membrane and thus to assess the viability of the cell (Müller, 1984, Steffenson *et al.,* 1994; Dacasto *et al.*, 2001). It is evident that earthworms accumulate high levels of metals such as lead (Martin and Coughtrey, 1975; Beyer *et al.*, 1982) and lead is also known to damage cell membranes. This damage can alter the permeability of the plasma membrane resulting in cell death (Müller, 1984; Steffenson *et al.*, 1994; Koizumi *et al.*, 1996; Herak-Kramberger and Sabolic, 2001; Tàtrai *et al.*, 2001).

Evaluation of cell viability through cytotoxicity measurements have become very important in assessment of the activity of chemicals (Mossman, 1983) and again one of the most common methods used is the trypan blue exclusion assay (Jones and Senft, 1985; MacCoubrey *et al.*, 1990). The assay is based on the fact that a damaged cell membrane will let the trypan blue dye through to consequently stain the cytoplasm as apposed to healthy cells with intact membranes that will exclude the blue dye (Harbell *et al.*, 1997). However, the trypan blue exclusion assay's potential as a biomarker technique in ecotoxicological research has not been investigated thouroughly (Snyman and Odendaal, *submitted*).

The principle aim of this study was to determine whether lead along the road verges in the CCT cause toxic stress / negative effects to soil organisms (*i.e.* earthworms). This was done by setting the following objectives:

- Firstly, to determine the accumulation of lead in soil at various sites along the three major highways of the CCT (N1, N2 and N7) by sampling of soil for a period of 12 months.
- Secondly, to determine if earthworms accumulated lead after being exposed to the contaminated soil from the sampling sites.
- Thirdly, to apply a potential biomarker (cell membrane integrity) by means of the trypan blue exclusion assay to determine whether the earthworms experienced toxic stress as a result of the exposure to lead contaminated soil.

CHAPTER 2: MATERIALS AND METHODS

2.1) FIELD SURVEY

2.1.1) Sampling area

The N1, N2 and N7 are the main routes to and from Cape Town and were chosen as the sampling area because of the high volumes of traffic these highways carry on a daily basis. The first (closest) sites were between 6 km (N1 highway) and 13 km (N7 highway) from the Cape Town CBD and the furthest sites between 31 km (N1 highway) and 45 km (N2 highway) from the Cape Town CBD. All the sites were chosen in the vicinity of busy intersections, housing, schools, parks and rivers and were between 2 and 12 kilometers apart (Figs 1, 2, 3, 4).

2.1.2) Sampling sites

Sites on the highways were numbered from 1 to 5 on the N7, 6 to 10 on the N2 and 11 to 16 on the N1. The lower number indicated the site closest to the Cape Town CBD and the higher number the site furthest away from Cape Town CBD. At each of the sampling sites soil samples were taken on the incoming (traffic into the city) and outgoing (traffic leaving the city) sides of the roads. These samples were treated separately for the purpose of this study.

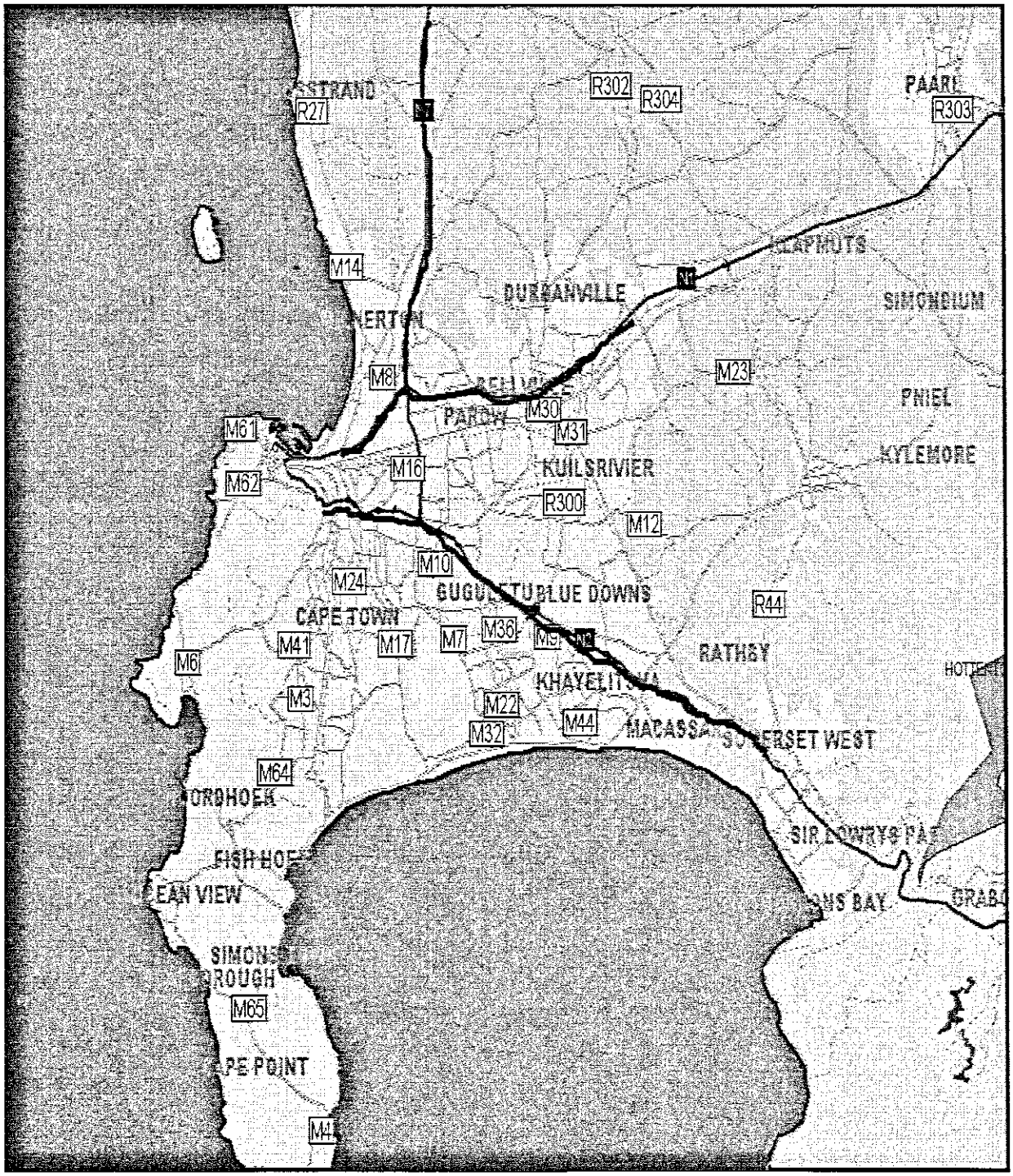


Figure 1: Map of the N7, N2 and N1 highways in the CCT (VeZa Route planner Version 2 Beta).

2.1.2.1) N7 highway

Soil: The type of soil found at most of the sites was sandy, but a more reddish clay soil was observed at site 5, which was furthest away from the Cape Town CBD (Sheat, 1984).

Structures, buildings and natural features: Situated in the vicinity of site 1 and 2 were residential areas and schools, as well as a refinery. Site 3 was close to a river and wheat and chicken farms were found between sites 4 and 5.

Vegetation: Blue gum and Port Jackson trees were a main feature alongside the N7 highway and *Phragmites* (reeds) grew vigorously at site 3 on the riverside.

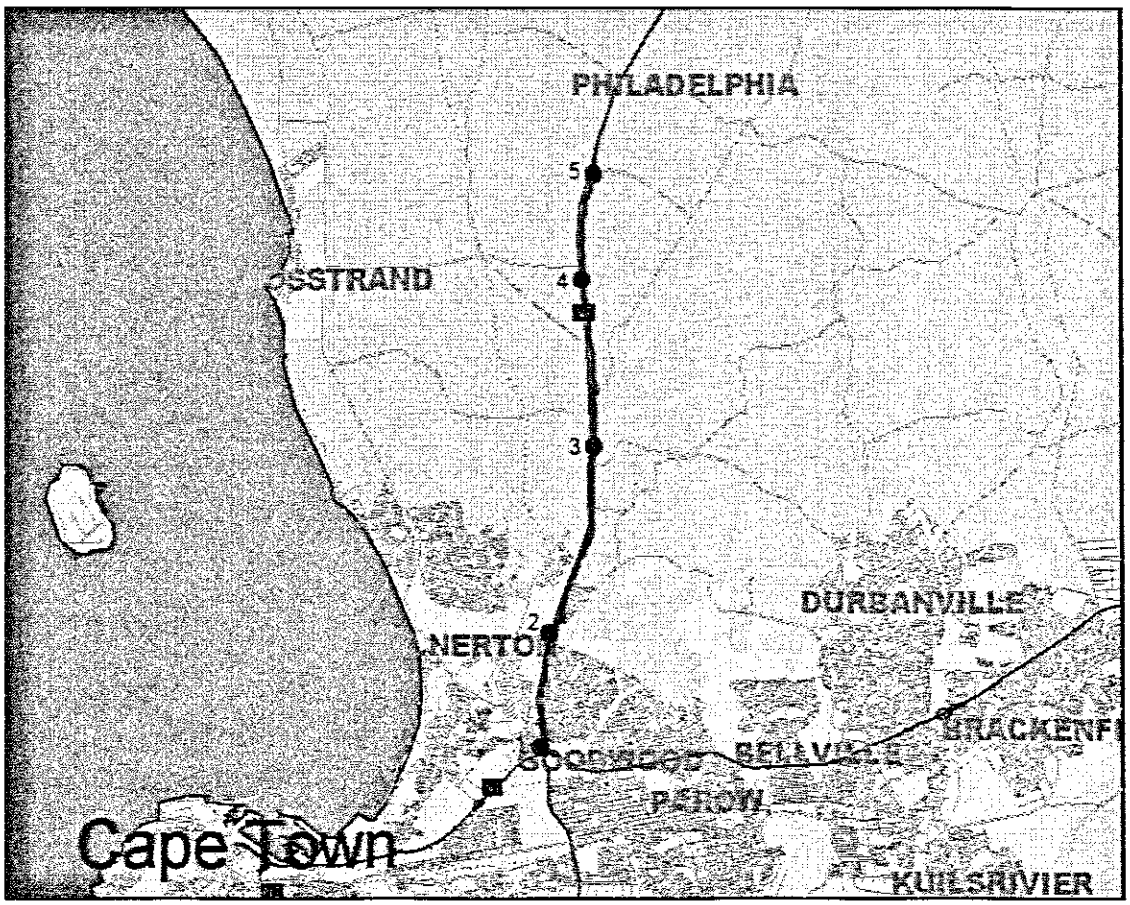


Figure 2: Map of sampling sites 1 to 5 (outgoing and incoming) on the N7 highway. Sites 1, 2, 3, 4 and 5 were respectively 13, 17, 23, 30 and 34 km away from the Cape Town CBD (VeZa Route planner Version 2 Beta).

2.1.2.2) N2 highway

Soil: A sandy soil was found along this highway (Sheat, 1984).

Structures, buildings and natural features: Situated along the N2 were residential areas and business parks in the vicinity of site 6 and a power station and sewage farm at site 7. Informal settlements from there on were very close to the highway from site 7 to site 9. Site 9 was close to a river and dams and site 10 was in the vicinity of a play park, residential areas, business parks and a sports field.

Vegetation: Pampas grass *(Cortaderia)* grew vigorously in dams and rivers along the N2.

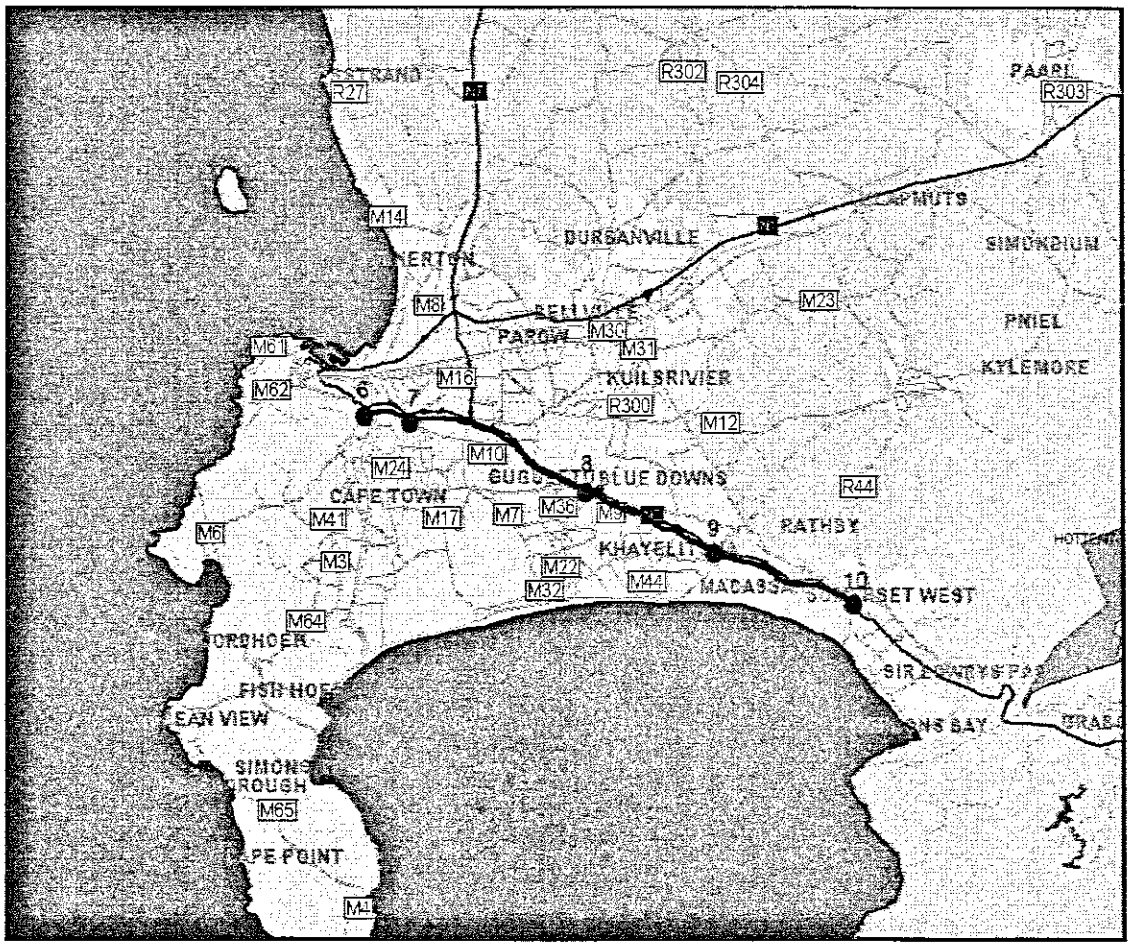


Figure 3: Map of sampling sites 6 to 10 (outgoing and incoming) on the N2 highway. Sites 6, 7, 8, 9 and 10 were respectively 8, 10, 20, 33 and 45 km away from the Cape Town CBD (VeZa Route planner Version 2 Beta).

2.1.2.3) <u>N1 highway</u>

Soil: The soil along this highway was mostly Sandy (Sheat, 1984).

Structures, buildings and natural features: Site 11 was situated close to an industrial area and very busy intersection. Residential areas were seen from site12 up to site 16, a school at site 14 and a river and nursery close to site 15.

Vegetation: Manatoka trees and grass were a common sight from site 1 onwards and *Poplar*, Blue gum and *Oleander* trees again from site 14 onwards.

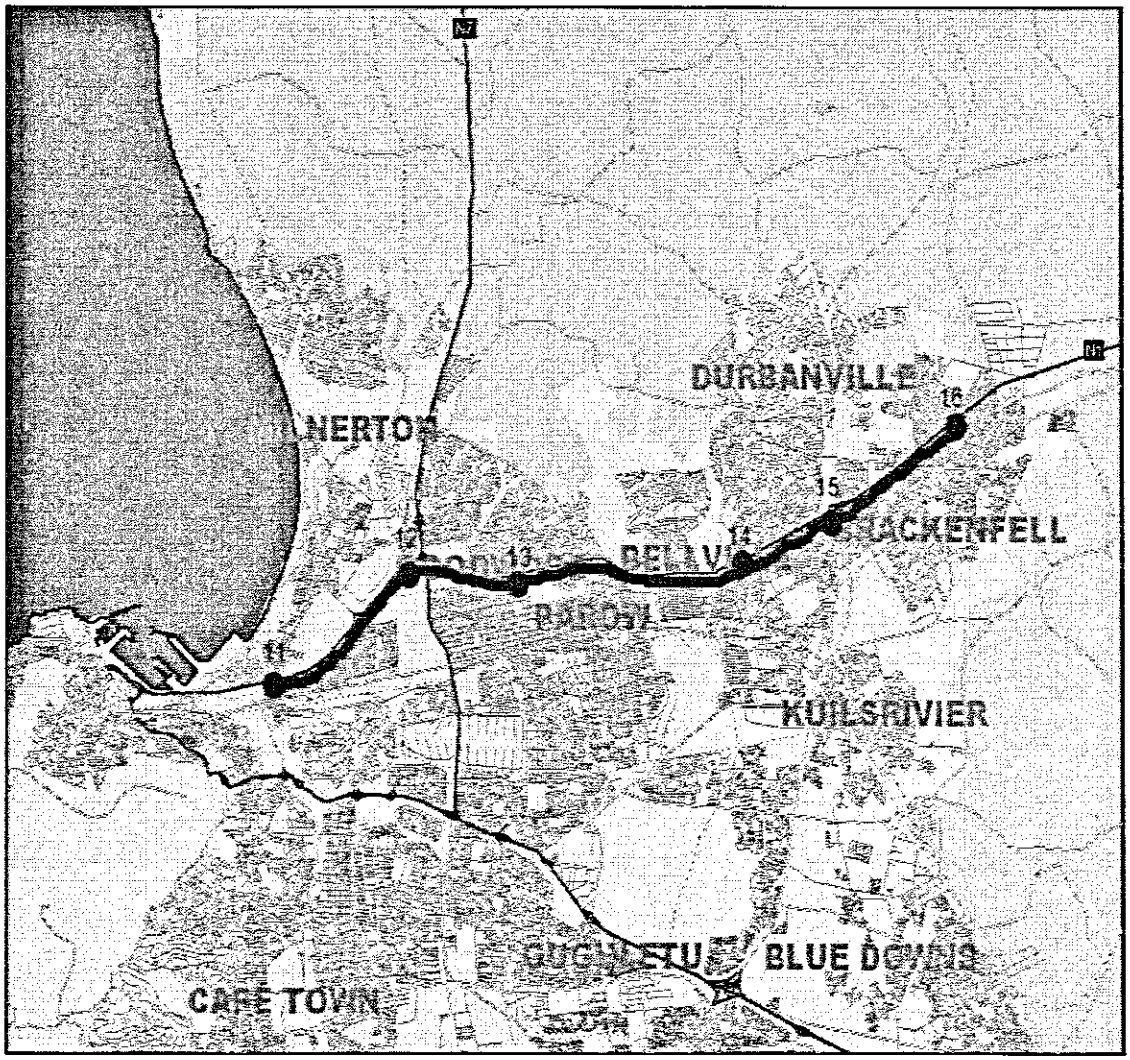


Figure 4: Map of sampling sites 11 to 16 (outgoing and incoming) on the N1 highway. Sites 11, 12, 13, 14, 15 and 16 were respectively 6, 12, 15, 23, 26 and 31 km away from the Cape Town CBD (VeZa Route planner Version 2 Beta).

2.1.3) Sampling procedure

This sampling was done every second month for a period of one year and for this purpose each sampling site was issued a name and a number. This sampling period commenced on the 23rd of July 2004 and ended on the 25th of July 2005. Five soil samples were taken at each site approximately three meters from the road verges and at a depth of approximately 5cm. The soil from each sample site was collected in labeled plastic vials and taken to the laboratory for analysis. Record was kept of the weather conditions and activities on and adjacent to sampling sites, prior and at the time of sampling that could possibly influence the outcome of the results.

2.2) DETERMINATION OF pH AND MOISTURE

pH and moisture were determined for soil samples. To test the pH of the soil, approximately 2 g of the soil was put into a 30 ml glass beaker and diluted with distilled water. A Hanna portable pH meter was used. A Precisa HA 300 310M moisture meter was used to measure the moisture content in each soil sample. Approximately 1g of the soil sample was used.

2.3) ACID DIGESTION

The rest of the soil samples were placed in separate, labeled petri dishes and into a Memmert oven to dry out for 48 hours at 60°C in order to obtain the dry weight of the soil. The dried soil was then sifted through a 1 mm sieve and placed into clean, labeled petri dishes and weighed on a Precisa XB 220A balance to obtain a weight of between 0.2 and 0.3 g.

The weighed soil was then placed into labeled, metal free test tubes for digestion. The test tubes with the soil samples, as well as a blank (test tube with only the 10 ml nitric acid, to measure for possible contamination) were placed in a Grant UBD digester in a fume cabinet and digested with 10 ml 55% nitric acid at a temperature of 40°C for one hour. The temperature was then increased to 120°C for a period of four hours. This method as used by Odendaal and Reinecke (1999) was used in this study. After cooling, the samples were filtered through Whatman no 6 (90 mm) filter paper and diluted to 20 ml with distilled

water using labeled 20 ml volumetric flasks. The samples were finally filtered through Whatman 0.45 µm cellulose nitrate membrane filter paper using a syringe and Millipore filter holders. The prepared samples were stored in a fridge in 30 ml plastic vials until it was ready to take to the ICP-AES laboratory at the University of Stellenbosch to determine the lead concentrations in the samples.

2.4) LEAD ANALYSIS

The lead concentrations in the samples were determined with an Inductively Coupled Plasma Atomic Emission Spectrophotometer (ICP–AES) and calculated using the following formula:

$$\frac{(\text{ICP reading}-\text{Blank}) \times [20 \text{ dilution factor}]}{\text{Dry mass from soil sample (g)}}$$

Lead concentration was expressed as mg/kg.

2.5) LABORATORY EXPERIMENTS

2.5.1) Exposure soil

The data from the sampling were used to identify which sites had the highest and lowest lead concentration on each specific highway. Two sites on each highway were then chosen for the exposure procedure. Two liters of soil sample were collected at each of the six identified sites.

2.5.2) Earthworm species used in the study

Eisenia fetida is the earthworm used in the study. Their resilience, wide temperature and moisture tolerance, short life cycles and wide distribution make them excellent study models (Dominguez, 2004). Only uncontaminated earthworms, bred under sterile conditions and fed on cow manure from grass eating cows were used in this study. Also, only adult clitellate earthworms were used.

2.5.3) Exposure procedure

The earthworms were exposed to the contaminated soil for five weeks, which was in accordance with Saint-Denis *et al.* (1999) that advised that earthworms be exposed to metal contaminated soil for at least 14 days and discrimination between doses were best after 14 to 28 days. Also, significant changes could best be observed after exposure to soil lead concentrations of 30 mg/kg or more (Saint-Denis *et al.* 1999). Five hundred grams of soil was used per container. The soil was collected from the sampling sites, weighed on a Mettler balance and placed in 6 individual, clean plastic containers. Each container, filled with 500 g of soil from its site, was labeled according to the sampling site from which the soil had been collected. To ensure a more or less ideal soil moisture 140 ml distilled water was added to each of the soil samples. Soil moisture of between 20 and 23% was then obtained. Thirteen healthy, uncontaminated earthworms were used per exposure container and they were weighed individually on a Precisa XB 220A balance before being placed in the separate plastic containers of contaminated soil. One hundred grams of cow manure was loosely scattered on the soil.

Lids were put on the containers to prevent the earthworms from crawling out and tiny holes were made in the lids for aeration. The six containers were placed in a box together with a thermometer to monitor the temperature during the exposure period. A lid on the box kept the area dark, a favourable situation for the earthworms. The earthworms were fed weekly with 50g of fresh cow manure.

The mass of the earthworms were measured at the end of the experiment. Six of the healthy, unexposed earthworms were weighed separately on a Precisa XB 220A balance. Also, after the exposure period six contaminated earthworms per exposure group were taken out of their respective exposure groups and weighed separately on a Precisa XB 220A balance before being put through the coelomocyte-extraction process (described on page 18) to determine the percentage viable cells.

2.6) BIOMARKER

2.6.1) Trypan blue exclusion assay

The objective of this investigation was to determine the percentage viable and non-viable coelomocyte cells in the earthworms, before and after exposure to the lead contaminated soil, as well as to determine the difference in cell viability in the earthworms between the high lead contaminated soil and low lead contaminated soil.

A buffer solution, PBS (sigma Dulbecco's phosphate buffered saline) was used to keep the cells alive during the cell counting procedure. The PBS solution was used at room temperature to prevent cell shock, but stored in the fridge after usage. A table in the laboratory was set up with the following materials for this procedure: A Nikon microscope and Neubauer haemocytometer chamber; pipette (Gilson 200) and tip; 1 ml sterile syringe; eppendorf microcentrifuge tubes and holder; trypan blue solution; PBS solution; glass beakers for waste; pipette; earthworm placed in petri dish with a little bit of distilled water to keep it moist; towel paper.

After being weighed, the six control earthworms were put through the coelemocyte-extraction procedure (described below). Six earthworms from each specific exposure group were put through the same process after the exposure period.

Fifty microliters (µl) of trypan blue were placed into an eppendorf microcentrifuge tube using a pipette (Gilson 200) with a tip. Thirty microliters (µl) of coelomic fluid were drawn from each earthworm into 20 µl of temperature adjusted PBS, using a 1 ml needle and syringe. This cell suspension was then added to the equal volume of trypan blue in the eppendorf microcentrifuge tube. The mixture in the eppendorf microcentrifuge tube was mixed thoroughly and left for 5 minutes. After 5 minutes the solution mixture was placed on the haemocytometer and covered with a cover slip. The blocks were numbered from 1 to 5 on each grid and all the viable and non-viable cells were counted in

each block. The viable cells appeared greenish and the non viable cells stained blue. The stained and unstained cells were counted after 5 minutes under a light microscope, using a Neubauer haemocytometer chamber. The percentage viable cells were calculated by dividing the number of unstained cells by the total number of cells counted per sample.

All the earthworms used in this procedure were killed by placing them separately in labeled 10 ml polytops in the freezer to be digested at a later stage.

2.7) ACID DIGESTION

The earthworms were placed separately in labeled petri dishes and dried in a Memmert oven for 48 hours at 60°C. They were weighed on the Precisa XB 220 balance after they had been dried and then placed in labeled test tubes. The soil and cow manure used in the laboratory experiments, as well as the earthworms were acid digested, filtered and stored according to the same method described on page 15 (No 2.3 Acid digestion).

2.8) LEAD ANALYSIS

The lead concentrations in the samples were determined according to the same method described on page16 (No 2.4 Lead analysis).

2.9) STATISTICAL ANALYSIS OF DATA

The data in this study was analyzed by using the Sigmastat 3.1 computer software package. The values are presented as the mean ± SD and the probability levels used for statistical significance were ($P<0.05$). T-tests and ANOVA on Ranks were used to compare the lead concentrations between the different sites and highways and in the earthworms.

CHAPTER 3: RESULTS

3.1) FIELD SURVEY

3.1.1) N7 HIGHWAY

3.1.1.1) Comparisons of lead concentrations in soil over the length of the highway

The mean lead concentrations for the outgoing and incoming sides of the N7 highway for the sampling period July 2004 to July 2005 are presented in Table 1 and illustrated in Figures 5 to 11. Lead concentrations are expressed in mg/kg.

Table 1: The mean lead concentrations (mg/kg) (± SD) for the outgoing and incoming sides of the N7 highway for the sampling period Jul 04 to Jul 05. Numbers 1 to 5 in the left column represent the sampling sites.

SAMPLING SITE N7		SAMPLING OCCASION						
		Jul-04	Sep-04	Nov-04	Jan-05	Mar-05	May-05	Jul-05
1) Out	Mean	a 74.78 1	a 130.55 2	a 74.82 1	a 72.01 1	a 49.37 3	a 125.80 2	a 79.63 1
	SD	8.60	7.46	36.32	11.49	4.44	14.77	5.01
1) In	Mean	a 81.39 1	a 111.61 1	a 114.66 1	a 86.73 1	a 208.08 2	a 206.44 2	a 87.94 1
	SD	21.01	9.94	16.93	17.83	59.96	93.81	23.76
2) Out	Mean	b 32.05 1	b 19.66 2	a 78.16 3	a 71.65 3	a 55.29 3	a 76.12 3	b 37.84 1
	SD	5.65	2.73	23.40	34.47	14.22	42.29	5.59
2) In	Mean	b 28.65 1	b 18.18 2	b 8.63 3	b 16.38 2	b 14.89 2	b 21.12 2	a 29.17 1
	SD	9.08	0.58	0.89	0.94	2.13	17.72	16.69
3) Out	Mean	c 184.73 1	c 52.84 2	a 51.92 2	a 93.21 3	b 109.42 3	b 19.22 4	c 226.77 5
	SD	47.36	3.23	9.32	7.45	32.10	3.20	60.11
3) In	Mean	b 15.41 1	c 46.50 2	c 56.23 2	c 48.99 2	c 49.44 2	b 63.08 2	b 18.29 1
	SD	2.94	8.22	12.20	12.18	4.58	59.11	2.53
4) Out	Mean	d 21.98 1	d 12.33 2	b 24.70 1	b 27.17 1	a 38.07 3	a 58.15 4	d 24.11 1
	SD	2.59	1.43	2.27	2.03	5.78	7.77	2.98
4) In	Mean	b 38.18 1	b 13.96 2	d 22.09 3	c 55.52 1	d 67.92 1	b 41.45 1	a 47.75 1
	SD	39.07	3.01	7.39	8.08	10.10	6.98	48.89
5) Out	Mean	d 17.51 1	c 46.01 2	b 24.52 3	c 5.01 4	a 46.17 2	c 40.50 2	d 21.96 5
	SD	2.34	4.67	1.82	0.73	16.21	2.47	2.50
5) In	Mean	b 17.41 1	d 67.07 2	c 57.54 3	a 96.44 4	d 73.43 2	b 40.15 5	a 22.76 6
	SD	2.36	4.74	6.70	6.34	8.51	14.33	2.74

Statistical significant differences are indicated with different letters or numbers. a = Comparisons of outgoing side over length of highway and *a* = comparisons of incoming side over length of highway. 1 = Comparisons over time on outgoing side and *1*= comparisons over time on the incoming side

a) Outgoing

In Jul 04 there were statistically significant differences ($P<0.05$) between most of the sites but no statistical differences between sites 4 and 5 ($P<0.05$). The mean lead concentration for site 3 (184.73 ± 47.36 mg/kg) was significantly higher than the concentrations found at the other sites (Fig 5).

There were statistically significant differences ($P<0.05$) during Sep 04 between most of the sites but no significant differences between sites 3 and 5 ($P>0.05$). During Sep 04 the mean lead concentration for site 1 (130.55 ± 7.46 mg/kg) was significantly higher than at sites 2, 3, 4 and 5 (Fig 6).

Pairwise multiple comparisons for the Nov 04 sampling occasion showed statistically significant differences ($P<0.05$) between most of the sites. No differences were however found for the following site comparisons: 2 and 3, 1 and 2, 1 and 3 & 4 and 5 ($P>0.05$) (Fig 7).

There were statistically significant differences ($P<0.05$) found between most sites in Jan 05 but site 3 did not differ significantly from sites 1 and 2 ($P>0.05$). The lowest mean lead concentration over the entire period on the N7 highway was found at site 5 (5.01 ± 0.73 mg/kg) (Fig 8).

In Mar 05 a mean lead concentration of 109.42 ± 32.10 mg/kg were found at site 3, which was significantly higher than the lead concentrations found at other sites ($P<0.05$). Comparisons showed no significant differences for other site comparisons (Fig 9).

Most of the sites in May 05 differed significantly ($P<0.05$) from each other and no differences were found between sites 1 and 4, 1 and 2, & 2 and 4 ($P>0.05$) (Fig 10).

In terms of lead concentration in soil, all the sites showed significant differences ($P<0.05$) from each other during Jul 05, except for sites 4 and 5. Site 3 (226.77 ± 60.11 mg/kg) had the highest mean concentration for this month (Fig 11).

b) Incoming

For Jul 04 there were statistically significant differences ($P<0.05$) in lead concentrations between sites. Lead concentrations at site 1 (81.39 ± 21.01 mg/kg) were statistically significantly higher than at any of the other sites ($P<0.05$). For the rest of the site comparisons there were no significant differences found ($P>0.05$) (Fig 5).

There were statistically significant differences ($P<0.05$) during Sep 04 between all of the sites with the exception of sites 2 and 4 ($P>0.05$). The mean lead concentration for site 1 (111.61 ± 9.94 mg/kg) was the highest for this month (Fig 6).

During Nov 04 there were statistically significant differences ($P<0.05$) between most sites but not between sites 3 and 5. Site 1, as in the previous two sampling occasions, had the highest mean lead concentration of 114.66 ± 16.93 mg/kg (Fig 7).

Statistically significant differences in lead concentrations were found ($P<0.05$) between most sites in Jan 05. The comparisons of sites 1 vs 5 & 3 vs 4 did not differ significantly from each other ($P>0.05$) (Fig 8).

In Mar 05 there were statistically significant differences ($P<0.05$) between most sites. Comparisons showed no significant differences between sites 4 and 5 ($P>0.05$). Site 1, with a mean lead concentration of 208.08 ± 59.96 mg/kg, was again significantly higher than the other sites ($P<0.05$) (Fig 9).

The lead concentrations found at site 1 were statistically significantly higher than those from the other sites in May 05 ($P<0.05$). No differences were found between any of the other sites ($P>0.05$). During this month, as well as for most of the other sampling occasions, site 1 (206.44 ± 93.81 mg/kg) showed the highest mean lead concentration (Fig 10).

Site comparisons 1 vs 3, 1 vs 5, 2 vs 3, 3 vs 4 & 3 vs 5 were found to be significantly different ($P<0.05$) from each other during Jul 05, in terms of soil lead concentrations. The other site comparisons did not reveal any other significant differences ($P>0.05$) (Fig 11).

3.1.1.2) Comparisons of soil lead concentrations over the sampling period per sampling site

See Table 1 on page 20 and Figures 5 to11 on page 27 to 30 for the following results.

a) Outgoing

Lead concentrations found at site 1 differed statistically ($P<0.05$) from each other during most of the sampling period. There were no significant differences in lead concentrations at site 1 between the following sampling occasions: Sep 04 and May 05, Nov 04 and Jul 05, Jan 04 and Jul 05, Jul 04 and Nov 04, Jul 04 and Jan 05, Nov 04 and Jan 05 ($P>0.05$). At site 1 the sampling occasions Sep 04 (130.55 ± 7.46 mg/kg) (Fig 6) and May 05 (125.80 ± 14.77 mg/kg) (Fig 10) had the highest mean lead concentrations for the sampling period.

Pairwise multiple comparisons showed statistical significant differences ($P<0.05$) in lead concentrations when the different sampling occasions of site 2 were compared. These significant differences were for the following comparisons: Sep 04 vs Nov 04, Jul 04 vs Nov 04, Nov 04 vs Jul 05, Sep 04 vs May 05, Jul 04 vs May 05, May 05 vs Jul 05, Sep 04 vs Jan 05, Jul 04 vs Jan 05, Jan 05 vs Jul 05, Sep 04 vs Mar 05, Jul 04 vs Mar 05, Mar 05 vs Jul 05, Sep 04 vs Jul 05 & Jul 04 vs Sep 04. No significant differences for the other occasion comparisons were found ($P>0.05$).

When the different sampling occasions at site 3 were compared in terms of lead concentrations, statistical significant differences ($P<0.05$) were found between most of the sampling occasions. There were no significant differences found between occasions: Jul 04 and Jul 05, Jan 05 and Mar 05, Sep 04 and Nov 04

(P>0.05). The highest mean lead concentration at site 3 for the sampling period was during Jul 05 (226.77 ± 60.11 mg/kg). It was also the highest concentration found over the sampling period (Fig 11).

When the different sampling occasions at site 4 were compared in terms of lead concentrations there were no statistical significant differences found for the following occasion comparisons: Nov 04 and Jan 05, Jan 05 and Jul 05, Jul 04 and Jul 05, Nov 04 and Jul 05 & Jul 04 and Nov 04 (P>0.05).

Statistically significant differences (P<0.05) between lead concentrations of different sampling occasions were found at site 5 but no differences were found for the following occasion comparisons: Sep 04 and May 05, Sep 04 and Mar 05 & Mar 05 and May 05 (P>0.05).

b) Incoming

At site 1, lead concentrations differed statistically (P<0.05) from those of other sites during the sampling period. However, there were no differences observed at site 1 for the following sampling occasion comparisons: Mar 05 and May 05, Jul 04 and Sep 04, Sep 04 and Jan 05, Sep 04 and Jul 05, Sep 04 and Nov 04, Jul 04 and Nov 04, Nov 04 and Jan 05, Nov 04 and Jul 05, Jul 04 and Jul 05, Jan 05 and Jul 05 & Jul 04 and Jan 05 (P>0.05). Sampling occasions Mar 05 (208.08 ± 59.96 mg/kg) and May 05 (206.44 ± 93.81 mg/kg) had the highest mean lead concentrations at site 1 over the sampling period (Figs 9, 10).

Most comparisons showed statistical significant differences (P<0.05) when the lead concentrations on the different sampling occasions at site 2 were compared. These comparisons were: Jul 05 vs Sep 04, Nov 04, Jan 05, Mar 05, May 05, & Jul 04 vs Sep 04, Nov 04, Jan 05, Mar 05, May 05. Also, Nov 04 vs Jan 05, Nov 04 vs May 05 & Nov 04, Mar 05.

Site 3's lead concentrations differed significantly ($P<0.05$) during the sampling period except between the following occasions: Nov 04 and May 05, Sep 04 and Nov 04, Nov 04 and Jan 05, Nov 04 and Mar 05, Mar 05 and May 05, Sep 04 and Mar 05, Mar 05 and Jan 05, Jan 05 and May 05, Sep 04 and Jan 05, Sep 04 and May 05, Jul 04 and Jul 05 ($P>0.05$).

Statistical significant differences ($P<0.05$) were found between most of the sampling occasions at site 4 but no differences were found in the following occasion comparisons: Jan 05 and Mar 05, Jul 04 and Mar 05, May 05 and Jul 05, Jul 04 and Jul 05 ($P\geq0.05$).

Most occasion comparisons for site 5 showed statistical significant differences ($P<0.05$) during the sampling period. Only Sep 04 and Mar 05 did not differ significantly from each other in terms of lead concentrations ($P>0.05$).

3.1.1.3) Comparisons between the corresponding outgoing and incoming sides of the N7

The total average lead concentrations of the outgoing sides of sites 1 to 5 were compared to the total average lead concentrations of the incoming sides of sites 1 to 5. A statistical significant difference ($P=0.038$) was found between the outgoing and incoming sites during the Jul 04 occasion but no significant differences were found during Sep 04 ($P=0.892$), Nov 04 ($P=0.698$), Jan 05 ($P=0.467$), Mar 05 ($P=0.222$), May 05 ($P=0.352$) or Jul 05 ($P=0.071$).

The lead concentrations of the outgoing sides of the individual sites were compared with those of the incoming sides of the individual sites, per sampling occasion. For sampling occasion Jul 04 most sites did not show any significant differences for outgoing vs incoming comparisons. A statistical significant difference was found at site 3 ($P=0.008$). The outgoing mean lead concentration for site 3 in Jul 04 was 184.73 ± 47.36 mg/kg as apposed to the incoming mean concentration of 15.41 ± 2.94 mg/kg (Fig 5).

During Sep 04 statistical differences were found at site 1 (P=0.009) and 5 (P=<0.001) but no significant differences between found at sites 2 (P=0.548), 3 (P=0.148) and 4 (P=0.304) (Fig 6).

Most sites did not show any significant differences in Nov 04. Sites 2 (P=0.008) and 5 (P=<0.001) showed statistical significant differences for outgoing vs incoming comparisons (Fig 7).

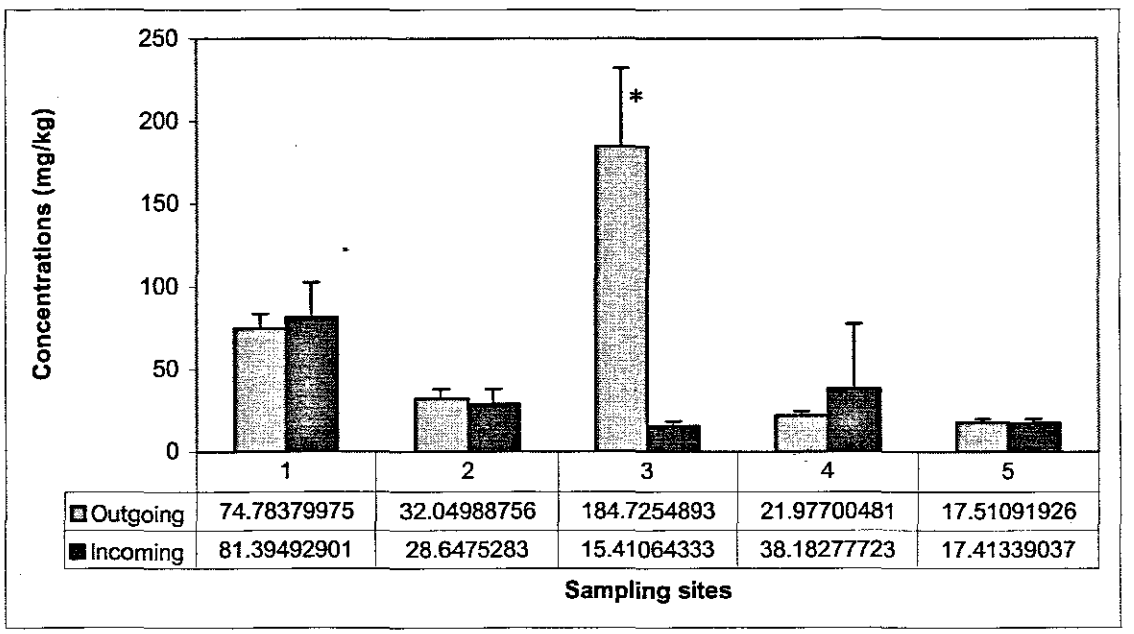
In Jan 05, outgoing vs incoming comparisons differed statistically significantly at sites 2 (P=0.008), 3 (P=<0.0001), 4 (P=0.008) and 5 (P=0.008). Only site 1 (P=0.056) did not show a significant difference (Fig 8).

Outgoing vs incoming comparisons done for Mar 05 showed statistical significant differences at most sites. There was no significant differences at site 5 (P=0.056). The mean lead concentration of the incoming side of site 1 (208.08 ± 59.96 mg/kg) during Mar 05 was significantly higher than the outgoing side's mean lead concentration of 49.37 ± 4.44 mg/kg. At site 3 the outgoing mean concentration (109.42 ± 32.10 mg/kg) for the same month was significantly higher than the mean concentration from the incoming side (49.44 mg/kg) (Fig 9).

In May 05 significant differences were found at sites 1(P=0.016), 2 (P=0.032), 3 (P=0.008) and 4 (P=0.007). There were no significant differences found at site 5 (P=0.151) in terms of lead concentration (Fig 10).

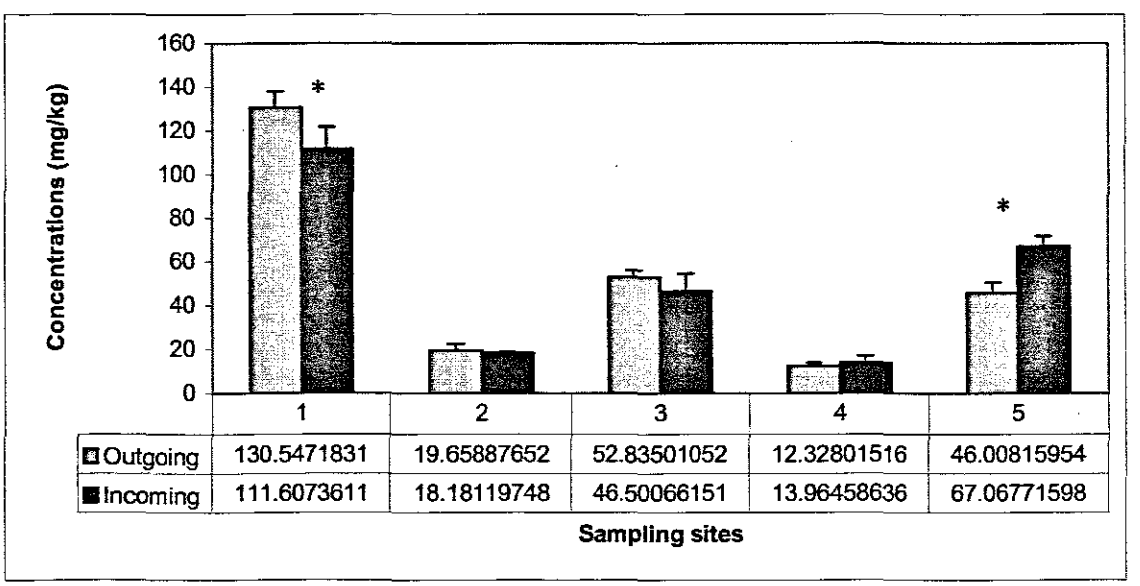
Only site 3 (P=0.008) showed a significant difference in Jul 05. No statistical differences were found at other sites. The mean lead concentration for the outgoing side of site 3 (226.77 ± 60.11 mg/kg) differed greatly from the incoming side's mean concentration of 18.29 ± 2.53 mg/kg (Fig11).

Graphs of the soil lead concentrations of the N7 highway over the sampling period Jul 04 to Jul 05. Statistically significant differences between the incoming vs outgoing sides of sites are indicated with an asterix above the graph bars.



	1	2	3	4	5
Outgoing	74.78379975	32.04988756	184.7254893	21.97700481	17.51091926
Incoming	81.39492901	28.6475283	15.41064333	38.18277723	17.41339037

Figure 5: Mean lead concentrations (mg/kg) (± SD) for the N7 highway for the Jul 04 sampling occasion. N = 5.



	1	2	3	4	5
Outgoing	130.5471831	19.65887652	52.83501052	12.32801516	46.00815954
Incoming	111.6073611	18.18119748	46.50066151	13.96458636	67.06771598

Figure 6: Mean lead concentrations (mg/kg) (± SD) for the N7 highway for the Sep 04 sampling occasion. N = 5

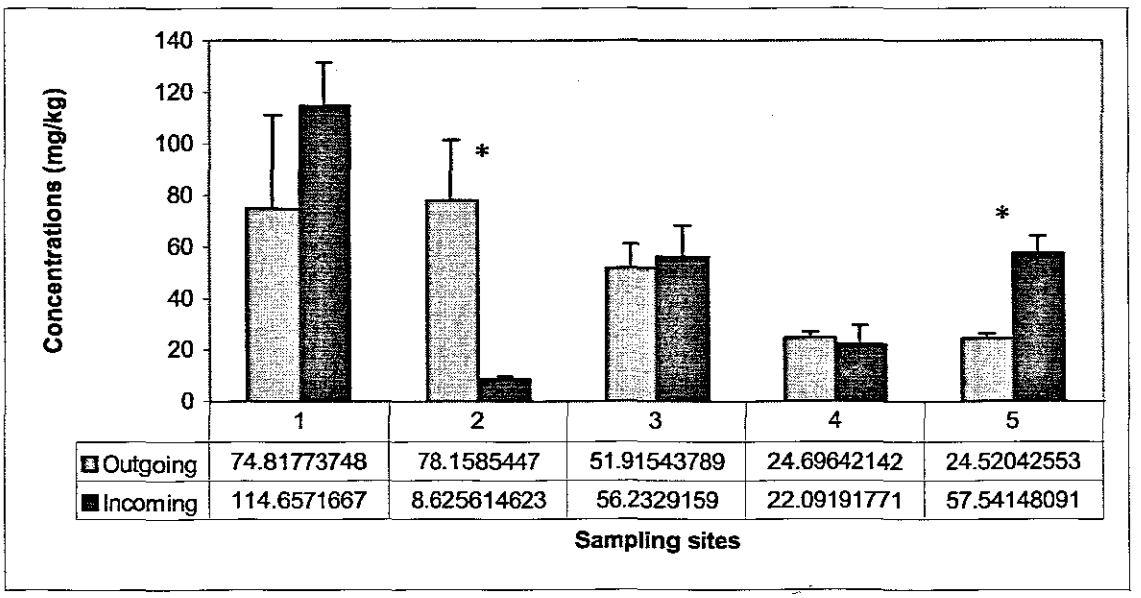


Figure 7: Mean lead concentrations (mg/kg) (± SD) for the N7 highway for the Nov 04 sampling occasion. N = 5.

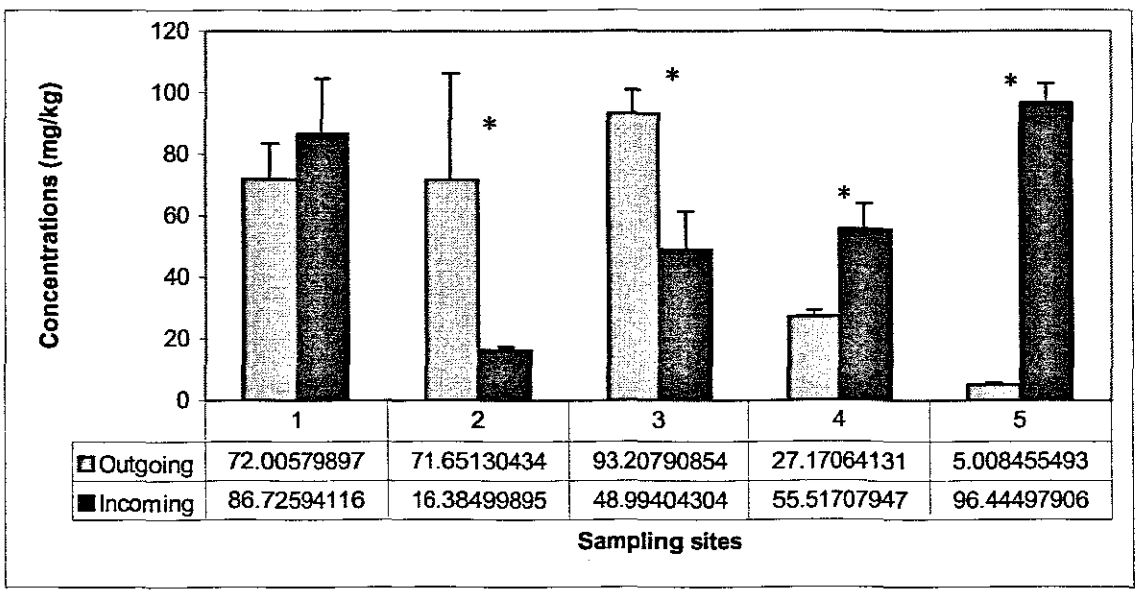


Figure 8: Mean lead concentrations (mg/kg) (± SD) for the N7 highway for the Jan 05 sampling occasion. N = 5.

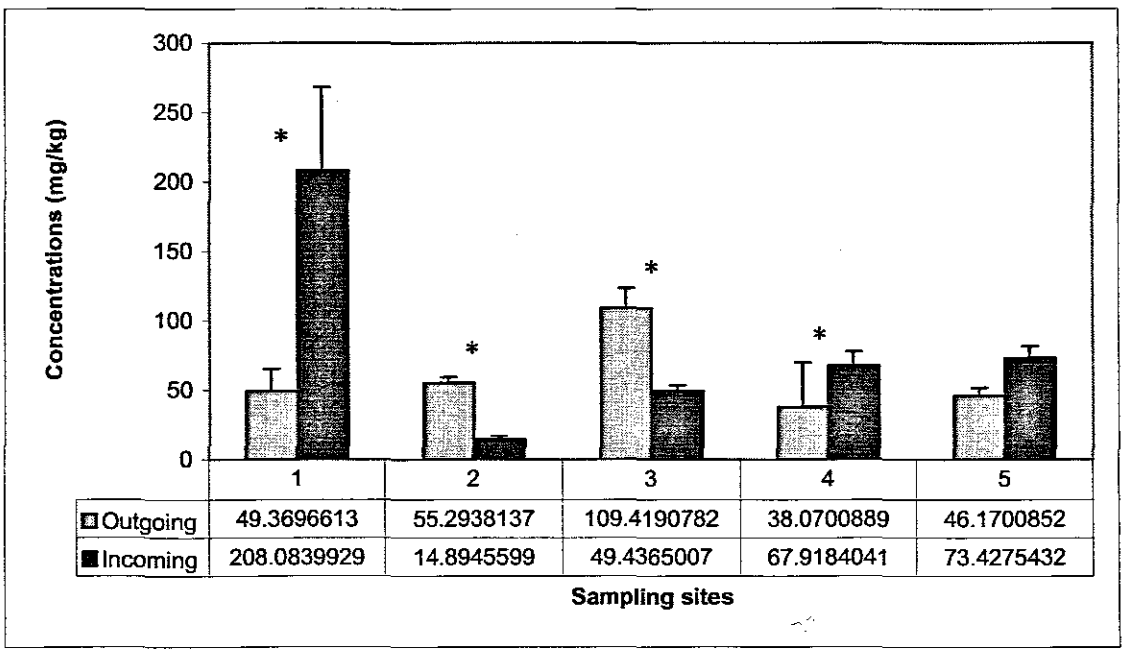


Figure 9: Mean lead concentrations (mg/kg) (± SD) for the N7 highway for the Mar 05 sampling occasion. N = 5.

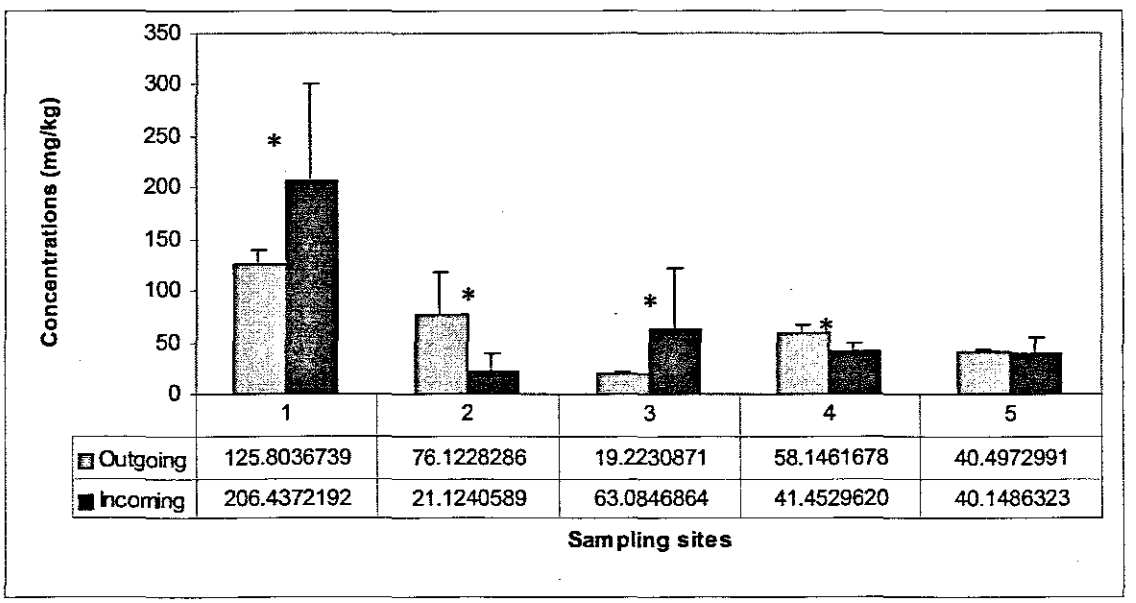


Figure 10: Mean lead concentrations (mg/kg) (± SD) for the N7 highway for the May 05 sampling occasion. N = 5.

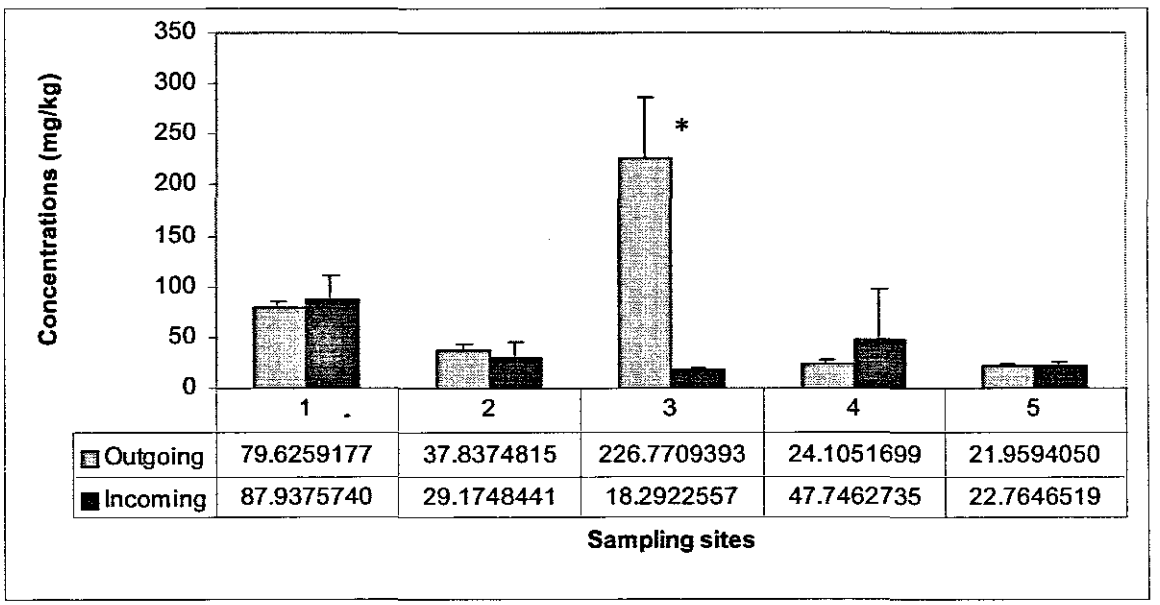


Figure 11: Mean lead concentrations (mg/kg) (± SD) for the N7 highway for the Jul 05 sampling occasion. N = 5.

3.1.1.4) pH and Moisture

pH ranged from a very acid of 2.3 to an alkaline 9.7 and the moisture % of the soil ranged from a relatively moist 24.7% to a dry 0.2%.

Table 2: pH and moisture % of soil of the N7 highway over the sampling period Jul 04 to Jul 05. Numbers 1 to 5 in the left column represent the sampling sites.

SAMPLING SITE N7		SAMPLING OCCASION						
		Jul-04	Sep-04	Nov-04	Jan-05	Mar-05	May-05	Jul-05
1) Out	pH	8	6.5	5	5.2	8.5	9	9.3
	Moisture	*2.5*	*6.5*	*2.4*	*2.7*	*1.3*	*3.7*	*9.5*
1) In	pH	2.3	6.5	4.7	5.9	7.5	8.8	8.8
	Moisture	*3.5*	*2.5*	*2.9*	*0.9*	*0.8*	*4.2*	*9.1*
2) Out	pH	7.5	6.4	4.8	5.3	8.2	8.6	8.4
	Moisture	*1.6*	*4.4*	*3.8*	*0.7*	*1.1*	*8.74*	*4.5*
2) In	pH	7.2	6.3	4.6	6	7.2	8.4	8.2
	Moisture	*2.4*	*1.8*	*0.3*	*0.9*	*0.4*	*1.5*	*7.1*
3) Out	pH	7.4	6.4	4.9	5.3	7.9	9.7	8.1
	Moisture	*5.7*	*5.9*	*2.4*	*1.2*	*0.9*	*8.8*	*24.7*
3) In	pH	7.1	6.3	4.6	5.7	7.3	9.3	8
	Moisture	*2.6*	*3.2*	*3.5*	*0.6*	*0.6*	*6.1*	*5*
4) Out	pH	7.2	6.2	4.8	5.6	7.7	8.9	8
	Moisture	*0.2*	*1.8*	*2.2*	*0.6*	*0.5*	*6.8*	*4.8*
4) In	pH	7.1	6.4	4.5	5.7	7.4	8.7	8
	Moisture	*0.8*	*1.8*	*3.7*	*0.4*	*0.5*	*3*	*3.4*
5) Out	pH	6.9	5.9	4.7	5.8	7.3	8.5	8.8
	Moisture	*0.8*	*14.2*	*5.3*	*0.6*	*0.7*	*13.7*	*4.3*
5) In	pH	6.9	6.3	4.6	3.6	7.2	8.2	9.2
	Moisture	*5.4*	*7.7*	*8*	*0.7*	*0.6*	*15.8*	*12.2*

3.1.1.5) Rainfall and vehicle densities in the vicinity of the sampling sites of the N7 highway over the sampling period Jul 04 to Jul 05

Rainfall in the vicinity of sites 1, 2 and 3 was taken at the Altydgedacht station and sites 4 and 5 at the Atlantis station.

Table 3: Rainfall (mm) in the area of the N7 highway for the sampling period Jul 04 to Jul 05 (The Cape Town Weather Office). Numbers 1 to 5 in the left column represent the sampling sites.

SAMPLING SITES	SAMPLING OCCASION						
	Jul-04	Sep-04	Nov-04	Jan-05	Mar 05	May 05	Jul-05
1	104.5	28.2	10	27.3	6.1	99.6	44.2
2	104.5	28.2	10	27.3	6.1	99.6	44.2
3	104.5	28.2	10	27.3	6.1	99.6	44.2
4	75.3	23.5	6.5	21	3	75.9	27.5
5	75.3	23.5	6.5	21	3	75.9	27.5

Vehicle counts on the outgoing side were taken north of Plattekloof road and on the incoming side the vehicle counts were taken south of Koeberg road. Light and heavy vehicles were presented separately.

Table 4: Vehicle counts taken on the N7 highway for a period within the sampling period of this study. The counts were taken by the CMC Directorate Transport from 3 to 5 Aug 04 and again on the 12th and 13th of Oct 04, and were the only data available.

N7 HIGHWAY	Vehicles	TRAFFIC COUNTS					
		03-Aug-04	04-Aug-04	05-Aug-04	12- Oct- 04	13- Oct- 04	TOTAL
Car density: Outgoing	Light	8149	8276	6395	15424	15879	54123
North	Heavy	1282	1333	741	2278	2410	8044
TOTAL		**9431**	**9609**	**7136**	**17702**	**18289**	**62167**
Car density: Incoming	Light	7819	7904	6517	6490	6521	35251
South	Heavy	1124	1187	700	1329	1337	5677
TOTAL		**8943**	**9091**	**7217**	**7819**	**43099**	**76169**

3.1.2) N2 HIGHWAY

3.1.2.1) Comparisons of lead concentrations in soil over the length of the highway

The mean lead concentrations for the outgoing and incoming sides of the N2 highway for the sampling period July 2004 to July 2005 are presented in Table 5 and illustrated in Figures 12 to 18. Lead concentrations are expressed in mg/kg.

Table 5: The mean lead concentrations (mg/kg) (± SD) for the outgoing and incoming sides of the N2 highway for the sampling period Jul 04 to Jul 05. Numbers 6 to 10 in the left column represent the sample sites.

SAMPLING SITE N2		SAMPLING OCCASION						
		Jul-04	Sep-04	Nov-04	Jan-05	Mar-05	May-05	Jul-05
6) Out	Mean	a 277.97 1	a 66.28 2	a 1522.59 3	a 208.20 4	a 513.32 1	a 885.77 5	a 366.17 7
	SD	82.29	8.36	92.38	32.45	71.70	224.73	100.59
6) In	Mean	*a* 161.24 *1*	*a* 99.18 *2*	*a* 204.50 *1*	*a* 115.81 *2*	*a* 232.98 *1*	*a* 520.17 *1*	*a* 208.16 *1*
	SD	47.47	8.55	13.33	12.73	14.72	31.85	63.52
7) Out	Mean	a 254.90 1	a 93.73 2	b 138.42 3	b 297.60 1	a 541.46 1	a 995.76 1	a 321.69 1
	SD	46.71	3.39	21.53	11.13	393.83	276.59	49.33
7) In	Mean	*b* 517.23 *1*	*b* 58.79 *2*	*b* 83.14 *2*	*b* 28.88 *3*	*b* 46.42 *1*	*b* 79.49 *2*	*b* 640.68 *1*
	SD	128.02	8.77	35.35	7.40	9.50	51.43	147.35
8) Out	Mean	b 27.78 1	b 19.03 1	c 59.71 2	c 236.33 3	b 103.88 1	b 290.17 4	b 32.56 1
	SD	5.28	8.92	7.94	41.00	6.06	92.90	4.38
8) In	Mean	*a* 73.22 *1*	*c* 18.55 *2*	*c* 44.49 *3*	*a* 113.86 *4*	*b* 59.92 *1*	*b* 141.19 *1*	*c* 84.25 *1*
	SD	31.11	0.38	7.48	40.36	8.53	36.40	35.05
9) Out	Mean	c 71.57 1	a 80.07 1	c 45.48 1	c 47.23 1	c 44.68 1	b 213.50 1	c 79.74 1
	SD	13.33	20.79	9.32	9.56	20.07	24.79	17.63
9) In	Mean	*a* 64.51 *1*	*c* 16.47 *2*	*d* 23.76 *3*	*c* 57.33 *1*	*c* 23.62 *1*	*b* 107.68 *3*	*c* 78.35 *1*
	SD	56.16	4.96	6.39	20.45	3.74	40.83	74.27
10)Out	Mean	a 300.49 1	b 20.12 2	d 260.30 3	d 458.70 4	a 317.01 1	b 233.13 1	a 365.12 6
	SD	17.82	0.98	21.89	35.72	33.55	30.35	12.13
10) In	Mean	*c* 313.81 *1*	*c* 40.55 *2*	*e* 407.70 *3*	*c* 79.52 *4*	*c* 16.08 *1*	*c* 276.20 *1*	*d* 357.2 *6*
	SD	35.02	2.48	18.49	4.17	2.99	51.76	19.72

Statistical significant differences are indicated with different letters or numbers. a = Comparisons of outgoing side over length of highway and *a* = comparisons of incoming side over length of highway. 1 = Comparisons over time of outgoing side and *1*= comparisons over time on the incoming side

a) Outgoing

In Jul 04 there were statistically significant differences ($P<0.05$) between most of the sites but no statistical differences between sites 7 and 10, 6 and 10 & 6 and 7. The mean lead concentrations found at sites 6 (277.97 ± 82.29 mg/kg), 7 (254.90 ± 46.71 mg/kg) and 10 (300.49 ±17.82 mg/kg) were significantly higher than the concentrations found at the other sites for Jul 04 (Fig 12).

There were statistically significant differences ($P<0.05$) during Sep 04 between most of the sites but no significant differences between sites 6 and 7, 7 and 9, 6 and 9 & 8 and 10. The lowest mean lead concentration found on the N2 highway over the sampling period was measured in this month at site 8 (19.03 ± 8.92 mg/kg) (Fig 13).

Pairwise multiple comparisons for the Nov 04 sampling occasion showed statistically significant differences ($P<0.05$) between most of the sites but no differences between sites 8 and 9. The mean lead concentration for sites 6 (1522.59 ± 92.38 mg/kg) and 10 (260.30 ± 21.89 mg/kg) were significantly higher than at sites 7, 8 and 9 ($P<0.05$) (Fig 14).

There were statistically significant differences ($P<0.05$) found between most sites in Jan 05 but sites 1 and 8 did not differ significantly from each other ($P>0.05$). The mean lead concentration for site 10 (458.70 ± 35.72 mg/kg) were the highest for this sampling occasion (Fig 15).

In Mar 05 there were statistically significant differences ($P<0.05$) between the following sites: 6 and 9, 6 and 8, 7 and 9, 7 and 8, 9 and 10, 8 and 10 & 8 and 9. Comparisons showed no significant differences in lead concentration between sites 6 and 10, 2 and 6 & 7 and 10. The mean lead concentrations at site 6 (513.32 ± 71.70 mg/kg), 7 (541.46 ± 393.83 mg/kg), and 10 (317.01 ± 33.55 mg/kg) were significantly higher than at the other sites ($P<0.05$) (Fig 16).

Most of the sites in May 05 differed significantly ($P<0.05$) from each other and no significant differences were found between sites: 6 and 7, 8 and 9, 8 and 10 & 9 and 10 ($P>0.05$). The mean lead concentration at site 6 (885.77 ± 224.73 mg/kg) and 7 (995.76 ± 276.59 mg/kg) were significantly higher than those found at other sites ($P>0.05$) (Fig 17).

In terms of lead concentrations in soil, the sites showed significant differences ($P<0.05$) from each other during Jul 05. In the site comparisons: 7 vs 10, 6 vs 10 & 6 vs 7 no significant differences were found ($P>0.05$). The mean lead concentrations at sites 6 (366.17 ± 100.59 mg/kg), 7 (321.69 ± 49.33 mg/kg) and10 (365.12 ± 12.13 mg/kg) showed the highest mean lead concentration for this month (Fig 18).

b) Incoming

In Jul 04 there were statistically significant differences ($P<0.05$) in lead concentrations between most of the sites. However, no differences were found for the following site comparisons: 6 vs 9, 6 vs 8 & 8 vs 9 ($P>0.05$). Site 7 (517.23 ± 128.02 mg/kg) and 10 (313.81 ± 35.02 mg/kg) showed significantly higher mean lead concentrations than the other sites ($P<0.05$) (Fig 12).

There were statistically significant differences ($P<0.05$) in terms of lead concentrations during Sep 04 between most sites but no significant differences were found when sites 8 and 10, 9 and 10 & 8 and 9 were compared ($P>0.05$) (Fig 13).

During Nov 04 there were statistically significant differences ($P<0.05$) between all of the sites. The mean lead concentrations at site 10 (407.70 ± 18.49 mg/kg) were significantly higher than at the rest of the sites (Fig 14).

Statistically significant differences ($P<0.05$) in lead concentrations were found between most sites in Jan 05. The site comparisons 6 vs 8 & 9 vs 10 did not differ significantly from each other ($P>0.05$). The mean lead concentration at site 6 (115.81 ± 12.73 mg/kg) and 8 (113.86 ± 40.36 mg/kg) were significantly higher than at site 7, 9 and 10 ($P<0.05$) (Fig 15).

In Mar 05 comparisons showed statistically significant differences ($P<0.05$) between most sites. Comparisons showed no significant differences between lead concentrations for the site comparisons 7 vs 8 & 9 vs 10 ($P>0.05$). The mean lead concentration at site 6 (232.98 ± 14.72 mg/kg) was significantly higher than the other sites ($P<0.05$). The lowest mean lead concentration found on the incoming side of the N2 highway over the sampling period was 16.08 ± 2.99 mg/kg at site 10 (Fig 16).

Site comparisons in May 05 that differed significantly ($P<0.05$) from each other were: 6 vs 7, 8 and 10 & 10 vs 7, 8 and 9. No significant differences were found in lead concentrations when sites 8 and 9, 7 and 8 & 7 and 9 were compared to each other ($P>0.05$). The mean lead concentrations at site 6 (520.17 ± 31.85 mg/kg) were significantly higher than at other sites ($P<0.05$) (Fig 17).

Comparisons showed sites to be significantly different ($P<0.05$) from each other during Jul 05 in terms of soil lead concentration except between sites 8 and 9. The mean lead concentration at site 7 (640.68 ± 147.35 mg/kg) were significantly higher than at the other sites (Fig 18).

3.1.2.2) Comparisons of soil lead concentrations over the sampling period, per sampling site

See Table 5 on page 33 and Figures 12 to18 on page 41 to 44 for the following results.

a) Outgoing

Lead concentrations found at site 6 differed statistically ($P<0.05$) from each other through the entire sampling period. The Nov 04 (1522.59 ± 92.38 mg/kg) sampling occasion had the highest mean lead concentration over the sampling period (Fig 14).

Pairwise multiple comparisons showed statistical significant differences ($P<0.05$) in lead concentrations when the different sampling occasions of site 7 were compared. These differences were for the comparisons: May 05 vs Jul 04, Sep 04, Nov 04, Jan 05, Mar 05, Jul 05, as well as Mar 05 vs Sep 04 and Nov 04. Also, for: Jul 04 vs Jul 05, Nov 04 vs Jul 05, Jul 04 vs Sep 04, Jul 04 vs Nov 04 & Sep 04 vs Nov 04 ($P<0.05$). No significant differences for the other occasion comparisons were found ($P>0.05$). The highest mean lead concentration during the sampling period was found at site 7 in May 05 (995.76 ± 276.59 mg/kg) (Fig 17).

When the different sampling occasions at site 8 were compared, in terms of lead concentrations, there were statistical significant differences ($P<0.05$) between most of the sampling occasions. No significant differences were found for occasion comparisons Jan 05 vs May 05, Jul 04 vs Jul 05 & Jul 04 vs Sep 04 ($P>0.05$). The highest mean lead concentration at site 8 as in the previous sampling site was in May 05 (290.17 ± 92.90 mg/kg) (Fig 17).

When the different sampling occasions for site 9 were compared, in terms of lead concentrations, statistical significant differences ($P<0.05$) between the following sampling occasions were found: May 05 vs, Jul 04, Sep 04, Nov 04, Jan 05, Mar 05, Jul 05, as well as Sep 04 vs Mar 05, Mar 05 vs Jul 05, Sep 04 vs Nov 04, Sep 04 vs Jan 05. No significant differences were found for the other occasion comparisons ($P>0.05$). As in the previous two occasions, May 05 had the highest mean lead concentration (213.5 ± 24.79 mg/kg) for site 9 (Fig 17).

Statistically significant differences ($P<0.05$) between lead concentrations of different sampling occasions were found at site 10 but no differences were found for the following occasion comparison: Jul 04 vs Mar 05 ($P>0.05$). Jan 05 with a mean lead concentration of 458.70 ± 35.72 mg/kg had the highest concentrations for site 10 (Fig 15).

b) Incoming

At site 6, lead concentrations differed statistically ($P<0.05$) throughout the entire sampling period. However, there were no statistical differences observed at site 6 for the following occasion comparisons: Mar 05 vs Jul 05, Nov 04 vs Mar 05, Jul 04 vs Nov 04, Nov 04 vs Jul 05, Jul 04 vs Jul 05 & Sep 04 vs Jan 05 ($P>0.05$). The highest mean lead concentration for this site was 520.17 ± 31.85 mg/kg in May 05 (Fig 17).

Most comparisons showed statistical significant differences ($P<0.05$) when the lead concentrations on the different sampling occasions at site 7 were compared. No significant differences between the following occasions were found: Jul 04 and Jul 05, Nov 04 and Mar 05, Nov 04 and May 05, Sep 04 and Nov 04, Sep 04 and Mar 05, Sep 04 and May 05, Mar 05 and May 05 ($P>0.05$). Jul 05 had the highest mean lead concentration for this sampling site (640.68 ± 147.35 mg/kg) (Fig 18).

There were statistically significant differences ($P<0.05$) found at site 8 for most of the pairwise comparisons of lead concentrations measured for the different sampling occasions, except for the following occasion comparisons: Jan 05 vs May 05, Mar 05 vs Jul 05, Jul 04 vs Jul 05, Jul 04 vs Mar 05 ($P>0.05$). May 05 (141.19 ± 36.40 mg/kg) had the highest mean lead concentration for this site (Fig 17).

The following occasion comparisons differed significantly at site 9, in terms of lead concentrations: May 05 vs Sep 04, Nov 04, Mar 05, as well as Jul 05 vs Sep 04, Nov 04, Mar 05, as well as Jan 05 vs Sep 04, Nov 04, Mar 05, as well as Jul 04 vs Sep 04, Nov 04, Mar 05. Sep 04 also differed significantly ($P<0.05$) from Nov 04 and Mar 05. The highest mean lead concentration was in May 05 (107.68 ± 40.83 mg/kg) (Fig 17).

Statistically significant differences ($P<0.05$) at site 10 were found between most of the sampling occasions apart from comparisons: Jul 04 vs Jul 05 and Jul 04 vs May 05 ($P<0.05$). Nov 04 had the highest mean lead concentration for this site of 407.70 ± 18.49 mg/kg (Fig 14).

3.1.2.3) Comparisons between the corresponding outgoing and incoming sides of the N2

The total average lead concentrations of the outgoing sides of sites 6 to 10 were compared to the total average lead concentrations of the incoming sides of sites 6 to 10. Most of the comparisons showed no statistical significant differences but significant differences were found for the Jan 05 (P=<0.001), Mar 05 (P=<0.001) and May 05 (P=<0.001) comparisons.

The lead concentrations of the outgoing sides of the individual sites were compared with those of the incoming sides of the individual sites, per sampling occasion. For sampling occasion Jul 04 most sites did not show any significant differences for outgoing vs incoming comparisons. A statistical significant difference was found at site 7 (P=0.003). The mean lead concentration at site 7 on the incoming side (517.23 ± 128.02 mg/kg) was significantly higher than that of the outgoing side of site 7 (254.90 ± 46.71 mg/kg) (Fig 12) (P=<0.05).

During Sep 04 statistical differences in lead concentrations were found at most sites. No significant differences were found at site 8 (P=0.151) (Fig 13).

All the sites showed statistical significant differences in terms of lead concentration in the Nov 04 comparisons. The mean lead concentration on the outgoing side of site 6 (1522.59 ± 92.38 mg/kg) was significantly higher than the lead concentration on the incoming side (204.50 ± 13.33 mg/kg) (P=<0.05) (Fig 14).

In Jan 05 outgoing vs incoming comparisons differed statistically significantly at most of the sites. Only site 9 (P=0.346) did not show a significant difference (P>0.05) in lead concentrations. The mean lead concentration of the outgoing side of site 10 (458.7 ± 35.72 mg/kg) was significantly higher when compared with the incoming side of the site (79.52 ± 4.17 mg/kg) (P=<0.05) (Fig 15).

Outgoing vs incoming comparisons done for Mar 05 showed statistical significant differences at most sites: 6 (P=<0.001), 7 (P=0.008), 8 (P= <0.001) and 10 (P=0.008), except for at site 9 (P=0.056) (Fig 16).

There were no significant differences found at site 10 (P=0.147) in terms of lead concentration in the May 05 sampling occasion. All of the other outgoing vs incoming comparisons per site differed significantly from each other: 6 (P=0.008), 7 (P=0.008), 8 (P=0.008), 9 (P=0.001). Site 7, on the outgoing side, had a relatively high mean lead concentration of 995.76 ± 276.59 mg/kg as apposed to the incoming side's relatively low mean lead concentration of 79.49 ± 51.43 mg/kg (Fig 17).

Most of the outgoing vs incoming comparisons showed a significant difference in lead concentrations in the Jul 05 sampling occasion. No statistical differences were found in the site 9 (P=0.151) and 10 (P=0.467) outgoing vs incoming comparisons. The mean lead concentration for site 7, on the incoming side, (640.68 ± 147.35 mg/kg) differed significantly from that of the outgoing side's (321.69 ± 49.33 mg/kg) (Fig 18).

Graphs of the soil lead concentrations of the N2 highway over the sampling period Jul 04 to Jul 05. Statistically significant differences between the incoming vs outgoing sides of sites are indicated with an asterix above the graph bars.

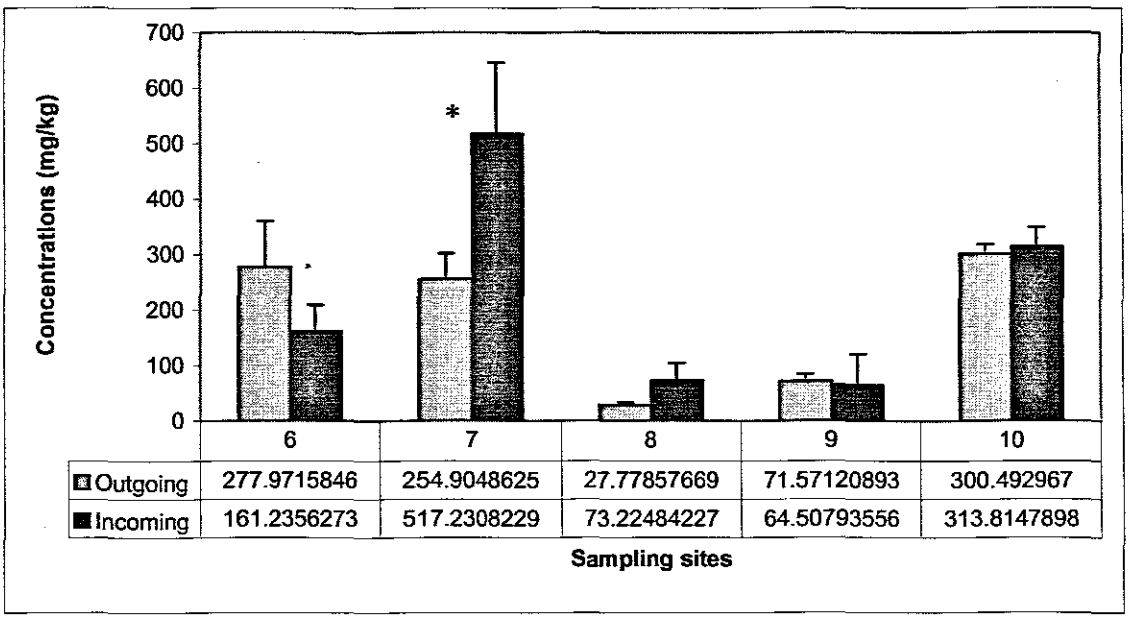


Figure12: Mean lead concentrations (mg/kg) (± SD) for the N2 highway for the Jul 04 sampling occasion. N = 5.

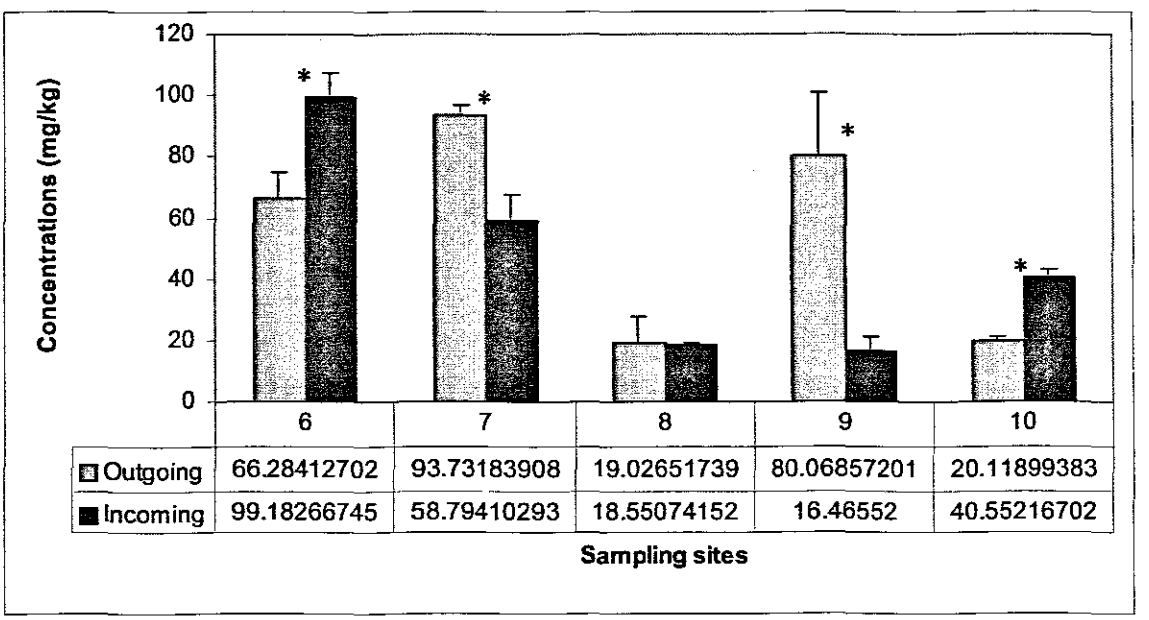


Figure 13: Mean lead concentrations (mg/kg) (± SD) for the N2 highway for the Sep 04 sampling occasion. N = 5.

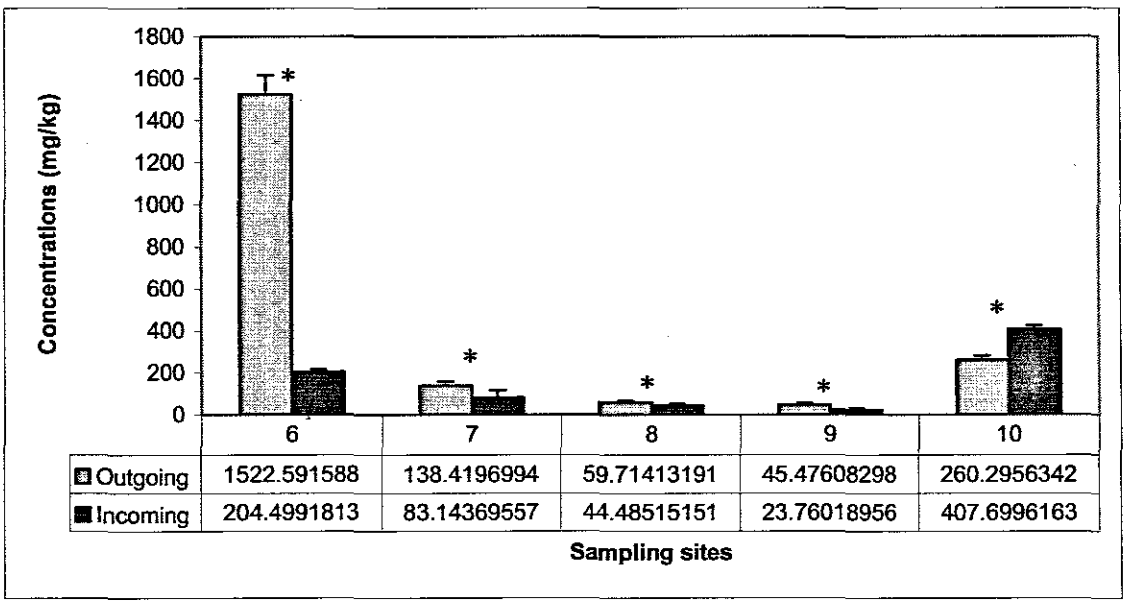


Figure14: Mean lead concentrations (mg/kg) (± SD) for the N2 highway for the Nov 04 sampling occasion. N = 5.

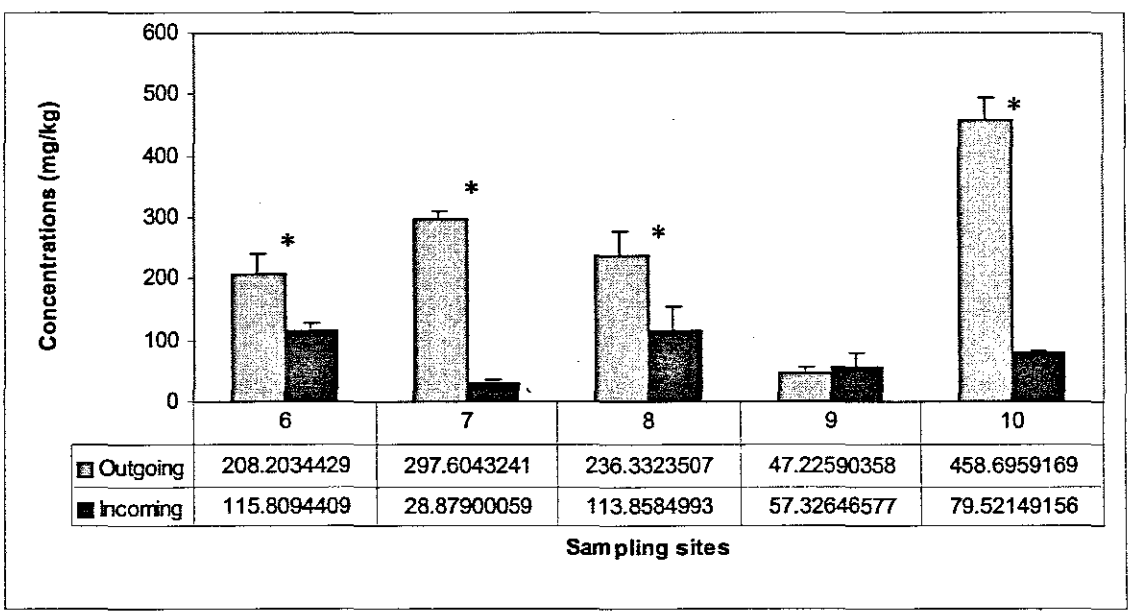


Figure 15: Mean lead concentrations (mg/kg) (± SD) for the N2 highway for the Jan 05 sampling occasion. N = 5.

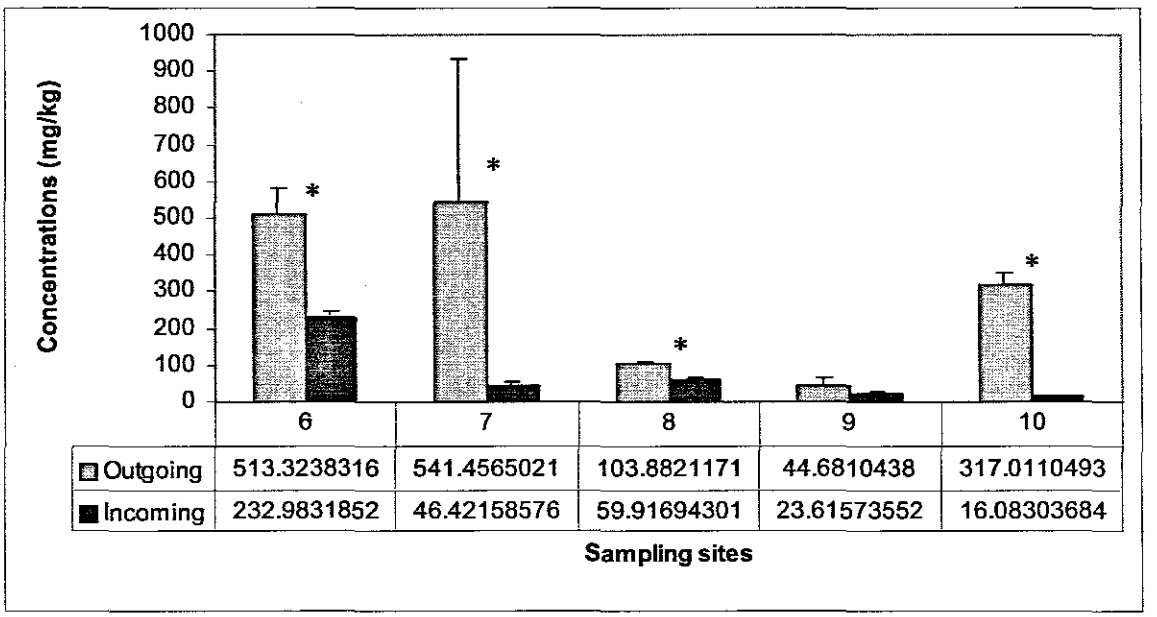


Figure 16: Mean lead concentrations (mg/kg) (± SD) for the N2 highway for the Mar 05 sampling occasion. N = 5.

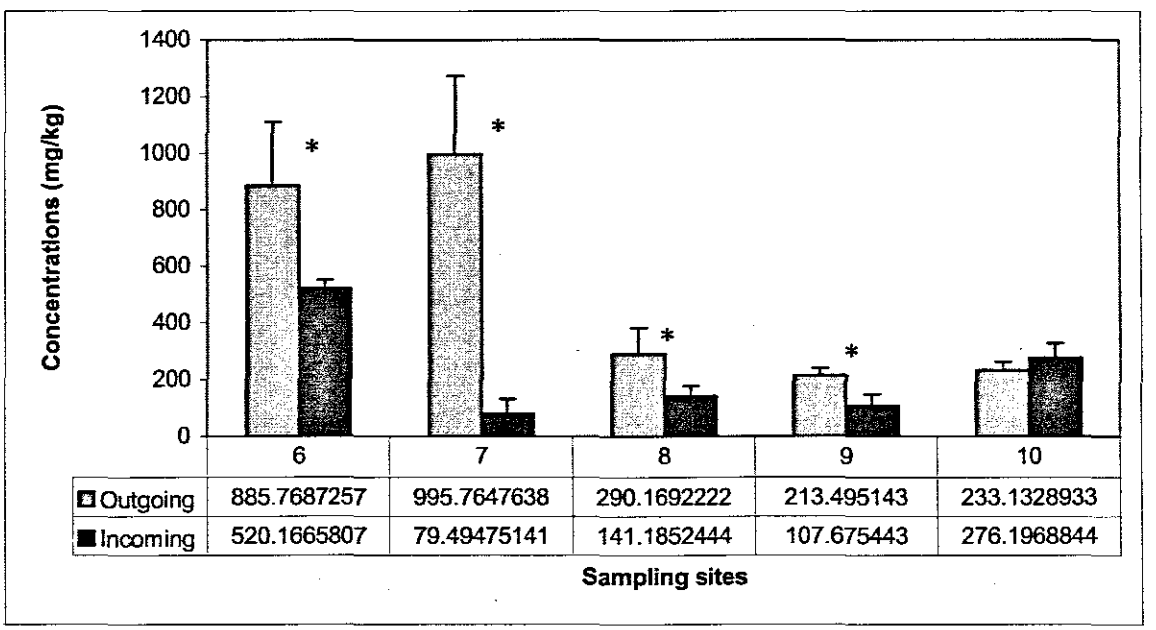


Figure 17: Mean lead concentrations (mg/kg) (± SD) for the N2 highway for the May 05 sampling occasion. N = 5.

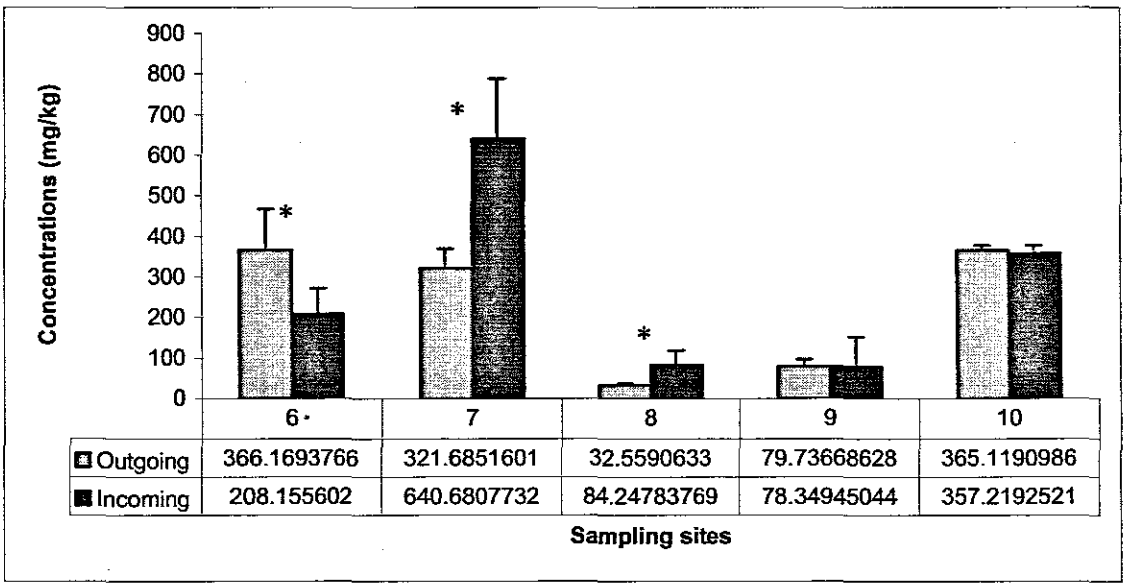


Figure 18: Mean lead concentrations (mg/kg) (± SD) for the N2 highway for the Jul 05 sampling occasion. N = 5.

3.1.2.4) pH and moisture

pH ranged from a very acid 2.9 to an alkaline 9.7 and the moisture % of the soil ranged from a relatively moist 26.5% to a dry 0.7%.

Table 6: pH and moisture % of soil of the N2 highway over the sampling period Jul 04 to Jul 05. Numbers 6 to 10 in the left column represent the sampling sites.

SAMPLING SITE		SAMPLING OCCASION						
		Jul-04	Sep-04	Nov-04	Jan-05	Mar-05	May-05	Jul-05
6) Out	pH	6.8	6.4	4.9	3.1	9.1	9.1	9.1
	Moisture	*11.6*	*3.7*	*2.9*	*2.3*	*1.5*	*5.7*	*12.6*
6) In	pH	8.6	8	5.7	2.9	8.3	8.7	9.2
	Moisture	*18.4*	*10.6*	*6*	*4.9*	*1.7*	*12.4*	*14.8*
7) Out	pH	7.5	6.6	5.1	3.1	8.9	8.8	8.8
	Moisture	*6.1*	*7.5*	*4.5*	*0.8*	*0.7*	*9.5*	*7.13*
7) In	pH	9.4	7.7	5.8	2.9	8.3	8.9	8.6
	Moisture	*5.6*	*1.3*	*5.4*	*2.4*	*1*	*5.8*	*4.6*
8) Out	pH	8.8	8.3	5.5	2.9	9.1	9.2	9.5
	Moisture	*7.8*	*3.9*	*5*	*5.3*	*5.8*	*2.8*	*6.1*
8) In	pH	9.7	8.1	6.8	2.9	8.1	9.1	9.3
	Moisture	*7.1*	*2.7*	*4.9*	*0.9*	*1.3*	*3.1*	*6.6*
9) Out	pH	9.3	8	5.6	3	8.8	9.5	9.4
	Moisture	*4.9*	*2.2*	*4.5*	*4.1*	*0.7*	*5.3*	*8*
9) In	pH	9.7	8.2	7.1	2.8	8	9.5	9.4
	Moisture	*8.8*	*2.8*	*3.6*	*9.3*	*1*	*13.6*	*11.5*
10) Out	pH	8.7	8.1	5.7	2.9	8.6	9.5	9
	Moisture	*23.7*	*7.1*	*5.7*	*22.1*	*26.5*	*8.8*	*15.5*
10) In	pH	9	8.3	7.3	2.8	8.1	9.3	8.8
	Moisture	*13.7*	*6.1*	*12.4*	*8.6*	*1.8*	*18.3*	*13.3*

3.1.2.5) Rainfall and vehicle densities in the vicinity of the sampling sites of the N2 highway over the sampling period Jul 04 to Jul 05

Rainfall in the vicinity of sites 6 and 7 was taken at the Molteno station, sites 8, 9 at the Airport and site 10 at the Strand station.

Table 7: Rainfall (mm) in the area of the N2 highway for the sampling period Jul 04 to Jul 05 (The Cape Town Weather Office). Numbers 6 to 10 in the left column represent the sampling sites.

SAMPLING SITES	SAMPLING OCCASION						
	Jul-04	Sep-04	Nov-04	Jan-05	Mar-05	May-05	Jul-05
6	128.4	48	9.6	22.6	15.8	14.2	57.4
7	128.4	48	9.6	22.6	15.8	14.2	57.4
8	64.7	25.1	3.4	24.5	8.7	77.7	64.6
9	64.7	25.1	3.4	24.5	8.7	77.7	64.6
10	94.2	42.6	5.4	50.6	16.2	68.8	44.2

Vehicle counts on the incoming side in June were taken west of Jan Smuts drive and Borchards quarry and on the outgoing side the vehicle counts were taken east of Blackriver Parkway and Modderdam road. In August the counts were taken on the Eersteriver Bridge. Light and heavy vehicles were presented separately.

Table 8: Vehicle counts taken on N2 highway for a period within the sampling period of this study. Counts include heavy and light vehicles. The counts were taken by the CMC Directorate Transport from 23 to 28 Jun 04 and again on the 3rd of Aug 04, and were the only data available.

N2 HIGHWAY	Vehicles	TRAFFIC COUNTS					
		23-Jun-04	24-Jun-04	28-Jun-04	29-Jun-04	03-Aug-04	TOTAL
Car density: Incoming West	Light	19027	42728	18934	34037	12470	**127196**
	Heavy	790	1950	1357	2702	532	**7331**
TOTAL		19817	44678	20291	36739	13002	**134527**
Car density: Outgoing East	Light	27008	46607	20751	34134	12478	**140978**
	Heavy	918	1942	1701	2812	738	**8111**
TOTAL		**27926**	**48549**	**22452**	**36946**	**13216**	**149089**

3.1.3) N1 HIGHWAY

3.1.3.1) Comparisons of lead concentrations in soil over the length of the highway

The mean lead concentrations for the outgoing and incoming sides of the N1 highway for the sampling period July 2004 to July 2005 are presented in Table 9 and illustrated in Figures 19 to 25. Lead concentrations are expressed in mg/kg.

Table 9: The mean lead concentrations (mg/kg) (± SD) for the outgoing and incoming sides of the N1 highway for the sampling period Jul 04 to Jul 05. Numbers 11 to 16 in the left column represent the sampling sites.

SAMPLING SITE N1		SAMPLING OCCASION						
		Jul-04	Sep-04	Nov-04	Jan-05	Mar-05	May-05	Jul-05
11) Out	Mean	a 457.38 1	a 552.57 2	a 682.89 3	a 609.91 3	a 404.63 1	a 368.00 1	a 424.79 1
	SD	85.13	185.49	80.87	94.95	52.75	77.01	61.70
11) In	Mean	a 666.83 1	a 362.40 2	a 480.66 3	a 495.25 3	a 630.11 1	a 322.47 2	a 397.75 2
	SD	113.10	75.11	34.26	43.69	99.08	59.15	60.88
12) Out	Mean	b 28.37 1	b 128.76 2	b 1274.30 3	b 8.63 4	b 82.79 2	b 100.88 2	b 23.21 1
	SD	3.49	26.62	35.85	2.13	49.34	26.67	6.11
12) In	Mean	b 1990.31 1	a 557.37 2	a 254.02 3	b 584.33 2	b 382.56 4	b 598.37 2	b 1138.12 5
	SD	172.42	92.42	42.38	30.61	49.21	43.46	115.71
13) Out	Mean	c 102.19 1	c 56.61 2	c 479.95 3	c 247.19 4	c 260.89 4	c 1270.48 5	c 195.93 6
	SD	10.46	10.10	37.48	18.98	42.52	226.27	12.56
13) In	Mean	c 76.59 1	b 76.81 1	a 349.28 2	c 163.52 5	c 112.66 4	c 754.66 5	a 583.90 6
	SD	3.14	18.51	10.26	6.91	5.70	91.33	58.35
14) Out	Mean	a 593.39 1	a 440.03 2	d 199.69 3	d 135.21 4	a 382.19 2	c 1300.25 5	a 359.89 2
	SD	7.22	59.65	35.65	19.14	96.74	133.88	50.27
14) In	Mean	d 12.96 1	b 56.64 2	b 1279.47 3	d 100.35 4	d 21.38 5	b 518.13 6	a 859.32 7
	SD	3.24	3.25	99.78	13.11	1.33	73.32	184.81
15) Out	Mean	c 81.90 1	c 41.52 1	e 64.91 1	e 77.62 1	b 110.01 2	d 201.43 3	a 281.38 3
	SD	18.46	27.94	8.30	6.62	12.17	27.03	89.50
15) In	Mean	e 59.27 1	b 45.26 2	a 205.83 3	e 58.82 1	c 80.88 4	d 142.13 3	c 200.78 5
	SD	4.87	6.39	246.27	1.58	3.19	23.06	14.13
16) Out	Mean	b 22.15 1	c 26.71 1	f 108.51 2	f 24.80 1	b 128.66 2	d 253.13 3	a 396.54 4
	SD	2.71	2.83	5.53	1.56	45.79	50.37	50.87
16) In	Mean	e 50.48 1	c 130.93 2	c 52.55 1	e 47.53 1	c 119.88 1	a 283.96 3	a 422.22 4
	SD	3.62	10.42	11.99	5.76	132.99	76.46	111.53

Statistical significant differences are indicated with different letters or numbers. a = Comparisons of outgoing side over length of highway and a = comparisons of incoming side over length of highway. 1 = Comparisons over time of outgoing side and 1= comparisons over time on the incoming side

a) Outgoing

In Jul 04 there were statistical significant differences ($P<0.05$) between most of the sites but no statistical differences between sites 11 and 14, 13 and 15 or 12 and 16 ($P>0.05$). The mean lead concentrations at site 11 (457.38 ± 85.13 mg/kg) and 14 (593.39 ± 7.22 mg/kg) were significantly higher than concentrations found at other sites (Fig 19).

There were statistical significant differences ($P<0.05$) found between most of the sites in Sep 04 but no significant differences between sites 11 and 14, 13 and 16, 13 and 15 or 15 and 16. The highest mean lead concentrations were found at sites 11 (552.57 ± 185.49 mg/kg) and 14 (440.03 ± 59.65 mg/kg) (Fig 20).

Pairwise multiple comparisons for the Nov 04 sampling occasion showed statistically significant differences ($P<0.05$) between all of the sites. The mean lead concentration for site 12 (1274.30 ± 35.85 mg/kg) were the highest for this sampling occasion (Fig 21).

There were statistically significant differences ($P<0.05$) found between all sites in Jan 05. Site 11 (609.91 ± 94.95 mg/kg) had the highest mean lead concentrations (Fig 22).

In Mar 05 mean lead concentrations for sites 11 (404.63 ± 52.75 mg/kg), 13 (260.89 ± 42.52 mg/kg) and 14 (382.19 ± 96.74 mg/kg) were significantly higher than at other sites. There were statistically significant differences ($P<0.05$) found between most sites. Comparisons showed no significant differences for the following pairwise site comparisons: 11 and 14, 12 and 15, 15 and 16 & 12 and 16 ($P>0.05$) (Fig 23).

Most of the sites in May 05 differed significantly ($P<0.05$) from each other and no differences were found between sites 13 and 14 or 15 and 16 ($P>0.05$). The mean lead concentration at site 13 (1270.48 ± 226.27 mg/kg) and site 14 (1300.25 ± 133.88 mg/kg) were higher than at the other sites (Fig 24).

In terms of lead concentrations in soil, pairwise comparisons showed significant differences ($P<0.05$) in Jul 05. The following pairwise site comparisons did not differ significantly from each other: sites 11 and 15, 11 and 14, 11 and 16, 15 and 16, 14 and 16, & 14 and 15 ($P>0.05$). The mean lead concentrations at sites 11 (424.79 ± 61.70 mg/kg), 14 (359.89 ± 50.27 mg/kg) and 16 (396.54 ± 50.82 mg/kg) were significantly higher than at the other sites on the N1 highway in Jul 05 (Fig 25).

b) Incoming

For Jul 04 there were statistical significant differences ($P<0.05$) in lead concentrations between most of the site comparisons, with the exception of sites 15 and 16 ($P>0.05$). Significantly higher mean lead concentrations were found at sites 11 (666.83 ± 113.10 mg/kg) and 12 (1990.31 ± 172.42 mg/kg) ($P<0.05$) (Fig 19).

There were statistical significant differences ($P<0.05$) during Sep 04 between most of the sites but no significant differences in lead concentrations in the pairwise comparisons for sites 11 vs 12, 13 vs 14 or 14 vs 15 ($P>0.05$). The mean lead concentration at sites 11 (362.40 ± 75.11 mg/kg) and 12 (557.37 ± 92.42 mg/kg) were significantly higher than at other sites (Fig 20).

During Nov 04 there were statistical significant differences ($P<0.05$) between most of the sites. These were for the following site comparisons: 13 vs 16 & 14 vs 11, 12, 13, 15. Also: 11 vs 16, 13 vs 16, 12 vs 16 & 15 vs 16. No differences were found between the other site comparisons ($P>0.05$). The mean lead concentration at site 14 (1279.47 ± 99.78 mg/kg), was the highest for this sampling occasion (Fig 21).

Statistical significant differences ($P<0.05$) in lead concentrations between most of the site comparisons in Jan 05 were found, except for site 15 vs 16 ($P>0.05$). Sites 11 (495.25 ± 43.69 mg/kg) and 12 (584.33 ± 30.61 mg/kg) showed significantly higher mean lead concentrations than sites 13, 14, 15 and 16 (Fig 22).

In Mar 05 there were statistical significant differences ($P<0.05$) between most

sites. Pairwise comparisons showed no significant differences between sites 13 and 16, 13 and 15 or 15 and 16 ($P>0.05$). Site 11 (630.11 ± 99.08 mg/kg) showed a mean lead concentration that was significantly higher than at other sites (Fig 23).

In the May 05 sampling occasion, the following pairwise site comparisons did not differ from each other: 12 vs 14 & 11 vs 16 ($P>0.05$). However, most of the other site comparisons differed statistically significantly ($P<0.05$) from each other. The highest mean lead concentrations for this sampling occasion were found at site 13 (754.66 ± 91.33 mg/kg) (Fig 24).

Statistical differences ($P<0.05$), in terms of soil lead concentration, were found at most sites in Jul 05 except for the 13 vs 14, 11 vs 13, 13 vs 16 & 11 vs 16 ($P>0.05$) pairwise site comparisons. The highest mean lead concentrations for this sampling occasion Jul 05 were found at site 12 (1138.12 ± 115.71 mg/kg) (Fig 25).

3.1.3.2) Comparisons of soil lead concentrations over the sampling period, per sampling site

See Table 9 on page 47 and Figures 19 to 25 on pages 55 to 58 for the following results.

a) Outgoing

Lead concentrations found at site 11 differed statistically ($P<0.05$) from each other during most of the sampling period. These differences were for the occasion comparisons: Nov 04 vs Jul 04, Sep 04, Mar 05, May 05, Jul 05, as well as Jan vs Jul 04, Sep 04. Mar 05, May 05, Jul 05. Also: Sep 04 vs Jul 04, Mar 05, May 05, Jul 05. There were no significant differences in lead concentrations for the other sampling occasion comparisons ($P>0.05$).

Pairwise multiple comparisons showed mostly statistical significant differences ($P<0.05$) in lead concentrations when the different sampling occasions of site 12 were compared. No significant differences between the following pairwise occasion comparisons were found: Sep 04 vs Mar 05, Sep 04 vs May 05, Mar 05 vs May 05 & Jul 04 vs Jul 05 ($P>0.05$). The mean lead concentration at site 12 in Nov 04 (1274.30 ± 35.85 mg/kg) was significantly higher when compared to the other sampling occasions (Fig 21).

When the different sampling occasions at site 13 were compared, in terms of lead concentrations, there were statistical significant differences ($P<0.05$) between most of the sampling occasions. There was no significant differences found between occasions Jan 05 and Mar 05 ($P>0.05$) (Fig 24).

When the different sampling occasions at site 14 were compared, in terms of lead concentrations, significant differences ($P<0.05$) were found between most of the sampling occasions. There were no significant differences found between the following occasion comparisons: Sep 04 vs Jul 05, Sep 04 vs Mar 05 & Mar 05 vs Jul 05 ($P>0.05$). The highest mean lead concentration at site 14 was during sampling occasion May 05 (1300.25 ± 133.88 mg/kg) (Fig 24).

Statistical significant differences ($P<0.05$) at site 15 between lead concentrations of different sampling occasions were found but no significant differences were found for the following occasion comparisons: May 05 vs Jul 05, Sep 04 vs Jan 05, Nov 04 vs Jan 05, Jul 04 vs Jan 05, Jul 04 vs Sep 04, Jul 04 vs Nov 05 & Sep 04 vs Nov 04 ($P>0.05$).

Site 16 showed statistical significant differences ($P<0.05$) in soil lead concentration during the sampling period for most of the occasion comparisons: No significant differences were found for the following occasion comparisons: Nov 04 vs Mar 05, Jul 04 vs Sep 04, Sep 04 vs Jan 05 and Jul 04 vs Jan 05 ($P>0.05$).

b) Incoming

At site 11 lead concentrations differed statistically ($P<0.05$) during the sampling period. However, there were no differences observed at site 11 for the following occasion comparisons: Jul 04 vs Mar 05, Nov 04 vs Jan 05, May 05 vs Jul 05, Sep 04 vs Jul 05 and Sep 04 vs May 05 ($P>0.05$).

Most comparisons showed statistical significant differences ($P<0.05$) when the lead concentrations on the different sampling occasions at site 12 were compared. No significant differences between the following occasions were found: Sep 04 vs May 05, Jan 05 vs May 05 & Sep 04 vs Jan 05 ($P>0.05$). Sampling occasion Jul 04 (1990.31 ± 172.42 mg/kg) had the highest mean lead concentration over the sampling period at site 12 (Fig 19).

There were statistical significant differences ($P<0.05$) found at site 13 for most of the pairwise comparisons of lead concentrations found on the different sampling occasions, except for the following that did not show any significant differences: Jul 04 vs Sep 05 ($P>0.05$). The May 05 (754.66 ± 91.33 mg/kg) sampling occasion had the highest mean lead concentration over the sampling period (Fig 24).

Site 14 showed significant ($P<0.05$) differences in all the occasion comparisons over the entire sampling period. The highest mean lead concentration of 1279.47 ± 99.78 mg/kg was found in Nov 04 (Fig 21).

Statistically significant differences ($P<0.05$) between most of the sampling occasions were found at site 15 but no differences were found in the following occasion comparisons: Nov 04 vs May 05 & Jul 04 vs Jan 05 ($P>0.05$).

Occasion comparisons for site 16 showed statistical significant differences ($P<0.05$) over the sampling period for most of the comparisons. No differences in lead concentrations were found in the following occasion comparisons: Nov 04 vs Mar 05, Jul 04 vs Jan 05, Jul 04 vs Nov 04 & Nov 04 vs Jan 05 ($P>0.05$).

3.1.3.3) Comparisons of lead concentrations between the corresponding outgoing and incoming sides of the N1

The total average lead concentrations of the outgoing sides of sites 11 to 16 were compared to the total average lead concentrations of the incoming sides of sites 11 to 16. Most of the sites showed no statistical significant differences except for a significant difference that was found in Jul 05 (P=<0.001).

The lead concentrations of the outgoing sides of the individual sites were compared to the corresponding incoming sides of the individual sites, per sampling occasion. For sampling occasion Jul 04 statistical significant differences for outgoing vs incoming side comparisons were found at all sites. Site 12 on the incoming side (1990.31 ± 172.42 mg/kg) had a mean lead concentration that was significantly higher than the concentration found on the outgoing side (28.37 ± 3.49 mg/kg) (Fig 19).

In Sep 04 statistical differences in lead concentration were found at most sites but no significant differences were found at sites 11 (P=0.066), 13 (P=0.064) and 15 (P=0.778) (Fig 20).

All the sites showed statistical significant differences between the each other during the Nov 04 sampling occasion. The lead concentration of the outgoing side of site 12 (1274.30 ± 35.85 mg/kg) was significantly higher than the incoming side (254.02 ± 42.38 mg/kg). Also, the incoming side of site 14 (1279.47 ± 99.78 mg/kg) was also significantly higher than the outgoing side of site 14 (199.69 ± 35.65 mg/kg) (Fig. 21).

At all the sites significant differences were found for outgoing vs incoming comparisons. In Jan 05 the incoming side of site 12 (584.33 ± 30.61 mg/kg) showed a significantly higher mean lead concentration than on the outgoing side (8.63 ± 2.13 mg/kg) (Fig 22).

Outgoing vs incoming comparisons for Mar 05 showed statistical significant differences at most sites with the exception of site 16 (P=0.151). The lead concentration at site 14 on the outgoing side (382.19 ± 96.74 mg/kg) was found to be significantly higher than on the incoming side of the site (21.38 ± 1.33 mg/kg) (Fig 22).

There were no significant differences found at the following sites during sampling occasion May 05 in terms of outgoing vs incoming lead concentrations: 12 (P=<0.001), 13 (P=0.008), 14 (P=<0.001), 15 (P=0.006), with the exception of site 11 (P=0.310) and 16 (P=0.473). In May 05 the site 14 on the outgoing side had a mean lead concentration of 1300.25 ± 133.88 mg/kg and was significantly higher than on the incoming side (518.13 ± 73.32 mg/kg) (Fig 24).

Comparisons done in Jul 05 showed statistical significant differences between outgoing vs incoming sides at most sites except for site 11 (P=0.505), 15 (P=0.095) and 16 (P=0.652). A high mean lead concentration of 1138.12 ± 115.71 mg/kg on the incoming side of site 12 was found and was significantly higher than on the outgoing side of site 12 (23.21 ± 6.11 mg/kg) (Fig 25).

Graphs of the soil lead concentrations of the N1 highway over the sampling period Jul 04 to Jul 05. Statistically significant differences between the incoming vs outgoing sides of sites are indicated with an asterix above the graph bars.

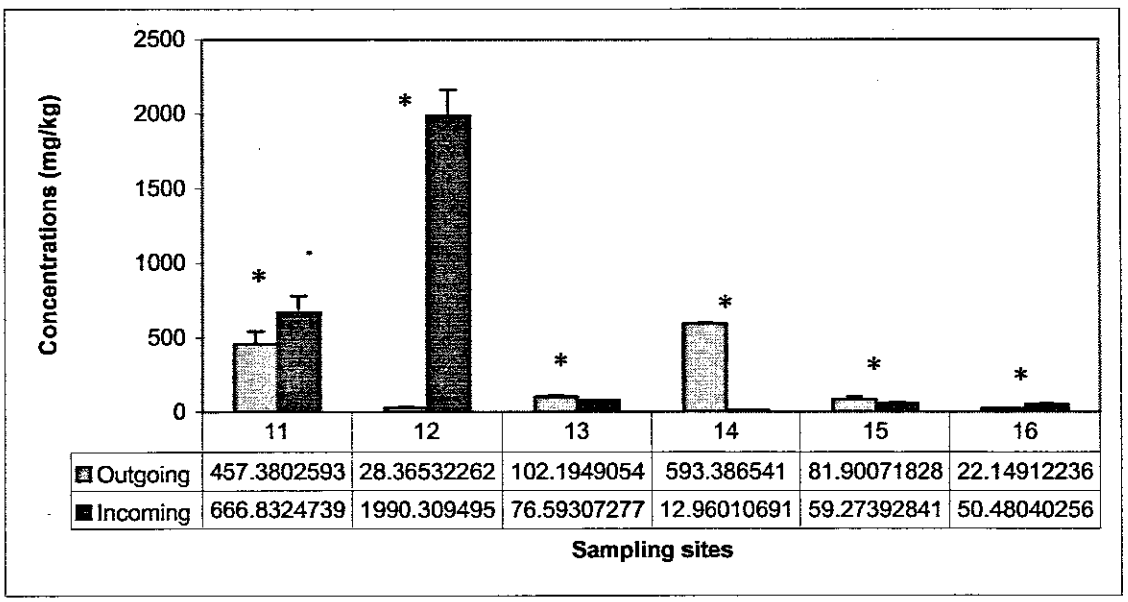


Figure 19: Mean lead concentrations (mg/kg) (± SD) for the N1 highway for the Jul 04 sampling occasion. N = 5.

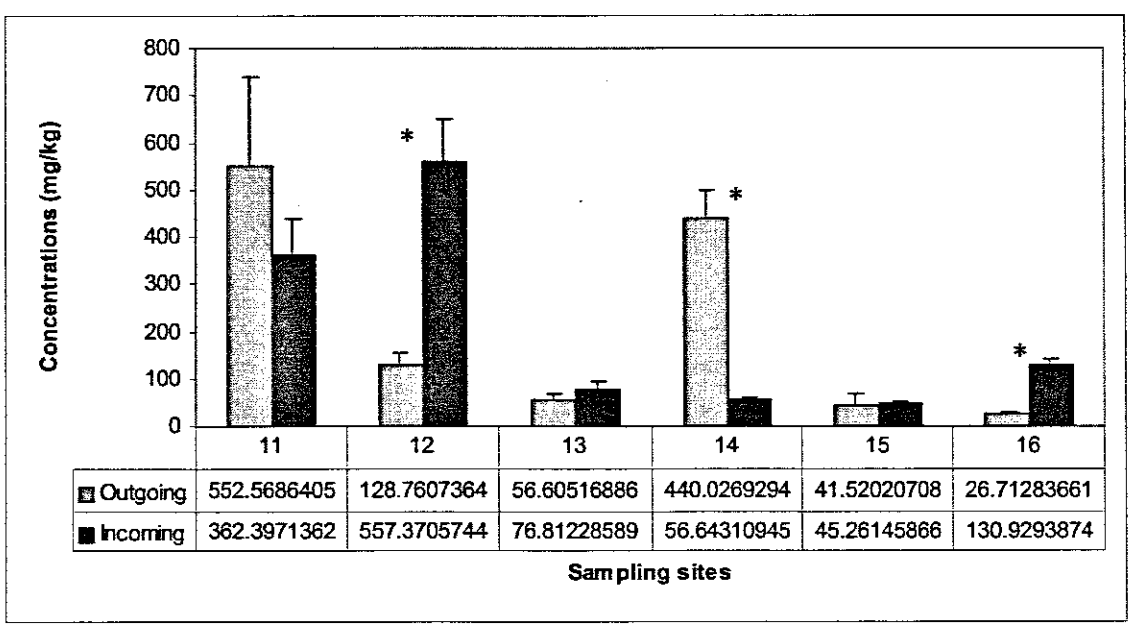


Figure 20: Mean lead concentrations (mg/kg) (± SD) for the N1 highway for the Sep 04 sampling occasion. N = 5.

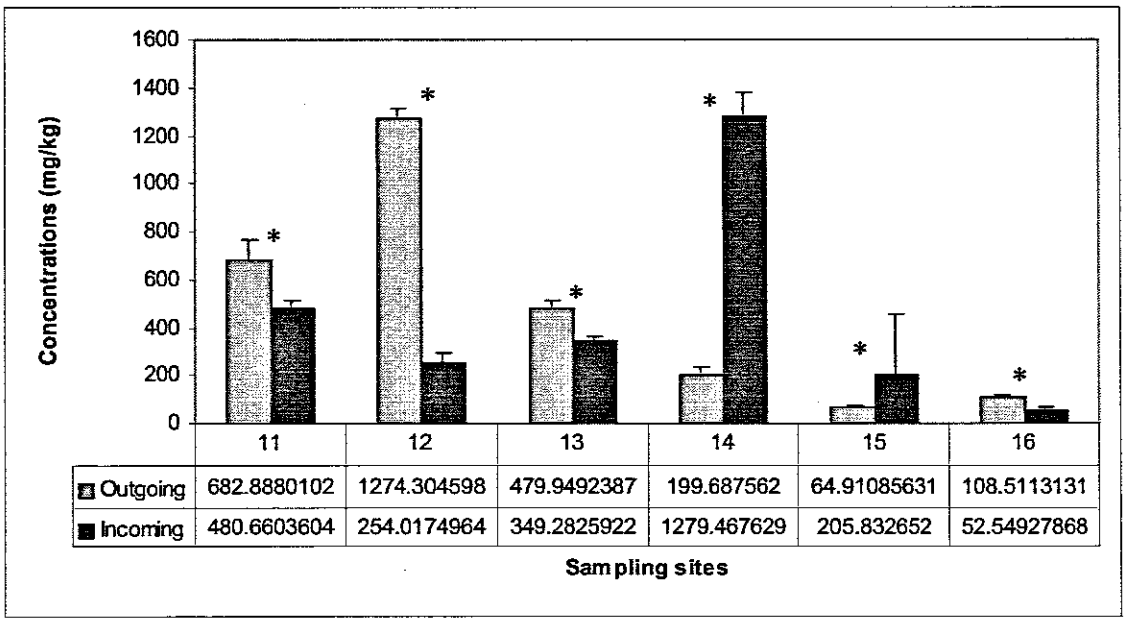


Figure 21: Mean lead concentrations (mg/kg) (± SD) for the N1 highway for the Nov 04 sampling occasion. N = 5.

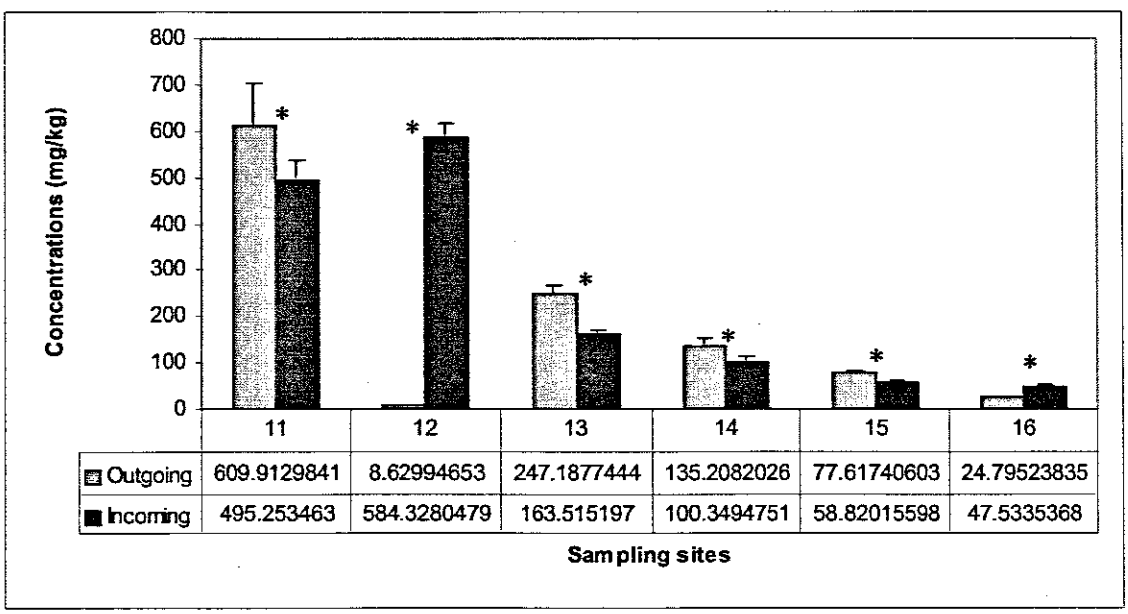


Figure 22: Mean lead concentrations (mg/kg) (± SD) for the N1 highway for the Jan 05 sampling occasion. N = 5.

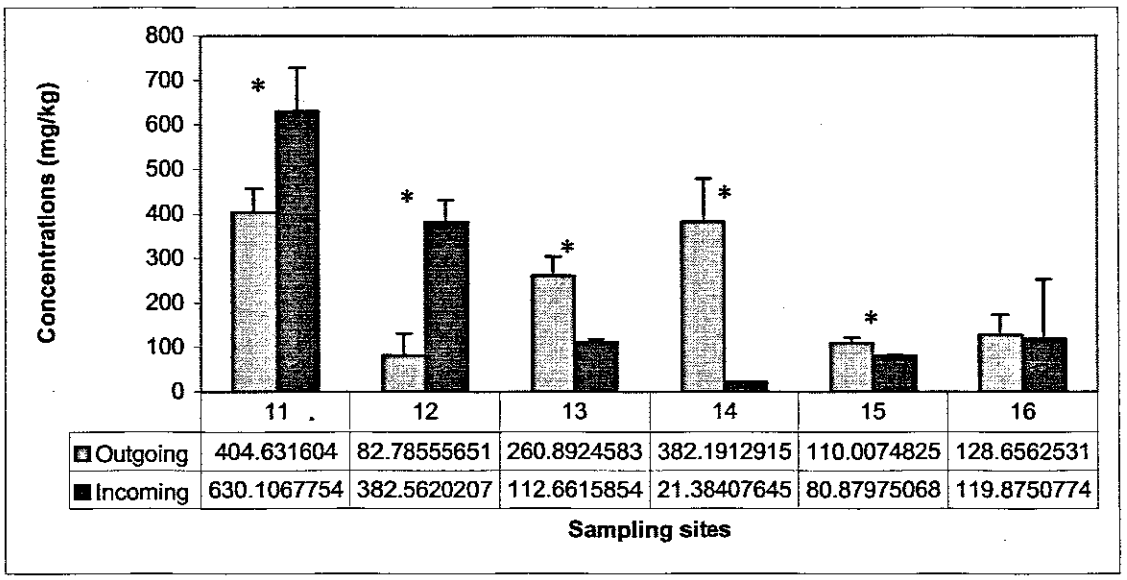


Figure 23: Mean lead concentrations (mg/kg) (± SD) for the N1 highway for Mar 05 sampling occasion. N = 5.

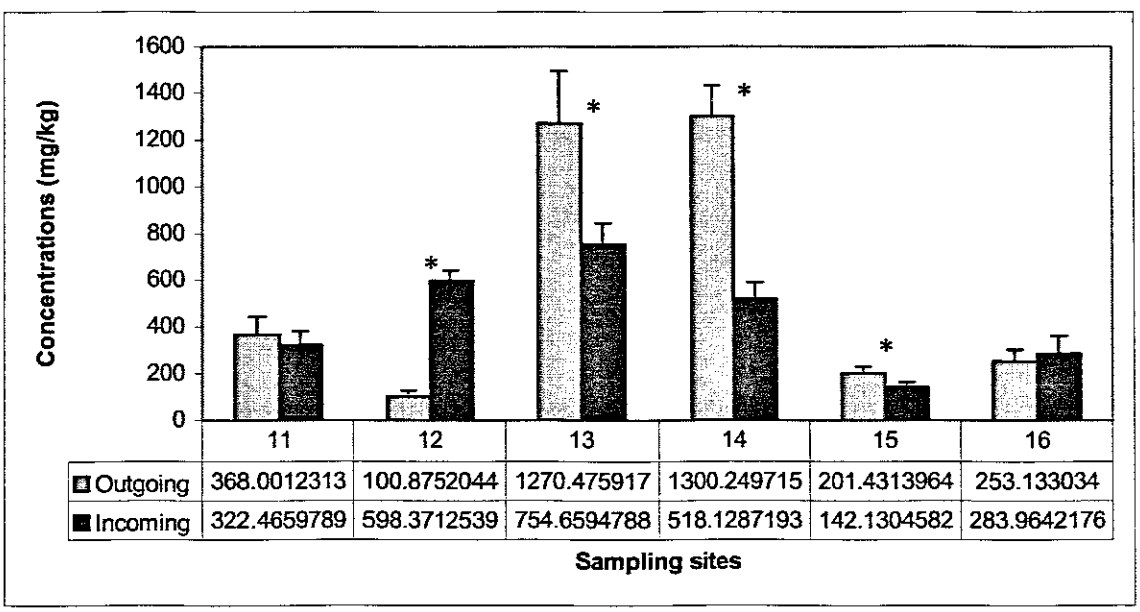
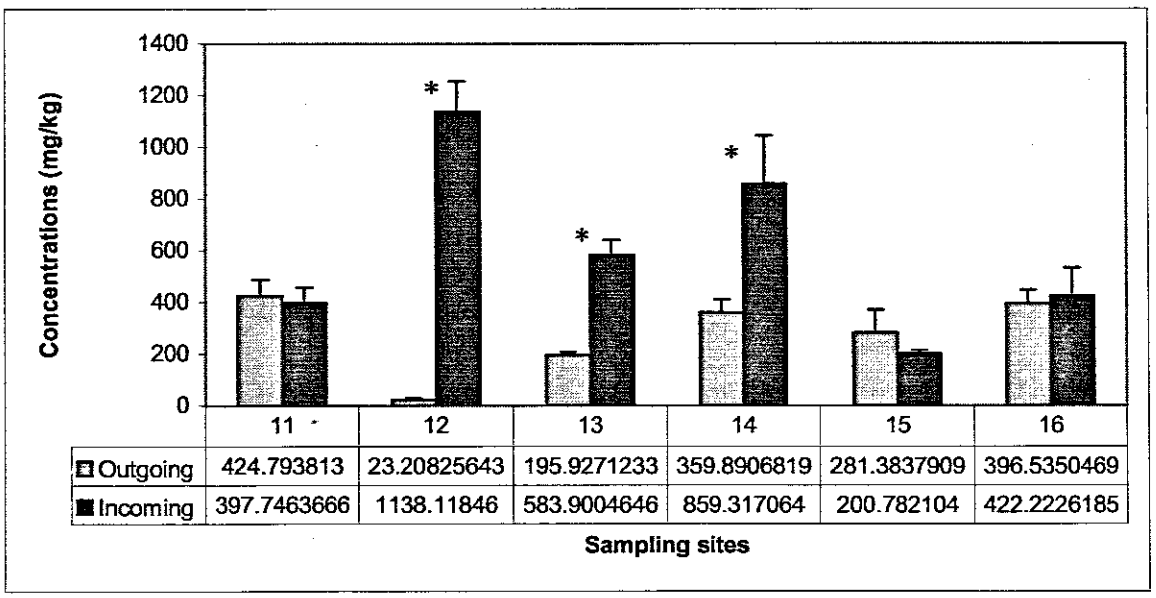


Figure 24: Mean lead concentrations (mg/kg) (± SD) for the N1 highway for the May 05 sampling occasion. N = 5.



	11	12	13	14	15	16
Outgoing	424.793813	23.20825643	195.9271233	359.8906819	281.3837909	396.5350469
Incoming	397.7463666	1138.11846	583.9004646	859.317064	200.782104	422.2226185

Figure 25: Mean lead concentrations (mg/kg) (± SD) for the N1 highway for the Jul 05 sampling occasion. N = 5.

3.1.3.4) pH and moisture

pH ranged from a very acid 2 to an alkaline 9.7 and the moisture % of the soil ranged from a relatively moist 29.3% to a dry 1.1%.

Table 10: pH and moisture % of soil of the N1 highway over sampling period Jul 04 to Jul 05. Numbers 11 to 16 in the left column represent the sampling sites.

SAMPLING SITE		SAMPLING OCCASION						
		Jul-04	Sep-04	Nov-04	Jan-05	Mar-05	May-05	Jul-05
11) Out	pH	8.3	6.4	5.3	6.1	7.7	8	8.8
	Moisture	*12.3*	*23.43*	*6.4*	*28*	*1.7*	*21.4*	*20.9*
11) In	pH	- 8	6.5	5.4	3	7.5	7.3	8.3
	Moisture	*6.4*	*20.9*	*5.5*	*10.8*	*1.6*	*15.3*	*21.4*
12) Out	pH	9.7	6.7	5.1	3.1	7.8	8.1	9.3
	Moisture	*7.5*	*3.5*	*3.2*	*3.7*	*0.9*	*2*	*5.8*
12) In	pH	8	6.7	5.5	2	7.6	7.3	8.2
	Moisture	*14.5*	*8.1*	*6*	*9.2*	*1.38*	*8.3*	*29.3*
13) Out	pH	8.7	6.4	5.4	3	7.5	7.8	8.9
	Moisture	*7*	*7*	*5.5*	*8.4*	*1.1*	*11.1*	*10.5*
13) In	pH	7.9	6.6	5.4	3.1	7.4	7.2	8
	Moisture	*14.1*	*5.7*	*3.9*	*12*	*1.2*	*18.2*	*17.7*
14) Out	pH	8.6	6.7	5.3	2.7	7.5	7.7	8.6
	Moisture	*23.6*	*10.4*	*3.8*	*12.7*	*1.6*	*16.6*	*32.7*
14) In	pH	8.6	6.7	5.4	3	7.3	7.1	8.1
	Moisture	*7.1*	*10.5*	*5.2*	*16*	*2.6*	*12.9*	*19.3*
15) Out	pH	8.3	6.4	5.2	2.4	7.4	7.6	8.5
	Moisture	*5.9*	*2.9*	*4.3*	*0.5*	*0.6*	*11.2*	*17.8*
15) In	pH	8.2	6.8	5.3	3.1	7.1	7.1	8
	Moisture	*13.7*	*8.7*	*2*	*8.4*	*1.3*	*11.2*	*24.1*
16) Out	pH	7.9	6.5	5.2	2.2	7.5	7.5	8.3
	Moisture	*1.6*	*2.1*	*3.2*	*8.3*	*0.8*	*8.5*	*19.2*
16) In	pH	7.6	6.6	5.2	3.1	7	7.1	8
	Moisture	*6.3*	*10.8*	*4.6*	*7.3*	*1.2*	*15.1*	*14.6*

3.1.3.5) Rainfall and vehicle densities in the vicinity of the sampling sites of the N1 highway over the sampling period Jul 04 to Jul 05

Table 11: Rainfall (mm) in the area of the N1 highway for the sampling period Jul 04 to Jul 05 (The Cape Town Weather Office). Numbers 11 to 16 in the left column represent the sample sites

SAMPLING SITE	SAMPLING OCCASION						
	Jul-04	Sep-04	Nov-04	Jan-05	Mar-05	May-05	Jul-05
11	128.4	48	9.6	22.6	15.8	140.2	57.4
12	128.4	48	9.6	22.6	15.8	140.2	57.4
13	128.4	48	9.6	22.6	15.8	140.2	57.4
14	104.5	28.2	10	27.3	6.1	99.6	44.2
15	104.5	28.2	10	27.3	6.1	99.6	44.2
16	104.5	28.2	10	27.3	6.1	99.6	44.2

Rainfall in the vicinity of sites 11, 12 and 13 was taken at the Molteno station and sites 14, 15 and 16 at the Altydgedaght station.

Vehicle counts on the incoming side in January were taken west of Mike Pienaar Drive and Montevista Boulevard and on the outgoing side the vehicle counts were taken east of Plattekloof Road and N7. Light and heavy vehicles were presented separately.

Table 12: Vehicle counts taken on N1 highway for a period within the sampling period of this study. The counts were taken by the CMC Directorate Transport from 20 to 29 Jan 04 and were the only data available.

N1 HIGHWAY	Vehicles	TRAFFIC COUNTS					
		20-Jan-04	21-Jan-04	27-Jan-04	28-Jan-04	29-Jan-04	TOTAL
Car density: Incoming West	Light	27742	62184	41620	74889	75033	281468
	Heavy	855	1992	1222	2419	2290	8778
TOTAL		**28597**	**64176**	**42842**	**77308**	**77323**	**290246**
Car density: Outgoing East	Light	35414	64105	39052	64826	66257	269654
	Heavy	1019	2086	1288	1922	1893	8208
TOTAL		**36433**	**66191**	**40340**	**66748**	**68150**	**277862**

3.2) LABORATORY EXPOSURES

3.2.1) EXPOSURE EXPERIMENT: N7, N2, N1

3.2.1.1) Lead concentrations in exposure soil

The soil used for the 5-week exposure experiment was collected from the 3 highways at the sites that were highest and lowest in mean lead concentrations over the entire sampling period. Each highway had a high lead and low lead contaminated site. Significant differences in lead concentrations were found in this soil between the different sites per highway when compared, as well as when all the sites on the 3 highways were compared with each other (P=<0.001).

Table 13: The mean lead concentration (mg/kg) (±SD) of the exposure soil at the start of exposure. Numbers in the left column indicate the sites on the specific highway. The highlighted areas had the highest mean lead concentration of a particular highway.

Site	pH	Moisture (%)	Lead conc. (mg/kg)	
			Mean	SD
N7-1	9	1	474.03	87.3
N7-5	7.9	1	34.53	5.33
N2-6	8.5	2.1	1140.74	167.01
N2-9	8.8	2.1	79.12	13.70
N1-12	8.1	1.6	914.19	91.69
N1-16	8.7	2.3	102.11	9.33

3.2.1.2) Lead concentrations in cow manure

Cow manure was obtained and fed to the earthworms on a weekly basis during the exposure period. There were no statistical differences in lead concentration between most of the batches of manure with the exception of batches 5 and 3 and 5 and 4 ($P<0.05$).

Table 14: Mean lead concentrations (± SD) (mg/kg) in cow manure that was fed to the earthworms during the exposure period. The weeks in the left column indicate the batch of manure collected.

Cow manure	pH	Moisture content (%)	Lead conc. (mg/kg)	
			Mean	SD
Week 1	8.6	82.33	6.59	1.52
Week 2	8.9	83.32	9.61	2.66
Week 3	8.4	81.2	3.02	1.04
Week 4	8.1	86.3	4.97	3.83
Week 5	9	85	23.79	0.72
Week 6	9	85.1	13.97	12.76

3.2.1.3) Percentage mass change of earthworms before and after exposure

The earthworms were exposed to the road verge soil for 5 weeks and their percentage mass change measured after exposure. Significant differences in mass change of earthworms were found between the before and after exposure masses: N2-6 (P=<0.001), N2-9 (P=0.026); N1-12 (P=<0.001), N1-16 (P=<0.001); N7-1 (P=<0.001), N7-5 (P=<0.001). However, there was no significant differences found between percentage mass changes of earthworms when all the exposure groups were compared to each other (P=0.416).

Table 15: Percentage mass changes of earthworms after a 5-week exposure period. The numbers in the left column represent the sample sites. The temperature and percentage moisture of the substrate (exposure soil) during exposure time is presented in columns 2 and 3. The highlighted areas had the highest mean soil lead concentration of a particular highway.

Exposure period (5 weeks)				
Site	Temp. °C	Moisture %	Growth	
			%	SD
N2-6	19-23	20-23	49.96	0.11
N2-9	19-23	20-23	20.3	0.15
N1-12	19-23	20-23	40.56	0.08
N1-16	19-23	20-23	65.96	0.13
N7-1	19-23	20-23	80.55	0.11
N7-5	19-23	20-23	71.95	0.13

3.2.2) BIOACCUMULATION OF LEAD IN EARTHWORMS: N7, N2, N1

The lead concentrations in the control group of earthworms that have never been exposed to lead were compared with the lead concentrations in the earthworms that have been exposed to the lead contaminated soil for 5 weeks.

Lead concentrations in earthworms per highway per sampling site were compared. There were no significant differences in lead concentrations found between the earthworms of the exposure groups: N7-1 vs N7-5 ($P=0.834$) & N2-6 vs N2-9 ($P=0.107$). Statistical differences were however found in lead accumulation between exposure groups N1-12 and N1-16 ($P= 0.033$) (Table 16).

When the lead concentrations in the earthworms of all the exposure groups were compared, the following exposure groups showed statistical differences ($P<0.05$) in lead concentrations: N1-12 vs N1-16 & N2-6 vs N1-16. No significant differences in lead concentration were found between other comparisons ($P>0.05$) (Table 16).

3.2.3) CELL VIABILITY: N7, N2, N1

The percentage viable cells of earthworms of the control group were compared to the percentage viable cells of earthworms exposed to the lead contaminated soil for 5 weeks.

Comparisons showed significant differences between the percentage viable cells of earthworms from exposure groups: N2-9 vs N1-12 & sites N2-9 vs N7-1 ($P<0.05$). No significant differences were found in the other comparisons ($P>0.05$). The highest percentage viable cells were found in earthworms from group N2-9 (58%) (Table 16).

When the percentage viable cells of the earthworms per highway per sampling site were compared statistical differences were found between comparisons: N1-12 vs N1-16 (P=0.048). There were no significant differences found in percentage viable cells of earthworms for the other exposure groups.

Comparisons showed significant differences between the percentage viable cells of the control earthworms and the percentage viable cells of earthworms from the lead exposed groups (P= < 0.001) (Table 16).

Table 16: The mean lead concentrations (± SD) (mg/kg) are presented in column 2 and 3 and the mean % viable cells in earthworms after the exposure period in column 4 and 5. Numbers in the left column represent the sites on the specific highway and the highlighted areas had the highest mean lead concentration of a particular site when the soil was sampled.

Site	Conc. (mg/kg)		Viable cells (%)	
	Mean	SD	Mean	SD
Control	47.04	37.26	91	0.05
N7-1	70.32	97.24	36	0.07
N7-5	44.79	63.68	42	0.07
N2-6	186.34	191.27	48	0.09
N2-9	18.26	5.84	58	0.07
N1-12	113.75	89.52	34	0.08
N1-16	13.43	16.39	46	0.09

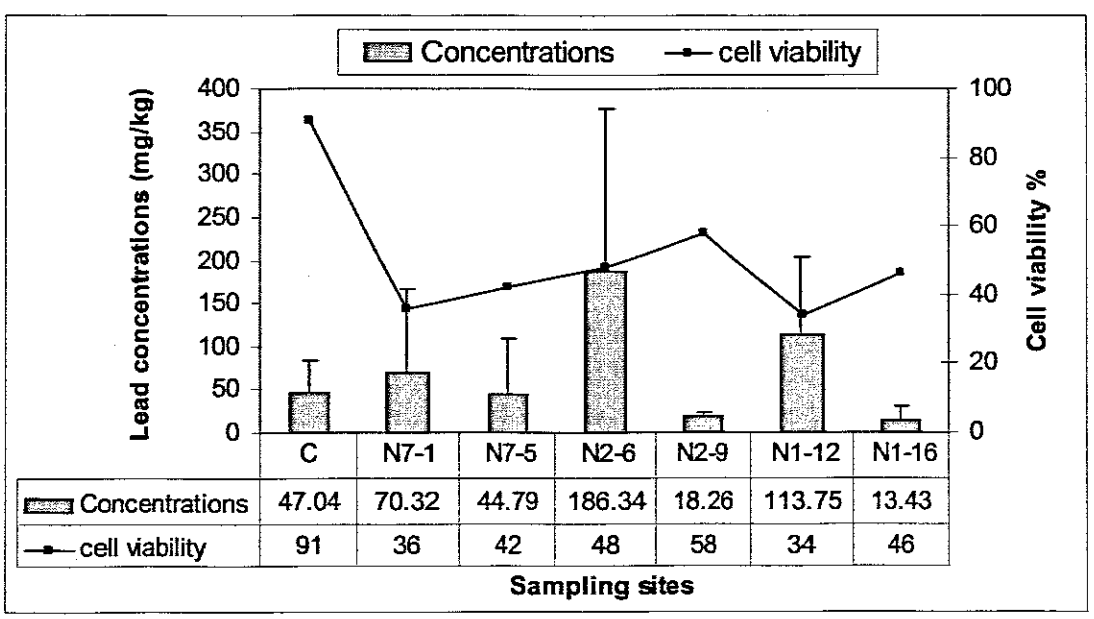


Figure 26: The mean lead concentrations (±SD) (mg/kg) accumulated by the earthworms and the % viable cell count after the 5 week exposure period.

CHAPTER 4: DISCUSSION

4.1) EVALUATION OF LEAD CONTAMINATION OF SOIL ALONG ROAD VERGES

The first part of the study was concerned with the determination of soil lead levels along the road verges of the 3 major highways in the City of Cape Town. The lead levels in the soil samples that were collected over the sampling period, July 2004 to July 2005, from the road verges of the N7, N2 and N1 are presented in Tables 1, 5 and 9.

4.1.1) Lead concentrations found along the N7 highway

The lead concentrations in the roadside soils on the N7 highway at sites 1 and 3 have been constantly higher than at the other sites. The highest mean lead concentrations over the sampling period were found at site 1 on the incoming side of the road in Mar 05 (208.08 ± 59.96 mg/kg) and in May 05 (206.44 ± 93.81 mg/kg), as well as at site 3 on the outgoing side of the road in Jul 04 (184. 73 ± 47.36 mg/kg) and Jul 05 (226.77 ± 60.11 mg/kg) (Table 1 & Figs 9, 10, 5, 11). The N7 highway receives morning and afternoon peak hour traffic and sites 1 and 3 on the outgoing and incoming sides of the road are situated close to busy intersections. Site 1 is especially busy because of the added influx of traffic from the N1 highway and a turn-off to an industrial area. The road close to site 3 receives additional traffic from the east and west side and the outgoing road is slightly uphill, which causes slower moving traffic (Fig 2). According to Othman *et al.* (1997) there is a correlation between lead levels in soil and the type of road (*i.e.* incline or decline).

The site with the lowest mean lead concentrations was found on the outgoing side of the highway at site 5 in Jan 05 (5.01 ± 0.73 mg/kg), which is furthest away from Cape Town and next to farmlands (Table 1 & Fig 8). This finding is in accordance with other studies (Harrison and Laxen, 1984; Onyari *et al.*, 1991;

Munch, 1993) that found lower lead concentrations further away from high activity areas. Site 5 does not receive peak hour traffic nor is it close to an intersection. The N7 highway had the lowest overall mean lead concentrations with only three sites that had concentrations of over 200 mg/kg and confirmed a correlation between lower lead concentrations and lower traffic density (See discussion pg 71).

4.1.2) Lead concentrations found along the N2 highway

The sites that stood out with continuous high mean lead concentrations during the sampling period were sites 6, 7 and 10 on the outgoing side of the N2 highway and sites 6 and 10 on the incoming side. Lead concentrations measured for the May 05 sampling occasion were the highest for the entire sampling period. Public holidays and school holidays from the 21st of Mar 05 to the 4th of Apr 05 and 1st and 2nd of May 05 could have added to the contamination load due to the mass of outgoing and incoming cars usually during the holiday period. However, the highest mean lead concentrations were found to be at site 6 on the outgoing side in Nov 04 (1522.59 ± 92.38 mg/kg) and in May 05 (885.77 ± 224.73 mg/kg). Also on the outgoing side, site 7 with a mean lead concentration of 995.76 ± 276.59 mg/kg in May 05 had amongst the highest mean concentrations found (Table 5 & Figs 14, 17).

The N2 receives morning and afternoon peak hour traffic and site 6 and 7, outgoing and incoming, is situated close to very busy intersections, causing slow moving traffic and a stop-start situation. The N2 has an extra load of traffic caused by the taxis commuting to and from the informal settlements, which is situated virtually next the N2 highway. Site 10 is situated in an urban area next to a busy main road and traffic lights, also causing the typical stop-start situation (Fig 3).

The lowest mean lead concentrations were found at site 9 in Sep 04 (16.47 ±4.96 mg/kg) and site 10 in Mar 05 (16.08 ± 2.99 mg/kg) on the incoming side of the N2 highway (Table 5 & Figs 13, 16). The latter was found to be significantly lower when compared to the other concentrations found at this site over the sampling period but could possibly be explained by the run-off

phenomenon. The site is situated on a slight slope starting at the edge of the road moving downwards. The rainfall measured in the vicinity of site 10 in Jan 05 (50.6 mm) and in Mar 05 (16.2 mm) before the soil sample was taken could have caused significant run-off from the road surface (Table 7). In a study done by Tiefenthaler *et al.* (2003) it was found that regardless of rainfall intensity or duration, the first ten minutes of run-off contained the highest concentrations of toxins. A sharp decrease in some metal concentrations within one meter of roadsides have been found by Bourcier and Hindin (1979) and Revitt and Ellis (1980) after rainfall and could very well be the reason for the sharp decrease in lead concentrations found at this site and during this study from one sampling session to the next.

4.1.3) Lead concentrations found along the N1 highway

Sites 11, 12, 13 and 14 on the outgoing side of the N1 highway and 11, 12 and 14 on the incoming side had continuous high mean lead concentrations during the entire sampling period. Very high mean lead concentrations were found in Jul 05 at all of the sites 11 to 16.

At the following sites on the N1 the highest mean lead concentrations of over a 1000 mg/kg were found: the outgoing side of site 12 in Nov 04 (1274.30 ± 35.85 mg/kg) and the incoming side of site 12 in Jul 04 (1990.31 ± 172.42 mg/kg); the outgoing side of site 13 in May 05 (1270.48 ± 226.27 mg/kg) and outgoing side of site 14 in May 05 (1300.25 ± 133.88 mg/kg) (Table 9 & Figs 21, 19, 24). In a study done in Finland by Peltola *et al.* (2005) soil lead concentrations of over 1000 mg/kg in an urban environment was considered hazardous. Similar and higher lead concentrations were found in soil of the verges of the N1 highway approximately 20 meters from residential areas and schools in some cases and is a cause for concern in terms of environment and human health.

The N1 highway receives peak hour traffic in the morning and afternoon. Sites 11, 12, 13 and 14 on the outgoing and incoming sides are situated at busy intersections and site 12 has the added load from vehicles getting onto the N7 highway. Site 14 is situated at a slope, which slows traffic down (Fig 4) (Othman *et al.*, 1997). Road works on the N1 during Sep 04, Oct 04 and Nov 04 may also

have added to the high levels of lead in the roadside soils because of the slow moving traffic it caused, especially at sites 13 and 14 (Fig 4).

Very low mean lead concentrations were found at the outgoing side of the highway at site 12 in Jan 05 (8.63 ± 2.13 mg/kg) and incoming side of site 14 in Jul 04 (12.96 ± 3.24 mg/kg) (Table 9 & Figs 22, 19). The rainfall that was measured in the vicinity of those sites was 9.6 mm in Nov 04 and 22.6 mm in Jan 05, before the soil samples were taken. At site 14 in Jul 04, 104.5 mm rainfall was measured (Table 11). Run-off due to heavy rain for long periods (Greenstein *et al.*, 2004) could explain the sudden drop in lead concentration at sites 12 and 14 as described in (4.1.2, page 68) at site 10 on the N2 highway.

Lead concentrations found in the roadside soils of the N1 ranged between 200 and 2000 mg/kg and these were of the highest concentrations found compared to the other two highways. In a study done by Davies (1995) rural areas had a contamination level of between 30-100 mg/kg and Alloway (2004) found concentrations of 266 mg/kg in urban soil from a survey done in Brittain. Lead concentrations of 422 mg/kg have been found in roadside soils in a study done by Swaileh *et al.* (2004) in Palestine and lead concentrations of 825.6 mg/kg and 832.2 mg/kg in Damascus City (Othman *et al.*, 1997) were found, which were higher than found in other studies (Tripathi *et al.*, 1989; Davies, 1990). The lead concentrations found along the roadsides in the present study were even higher than found in the aforementioned studies. It is therefore apparent that the road verges are quite contaminated.

4.1.4) Factors influencing lead concentrations in soil

According to Wheeler and Rolfe (1979) lead in roadside soils is influenced by many factors such as climate, wind conditions, traffic density, industrial activities, distance from road and topography. Their possible influences are discussed below.

a) Vehicular density

The N1, with a light vehicle count of 269654 units per day on the outgoing highway and 281468 on the incoming highway were carrying by far the highest load of vehicles in comparison with the other two highways (Table 12). The N2 had the second highest vehicle count of 140978 light vehicles on the outgoing side and 127196 light vehicles on the incoming side of the highway (Table 8). The N7 had the lowest number of light vehicles traveling its road with 54123 vehicles on the outgoing and 35251 vehicles on the incoming side (Table 4). The heavy vehicle count on the highways showed similar results. Their use of diesel might have had a minimal impact on the lead concentration in soil, as lead is not usually added to diesel (Ozaki *et al.*, 2004).

According to the vehicle count data, the traffic density on the highways, were extremely high. From the above comparison of lead concentrations and amount of vehicles on the respective highways a correlation between the amount of vehicles and lead concentrations in the roadside soil were found (Piron-Frenet *et al.*, 1994; Viard *et al.*, 2004)). The roadside soils on the N1 highway, with the most traffic, seemed more contaminated than the other two highways. Further, the roadside soil of the N2 highway with more traffic than the N7 highway also seemed more contaminated than the roadside soil in the N7 highway with less traffic. Also, the lead concentrations in the soil at busy intersections were found to be higher than at other areas along the roadsides and thus a correlation can be made between the lead levels at busy intersections and vehicular density at these intersections (CCT, 2002).

In a study done alongside Brittish motorways, where vehicles travel at high constant speeds (highways), tetraalkyl lead represents 1% of the total lead and in areas where vehicles frequently stops and starts, (urban areas, intersections), it represents 5 to 6% of the total lead (Harrison *et al.*, 1979). This scenario correlates with results that have been found on the highways and at busy intersections on the highways. In another study done by Chamberlain (1979) it was found that at open sites with freely moving traffic the roadside lead concentration was much lower than in narrow streets with slow moving traffic.

This is in accordance with the IPCS (1989) stating that the highest lead concentrations are found in soils and organisms close to roads where the traffic density is high. According to Sutherland and Tack (2000) a number of pollutants are introduced into the atmosphere by motor vehicles, hence the focus on studying metal contamination in roadside soils (Sutherland and Tolosa, 2001; Sutherland, 2003). In a study done by Harper *et al.* (2003), it was found that due to the weak public transport system, the reliance is on vehicles traveling the roads and that on average the cars in South Africa are older than in developed countries. Older vehicles are consequently associated with higher levels of pollution.

b) Rainfall and Run-off

Lead is transported to soils by rain and dew from vegetation and road surfaces coming from traffic and industrial emissions. The contaminated seepage and leachate, as well as flooding of water contributes to contamination of lead in soil coming from contaminated sites (Berrow and Webber, 1972).

Sharp decreases in lead concentrations were found at the following sites in the present study. On the N7 highway, site 3 on the outgoing side with a mean lead concentration of 184. 73 ± 47.36 mg/kg in Jul 04 dropped to a concentration of 52.84 ± 3.23 mg/kg in Sep 04. Rainfall for Jul 04 in the vicinity of site 3 was measured at 104.5 mm and 28.2 mm in Sep 04. Site 5 on the outgoing side with a mean lead concentration of 24.52 ± 1.82 mg/kg in Nov 04 dropped to a concentration of 5.01 ± 0.73 mg/kg in Jan 05. Rainfall for Nov 04 was 6.5 mm and 21 mm for Jan 05 (Tables 1, 3).

On the N2 highway site 6 on the outgoing side with a mean lead concentration of 1522.59 ± 92.38 mg/kg in Nov 04 dropped to a concentration of 208.20 ± 32.45 mg/kg in Jan 05. Rainfall measurements taken in Nov 04 were 9.6 mm and 22.6 mm in Jan 05. Site 7 on the outgoing side dropped from a mean lead concentration of 995.76 ± 276.59 mg/kg in May 05 to a mean lead concentration of 321.69 ± 49.33 mg/kg in Jul 05. Rainfall measured in May 05 was 14.2 mm and 57.4 in Jul 05 (Tables 5, 7).

On the N1 the mean lead concentrations dropped as follows: site 12 on the

outgoing side in Nov 04 dropped from a mean lead concentration of 1274.30 ± 35.85 ± 2.13 mg/kg to 8.63 ± 2.13 mg/kg in Jan 05. Rainfall measured in Nov 04 was 9.6 mm and 22.6 mm in Jan 05. Site 12 on the incoming side in Jul 04 with a mean lead concentration of 1990.31 ±172.42 mg/kg dropped to a lead concentration of 557.37 ± 92.42 mg/kg in Sep 04. Rainfall in Jul 04 was 128.4 mm and 48 mm in Sep 04. Site 14 incoming had a mean lead concentration of 1279.47 ± 99.78 mg/kg in Nov 04 and dropped to 100.35 ± 13.11 mg/kg in Jan 05. Rainfall of 10 mm in Nov 04 and 27.3 in Jan 05 was measured in the vicinity of site 14 (Tables 9, 11).

From this pattern the rainfall and run-off phenomena played a significant role in the fluctuating lead concentrations in the soil. In a toxicity study of parking run-off in urban areas it was found that the water runs into storm water pipes into sewerage plants or watercourses.

c) Mobility

Soils are known to act as "sinks" for lead and are relatively immobile compared to other environmental media. The limited mobility of soil pollution (Svendsen *et al.*, 2002) makes it simpler to investigate the contaminants in the soil. The accumulation of lead in soil results mainly from dry and wet deposition of atmospheric lead, especially close to emission sources (Harrison and Parker, 1977). The vast majority of atmospheric lead, especially lead in water due to the strong binding capacities of soil components for metals eventually turn up in soils (Little and Wiffen, 1977).

The origin of lead accumulation is important but the main concern is their eventual fate. Site 12 on the incoming side of the N1 highway is a constantly wet area with a dam approximately 100 meters from the roadside. Constant high soil lead concentrations were found at this site and the concern was that the lead could move to the water but it appears from the literature that surface soils retain most of the lead. There is not much evidence of substantial movement of lead through soil profiles or into watercourses (Semlali *et al.*, 2004; Watmough and Hutchinson, 2004).

Teutsch *et al.* (2001) found in their study that the petrol lead in roadside soils that were contaminated for over 40 years had penetrated the entire soil profile down to 30cm. An example of extreme lead contamination at a medieval smelting site showed that migration of lead was 4.5m down the soil profile in 555 years (Whitehead, 1997), which is evidence for limited migration.

Accumulated metals in soils are considered "chemical time bombs", (Stigliani *et al.*, 1991; Hekstra, 1997) especially lead, being the most widely dispersed and also given it's known toxicity. This "time bomb", waiting to be set off by the right climatic or environmental trigger makes for an imperative comprehension of the processes that control it's mobilization, especially when environmental conditions are of a sensitive nature.

d) Wind

Sharp increases and decreases in the mean lead concentrations were found at most of the sites over the sampling period during the time of the prevailing winds; south–easter in the summer and north–western in the winter. Most of the atmospheric emissions containing lead particles are deposited within 0 to 5 meters of the roadside but it can be carried further by wind (Othman *et al.*, 1997; Tong and Che Lam, 2000). Rural environments, as a result of winds, may be left with elevated lead levels (Boultron *et al.*, 1983). According to Pitt and Sutherland (1982), accumulation decreases with time because of dispersion by wind. The prevailing winds are extremely strong and blow over the N1, N2 and N7 from the Cape Flats, which is open and characteristically sandy (Sheat, 1984), causing soil to blow away in one area and settle somewhere else (Tong and Che Lam, 2000). In this study wind may have been another cause for fluctuating concentrations in the roadside soils.

4.2) LABORATORY EXPOSURES

The second part of the study was to determine the bioaccumulation of lead in earthworms and whether it caused toxic stress. The percentage earthworm growth, mean lead concentrations in the soil, cow manure and earthworms in this experimental procedure, as well as cell viability percentages are presented in Tables 13, 14, 15, 16 and Fig 26.

In a study done by Davies *et al.* (2003) the earthworm *Eisenia fetida* was subjected to a soil contaminated with lead nitrate. For the first 14 days the lead was not in equilibrium with the soil and the results showed that the lead changed from a mobile to a less mobile form. When extrapolating field data to laboratory exposures the implication for toxicity testing should be considered, as quite often those laboratory experiments will produce a much higher toxicity LC_{50}'s than similar concentrations found in the field (Spurgeon and Hopkin, 1995).

4.2.1) Bioaccumulation

The earthworms were exposed to the following lead concentrations in the more contaminated soil that were collected from the following sites: The N7 site 1 (474.03 ± 87.3 mg/kg), N2 site 6 (1140.74 ± 167.01 mg/kg) and the N1 site 12 (914.19 ± 91.69 mg/kg). The earthworms were exposed to the following lead concentrations in the less contaminated sites: The N7 site 5 (34.53 ± 5.33 mg/kg), the N2 site 9 (79.12 ± 13.70 mg/kg) and the N1 site 16 (102.11 ± 9.33 mg/kg) (Table 13). They were exposed to the contaminated soil for five weeks, which was in accordance with Saint-Denis *et al.* (1999) that advised that earthworms be exposed to metal contaminated soil for at least 14 days and discrimination between doses were best after 14 to 28 days. Also, significant changes could best be observed after exposure to soil lead concentrations of 30 mg/kg or more (Saint-Denis *et al.* 1999).

The control earthworms that had not been exposed to lead had a mean lead concentration of 47.04 ± 37.26 mg/kg. The mean lead concentrations accumulated by the earthworms during the 5-week exposure period ranged from a relatively high 186.34 ± 191.27 mg/kg (soil from site 6 on the N2

highway), to a lower mean concentration of 13.43 ± 16.39 mg/kg (soil from site 16 on the N1 highway). The earthworms in the highly contaminated soil accumulated on average much higher concentrations of lead than the earthworms in the lower lead contaminated soil.

Davies *et al.* (2003) found in their study that the earthworms accumulated much more lead in the experiment with a soil lead concentration of 5000 mg/kg than in the experiment with a soil lead concentration of 3000 mg/kg and suggested that the bioaccumulation of lead by *Eisenia fetida* is controlled by external environmental concentrations. They also found that the earthworms can regulate the uptake of lead at lower lead concentrations but at higher concentrations, above 5000 mg/kg, widespread mortality sets in as a result of a breakdown in the regulatory mechanism. The earthworms that survive such high lead concentrations have strong and efficient regulatory systems or can tolerate higher concentrations of lead (Davies *et al.*, 2003). In the case of sites 1 (70.32 ± 97.24 mg/kg), 6 (186.34 ± 191.27 mg/kg), 9 (18.26 ± 5.84 mg/kg) and 16 (13.43 ± 16.39 mg/kg) the lead concentrations in the earthworms did not exceed the concentration found in the soil and the assumption can be made that the lead is either not accumulated or it is excreted (Table 13 and 16). It has been found that earthworms tolerate high environmental concentrations of toxic metals and they do it by accumulating or storing it in a non-toxic form, not absorbing the metal or by excreting it efficiently, which may explain fluctuations in lead accumulation (Ireland, 1979) and also why different species at the same site can contain different concentrations of lead (Raw and Dobson, 1959; Morgan and Morgan, 1998; Morgan *et al.*, 1999).

The lead concentrations found in the control earthworms (47.04 ± 37.26 mg/kg) were similar to the lead concentrations accumulated by the earthworms at site 5 (44.79 ±63.68 mg/kg). This site 5 (34.53 ± 5.33 mg/kg in soil) on the N7 highway was furthest away from Cape Town and in a rural area surrounded by farms. Lead present in control earthworms can be explained by low levels of lead that were found in the substrate, which can also be explained by the natural occurrence of lead in the substrate on the farm (Maboeta *et al.*, 1999). At this site the soil was a reddish clay (Sheat, 1984), abundant in spiders and insects

and seemed like a much healthier soil (Table 13 & 16). At site 9 the lead concentrations measured in the exposure soil were 79.12 ± 13.70 mg/kg. The earthworms exposed to that soil accumulated a relatively low concentration of lead (18.26 ± 5.84 mg/kg). A similar phenomenon was found in soil from site 16 where the lead concentration found in the exposure soil at this site was 102.11 ± 9.33 mg/kg and the earthworms in this soil also accumulated a low lead concentration of 13.43 ± 19.39 mg/kg. This phenomenon could be explained by "hyper regulation" in which the earthworms work very hard to regulate the uptake of lead (Davies *et al.*, 2003) (Table 13 & 16). The most likely explanation in the present study may be that lead was excreted efficiently.

Maximum activity of soil dwelling earthworms occur at cooler temperature and high moisture content and it has been found that the highest temporal body burden of lead in earthworms was obtained when the temperature and moisture content was conducive to maximum activity (Ireland and Wooten, 1976). The temperature measured during the exposure period in this study was between 19 and 23 °C and the moisture content ranged between 20 and 23%, which were both favourable conditions for the earthworms (Table 14). Temperature and moisture percentage did therefore not play a significant role in the laboratory exposures.

The pH measured in the soil along the roadsides ranged from 2.0 to 9.7 over the sampling period, with the pH in the soils of the N1 and N2 highway in Jan 05 being extremely low (2.8 and 3.1) (Tables 6 & 10). In a study done by Kaplan *et al.* (1980) mortality of *Eisenia fetida* occurred in laboratory experiments that tested the effects of pH. The pH levels were below 5.0 and above 9.0. No mortalities were found during the five week exposure period in this study, where pH in the laboratory exposure soil ranged between 7.9 and 9.0 and cow manure pH that was used for feeding, ranged between 8.1 and 9.0 (Tables 13, 15). It is difficult to predict accurately concerning the overall toxicity of a soil from chemical analysis alone because of the complex nature of the contamination at a site in the soil. Interactions between the soil characteristics that influence the bioavailability of lead (p H, organic content, ionic composition) and the individual

chemicals make it difficult to make definite predictions (Lavelle *et al.*, 1997). Elevated lead levels are found in waters with low pH and high chloride content with high temperatures. pH did probably not play a significant role in the laboratory exposures but could have influenced the bioavailability of lead to the earthworms.

4.2.2) Earthworm response to exposure to lead contaminated soil

4.2.2.1) Cell viability

The total concentration of metals in soils is not directly available to organisms and the total concentration is also not necessarily a threat to soil organisms, humans or animals (Ure and Davidson, 2002). The physical and chemical characteristics of soil in conjunction with physiology and behaviour determine the bioavailability of chemicals to earthworms (ASTM, 1999). After absorption lead may be metabolized or excreted or accumulated in other tissues as discussed in the bioaccumulation section (4.2.1, page 75). It may also be sequestered internally or transported in the earthworm to the site of toxic action (STA). Toxic effects may only occur when the amount of lead present in the STA exceeds the threshold (McCarty and Mackay, 1993).

In the present study the trypan blue exclusion assay was used to measure the effect of lead exposure on the membrane stability of the coelomocytes in the coelomic fluid of the earthworm, *Eisenia fetida.* When these cells of the control earthworms of the present study that have not been exposed to lead before were observed under the microscope, most of the cells had a green brownish appearance. When the cells were counted the result was 91 ± 0.05% cell viability. A blotchy blue colour observed in most of the cells of all the exposed earthworms were corroborated by the relatively low percentage viable cells of between 34 and 58% that were found in the present study (Table 16).

According to Snyman and Odendaal (*submitted*) only cells with impaired plasma membrane functioning take up the trypan blue dye and from the above results it

is clear that the accumulated lead caused an alteration in the plasma membrane integrity. A similar result was also found by Reinecke and Reinecke (2003) for lysosomal membrane integrity, where clean clitellate earthworms were exposed to relatively low lead concentrations for 4 weeks. This exposure resulted in differences between the NRRT's between the control and exposed earthworms and clearly demonstrated the sensitivity of the lysosomal assay. The NRRT's of exposed earthworms were compared with the controls and it was found to be significantly lower than that of the control earthworms. The same was found in this present study when the trypan blue exclusion assay and coelomocyte membranes were used.

In the highest exposure groups per highway, a significant decrease in percentage viable cells was seen (N7, 36 ± 0.07%; N2 48 ± 0.09%; N1, 34 ± 0.08%) and in the lowest exposure groups per highway again there was a significant increase in cell viability percentages observed (N7, 42 ± 0.07%; N2, 58 ± 0.07%; N1, 46 ± 0.09%). Similar findings were made by Reinecke and Reinecke (2003) for lead exposed earthworms. They have found that the NRRT's decreased in earthworms with higher body burdens of lead in comparison with earthworms with lower body burdens. This type of dose response relationship was found for copper in the earthworms *Lumbricus sp.* and *Eisenia andrei* (Weeks and Svendsen, 1996; Svendsen and Weeks 1997a). Site 12 (N1), had the lowest cell viability count of 34 ± 0.08%. This site's earthworms showed morphological changes as discussed in (4.2.2.2 page 80) (Table 16).

The fact that clear statistically significant responses were seen after the five week exposure period in the highest, as well as lowest exposure groups indicate that these responses could serve as an early warning system of lead exposure.

In conclusion, the coelomocyte viability of *Eisenia fetida*, measured with the trypan blue exclusion assay, may possibly serve as a useful biomarker of lead exposure.

4.2.2.2) Morphological and body mass changes of earthworms

There is evidence that the morphology of earthworms is affected by lead exposure (Kalaiselvan *et al.*, 1996). Morphological changes in this study were not quantified but such changes in the earthworms after the 5 week exposure period were observed in the earthworms exposed to soil from a relatively highly contaminated site (site 12). Abnormal swellings at the clitellar and other areas and a yellowish tinge in the earthworms were observed. The earthworms also became slow and unresponsive as apposed to their initial fast movements, including lifting of the body, coiling and curling. In a paper by Rao *et al.* (2003) the effect of lead exposure at 10.98 mg/kg, with an exposure period of 14 days, to earthworms caused the same morphological changes.

In terms of weight no negative effect was observed, in fact the percentage mass increase was significantly higher than at the start of the experiment for all the earthworms of all the exposure groups. The highest percentage mass gain (81%) was found for earthworms exposed to soil from site 1 on the N7 highway, which was a high lead contaminated site. The lowest percentage mass increase was 20% for earthworms exposed to soil from site 9 on the N2, which was a less contaminated site (Table 14). In Reinecke *et al.* (1997) no negative effect on growth was found when *Eisenia fetida* was exposed to lead nitrate concentration and could be explained by the hormesis phenomenon (Spurgeon *et al.*, 2004), which is a physiological response to lead that have accumulated in the body. The lead had a possible stimulating effect on the earthworms.

4.3) REHABILITATION OF LEAD CONTAMINATED SOILS

Society has become more aware of the dangers of lead and this resulted in measures to reduce their contribution to the environmental load. Already lead additives have been removed from petrol and the lead tanks and pipes carrying drinking water, is replaced by plastic and copper pipes (Duffus, 1980). In South Africa unleaded petrol had been introduced in 1996 and the phasing out of leaded petrol followed.

As from January 2006 leaded petrol was no longer available in this country. In a study done by Yang and Mosby (2005) lead contaminated urban soil was treated by phosphoric acid application. It lowers the pH of the soil and provides highly soluble phosphate and has proved to be most effective in remedial treatment for maximum reduction of lead exposure to the ecosystem and humans (Casteel *et al.*, 1997).

There is an increase in popularity to immobilize metals in soils from contaminated environments. Singh *et al.* (2006) in their study used a waste by-product from the phosphate mining industry, phosphatic clay, as a sorbent for lead from aqueous effluents. They have found this method to be a successful and cost effective way to remediate lead from water, soil and sediment.

Shooting ranges in Finland have become a great cause for concern in terms of soil lead-, and groundwater lead contamination. Excavation in combination with disposal, as well as banning lead shot activities are remedial methods currently used but they are in search of new and more cost effective methods (Sorvari *et al.*, 2006).

The application of bone char (BC) to lower the bioavailability of lead in contaminated soil proved to be a potential remediation technique. This study was done in China by Chen *et al.* (2006) and they determined the bioavailability in terms of uptake by Chinese cabbage. They found that the lead concentrations in the roots and shoots decreased with the application of increased bone char.

Phytoremediation techniques as an alternative for more conventional methods of remediation for moderately contaminated soils are becoming more popular. In a previous study metal accumulation in the flora associated with ultra-mafic and non ultra-mafic soils in Portugal was determined. The plant species *Lavandula stoechas*, *Thymus mastichina* and *Cistus ladanifo* were used for phytoremediation and phytoextraction (Lázaro *et al.*, 2006).

A bacterial strain (*Bacillus sp*) isolated from metal polluted soils can be used for bioabsorbsion of copper and lead from aqueous solutions (Tunali *et al.*, 2005). Microwave (MW) technology has been used effectively to vitrify contaminated soil wastes, as well as for the immobilization of metals in soils (Jou, 2006).

The excavation and disposal method, being labour intensive, could be a possible rehabilitation method used in Cape Town. This could provide employment opportunities for many unemployed people. The onus is on municipal and state agencies to decide on proper soil remediation but these management decisions are often very difficult due to high and uncertain costs of remediation, uncertainty regarding the contamination and the multiple impacts on the environment. Because of the importance of remediation of contaminants, Scholtz and Scnabel (2006) gave guidelines on management, decision making and remedial alternatives to assist in these difficult decisions.

CHAPTER 5: CONCLUSION

1) Lead accumulated to high concentrations in soil along side Cape Town highways when compared to data from other countries.

2) It is concluded that there is a possible correlation between lead levels in roadside soil and traffic density, especially in the area of busy intersections.

3) Factors such as run-off (related to rainfall) and distance from Cape Town CBD (related to traffic density) was at least partly responsible for the fluctuations in soil lead concentrations from one sampling occasion to the next.

4) Earthworms experimentally exposed to soil from the road sides have accumulated lead over the exposure period, indicating that some of the soil lead was bioavailable.

5) Behavioural and morphological changes in the earthworms were observed after being exposed to the lead contaminated soil and could serve as an indication of toxic stress due to lead contamination.

6) The percentage cell viability (biomarker) used in this study has been useful in identifying toxic stress in earthworms caused by lead in roadside soils. The additional information obtained by using biomarkers could not be obtained by chemical analysis of lead concentrations alone.

7) A high percentage mass increase in the experimentally exposed earthworms was observed in which lead had a possible stimulating effect on the earthworms (hormesis). This phenomena could possibly explain the relationship found between a higher percentage mass gain found in earthworms from higher lead contaminated soil and lower percentage mass increase found for earthworms exposed to soil from less contaminated sites.

CHAPTER 6: REFERENCES

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