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# THE USE OF SENTINEL ORGANISMS TO EVALUATE THE HEALTH OF METAL CONTAMINATED FOREST ECOSYSTEMS IN THE WESTERN CAPE, SOUTH AFRICA

**by**

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**Thesis submitted in fulfilment of the requirements for the degree**

**Doctor of Philosophy: Environmental Health**

**in the Faculty of Applied Sciences**

**at the Cape Peninsula University of Technology**

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**Cape Town**
December 2019

**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**  1 December 2019 **Date**

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## ABSTRACT

Chronic exposure to high levels of metals in the environment can cause severe damage to ecosystems and human health. The City of Cape Town, an immense contributor to atmospheric pollution lies beneath the Table Mountain range, one of the Seven Wonders of the World and renowned for its biodiversity and ancient, indigenous forests, yet no plan exist to monitor metal concentrations. These ecosystems are subjected to considerable metal inputs all year round, which greatly increases during brown haze episodes in winter. Soil, leaf litter, moss, lichen and millipedes are key organisms in forests and reliable indicators for determining critical loads and preventing broader impacts to forests, which leads to the main purpose of this study: to determine the health of metal-contaminated forest ecosystems in the western cape, the smallest biome in South Africa, yet of utmost importance to our environment and ultimately human health. The objectives of this study were a) to determine concentrations of prominent metals in soil, leaf litter and sentinel organisms in a pilot study b) to compare the dry and wet season with regard to: (i) seasonal fluctuations of the concentrations of the metals (ii) the oxidative stress effect of metals, using two markers indicative of induced oxidative stress (oxidative lipid damage products and glutathione (total GSH levels) in the pill millipede, *Spaerotherium compressum*, the moss, *Hypnum cupressiforme* and the lichen, *Parmotrema sp.* and c) to expose millipedes to metal contaminated soil for a period of six weeks: (i) to determine whether they accumulated metals and (ii) to assess the induced oxidative damage to lipids and redox status of glutathione in the pill millipede, *Spaerotherium compressum*. Three sampling sites in three afromontane forests in the Western Cape, Platbos, Orange Kloof and Newlands forest formed the study area. Five subsamples of soil, leaf litter and each sentinel organism were collected and chemically analysed to determine the metal concentrations, using an Inductively Coupled Plasma Mass Spectrophotometer (ICP–MS). The oxidative stress effect was determined in freeze dried samples. Millipedes were exposed in terrariums to a cocktail of metals Al, Fe and Mn for six weeks at control, low and high concentrations and analysed thereafter. Significant ($P<0.05$) findings in this study were the metal contamination patterns observed at the sites and forests in closest proximity of the City of Cape Town and related pollutant sources, especially vehicle volumes, and traffic behaviour. Even more significant ($P<0.05$) was the enhanced

metal concentrations found during winter in the forest in closest proximity of the city, impacted by the brown haze phenomena. Metals may further have triggered an overproduction of ROS (reactive oxygen species) judging from the activated antioxidant, tGSH levels in an effort to scavenge ROS, as well as the MDA (malondialdehyde) levels (measured as TBARS), which indicated damage to cells. An important finding was that MDA levels were mostly higher in winter during the brown haze episodes signifying more damage in the organisms during that season. A programme to monitor metals in these forests was also suggested in view of a concern for the survival of forests and human health, as a result of the growing population, vehicle traffic and urbanization.

## ACKNOWLEDGEMENTS

**I wish to thank:**

- My supervisor, Prof James P Odendaal for making this research in the forests possible, giving me free reign to experiment in the laboratory and through his exstensive knowledge in the field of ecotoxicology, guided the many different components of this process to completion.
- My co-supervisor, Prof Reinette G Snyman for her insight in seeing and providing severel avenues to explore and experiment with in terms of the biomarker research with organisms not yet experimented on in this laboratory.
- Professor Jeanine L Marnevick from the Oxidative Stress Research Centre for her scientific advice, knowledge and insightful discussions with regard to the antioxidant study, a field I new nothing about, but was made comfortable with by her.
- Fanie Rautenbach from the Oxidative Stress Research Centre, for his excellent help with the antioxidant experiments and the extra mile he has gone to help solve problem areas.
- Deborah J Winterton from SANParks for granting research permits for sampling of soil, leaf litter and sentinel organisms and her good nature throughout the process.
- Francois and Melissa Krige, the owners of Platbos forest, for permission to sample soil, leaf litter and sentinel organisms. They have been over accommodating, giving me open access, trusting me in their beautiful forest, of which I am extremely thankful.
- The enthusiastic and very helpful Professor Terry Hedderson from the University of Cape Town for the identification of the moss species used in this study.
- Dr Andre Aptroot from the ABL Herbarium in the Netherlands for his eagerness to identify the lichen species used in this study, after a long search to find someone to identify it in South Africa.
- Michelle Hamer from SANBI for the identification of the pill millipede species used in this research. Thank you so much, as it formed such an important part of this study.
- Riana Rossouw from the ICP laboratory at the University of Stellenbosch that could always be counted on for her prompt analysis of samples.
- The helpful and friendly Bemlab staff for the soil analysis.

The financial assistance of the Cape Peninsula University of Technology towards this research is acknowledged. Opinions expressed in this thesis and the conclusions arrived at, are those of the author, and are not necessarily to be attributed to the University.

"What we are doing to the forests of the world is but a mirror reflection of what we are doing to ourselves and to one another."

Mahatma Gandhi

## DEDICATION

Soli Deo Gloria

For

Nicoline Holtzhausen, a true friend

A project of this magnitude is not possible to accomplish in a vacuum, as can be seen from the list of acknowledgements. Also, the moral support of others are invaluable and therefore, with my whole heart, thank you for your unfailing moral support throughout this whole process.

## GLOSSARY

| Terms/Acronyms | Definition/Explanation |
|---|---|
| **CMA** | **Cape Metropolitan Area.** |
| **CBD** | **Central Business District.** |
| **Southern Afromontane forests** | **These forests are tall, shady, multi-layered and indigenous and occur in ravines and fire-safe habitats associated with Sandstone Fynbos (De Villiers et al., 2005).** |
| **Ecotoxicology** | **The study of toxic effects of various agents on living organisms, especially on the population and communities within ecosystems (Connel, 1999).** |
| **Brown haze** | **A metereological condition, which occurs when cooler air just above the surface of the ground – air that's full of city pollutants – becomes trapped by a layer of warm air above it. It cannot rise and mix with the atmosphere, until heating or winds break up the inversion layer (City of Cape Town - Air Quality, 2007).** |
| **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 and Van Brummelen, 1996).** |
| **Bioindicator** | **A sensitive, representative organism of functional importance in the ecosystem, which is easily collected, identified and analysed (Greensdale, 2007).** |
| **Sentinel organisms** | **Indicator species (biomonitors), capable of accumulating persistent pollutants like metals in their tissues. They are used to measure the biologically available concentration of a specific pollutant in a specific ecosystem and also reflects ambient pollutant levels (Beeby, 2001).** |
| **Millipedes** | **Diplopoda are nocturnal arthropods, ecologically important as detritivores (saprophages, or consumers of dead plant material) and a major component of terrestrial ecosystems throughout the world (Hopkin and Read, 1992).** |

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| **Reactive oxygen species (ROS)** | **ROS are a group of free radicals, which are produced as a normal product of plant cellular metabolism. Environmental stresses may cause excessive production of ROS, resulting in oxidative damage and ultimately cell death (Sharma et al., 2012).** |
| **Oxidative stress** | **Refers to the situation where an endogenous antioxidant defence system is overwhelmed by the production of free radicals, in favour of the free radical oxidants (Halliwell, 1992).** |
| **Lipid peroxidation (LPO)** | **Oxidative damage to lipids in an organism's bio-system (Halliwell, 1992).** |
| **Glutathion (tGSH)** | **A non-enzymatic endogenous antioxidant that performs an essential role in neutralizing and/or detoxifying the oxidative damage caused by ROS (Regoli et al., 2011).** |
| **MDA** | **A decomposition product of polyunsaturated fatty acids, produced during peroxidation of membrane lipids (Mittler, 2002).** |

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# CHAPTER ONE

# THE USE OF SENTINEL ORGANISMS TO EVALUATE THE HEALTH OF METAL CONTAMINATED FOREST ECOSYSTEMS IN THE WESTERN CAPE, SOUTH AFRICA.

## 1.1 INTRODUCTION

### 1.1.1 Background and Literature study:

Air pollution is a key threat to human health, quality of life and the biophysical environment (State of the Environment Outlook Report for the Western Cape Province, 2013). Although a large range of toxic chemicals are found in the environment, heavy metals are considered one of the most serious and widespread forms of environmental contamination (Pruski and Dixon, 2002). However, little information exists on the concentrations of pollutant metals, either in the atmosphere or in atmospheric deposition or of its emissions in the Southern Hemisphere, especially in Africa (Slemr et al., 2011).

South Africa is the largest producer of primary metals in the world (Masekoameng et al., 2010) and the potential for these emissions from human activity to contribute to local and regional contamination is substantial. South Africa holds overall, 40% of the world's gold reserves and is responsible for 12% of its global production. Much of South Africa's electricity production relies on coal combustion (64% of the energy supply) (Dabrowski et al., 2008), and for industry, which could contribute hugely to mercury emissions (Sprovieri et al., 2010). China has been ranked number one and South Africa second in the world as mercury emitter (Dabrowski et al., 2008; Leaner et al., 2009) in a global anthropogenic mercury emissions inventory for the year 2000 (Pacyna et al., 2006).

In the City of Cape Town monitoring data showed that air quality is still deteriorating due to industrial activities, increased vehicle traffic, the burning of waste, power generation and the use of wood and coal fuels by a large sector of the population (CMA, 1998; Sucharova et al. 2011). Provincial traffic volumes are highest within the City of Cape Town, which is a major source of pollution such as lead. This town is also the biggest contributor to atmospheric pollution in South Africa as it is the area

of highest population density and economic activity (State of the Environment Outlook Report for the Western Cape Province, 2013). In 2006 the City of Cape Town experienced over 150 days where air pollution levels were higher than internationally accepted standards. This means that the people of this city were breathing in smoke and gases that are harmful to their health for nearly half of 2006. Heavy metal exposure in humans is known to have toxic effects that could be acute, chronic or sub-chronic, (neurotoxic, carcinogenic, mutagenic or teratogenic) (McCluggage, 1991; INECAR, 2000; European Union, 2002; Young, 2005). Pollution monitoring data taken recently in the City of Cape Town Metropolitan area shows that pollutant concentrations are now at or above acceptable standards (State of the Environment Outlook Report for the Western Cape Province, 2013). For a small city (on an international scale), Cape Town's air pollution levels are unusually high. This is due to a meteorological (weather) condition called "low-level temperature inversions," better known as the brown haze, which means that cooler air just above the surface of the ground – air that's full of city pollutants – becomes trapped by a layer of warm air above it. This trapped layer of concentrated pollution can sometimes be as low as 30 m (particularly in winter), which cannot rise and mix with the atmosphere, until heating or winds (the southeaster) break up the inversion layer (City of Cape Town - Air Quality, 2007).

Air pollution is not confined to the city. It easily crosses boundaries, being dispersed over large ranges, hundreds of kilometres from their source, where they affect many different ecosystems and remain toxic in the environment for extended periods of time. Pollutants then continue to affect water resources (i.e. ponds, streams, wetlands, lakes, dams), are taken up by plant material and accumulate in faunal species (including humans) (State of the Environment Outlook Report for the Western Cape Province, 2013), as well as metabolically in the soil biota (Van Straalen et al., 2001).

Studies showed that natural ecosystems such as forests receive considerable metal inputs through atmospheric deposition (Mason et al., 2000; Schwesig and Matzner, 2000) and a consequence of atmospheric deposition is accumulation of metals in forest ecosystems (Rasmussen, 1998; Reimann and De Caritat, 2000; Brannval et al., 2001). Forest canopies have the ability to intercept aerosols in the atmosphere.

The aerosols reach the soil by being either washed down by rain or through fallen leaves (Ettler et al., 2005; Driscoll et al., 2007), which is often the reason why the topsoil in forests, where most of the forest animals live, contain relatively high levels of metals. Metal contamination can originate from natural sources as well, such as *in situ* weathering of rock minerals, but the increasing quantity of metals found in ecosystems is mainly as a result of the polluted environment (Nriagu, 1994; Ayräs and Kashulina, 2000; Renberg et al., 2000). A substantial fraction is also released and redistributed through physical and biogeochemical migration processes in the soil profile (Dahmani-Muller et al., 2000; Klaminder et al., 2005; Steinnes et al., 2005), explaining the rising metal burden to soils (De Vries et al., 2002). Metals have severe impacts and are a major threat to ecosystems (Kozlov and Zvereva, 2007) and human health (Järup**,** 2003)**.** They reach toxic concentrations (Fosmire, 1990; Nolan, 2003; Young, 2005) in densely populated areas and beyond, cannot be degraded and accumulate in soil and trophic chains for years (Causey et al., 2003), even if their release to the environment can be restricted (Brusseau, 1997). In fact, accumulation of metals in the organic soil layers range over time periods from several years to a few decades (Suchara and Sucharova, 2002). In a study done in Poland (Sawicka-Kapusta et al., 2003), lead and other metal concentrations did not decrease over the years, even after the introduction of unleaded petrol.

Toxic metal levels in ecosystems affect abundance, diversity and distribution of soil animals (Hopkin, 1989), and according to Marschner et al. (1995), interfere with respiratory processes, photosynthesis and protein synthesis. Decreases in respiration rates in forests have been reported, indicating extreme metal contamination (Bewly and Stotzky, 1983; Laskowski et al., 1994; Fritze et al., 1996). Tree growth in forests could be reduced (Aznar et al., 2007) and contamination could have direct toxic effects on plants (Singh et al., 1997) and fauna (Vyas et al., 2000; Lewis et al., 2001). Metal pollution may impact natural populations (Van Hook and Yates, 1975; Hunter et al., 1987b; Posthuma and Van Straalen, 1993; Lock et al., 2003) and in turn show effects at the community level and alter critical ecosystem functions (Coughtrey et al., 1979; Hunter et al., 1987a; Tyler et al., 1989; Read et al., 1998; Nahmani and Lavelle, 2002; Creamer et al., 2008; Clements and Rohr, 2009). Excessive amounts of toxic metal ions also induce several stress responses and

damage to different cell components such as membranes, proteins and DNA (Waisberg et al., 2003; Jimi et al., 2004).

Soil is one of the most valuable non-renewable resources in the world and forms an integral part of all terrestrial ecosystems (De Bruyn, 1997; Bedano et al., 2006). The litter layer has the greatest accumulation of metals, (Martin et al., 1982), as it receives metal imputs from the atmoshere and throughfall, but also by fungi through microbial translocation and immobilization of metals from underlying contaminated soil layers (Lomander and Johansson, 2001; Lomander, 2002; Tyler, 2005).

although it can also penetrate deeper into the soil (15-30 cm) as were found in Sweden, indicating a greater influence of anthropogenic metal pollution also at this depth (Alriksson, 2001). However, organisms in the top litter layer are often exposed to higher concentrations of metals (Hopkin, 1989).

Soil organisms are in close contact with the surrounding soil and the contaminants therein, therefore soil biota are at risk of being detrimentally affected. The performance of primary producers depend on the health and the proper functioning of soil biota (Wardle, 2002). Vital microbial processes such as decomposition, element cycling, nitrogen fixation, energy transfer, formation of humus and soil structure, plant growth and degradation of organic pollutants take place in the organic surface layer of the soil (Kennedy, 1999; Verstraete and Top, 1999). Most plant roots are also found in this layer. This layer is vital for organisms that form the base for food chains. The disturbance of this soil layer may have considerable ecological consequences for forests (Tyler et al., 1989). Genetic constitution of soil biota has low renewability and if an entire species is lost, it will probably never be recreated. Soil is the least renewable forest resource. Biota can be reintroduced from other areas on a limited basis, but it is not usually possible for soil (Kimmins, 1997). Thus, sustaining soil function is extremely important (De Vries et al., 2002).

The Table Mountain chain lies within the City of Cape Town: It is an international tourism icon and Natural World Heritage Site, recognised globally for its extraordinarily rich, diverse and unique fauna and flora - with rugged cliffs, steep slopes and sandy flats. Situated at the south-western tip of Africa, it stretches from

Signal Hill in the north to Cape Point in the south (SANParks, 2012), is an important component of the Cape Floristic Region (CFR) (Myers et al., 2000) and a member of the Mediterranean Biome (Fynbos biome). It is also one of the world's most imperilled ecosystems (Underwood et al., 2009). Four of the 56 vegetation subtypes in Cape Town are globally extinct, 19 are critically endangered; seven are endangered and nine are vulnerable. Six are least concern, however, of the six least concern subtypes, (of which one is the afromontane forests), four are nationally critically endangered and one is nationally endangered. Thirteen plant species are already globally extinct, making the City of Cape Town one of the most acute areas in the world for plant extinction (Rebelo et al., 2006).

Southern afromontane forests on the Table mountain chain are tall, shady, multi-layered and indigenous. These forests occur in ravines and fire-safe habitats associated with Sandstone Fynbos (De Villiers et al., 2005). However, they are, surrounded by Cape Town's CBD and urban areas, as well as industrial sources of pollution. Metals could be transported (State of the Environment Outlook Report for the Western Cape Province, 2013) by Cape Town's prevailing winds and rain (Jastrzębski, 1974) through the atmosphere and accumulate in these forest ecosystems. Thus, there is a significant chance that these forest ecosystems could also be contaminated with metals and suffer the same risk of deterioration as other forests globally. Being one of the most acute areas in the world for plant extinction (Rebelo et al., 2006), the health of southern afromontane forest ecosystems in the Western Cape are in urgent need of evaluation. No study to investigate this has been done to date.

Forest ecosystem health has been defined as having the capacity to supply and allocate water, nutrients and energy efficiently enough that productivity is increased or maintained, while still maintaining resistance to biotic and abiotic stresses. Two key threats to forest health and sustainability are pollution and climate change (McLaughlin and Percy, 1999; Innes and Oleksyn, 2000; Percy et al., 2000; Innes and Haron, 2001). Thus, to sustain the ecosystem function is especially important with regard to forest response to air pollution (McLaughlin and Percy, 1999).

Millions of people around the world depend on forests for their livelihood, food and security. Forests provide home to 80% of the world's terrestrial biodiversity. At least 40% of the world's oxygen is produced by forests. More than a quarter of modern medicines originate from forest plants. Catchments in forests supply many regions world wide of drinking water (Sauvé et al., 1998, 2003). Tropical forest ecosystems contain around 25% of the carbon in the terrestrial biosphere (Bonan, 2008) and their clearance and degradation account for about 17% of annual $CO_2$ emissions worldwide (IPCC, 2006).

An estimation done in South Africa showed that approximately 2–3 million households gain some significant benefit from key forest components, which include (i) savannas, (ii) plantations, (iii) indigenous forests, and (iv) woodlots (NFAP, 1997). Savannas, characterised by a co-dominance of trees and grasses are the largest biome in South Africa, (Thompson et al., 2001) and indigenous forests the smallest (NFAP, 1997; Mayers et al., 2001). Many communities live adjacent to indigenous forests, extracting multiple resources for subsistence and income generation (Von Maltitz and Grundy, 2000; Lawes et al., 2004b). However, urban populations also utilize forests and forest products extensively for ecotourism, as markets for natural resources and also for maintaining spiritual and cultural beliefs (Mander, 1998; Cocks and Wiersum, 2003). Three hundred thousand traditional healers in South Africa, serve 27 million customers, with two thirds of their plant medicines coming from forests (Mander, 1998). At least 800 000 people are involved in the craft industry (DACST, 1998) and in some areas even more than 70% of households (Marcus, 2000).

Tourism to forested areas includes walks, bird watching, waterfalls and streams, which offers a variety of experiences to eco-tourists. The Knysna State forest is an example of eco-tourism, as it attracts at least 200 000 visitors a year (a conservative estimate), where they use two hiking trails, over a dozen day walks, scenic routes for driving, picnic sites, a camping site, a horse trail, four mountain bike trails and a youth hostel. In that same forest, the Big Tree (a particularly large *Podocarpus falcatus* tree for that forest) is visited by over 75 000 people a year. Eco-tourism from the state-owned forests in the Knysna/Tsitsikamma forests provides approximately 30% of income, which is worth millions to the regional economy of Knysna

(Vermeulen, 2004). Table Mountain National Park similarly benefits people, locally, nationally and internationally with its hikes, beautiful forest walks to pristine picnic and day-visit spots and accommodation facilities (SANParks, 2012).

National and European monitoring programmes have been established in the last few decades, not only to assess the ecological and economic value of forest ecosystems, but also to evaluate the risks posed by human-related factors to ecosystem function and biodiversity conservation (Granke et al., 2009). The Italian National Forest Inventory monitors forest ecosystems intensively (Petriccione and Pompei, 2002) and their aim is to evaluate the effects of human-related stressors and detection of long-term ecological processes, which is based on an integrated and combined evaluation of forest structure, atmospheric deposition, crown condition, climatic parameters and biodiversity (Ferretti, 2002). The United Nations have also proclaimed 2011 the International Year of Forests in order to make people aware of the important role forests play in survival of civilization and more importantly how to protect these “green lungs” of the earth for future generations (SOFO, 2011).

For a proper assessment of soil quality and health in forest ecosystems, chemical analysis should be supported with biological assays involving indicator or monitor organisms (Nahmani and Rossi, 2003; Jänsch et al., 2005; Yang et al., 2006). The reason being that metal bioavailability is mostly controlled by physico-chemical biological factors such as organic matter and clay mineral content, soil reaction, oxygen status or living organism activity (Ernst, 1996; Van Gestel, 2008; Smolders et al., 2009). A lot of these organisms live their entire lives in a few square meters of soil, making them good representatives of local conditions (Migliorini et al., 2004).

Many authors concur that a good bioindicator is sensitive, representative and of functional importance in the ecosystem. It is also easily collected, identified and analysed (Greensdale, 2007). Sentinel organisms are indicator species (biomonitors) capable of accumulating persistent pollutants, like metals in their tissues. These organisms are used to measure the biologically available concentration of a specific pollutant in a specific ecosystem and a key feature is that it reflects ambient pollutant levels (Beeby, 2001). To detect changes in the environment biological monitoring,

using sentinel organisms, has been extremely effective as an early warning system (Keddy, 1991) and can even detect decreasing metal bioaccumulation from pollution control measures (Bealy et al., 2008).

Bryophytes and lichens are common forest floor components and are important as carbon budget models, contributing significantly to the overall forest $CO_2$ fluxes (Goulden and Crill, 1997; Morén and Lindroth, 2000; Swanson and Flanagan, 2001).

Lichens have been used in many studies as biomonitors of atmospheric trace elements, due to their ability to absorb elements directly from the air and accumulating them in their tissues (Guidotti et al., 2009; Cansaran-Duman et al., 2011). Lichen thallus does not possess waxy cuticles or roots and depends mainly on an atmospheric input of mineral nutrients. They have an extraordinary capability to grow over a large geographical range where they accumulate mineral elements far above their own needs. These features rank them among the best bioindicators of air pollution (Aras, et al., 2010; Cansaran-Duman et al., 2011; Cansaran-Duman, et al., 2013). The advantages of biomonitoring with lichens as opposed to instrumental analyses are that they accumulate most of the elements of the periodic table and they are cost-effective, do not need any kind of treatment and do not depend on electricity for their operation (Citterio et al., 2002). Lichens can also be monitored during the entire year (Asta et al., 2002; Scheidegger et al., 2002). According to Dettki and Esseen (1998) and Will-Wolf et al. (2002), lichens are widespread in many forest ecosystems, take part in nutrient cycling and are an important component of forest ecosystems (Knops and Nash, 1996).

Bryophytes have a buffering effect on soil temperature (Skre and Oechel, 1979; Startsev et al., 2007), are an important link in the ecosystem budget of nitrogen and phosphorous (Tamm, 1953; Chapin et al., 1987) and also affects seedling establishment and growth. These processes are linked to underground interactions via mycorrhiza (Zackrisson et al., 1997), thus acting as ecosystem engineers (Jones et al., 1994). Tissue analysis of mosses has been used extensively in the biomonitoring of pollution, including metals (Tyler, 1990; Fernandez et al., 2002; Harmens and Norris, 2008) and tissue chemistry is closely correlated with atmospheric inputs (Bates, 2000; Pitcairn et al., 1995, 1998, 2003). Mosses rely on

wet and dry deposition for their nutrient supply as they lack a conductive root system (Bates, 2000) and absorb nutrients across the entire surface of the plant due to the lack of a cuticle. They also have the ability to remobilize nutrients. Their characteristics therefore make them excellent subjects for biomonitoring and superior in this regard to vascular plants (Peñuelas and Filella, 2001).

Soil invertebrates may be used as indicators of soil quality, because they reflect ecological processes (Coleman, 2008). They are also effective biomonitors of metal contamination in terrestrial ecosystems (Hopkin, 1989; Hopkin et al., 1989; Dallinger, 1991; Dallinger and Rainbow, 1993; Berger and Dallinger, 1993). Investigations showed that up to several km' s away from emission sources, the density of soil invertebrates are reduced in metal polluted soils (Bengtsson and Rundgren, 1982; Bengtsson et al., 1983; Spurgeon et al., 1994; Spurgeon and Hopkin, 1995, 1999; Haimi and Siira-Pietikäinen, 1996). Changes in soil quality may be indicative of change in the soil's structural and biological integrity, which may reflect degradation from environmental stresses (Wander and Drinkwater, 2000). Invertebrates affect soil carbon (C) and nitrogen (N) cycles by their interactions and effects on microbial biomass, inorganic and organic N pools, as well as soil organic matter pools (Yeates, 2003; Osler and Sommerkorn, 2007). They further contribute to critical ecological processes in soil, such as decomposition and nutrient cycling (De Ruiter et al., 1993a, b, 1994; Osler and Sommerkorn, 2007).

Some taxonomic groups of invertebrates, due to their close contact with soil such as Diplopoda, have been proposed as bioindicator organisms. Diplopods are commonly found underneath decayed, fallen stumps and leaves and feed on detritus, organic matter, fruits and mineral material. They aerate soil and facilitate decomposing of organic matter by fungi and bacteria. As a result of the breakdown of their excrements, which is rich in ammonia and uric acid, they are an important source of nitrates in the soil (Schubart, 1942; Hopkin and Read, 1992). Millipedes are continuously exposed to soil contaminants, thus considered good environmental indicators and are successfully used in ecotoxicological research and evaluations (Triebscorn et al., 1991).

Chemical pollution occurs as complex mixtures in the field, which makes prediction of the resulting effects difficult and therefore requires using multiple biological endpoints (Devaux et al., 1998; Flammarion et al., 2002). There are new biological approaches to soil monitoring, such as the measurement of biochemical and cellular responses to pollutants (i.e. biomarkers) on organisms living in the soil (bioindicators) (Kammenga et al., 2000). Biomarkers to evaluate the effects of contaminants on organisms are becoming increasingly important (Svendsen and Weeks, 1997; Spurgeon et al., 2000; Morgan et al., 2002). More specifically, biomarkers of exposure are early reversible cellular changes in the organism, offering an early signal of exposure to micropollutants. Biomarkers of effect give an assessment of a toxicological effect on the organisms and are directly related to the risk of adverse health effects (Suter, 2006). 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). 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).

Oxidative stress biomarkers have been used in studies of environmental impact, including ecotoxicological analyses of the soil with great success, as they respond to a broad range of contaminants (Tsangaris et al., 2011), such as metals. Metal toxicity in biological systems is known to cause the production of reactive oxygen species (ROS), which may affect various cellular processes, of which the functioning of the membrane system is most significant (Pinto et al., 2003; Valko et al., 2005). Oxidative stress occurs when the endogenous antioxidant system is overwhelmed by the production of oxidants, such as ROS (Halliwell, 1992). Oxidative stress biomarkers can thus be used to assess the impact of pollutants on organisms in field situations (Regoli and Principato, 1995; Verlecar et al., 2008). Living organisms have

developed an antioxidant defence system to balance the ROS that have formed naturally (Valavanidis et al., 2006). Enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POX), and ascorbate peroxidase, as well as non-enzymatic antioxidants with low molecular weights, such as proline, cysteine, non-protein thiol, ascorbic acid, and glutathione are able to reduce oxidative stress by scavenging ROS (Choudhury and Panda, 2004, 2005; Singh et al., 2006). Membrane lipid peroxidation in the organisms can be estimated by measuring the content of malondialdehyde (MDA), which serves as an indicator of induced oxidative stress (Sujetovien and Galinyt, 2016).

The sequential order of alterations in a biological system, due to the presence of pollutants occur in crescent levels of biological organization. It extends from the molecular or biochemical level to the physiological or individual level, until the population and ecosystem level (Stegeman et al., 1992) and when a significant alteration is evident, the ecosystem is already severely damaged. Therefore, responses at lower levels of biological organization are techniques considered to be more preventive (Nascimento et al., 2008).

Harmful substances are constantly being released into the terrestrial environment and in order to avoid triggering a possible unbalance in the ecosystems, causing, amongst other, extinction of species, it is necessary to know the effect of those substances on the organisms present there. The combined use of methods in the evaluation of damage in indicator organisms provide a more complete understanding of the effect of contaminants on exposed organisms, as well as more reliable results. Understanding the importance of this concept will greatly benefit the future work of researchers in ecotoxicology (Magalhães and Ferrão-Filho, 2008). This study therefore proposes to use a combination of methods and key sentinel organisms, proposed by many authors in order to obtain a clear and reliable picture of the health of forest ecosystems in the forest pockets of the Western Cape.

In South Africa, the conservation of forests are seen as high priority due to their particular floral and faunal communities and there is an attempt to protect and maintain both the ecosystems, as well as the forest biota diversity (Geldenhuys and MacDevette, 1989). This protection is however against human exploitation such as

logging and product extraction (Castly and Kerley, 1996) rather than air pollution caused by human activities. Virtually no information exists regarding the impact of air pollution and specifically metal contamination in these already stressed South African forests suffering from exploitation and the effects of climate change. In light of the increased pollution rates (CMA, 1998; State of the Environment Outlook Report for the Western Cape Province, 2013), global concern regarding forest decline, the utmost importance of forests in the existence of humanity, the importance of proactive management and the lack of studies in this field, this proposed research is of great scientific and practical importance.

### 1.1.2 Significance:

Metal accumulation in forest ecosystems may contribute to the decline of forests, and forest decline poses serious threats to the existence of mankind, due to the important role forests play in our environment. As mentioned earlier, millions of people around the world depend on forests for their livelihood, food and security. Forests produce, at least 40% of the world's oxygen and more than a quarter of modern medicines originate from forest plants. This study is important as it addresses metal contamination caused by human activities and the effect of that contamination on the wellbeing of our forests and subsequently, our livelihood.

### 1.1.3 Statement of the Research Problem:

Air pollution is a global threat (McCrink-Goode, 2014) to human health, quality of life and the biophysical environment (State of the Environment Outlook Report for the Western Cape Province, 2013). Even though a large mixture of pollutants in the environment are found, heavy metals are considered one of the most serious and widespread forms of environmental contamination (Pruski and Dixon, 2002). South Africa is the largest producer of primary metals in the world (Masekoameng et al., 2010) and the City of Cape Town the biggest contributor to atmospheric pollution, in South Africa as it is the area of highest population density and economic activity. Recent pollution monitoring data taken in the City of Cape Town Metropolitan area shows that pollutant concentrations are at or above acceptable standards (State of the environment Outlook Report for the Western Cape Province, 2013) and even if further improvements are made in urban air quality, long range transport of pollutants

from other regions may continue to add to the background concentrations, making further rehabilitation more difficult (Hopke, 2009).

The Table Mountain chain lies within the City of Cape Town. It is recognised globally for its extraordinarily rich, diverse and unique fauna and flora (SANParks, 2012), but it is also one of the world's most imperilled ecosystems (Underwood et al., 2009). Four of the 56 vegetation subtypes in Cape Town are globally extinct and 19 are critically endangered. Thirteen plant species are already globally extinct, making the City of Cape Town one of the most acute areas in the world for plant extinction (Rebelo et al., 2006). Indigenous southern afromontane forests on the Table mountain chain occurring in ravines (De Villiers et al., 2005) are surrounded by Cape Town's CBD and urban areas, as well as industrial sources of pollution. Forest ecosystems receive considerable metal inputs through atmospheric deposition (Mason et al., 2000; Schwesig and Matzner, 2000) and a consequence of atmospheric deposition is accumulation of metals in forest ecosystems (Rasmussen, 1998; Reimann and De Caritat, 2000; Brannval et al., 2001), especially in populated areas in the vicinities of large cities (Rieuwerts et al., 1999). Metals have severe impacts and are a major threat to ecosystems (Koslov and Zvereva, 2007) and human health (Järup**,** 2003)**.** There is a global decline in forests and forest ecosystems are suffering and are unstable (Royal Ministry for Foreign Affairs, 1971; UN/ECE, 1979; Smith, 1981; Schaub et al., 2010).

Forests, both internationally and within South Africa, offer numerous benefits to adjacent communities and society at large (Wollenberg and Ingles, 1998; Oksanen et al., 2003; Lawes et al., 2004a), but more importantly, tropical forest ecosystems contain around 25% of the carbon in the terrestrial biosphere (Bonan, 2008) and their clearance and degradation account for about 17% of annual $CO_2$ emissions worldwide (IPCC, 2006).

Metal pollution arising from Cape Town's CBD, urban and industrial areas may result in metals settling and accumulating in indigenous forest ecosystems associated with Table Mountain. This may cause serious ecological consequences that could contribute to the eventual decline of forests and the extinction of species (Tilman et al., 1994).

### 1.1.4 Research Question:

What is the effect of metal contamination caused by human activity on key forest organisms in the Western Cape and how will the health of such ecosystems be influenced?

### 1.1.5 Aims of the Study:

The principle aim of this study was to determine how metal contamination influences forest ecosystem health by measuring metal concentrations of various sentinel organisms in a forest ecosystem. Also, to evaluate the effect of the accumulated metals on those sentinel organisms by measuring parameters indicative of induced oxidative stress.

### 1.1.6 Research objectives:

- To determine the concentrations of prominent metals in the forest soil, leaf litter, mosses, lichens and millipedes at various sites by means of a once off pilot study.
- To apply biomarkers to a) determine whether sentinel organisms experience metal induced toxic stress and b) to determine the effect of accumulated metals on these organisms as a result of exposure to metal contamination due to air pollution arising from the CBD.
- To compare the dry and wet season with regard to: (a) seasonal fluctuations of the concentrations of the metals Al, Fe and Mn; (b) the oxidative stress-induced effect of metals, using two markers associated with induced oxidative damage (i.e. malondialdehyde (MDA) concentration and total glutathione (tGSH) levels) in the pill millipede, *Spaerotherium compressum*, the moss, *Hypnum cupressiforme* and the lichen, *Parmotrema sp.*
- To determine, by means of a laboratory exposure experiment: a) the metal concentrations accumulated in the millipedes after a period of 6 weeks and b) to assess whether responses linked to oxidative stress (oxidative lipid damage and altered levels of the andogenous antioxidant, tGSH) measured in the pill millipede, *Spaerotherium compressum* may be utilized as relevant biomarkers of exposure to the metals Al, Fe and Mn. To determine which of

the sentinel organisms exhibited the highest levels of metal bioaccumulation and why.

- To determine the location of sources of metal pollution causing the possible contamination.

# CHAPTER TWO

# MATERIALS AND METHODS

## 2.1 PILOT STUDY

### 2.1.1 Sampling area

Three afromontane forests (tall, shady, multi-layered and indigenous) in the Western Cape were selected as the sampling areas **(Fig 2.1)**. Platbos forest on the slopes of the Baviaanspoort Hills served as a control area for comparison. This ancient, indigenous forest is the largest remaining fragment of the Swartkransberg forests and located between Gansbaai and Hermanus, approximately 170 km from Cape Town (Platbos, 2016). No potential sources of pollution are close to this forest **(Fig 2.3)**. The other two areas were chosen because they are situated on Table Mountain, which is surrounded by the City of Cape Town and industrial and urban sources of pollution **(Fig 2.2)**. Orange Kloof is the most intact and oldest indigenous forest on Table Mountain and located on the eastern slopes of Table Mountain at the northern end of the Hout Bay valley **(Fig 2.7)**. Newlands Forest is situated on the eastern slopes of Table Mountain, beside the suburb of Newlands (SA-Venues.com, 2017) **(Fig 2.11)**.

### 2.1.2 Sampling sites

Three 20 x 20 $m^2$ sampling sites within each of the three selected forests were chosen, as recommended by Fernández et al. (2002) and Aboal et al. (2006), with the first sampling site at the lowest point at least 300 m away from highways and 100 m from other types of roads or structures. The remaining two sampling sites thereafter were at different altitudes on higher points on the mountain. Sites were planned between 150 m to 200 m apart, but it was not always possible, due to rough terrain and availability of the organisms to be sampled **(Platbos: Figs 2.3, 2.4, 2.5, 2.6, & Table 2.1; Orange Kloof: Figs 2.7, 2.8, 2.9, 2.10 & Table 2.2; Newlands: 2.11, 2.12, 2.13, 2.14 & Table 2.3)**.

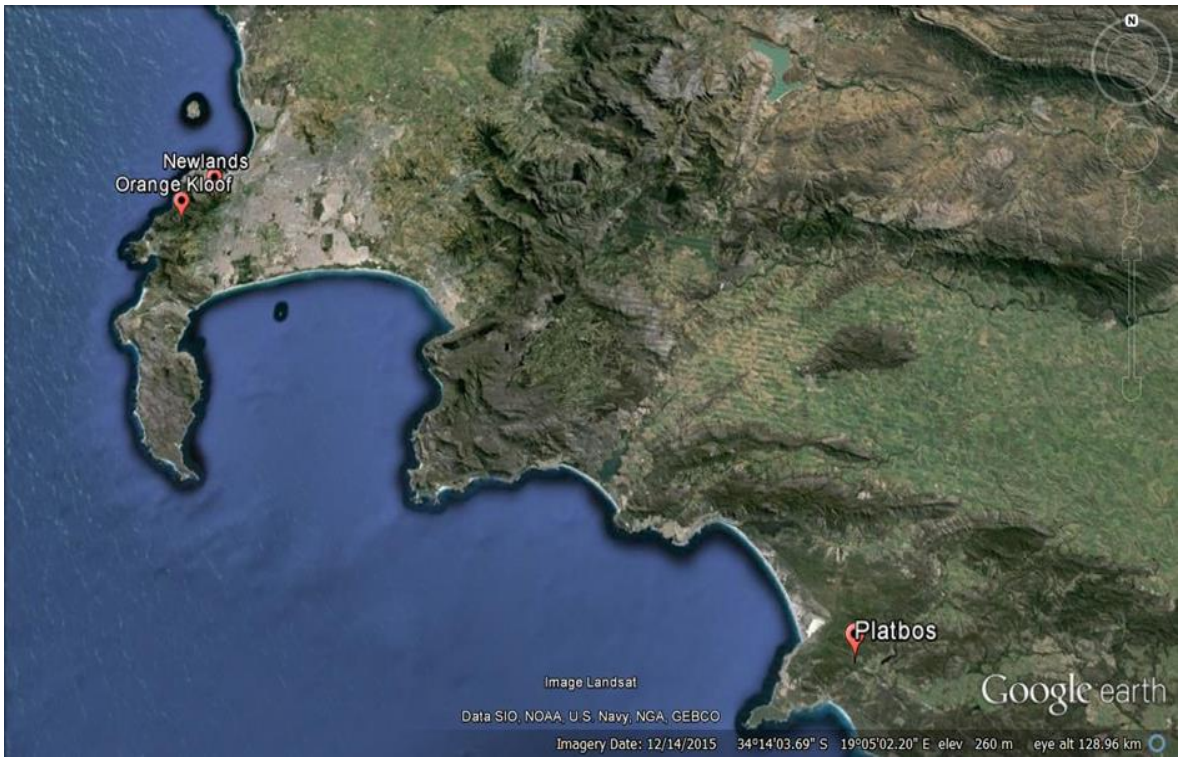


**Figure 2.1:** Platbos, Orange Kloof and Newlands forests in the Western Cape (GOOGLE earth).

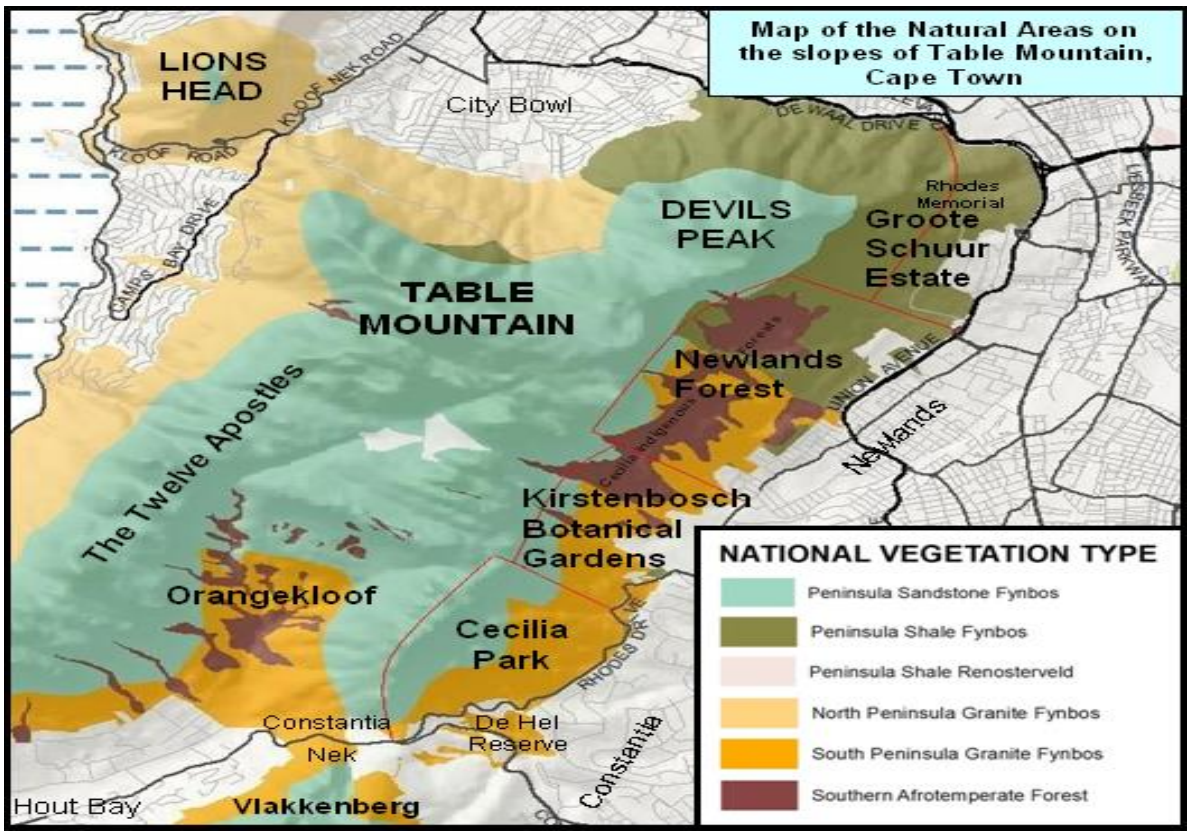


**Figure 2.2:** Map of the sampling areas on Table Mountain National Park. Southern afromontane forests are highlighted in purple (ABU Shawka–own work, CCBY–SA 3.0).

### 2.1.2.1) PLATBOS FOREST

***Soil:*** The ancient sand dune on which Platbos forests grows, comprises of a deep alkaline and sandy soil at all three sites, as determined by a 5 fraction analysis **(par. 2.1.5, p. 25))**

***Structures, buildings and natural features****:* The terrain is flat, the forest is relatively open and the canopy is low - a maximum of ~10m. Hiking trails, a whole sale nursery, private residences, Old Olive cabin, the Forest and Honey Bee camps, a sixty year old regrowth forest and a labyrinth are a part of this forest.

***Vegetation:*** The afromontane species *Celtis africana* (African White-stinkwood) and *Olinia ventosa* (Induqu Hard-pear), as well as the coastal forest species *Sideroxylon inerme* (Milkwood) and *Apodytes dimidiate* (White pear) are dominant. In the understorey, *Chionanthus foveolata* (Pock-Fewood) and epiphytes such as *Peperomia*, ferns, mosses, lichens, the old Man's Beard lichen *(Usnea cf barbata*) and woody climbers of lianas are common. An 800 year old Wild Olive and 1000 year old Milkwood tree still survives in this forest (Platbos, 2016).


**Figure 2.3:** Platbos forest: Location of sampling sites 1, 2 and 3 (GOOGLE earth)
P1- 34°33'58.99"S 19°26'54.48"E; P2- 34°34'04.56"S 19°26'47.59"E; P3- 34°34'00.34"S 19°27'10.60

**Table 2.1:** GPS coordinates of sampling sites within the Platbos forest (P):

| GPS COORDINATES | ELEVATION (M) | EYE ALTITUDE |
|---|---|---|
| P1- 34°33'58.99"S 19°26'54.48"E | 114 | 825 |
| P2- 34°34'04.56"S 19°26'47.59"E | 133 | 825 |
| P3- 34°34'00.34"S 19°27'10.60"E | 88 | 825 |

**Figure 2.4:** Platbos Site 1

**Figure 2.5:** Platbos Site 2

**Figure 2.6:** Platbos Site 3

### 2.1.2.2) ORANGE KLOOF FOREST

***Soil:*** The soil in this forest is classified at sites 1 and 3 as sandy and site 2 as loam sandy as per a 5 fraction analysis **(par. 2.1.5, p.25)**. The sandy soils are derived from the parent sandstone, which is sparsely distributed with oxides of Mn and Fe.

***Structures buildings and natural features*:** This forest overlooks Houtbay and Constantia and houses five Hoerikwaggo tented camps and forest trails, the Disa River and Woodhead Dam.

***Vegetation:*** Ancient Milkwoods *(Sideroxylon inerme)* are found here and the Blossom tree *(Virgillia divaricate)* and Butterspoon *(Cunnonia capesis)* are also abundant, together with Yellowwoods *(Podocarpus elongates),* Wild peach *(Kiggelaria Africana),* Bladdernut *(Diospyros whyteana)* and Fewood *(Olinia ventosa).* Mosses and lichen species are also abundant in this forest (SA-Venues.com, 2017).

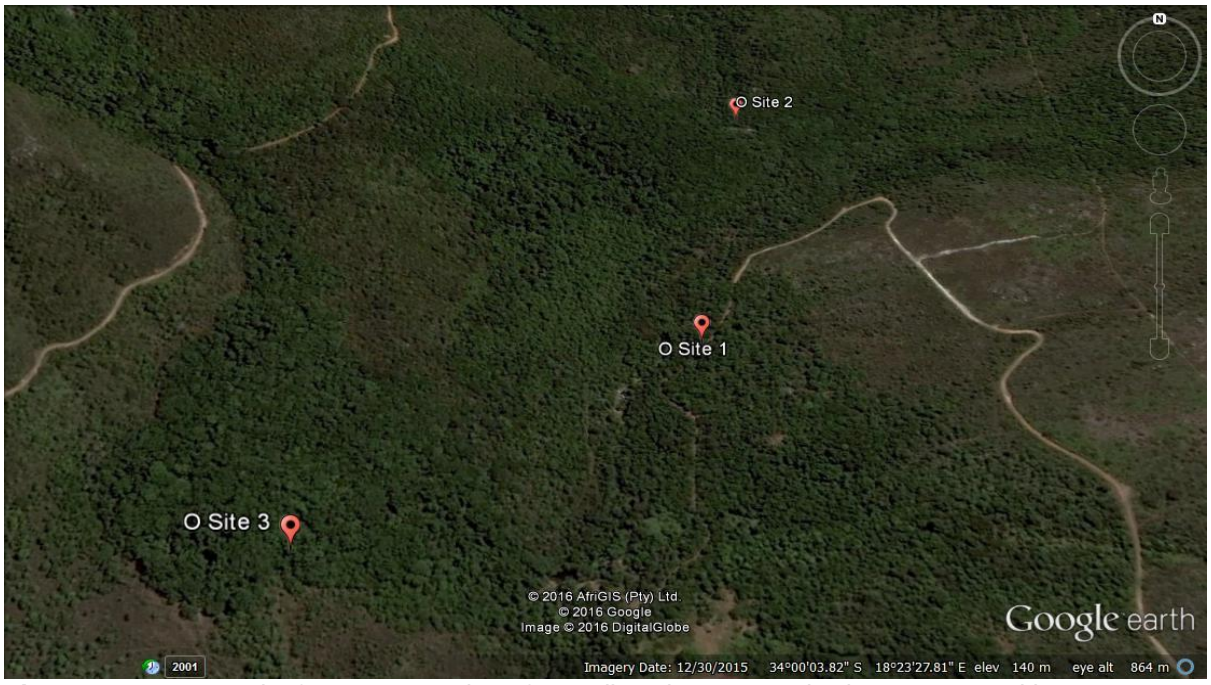


**Figure 2.7:** Orange Kloof Forest: Location of sampling sites 1, 2 and 3 (GOOGLE earth) O1-34°00'00.67"S 18°23'31.84"E; O2- 33°59'50.62"S 18°23'36.23"E; O3- 34°00'11.55"S 18°23'17.95"E

**Table 2.2:** GPS coordinates of sampling sites within the Orange Kloof forest (O).

| GPS COORDINATES | ELEVATION (M) | EYE ALTITUDE |
|---|---|---|
| O1- 34°00'00.67"S 18°23'31.84"E | 143 | 844 |
| O2- 33°59'50.62"S 18°23'36.23"E | 175 | 844 |
| O3- 34°00'11.55"S 18°23'17.95"E | 161 | 844 |

**Figure 2.8:** Orange Kloof Site 1

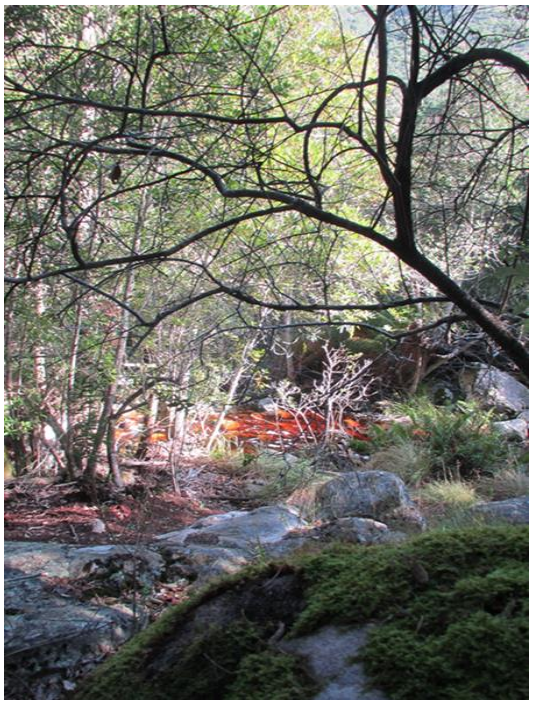

**Figure 2.9:** Orange Kloof Site 2

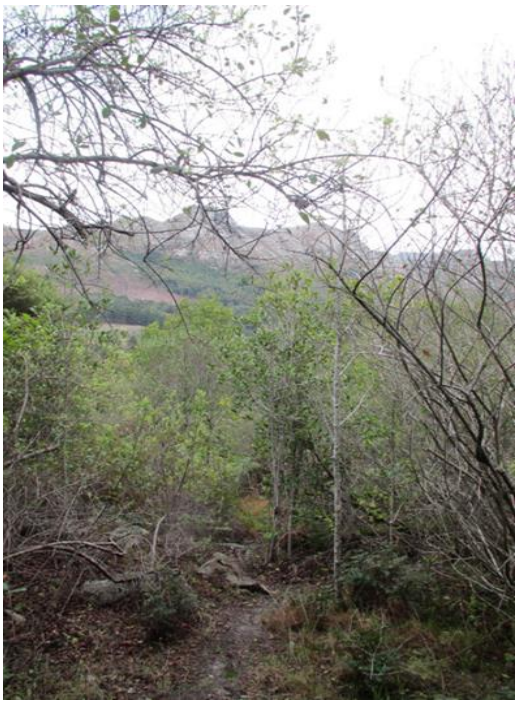

**Figure 2.10:** Orange Kloof Site 3

### 2.1.2.3) NEWLANDS FOREST

***Soil:*** The soils found in this forest are derived from Table Mountain sandstone and characterized by Bemlab laboratory through a 5 fraction analysis at sites 1 and 3 as loam sandy and site 2 as sandy **(par. 2.1.5, p, 25).**

***Structures, buildings and natural features*:** The fire station, City Parks Nursery and the Newlands reservoir are prominent features in this forest, but council houses are also situated here. The Krom River, streams and hiking trails are main attractions for visitors.

***Vegetation*:** The indigenous afromontane forests are combined with a series of pine and gum plantations. *Curtisia dentate* (Assegai), *Olea capensis* (Fewood), *Kiggelaria africana* (Wild peach) and *Podocarpus elongates* (Yellowwood) to name but a few are commonly found in this forest. Mosses and lichens also grow vigorously on rocks, trees and rocks (Sa-Venues.com, 2017).


**Figure 2.11:** Newlands Forest: Location sampling sites 1, 2 and 3 (GOOGLE earth) N1- 33°58'26.19"S 18°26'41.23"E; N2- 33°58'00.80"S 18°26'33.80"E; N3- 33°57'56.92"S18°26'30.10"E

**Table 2.3:** GPS coordinates of sampling sites within the Newlands forest (N).

| GPS COORDINATES | ELEVATION (M) | EYE ALTITUDE |
|---|---|---|
| N1- 33°58'26.19"S 18°26'41.23"E | 137 | 852 |
| N2- 33°58'00.80"S 18°26'33.80"E | 216 | 968 |
| N3- 33°57'56.92"S 18°26'30.10"E | 247 | 965 |

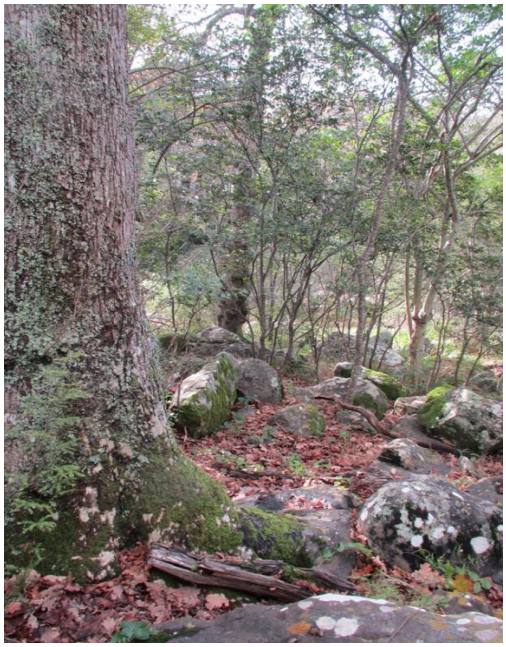

**Figure 2.12:** Newlands Site 1

**Figure 2.13:** Newlands Site 2

**Figure 2.14:** Newlands Site 3

### 2.1.3 Sampling procedure

Once off sampling was done in May 2014 and each sampling site was given co-ordinates, which were mapped, using a GPS-system **(Platbos: Figs 2.4, 2.5, 2.6, & Table 2.1; Orange Kloof: Figs 2.8, 2.9, 2.10 & Table 2.2; Newlands: 2.12, 2.13, 2.14 & Table 2.3)**. Eight samples (replicates per site) of equal weight or size of the soil, leaf litter and each organism at each of the sampling sites were randomly collected at least 50 cm apart using latex gloves and plastic knives and scoops, although only 5 samples of each were analyzed **(Figs 2.15, 2.16, 2.17)**.

***Soil***

The top 0-5 cm of soil under the leaf litter layer was collected and transported to the laboratory in clean 50 ml plastic vials (Rieuwerts et al., 1996; Martley et al., 2004; Kříbek et al., 2010; Stafilov et al., 2010), where it was subjected to pH and moisture tests. The remainder of the soil samples were frozen and kept in a freezer before further analysis.

***Leaf litter***

Decomposing leaf litter from various afromontane tree species were scraped from the top soil layer. Samples were transported separately in clean plastic bags to the laboratory, after which the moisture content was determined. The remainder of the leaf litter was kept in a freezer before further analysis.

***Moss***

Whole mosses were used in order to get an idea of metals that have accumulated over a long period of time. The species, *Hypnum cupressiforme* **(Fig 2.15)** was randomly collected from rocks at a height of 1 to 1.5 m from the ground to prevent any soil contamination (Bargagli and Nimis, 2002). Samples were transported separately in clean plastic bags and cleaned from extraneous material, but not subjected to any washing in order to prevent possible changes in metal contents (Wadleigh and Blake, 1999; Bargagli and Nimis, 2002; Conti, 2002). Moss samples were kept in a freezer before further analysis.

***Lichen***

The lichen, *Parmotrema sp.* **(Fig 2.16)** was randomly collected and carefully removed from rocks at a height of 1 to 1.5 m from the ground to prevent any soil contamination (Bargagli and Nimis, 2002). They were transported to the laboratory in clean plastic bags where they were cleaned to remove dust, leaf debris, fungus and degraded material deposited on the surface, but not rinsed in water to prevent possible changes in metal contents (Wadleigh and Blake, 1999; Bargagli and Nimis, 2002; Conti, 2002). To get an idea of metals that have accumulated over a long period of time, the whole lichen thalli was used for analysis. Lichen samples were brought back to the laboratory and kept in a freezer before further analysis.

***Millipedes***

Similar sizes of the adult millipedes (eight), *Sphaerotherium compressum* **(Fig 2.17)** were hand collected underneath the litter layer in the soil. They were transported separately in plastic containers in the forest soil in order to prevent unnecessary stress. In the laboratory the millipedes were killed by putting them separately in labelled 10 ml plastic vials in the freezer before further analysis.

### 2.1.4 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. Moisture percentage for each soil sample was determined by measuring the wet and dry weight and calculating the percentage, using the lost weight. The results are described in the Results section of each forest **(Platbos: Table 3.2; Orange Kloof: Table 3.7; Newlands: Table 3.12).**

### 2.1.5 Soil characterization

Five topsoil samples from each site were taken to make up a 1 kg composite sample per site and taken to a laboratory for a 5 fraction analysis.The results are described under the soil section of each forest, as well as in the Results section of each forest **(Platbos: Table 3.5; Orange Kloof: Table 3.10; Newlands: Table 3.15).**

### 2.1.6 Acid digestion

The lichen, moss, soil, leaf litter and millipedes were placed in separate, labelled petri dishes and into a Memmert oven to dry out for 48 hours at 60°C in order to obtain the dry weight. The dried samples were ground into powder, homogenized with a mortar and pestle and weighed to obtain a weight of approximately 0.2 and 0.3 g per sample. Each millipede was weighed separately and intact on a Precisa XB 220A balance.

The weighed samples were placed into labelled, metal free test tubes for digestion. The test tubes with the 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 65% nitric acid at a temperature of 40°C for one hour. The temperature was then increased to 120°C for a period of three 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 labelled 20 ml volumetric flasks. The samples were then filtered through Whatman 0.45 µm cellulose nitrate membrane filter paper using a syringe and Millipore filter holders. Finally, one ml was diluted with 9 ml of distilled water and the prepared samples were stored in centrifuge tubes and taken to the ICP-MS laboratory at the University of Stellenbosch to determine the metal concentrations in the samples.

Soil, leaf litter, moss, lichen and millipedes were analysed for twelve metals (Cd, Pb, Mo, Co, V, Ni, Cr, Al, Fe, Mn, Cu, Zn) in May 2014. However, only the latter five metals (Al, Fe, Mn, Cu, Zn) are discussed here, as they were the ones showing relatively high concentrations. The metal concentrations in the samples were determined with an Inductively Coupled Plasma Mass Spectrophotometer (ICP–MS) and calculated using the following formula:

$$\frac{(\text{ICP reading} - \text{Blank}) \times [200 \text{ dilution factor}]}{\text{Dry mass of sample (g)}}$$

Metal concentration is expressed as mg/kg.

### 2.1.7 Statistical analysis of data

Kruskal–Wallis one-way analysis of variance on ranks was carried out to compare the concentrations of metals at different sites during this study in soil, leaf litter, lichen, moss and millipede samples. The Student–Newman–Keuls method was used to do pairwise multiple comparisons and t-tests were used to compare soil and leaf litter, as well as moss and lichen concentrations. The values are presented as the mean ± SD and the probability levels used for statistical significance were $P<0.05$. Statistical analysis was done using the Sigma plot 13.0 software package.

**Figure 2.15:** Hypnum cupressiforme (Moss)

**Figure 2.16:** Parmotrema sp. (Lichen)

**Figure 2.17:** Sphaerotherium compressum (Pill millipede)

## 2.2 DRY AND WET SEASON STUDY

### 2.2.1 Sampling area

The two afromontane forests, Orange Kloof and Newlands in the Western Cape decribed in **par. 2.1.1, p. 16** were selected as the sampling areas, because of their location on Table Mountain, which is surrounded by the City of Cape Town, as well as industrial and urban sources of pollution **(Figs 2.1, 2.3).**

### 2.2.2 Sampling sites

The same three 20 x 20 m² sampling sites within each of the two selected forests that were used in the pilot study served as the study areas in this seasonal study as described in **par. 2.1.2, p. 16.** A reference control site (site C) **(Fig 2.19)** in a more remote part of Orange Kloof, the forest furthest away from the CBD of Cape Town was added (**Orange Kloof: Figs 2.8, 2.9, 2.10, 2.18, 2.19 & Table 2.4; Newlands: 2.11, 2.12, 2.13, 2.14 & Table 2.3)**.

#### 2.2.2.1) ORANGE KLOOF FOREST

***Soil:*** As described in **par. 2.1.2.2, p 20.** The soil at the additional site, site C, by means of a 5 fraction analysis, was characterised as loam sandy **(par. 2.1.5, p, 25 & 2.2.5, p. 32)**.

***Structures, buildings and natural features:*** As described in **par. 2.1.2.2, p 20.**

***Vegetation*:** As described in **par. 2.1.2.2, p 20.**

See **Figs 2.8, 2.9, 2.10, 2.18, 2.19 & Table 2.4** for the location of the sampling sites.


**Figure 2.18:** Orange Kloof forest: Location of sampling sites 1, 2, 3 (GOOGLE earth)
O1-34°00'00.67"S 18°23'31.84"E; O2- 33°59'50.62"S 18°23'36.23"E; O3- 34°00'11.55"S 18°23'17.95"E

**Table 2.4:** GPS coordinates of sampling sites within the Orange Kloof forest (O).

| GPS COORDINATES | ELEVATION (M) | EYE ALTITUDE |
|---|---|---|
| O1- 34°00'00.67"S 18°23'31.84"E | 143 | 844 |
| O2- 33°59'50.62"S 18°23'36.23"E | 175 | 844 |
| OC- 34°00'69.62"S 18°23'45.23"E | 159 | 844 |
| O3- 34°00'11.55"S 18°23'17.95"E | 161 | 844 |

**Figure 2.19:** Orange Kloof Site C (Control site)

**2.2.2.2) NEWLANDS FOREST**

***Soil:*** As described in **par. 2.1.2.3, p 19, par. 2.1.5, p, 24 & 2.2.5, p. 32)**.

***Structures, buildings and natural features:*** As described in **par. 2.1.2.3, p 21.**

***Vegetation*:** As described in **par. 2.1.2.3, p 22.**

See **Figs 2.11, 2.12, 2.13 & Table 2.3** for the location of the sampling sites.

**2.2.3 Sampling procedure**

The study comprised of two sampling periods: a dry season session from January to March 2015 and a wet season session from June to August 2015. A time frame of 3 months per dry (January, February, March) and wet (June, July, August) season was allocated for sampling as those were the months that were earmarked as, either hot and dry or cold and wet. The temperatures, wind and rainfall generally does/did not vary much within those 3 months of the dry or the wet season respectively **(Tables 4.4, 4.9 & 4.17, 4.22).** The climate in Cape Town is Mediterranean with dry warm summers and wet mild winters (WeatherSpark, 2015). It was also taken into account that 5 different types of samples needed to be collected in a mostly rough terrain in the mountain, which was quite a time consuming task.

Most of the sampling over the dry period was done within a month and a half, which was slightly longer than the 1 month that it took to collect all the samples in the wet season. The longer period in summer was nesassary due to the difficulty of finding millipedes in the warm dry soil. Millipedes prefer habitats with high moisture and dense leaf litter in a loam soil and tend to burrow deep into the soil during dry spells (SANBI, 2016). It would have been ideal to have collected the millipedes in the similar time periods in both seasons. However, the study areas were not situated in the vicinity of major industries that may have made a huge difference in the metal concentrations, if one of the sampling sessions were longer, but are impacted by air pollution over time, which lead us to believe that the results were not compromised by the slightly longer summer sampling session. Average temperatures during the dry season ranged from a minimum of 9°C in February to a maximum of 41°C in

March. January 2015 was the hottest month of the year with an average daily high temperature of 27°C. The longest dry spell was from 15 February to 16 March 2015 **(Fig 2.20)**.

Heavy, moderate, and light rain was observed in the wet season during the month of June 2015. Average temperatures during the wet season ranged from 2°C to 25°C **(Fig 2.21)**. The highest sustained wind speed of 15 m/s, occurred on July 29 and the highest daily mean wind speed was 11 m/s on July 21. The highest wind gust speed was 22 m/s on September 14 and the windiest month was January, with an average wind speed of 6 m/s. The month with the least wind was May, with an average wind speed of 4 m/s **(Fig 2.22)** (WeatherSpark, 2015).

The wet sampling sessions were done during regular spells of the visible brown haze hanging over the city. The haze occurs under stable atmospheric conditions with low-level temperature inversions mainly during March to September, but recently has extended into the summer months as well (City of Cape Town, 2016).

Each sampling site was given coordinates, which were mapped, using a GPS-system **(Tables 2.2, 2.3, 2.4)**. Eight replicates, using only five of equal weight or size of each organism at each of the sampling sites were randomly collected at least 50 cm apart using latex gloves and plastic knives and scoops.

***Soil***

As described in **par. 2.1.3, p. 24.**

***Leaf litter***

As described in **par. 2.1.3, p. 24.**

***Moss***

As described in **par. 2.1.3, p. 24.**

***Lichen***

As described in **par. 2.1.3, p. 25.**

***Millipedes***

As described in **par. 2.1.3, p. 25.**

### 2.2.4 Determination of pH and moisture

As described in **par 2.1.4 p.25.** The results are described in the Results section of each forest in both the dry and wet seasons **(Orange Kloof: Tables 4.2, 4.15 & Newlands: Tables 4.7, 4.20).**

### 2.2.5 Soil characterization

As described in **par. 2.1.5 p. 25 & 2.2.2.1, p. 28** for characteration of the site C soil. The results are described in the Results section of each forest in both the dry and wet seasons **(Orange Kloof: Tables 4.5, 4.18 & Newlands: Tables 4.10, 4.23).**

### 2.2.6 Acid digestion

As described in **par. 2.1.6, p. 26**. In this seasonal study the soil, leaf litter, moss, lichen and millipedes were analysed for twelve metals (Cd, Pb, Mo, Co, V, Ni, Cr, Al, Fe, Mn, Cu, Zn), but only the metals, Al, Fe and Mn are discussed here. These metals not only showed much higher concentrations than the other nine metals, as were determined in the pilot study, but are released anthropogenically in the atmosphere on a daily basis in the expanding City of Cape Town, via industries and vehicle traffic, amongst others. Aluminium and Fe are of great significance in the Western Cape due to their enhanced levels and the unknown influence it has on the nearby forest ecosystems. Aluminium and Mn contamination are of great significance to forests, as high Al concentrations and low soil acidity have been implicated in the dieback of trees, but so has high Mn bioavailability in soil that has lead to Mn toxicity in plants, also in forests. These metals are also not well studied and their impact on Cape Town forests, where they appear in high concentrations could lead to new information regarding the health of these forest ecosystems, impacted by them.

### 2.2.7 Biochemical analysis

Moss *(Hypnum cupressiforme),* lichen *(Parmotrema sp.)* and adult specimens of the pill millipede *Sphaerotherium compressum* were manually collected in Orange Kloof

and Newlands forests at the aforementioned sampling areas (see 2.1.1) during the dry season (January 2015) and the wet season (June 2015).

Samples of moss and lichen were transported to the laboratory in clean plastic bags, in cooler boxes and frozen immediately at -80ºC before further analysis. The millipedes were transported in separate plastic containers in the forest soil, leaf litter and decaying wood from their collection sites. Care was taken not to subject them to any additional stress. In the laboratory they were acclimated in tanks for a period of 15 days in the same soil, leaf litter and decaying wood. The environmental conditions of the collection site such as photoperiod, relative humidity and temperature of ± 21 to 22ºC were adhered to (Godoy and Fontanetti, 2010; Nogarol and Fontanetti, 2010). The millipedes were frozen at -80ºC after the acclimation period, before further analysis. All the samples were freeze dried for 48 hours. The exoskeleton of the pill millipedes were removed prior to being freeze dried.

The samples were prepared for tGSH and lipid peroxidation analysis by using approximately 2 g of the moss, lichen and millipede samples, respectively. Each sample was weighed using a Sartorius 2006 MP Balance and homogenized with 10 ml Sodium Phosphate monobasic buffer (7.5) (Sigma Aldridge). Added to the buffer solution was EDTA (Ethylenediamine-tetraacetic acid disodium salt-dihidrate) (99.1%), distilled water and NAOH (Sodium hydroxide) and Triton (0.2%) x 100. The homogenate was stored in eppendorphs and frozen at -80ºC before further analysis.

#### *2.2.7.1) Lipid peroxidation*

The method of Nair and Turner (1984) was used to determine the concentrations of malondialdehyde (MDA) as a marker of lipid peroxidation (LPO) and was based on the reaction with thiobarbituric acid (TBA). A fresh mixture of 3 ml of TBA reagent made up of 1:3 by volume of 0.8% TBA and 20% trichloroacetic acid (TCA) was prepared and mixed well with a 0.33 ml of the homogenate. A boiling water bath was used to incubate the mixture for 20 min, after which it was cooled. Thereafter the mixture was centrifuged at 4200 rpm for 20 min and the MDA level was measured spectrophotometrically at 532 nm. The results were expressed as nM of MDA $mg^{-1}$ of wet tissue.

### *2.2.7.2) Total Glutathione*

The method according to Owens and Belcher (1965) was used to measure the total glutathione level at 412 nm using 50, 5-dithio-bis-(2-nitrobenzoic acid) (DTNB). The assay mixture was made up of 0.1 ml of the homogenate, 1.5 ml of 0.5 M phosphate buffer, pH 8.0 followed by 0.4 ml of 3% metaphosphoric acid and 30 l L DTNB (0.01 M). The total glutathione present in the sample in terms of 1 g $g^{-}1$ wet weight tissue was calculated after it was calibrated against the standard curve of GSH.

### 2.2.8 Statistical analysis of data

As described in **par. 2.1.7, p 27.**

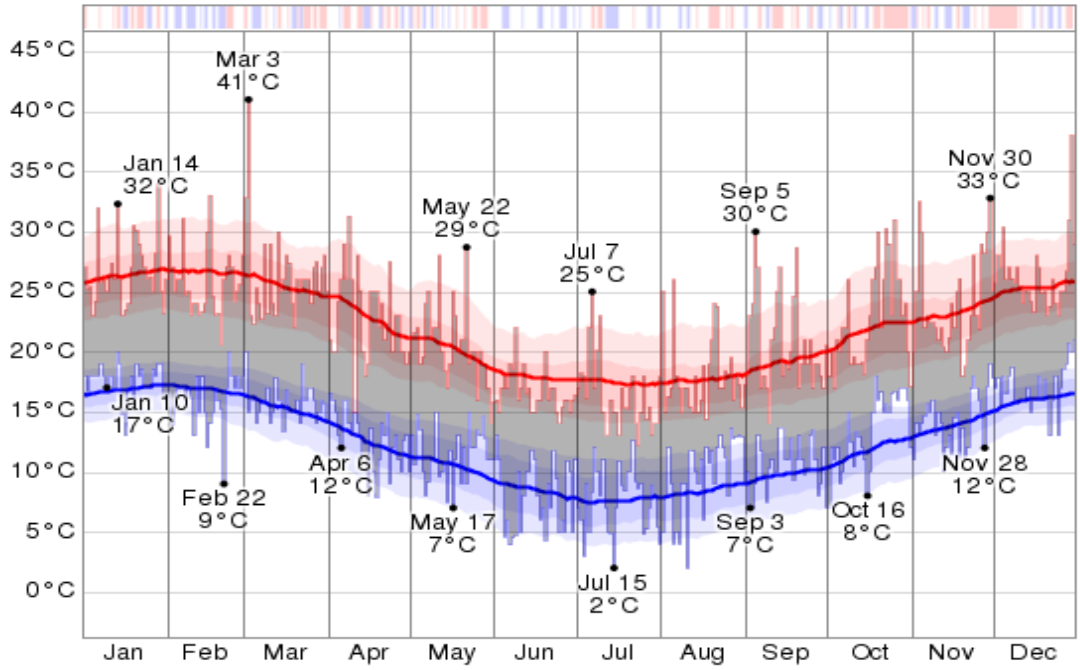


**Figure 2.20:** The daily low (blue) and high (red) temperature during 2015 (WeatherSpark, 2015).

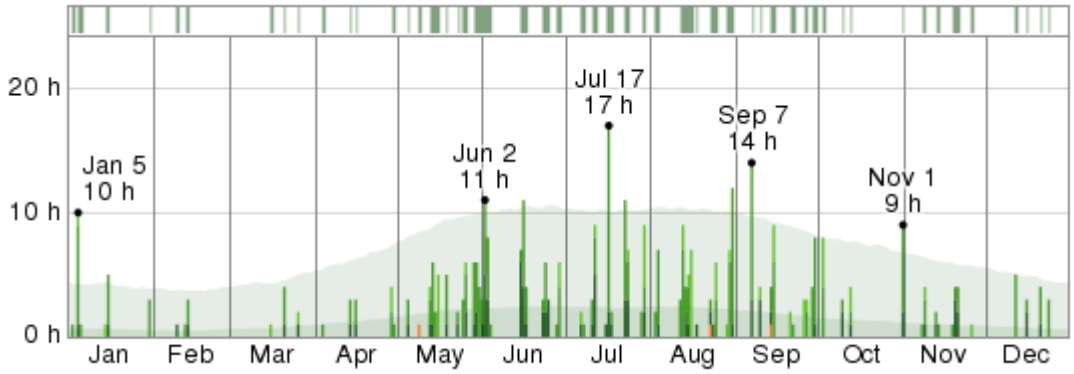


**Figure 2.21:** The daily number of hourly observed precipitation reports during 2015, colour coded according to precipitation type, and stacked in order of severity. From the bottom up, the categories are thunderstorms (orange); heavy, moderate, and light snow (dark to light blue); heavy, moderate, and light rain (dark to light green); and drizzle (lightest green). The faint shaded areas indicate climate normals. The bar at the top of the graph is green if any precipitation was observed that day and white otherwise (WeatherSpark, 2015).

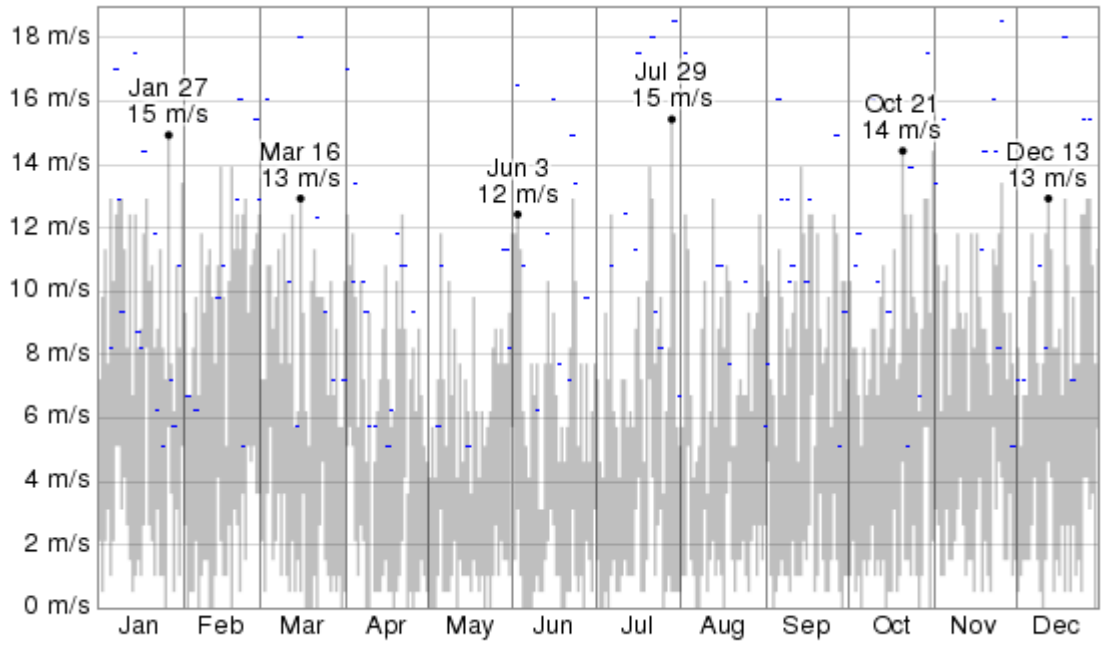


**Figure 2.22:** The daily low and high wind speed (light gray area) and the maximum daily wind gust speed (tiny blue dashes) (WeatherSpark, 2015).

## 2.3 EXPOSURE EXPERIMENT

### 2.3.1 Exposure soil

Soil, leaf litter and decaying pieces of wood were collected from site C at Orange Kloof forest, which served as the control area throughout the whole study **(Fig 2.18).** The decomposing leaf litter contained leaves from the various afromontane tree species and the decaying wood had species of the moss *Hypnum cupressiforme* and lichen *Parmotrema* sp. still growing on them.

Two kilograms of soil **(Fig 2.23)** and 200 g of the leaf litter **(Fig 2.24)**, weighed on a Metler balance were collected for each of the three exposure tanks. The decaying wood and branches covered in the moss and lichen **(Fig 2.25)** was added to the tanks as food, but more so to create the most natural environment possible for the millipedes, thus minimizing unnecessary stress (Sosinka et al., 2014).

### 2.3.2 Pill millipede species used in the study

As described in **par. 2.1.3 *Millipedes*, p 25 (Fig 2.26).**

### 2.3.3 Exposure procedure

The pill millipedes were acclimatized in the laboratory for fifteen days, which is advised in order for these diplopods to return to a relatively stress free condition after being handled and before being exposed to contaminated soil for six weeks. This procedure is in accordance with Nogarol and Fontanetti, (2010), although their exposure periods are generally as long as ninety days.

Soil pH, as well as moisture percentage of the soil and leaf litter were measured before the three tanks (20 cm wide x 25 cm long x 45 cm high) with perforated lids for aeration were filled with the forest soil, leaf litter, decaying branches and thirty millipedes per tank. The environmental conditions of the collection site such as photoperiod, relative humidity and temperature of ± 21 to 22ºC, which included a daily mist spray of distilled water was adhered to in the laboratory (Godoy and Fontanetti, 2010; Nogarol and Fontanetti, 2010) **(Fig 2.27)**.

After the acclimation period, twelve millipedes were randomly removed from the three tanks and weighed on a Precisa XB 220A balance. Six millipedes were killed by putting them separately in labelled 10 ml plastic vials in the freezer to be digested at a later stage. Samples of the soil and leaf litter were also frozen for digestion purposes. The remaining six millipedes were defecated and frozen at -80ºC for oxidative stress analysis, also at a later stage. This procedure marked week naught of the six week exposure period that followed.

The six week exposure period commenced by preparing three tanks with 2 kg of fresh, moist (20-23%) forest soil, 200 g of leaf litter, as well as decaying wood, all from site C where the millipedes were collected. Two separate cocktails of the metals, Al, Fe and Mn were made up, of which one had the low and the other one had the high dosage. The remaining tank served as the control tank. The low dosage consisted of 1.4 g of Al, 1.4 g of Fe and 0.06 g of Mn. The powders were mixed with distilled water and sprayed onto the soil, only once until wet, but not drenched. The same method was used to spike the soil in the other tank with a higher dosage, which consisted of 20 g of Al, 20 g of Fe and 1.2 g of Mn. The dosages were calculated according to the lowest and highest concentrations of the metals measured in the forest soils during the whole study and then made slightly higher. The lowest concentrations were as follows: Al (1400 mg/kg), Fe (1400 mg/kg and Mn (60 mg/kg). The highest concentrations were: Al (20 000 mg/kg), Fe (20 000 mg/kg) and Mn (1200 mg/kg).

The remaining pill millipedes from the acclimation period were divided between the three tanks (26/tank) and the same procedure with regard to photoperiod, relative humidity and temperature of ± 21 to 22ºC, including a daily mist spray of distilled water (Godoy and Fontanetti, 2010; Nogarol and Fontanetti, 2010) was adhered to in the laboratory during the exposure period of six weeks. Fresh leaf litter was collected from site C on a weekly basis and a thin layer added to the tanks.

After the six weeks of exposure, the procedure done, before exposure (week 0) was repeated. pH of the soil was taken, as well as the moisture percentage of the soil and leaf litter and the samples were frozen before further analysis. Twelve millipedes per tank were removed and weighed, after which six were killed by putting them

separately in labelled 10 ml plastic vials in the freezer for digestion purposes. The other six millipedes were, defecated and frozen at -80ºC for oxidative stress analysis. This procedure marked the end of the six week exposure period **(Fig 2.28).**

### 2.3.4 Determination of pH and Moisture

As described in **par. 2.1.4 on p. 25 & Results Table 5.4**

### 2.3.5 Soil characterization

As described in **par. 2.1.5, p 25; 2.2.5, p 32 & Results Table 5.6.**

### 2.3.6 Acid digestion

As described in **par. 2.1.6, p. 26 & 2.2.6, p. 32**. Soil, leaf litter and millipedes were analysed for the three metals, Al, Fe and Mn used in the cocktail.

### 2.3.7 Biochemical analysis

As described in **par. 2.2.7, p 32.**

#### *2.3.7.1) Lipid peroxidation*

As described in **par. 2.2.7.1, p. 33.**

#### *2.3.7.2) Total Glutathione*

As described in **par. 2.2.7.2, p. 34.**

### 2.3.8 Statistical analysis of data

As described in **par. 2.1.7, p. 27.**

**Figs 2.23-2.28:** Substrate and organisms collected from site C, used in the exposure tanks, acclimation and exposure experiment.

**Figure 2.23:** Exposure soil

**Figure 2.24:** Leaf litter

**Figure 2.25:** Decaying wood/moss/lichen

**Figure 2.26:** Pill millipedes

**Figure 2.27:** Acclimation period (15 days)

**Figure 2.28:** Exposure experiment (6 weeks)

# CHAPTER THREE

# PILOT STUDY

# METAL CONTAMINATION AND BIOACCUMULATION IN SOIL, LEAF LITTER AND SENTINEL ORGANISMS IN SOUTH AFRICAN FOREST POCKETS

## 3.1 INTRODUCTION

Forest ecosystems are crucial for the survival of civilization – they are the "green lungs" of the earth (SOFO, 2011), yet they are under serious threat from industrial pollution at local, regional and global levels (Fowler et al., 1999; Kozlov et al., 2009; Matyssek et al., 2012).

These ecosystems are highly sensitive to atmospheric pollution, regardless of the location of point source emissions (Mayer, 1983; Lovett and Lindberg, 1984; Gandois et al., 2010). Air pollution is not confined to the city (Singh et al., 2013), in fact forest ecosystems are reached via long range transport through the atmosphere (Steinnes and Friedland, 2006). Pollutants consequently accumulate in the soil and plants of these forests through wet or dry deposition (Migon et al., 1997; Wu et al., 2011; Gandois and Probst, 2012), which may continue to add to contaminant concentrations. Constant accumulation of contaminants makes rehabilitation of these ecosystems significantly more difficult (Hopke, 2009), as it may require large scale phytoremediation of soils, sediments, surface water and groundwater (Peuke and Rennenberg, 2005).

Natural forest areas receive metal inputs mainly from the atmosphere (Gandois and Probst, 2012). This is possible mostly, because forest canopies have the ability to intercept those aerosols through the large surface area of tree leaves and air turbulences, which are created by their structure (Tallis et al., 2011). Pollutants from the atmosphere reach the soil by being either washed down by rain or through fallen leaves (Ettler et al., 2005; Driscoll et al., 2007), resulting in forest soils that contain large terrestrial pools of metals (Kaste et al., 2006; Steinnes and Friedland, 2006; Driscoll et al., 2007; Juillerat et al., 2012; Richardson et al., 2013; Richardson et al., 2015).

Metals are considered one of the most serious and widespread forms of environmental contamination (Pruski and Dixon, 2002) and soil is one of the most valuable, non-renewable resources in the world, forming an integral part of all terrestrial ecosystems (De Bruyn, 1997; Bedano et al., 2006). A substantial fraction of metals are released and redistributed through physical and biogeochemical migration processes in the soil profile (Dahmani-Muller et al., 2000; Klaminder et al., 2005; Steinnes et al., 2005), explaining the rising metal burden to soils (De Vries et al., 2002). However, the increase in metal content is also caused by human activities, such as industries, construction and vehicle traffic (Steinnes and Friedland, 2005).

Millions of people around the world depend on forests for their livelihood, food and security (Sauvé et al., 1998, 2003) and many such communities live adjacent to indigenous forests, extracting multiple resources for subsistence (Von Maltitz and Grundy, 2000; Lawes et al., 2004). More than a quarter of modern medicines originate from forest plants (Sauvé et al., 1998, 2003) and two thirds of plant medicines coming from forests are used by three hundred thousand traditional healers in South Africa to serve 27 million customers (Mander, 1998). These ecosystems provide home to 80% of the world's terrestrial biodiversity (Sauvé et al., 1998, 2003), making them invaluable in terms of biodiversity, especially for rare and threatened species (Humphrey, 2005).

Furthermore, at least 40% of the world's oxygen is produced by forests. Catchments in forests supply many regions world wide of drinking water. Tropical forest ecosystems contain around 25% of the carbon in the terrestrial biosphere (Bonan, 2008) and their clearance and degradation account for about 17% of annual carbon dioxide emissions worldwide (IPCC, 2006).

Forests are Southern Africa's smallest biome, covering only 1% of South Africa's land surface (500 000 hectares), yet the diversity of plant and animal species it harbours is out of proportion to their size (418 species per ha compared to 98 species per ha for the Fynbos). Indigenous forests are rare and endangered ecosystems and cover less than 0.05% of the Western Cape, but their roles in the environment are tremendous as carbon sinks, in the functioning of water

catchments, erosion control and the provision of resources that aids in the survival of many rural households. It is also the most fragmented biome, which greatly increases its vulnerability to humaninduced impacts, as it is the most densely populated, of which the amount of human activities, place the forests under serious threat. The National Forests Act, 1998, therefore provides for the protection of the total natural forest biome. Protecting these forests may further contribute enormously to biodiversity conservation in the country (DAFF, 2016). Yet, there is a global decline in forests and those ecosystems are suffering and unstable. This is a worldwide cause for great concern (Royal Ministry for Foreign Affairs, 1971; UN/ECE, 1979; Smith, 1981; Schaub et al., 2010). It is now widely accepted that air pollution is a global threat (McCrink-Goode, 2014) to human health, quality of life and the biophysical environment (Singh et al., 2013). Indigenous afromontane forests are the main forest-type in the south-western part of South Africa and occur in the Table Mountain National Park, in ravines and fire-safe habitats, associated with sandstone fynbos (De Villiers et al., 2005). Evergreen trees that can reach up to 30 m in height dominate these forests (SANBI, 2016). The Table Mountain chain is a natural World Heritage Site and is recognised globally for its extraordinarily rich, diverse and unique fauna and flora, including four of the remaining seven ancient forests (SA-Venues.com, 2017). However, it is also one of the world's most imperilled ecosystems (Underwood et al., 2009). Four of the 56 vegetation subtypes in Cape Town are globally extinct and 19 are critically endangered. Thirteen plant species are already globally extinct, making the City of Cape Town one of the most acute areas in the world for plant extinction (Rebelo et al., 2006). Table Mountain lies within the City of Cape Town and is surrounded by Cape Town’s CBD and urban areas, as well as industrial sources of pollution (Singh et al., 2013).

Economic activity is extremely high within the City of Cape Town (Singh et al., 2013). Emissions from motor vehicles are associated with particulate matter containing complex mixtures of metals not only from tires, brakes and parts wear, but also resuspended road dust (Peden, 2002). Aluminium and Fe originate from vehicle component rust and brake lining material, Fe products in brake pads, but also from vehicle exhaust (Adachi and Tainoshob, 2004; Manno et al., 2006; Amato et al., 2011). Iron is also emitted when the cast Fe rotor or drum from a vehicle presses against the brake pad, which can wear simultaneously with the pad (Manno et al.,

2006). Fe and Cu are found in brake housing dust and crushed brake pads (Adachia and Tainoshob, 2004, Schauer et al., 2006; Kreider et al., 2010). Copper and Zn are related to brake linings (Legret and Pagotto, 1999; Thorpe and Harrison, 2008) and galvanized structures and fragments of car tire rubber (Huber et al., 2016), as well as traffic exhaust (Steiner et al., 2007). Also from the exhaust processes, emerging as a source of Mn pollution, is the gasoline additive methylcyclopentadienyl Mn tricarbonyl (MMT), an octane boosting and antiknock agent, which replaces or reduces the lead content in petrol (Nogueira and Röllin, 2011). High concentrations of these metals are thus constantly released from exhaust and non-exhaust processes in road dust (Schauer et al., 2006; Apeagyei et al., 2011).

A meteorological condition called low-level temperature inversions, better known as the brown haze, also adds to the unusual high levels of air pollutants. Haze is a sign of significant concentrations of atmospheric particulate matter (Cheng et al., 2013). The brown haze occurs when cooler air just above the surface of the ground – air that's full of city pollutants – becomes trapped by a layer of warm air above it. This trapped layer of concentrated pollution can sometimes be as low as 30 m and occurs mostly in winter. It cannot rise and mix with the atmosphere, until heating or winds break up the inversion layer (City of Cape Town - Air Quality, 2007), thereby continuing to add to the deterioration of the air quality. Monitoring data taken in Cape Town confirms this trend, which could accelerate if corrective action is not taken (Sucharova et al. 2011; CMA, 2015). $PM_{10}$ is currently a concern in Cape Town and thus measured with levels of approximately 25 µg/m³ to 10 µg/m³ over a four year measurement period, but reaching levels of 50µg/m³ in 2014 on certain days (SoAR, 2014). China is a good example of haze pollution indicating high concentrations of $PM_{10}$ and $PM_{2.5}$ in the atmosphere (Cheng et al., 2013), but $PM_{2.5}$ concentrations are of utmost concern with levels of >60 µg m−3, appearing in cities and lower concentrations (<40 µg m−3) in adjacent remote forested areas (Yang et al., 2011). Haze in China is is now considered to be a critical environmental air issue as a result of its detrimental environmental and public health effects (Tie et al., 2009; Chen et al., 2013; Yao et al., 2014; Huo et al., 2015). Examples include: nervous system- and internal organs damage, cardiovascular diseases, reproductive impairments and cancer (Raghunath et al., 1999; Waisberg et al., 2003; Li et al., 2014), as well as

adverse impacts on remote ecosystems (Chameides et al., 1999; Zhang et al., 2013) and the climate as a whole (Solomon et al., 2007; Tainio et al., 2013).

Model calculations forecasted severe regional problems associated with pollution in South Africa by 2050 (Fowler et al., 1999). Global consequences for carbon cycles, primary productivity and other characteristics of forest ecosystems have been predicted. Europe managed to accomplish a decrease in metal depositions from the atmosphere after having made deliberate economic changes during the last 50 years (Pacyna et al., 2009). Unfortunately continents, such as Africa and Asia may still significantly affect the global emissions of metals due to their increasing industrial activities (Pirrone et al., 2010; Zhang et al., 2011).

In the Southern Hemisphere, especially for Africa little information exists on the concentrations of pollutant metals, either in the atmosphere or in atmospheric deposition or of its emissions (Slemr et al., 2011). Little is also known of its effect on ecosystem processes (Selonen and Setälä, 2015), but it is well known that metals have a long lasting impact on forest ecosystems (Nilsson and Grennfelt, 1988; Raukas, 2010).
Organisms are not only relying, but are dependent on forest continuity (Nordén and Appelqvist, 2001; Humphrey, 2005; Hurme et al., 2008). Thus, to sustain the ecosystem function, is especially important with regard to forest response to air pollution (McLaughlin and Percy, 1999). Monitoring with sentinel organisms then become useful due to their ability to accumulate metals. They are sensitive, representative and of functional importance in the ecosystem. They are also easily collected, identified and analysed (Greensdale, 2007).

Soil is a catchment for contaminants and is commonly used to assess atmospheric pollution (Xing et al., 2004; Zhang et al., 2013) and increases in metal concentrations (Zhang et al., 2013; Daresta et al., 2015). Metals are also active metabolically in the soil biota (Van Straalen et al., 2001). Metals are recycled in vegetation by way of root assimilation and returned to the soil by means of litter fall (Bergkvist, 2001; Sevel et al., 2009). Decomposition of organic material in forest litter is regarded as a key process in the ecosystem that may affect its biodiversity (Berg and McClaugherty, 2008). Forest litter is a local and global receptacle of organic carbon (Jandl et al.,

2007; Galka et al., 2014), but acts as a protective layer against water erosion (Kabala et al., 2013). Forest litter is also a filter (Waroszewski et al., 2009; Szopka et al., 2011, 2013), limiting toxicity for soil organisms and plants (Medynska-Juraszek and Kabala, 2012).

Plant material such as lichens, mosses, tree barks and tree leaves (Salo et al., 2012; Chaparro et al., 2013) are also known to accumulate metals (Gautam et al., 2005; Magiera et al., 2008; Basavaiah et al., 2012; Magiera et al., 2011, 2013; Jordanova et al., 2013; Lourenco et al., 2014; Wawer et al., 2015). Tissue analysis of mosses have been used extensively in the biomonitoring of pollution, including metals (Tyler, 1990; Fernandez et al., 2002; Harmens and Norris, 2008) and tissue chemistry was found to be closely correlated with atmospheric inputs (Bates, 2000; Pitcairn et al., 1995, 1998, 2003). *Hypnum cupressiforme* is a small to medium-sized moss, about 2-10 cm long growing on tree trunks, logs, walls, rocks and other surfaces. The prostrate, creeping stems form smooth, dense mats and the overlapping leaves give the impression of a cypress tree. *Hypnum* species are also relatively tolerant of pollution (Edwards, 2012**)** and are also known to absorb pollutants, especially metals directly from atmospheric deposition, due to their dense carpet (Sacharová and Suchara, 1998).

Lichens have been used in many studies as biomonitors of atmospheric trace elements, due to their ability to absorb elements directly from the air and accumulating them in their tissues (Guidotti et al., 2009; Cansaran-Duman et al., 2011). *Parmotrema* is a genus characterized by foliose thalli, forming short and broad, often ciliate lobes (Crespo et al*.,* 2010).

Terrestrial invertebrates such as diplopods (Nakamura and Taira, 2005; Nakamura et al., 2005) accumulate metals as they are directly and continuously exposed to soil contaminants. They are thus considered good environmental indicators and are successfully used in ecotoxicological research and evaluations (Triebskorn et al., 1991). Pill millipedes feed on dead organic matter in soil and leaf litter, including decaying leaves, wood and fruits. They transform detritus into humus, which contributes to soil fertility. The *Sphaerotherium* species (Brandt, 1833), a diverse higher taxon of Diplopoda (Hoffman, 1980) are bulky and pill–shaped. They are

found from Malawi to South Africa. During mating the males produce a sound, called stridulating by rubbing certain body parts together. They are usually found in forest and coastal forest habitats with high moisture and dense leaf litter in a loam soil (SANBI, 2016).

Harmful substances are constantly being released into the terrestrial environment and in order to avoid triggering a possible unbalance in the ecosystems, causing amongst other, extinction of species, it is necessary to know which substances are present there, as well as the effects thereof on the organisms (Magalhães and Ferrão-Filho, 2008). Adverse effects on plant growth and productivity (Daresta et al., 2015), as well as human health via the food chain (Peralta-Videa et al., 2009; Seth et al., 2012) is a major concern. Still these issues are not getting the attention they deserve (Yang et al., 2011; Zhang, 2011b; Tan and Duan, 2013).

The objective of this study was to determine the concentrations of prominent metals arising from industrial and urban pollution in indigenous forests where the ecology is fragile, without pronounced anthropogenic activities and human intrusion using soil, leaf litter and key forest organisms (mosses, lichens and pill millipedes) in three of the seven ancient forests (Platbos, Orange Kloof and Newlands) in the Western Cape, South Africa.

It was the first part of a larger research project to evaluate the health of forest ecosystems in the Western Cape, due to the importance of forests, rising concern of the deteriorating air quality in Cape Town, as well as it being one of the most acute areas in the world for plant extinction. Studies of this kind are not often done in areas where the ecology is fragile without any pronounced anthropogenic activities, regardless of a rapidly growing human population, urbanization and industrialization.

The sentinel organisms: moss, *Hypnum cupressiforme* **(Fig 2.15),** foliose lichen, *Parmotrema sp.* **(Fig 2.16)** and pill millipede, *Sphaerotherium compressum* **(Fig 2.17)** were chosen for this study due to their common presence in the forests.

## 3.2 RESULTS

### 3.2.1) PLATBOS FOREST

#### *3.2.1.1) Comparisons of metal concentrations in soil, leaf litter and sentinel organisms between sites 1, 2 and 3*

The mean metal concentrations in soil, leaf litter and sentinel organisms for sites 1, 2 and 3 of Platbos forest for the sampling occasion in May 2014 are presented in **Table 3.1.** No millipedes were found in this forest. Metal concentrations are expressed in mg/kg.

**Table 3.1:** The mean metal concentrations (mg/kg) (± SD) in soil, leaf litter and sentinel organisms for sites 1, 2 and 3 of Platbos forest for the sampling occasion in May 2014.

| METAL | | SOIL | | | LEAF LITTER | | | MOSS | | | LICHEN | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Site 1 | Site 2 | Site 3 | Site 1 | Site 2 | Site 3 | Site 1 | Site 2 | Site 3 | Site 1 | Site 2 | Site 3 |
| Al | Mean | $^{a}$1722.48 | $^{a}$1047.18 | $^{a}$879.28 | $^{a}$815.59 | $^{b}$153.42 | $^{b}$152.49 | $^{a}$1275.41 | $^{a}$674.18 | $^{a}$1240.51 | $^{a}$986.22 | $^{a}$434.13 | $^{a}$734.41 |
| | *SD* | *327.71* | *126.54* | *154.37* | *311.89* | *75.69* | *98.76* | *389.04* | *207.44* | *651.46* | *618.35* | *145.91* | *259.97* |
| Fe | Mean | $^{a}$1885.75 | $^{b}$1023.12 | $^{b}$988.02 | $^{a}$785.36 | $^{b}$139.16 | $^{c}$71.03 | $^{a}$1021.19 | $^{a}$460.60 | $^{a}$1056.66 | $^{a}$855.20 | $^{a}$454.05 | $^{a}$680.84 |
| | *SD* | *331.17* | *138.75* | *236.99* | *352.36* | *55.53* | *20.32* | *420.12* | *113.23* | *753.12* | *507.25* | *176.4* | *256.46* |
| Mn | Mean | $^{a}$44.88 | $^{a}$76.28 | $^{a}$44.30 | $^{a}$70.72 | $^{a}$31.87 | $^{a}$26.43 | $^{a}$55.19 | $^{a}$40.64 | $^{a}$34.68 | $^{a}$18.42 | $^{a}$9.14 | $^{a}$22.81 |
| | *SD* | *14.01* | *17.29* | *39.77* | *41.34* | *11.31* | *15.06* | *7.91* | *11.51* | *15.1* | *8.66* | *1.28* | *20.16* |
| Cu | Mean | $^{a}$1.03 | $^{a}$1.13 | $^{a}$0.86 | $^{a}$3.95 | $^{b}$1.94 | $^{b}$2.54 | $^{a}$2.88 | $^{b}$0.90 | $^{b}$1.03 | $^{a}$1.86 | $^{a}$0.26 | $^{a}$0.40 |
| | *SD* | *0.91* | *0.57* | *0.54* | *1.39* | *0.95* | *1.17* | *1.24* | *0.15* | *0.77* | *1.43* | *0.51* | *0.69* |
| Zn | Mean | $^{a}$10.64 | $^{a}$12.62 | $^{b}$2.68 | $^{a}$16.04 | $^{a}$10.12 | $^{b}$6.45 | $^{a}$16.14 | $^{a}$12.69 | $^{b}$7.74 | $^{a}$16.59 | $^{a}$11.51 | $^{a}$10.45 |
| | *SD* | *5.4* | *4.52* | *4.24* | *6.77* | *2.21* | *1.34* | *3.37* | *3.23* | *2.9* | *7.1* | *2.36* | *3.65* |

Statistical signifcant differences (P<0.050) between sites are indicated with different superscripted letters. Comparisons were done separately for the soil, leaf litter and sentinel organisms. N=5.

***a) Soil***

In May 2014 there were no statistically significant differences found in the soil between any of the sites at Platbos in terms of Al (P=0.265), Mn (P=0.085) and Cu (P=0.779) concentrations **(Table 3.1)**.

Iron concentrations in soil at site 1 differed significantly from the concentrations found at sites 2 and 3 (P<0.05). No statistically significant differences were found between sites 2 and 3 (P>0.05). Site 1 had the highest mean Fe concentration of 1885.75 ± 331.17 mg/kg **(Table 3.1)**.

Soil Zn concentrations at site 3 differed significantly from the concentrations found at sites 1 and 2 (P<0.05). No statistically significant differences were found between sites 1 and 2 (P>0.05). The mean Zn concentration (12.62 ± 4.52 mg/kg) found at site 2 was higher than at sites 1 and 3 **(Table 3.1)**.

***b) Leaf litter***

Pairwise multiple comparisons of Al for the sampling occasion showed statistically significant differences in the leaf litter (P<0.05) between sites 1 and 3 and 1 and 2, but no significant differences between sites 2 and 3 (P>0.05) were found. The mean Al concentration at site 1 (815.59 ± 311.89 mg/kg) was significantly higher than the mean concentrations found at site 2 (153.42 ± 75.69 mg/kg) and site 3 (152.49 ± 98.76 mg/kg) **(Table 3.1)**.

Statistically significant differences in Fe concentrations of leaf litter between all the sites (P<0.05) were found, with site 1 having the highest mean Fe concentration of 785.36 ± 352.36 mg/kg **(Table 3.1)**.

No significant differences were found in the leaf litter between the three sites in terms of Mn concentrations (P=0.059) (**Table 3.1)**.

Pairwise multiple comparisons of Cu concentrations for sites 1 vs 2 and 1 vs 3 (P<0.05) showed significant differences between them, with the exception of site 2

vs 3 (P>0.05). The mean Cu concentration at site 1 (3.95 ± 1.39 mg/kg) was higher than at the other sites **(Table 3.1)**.

When Zn concentrations in leaf litter were compared, significant differences were found when sites 1 vs 3 and 2 vs 3 were compared (P<0.05). No statistically significant differences were found between sites 1 and 2 (P>0.05). Site 1 had the highest mean concentration of 16.04 ± 6.77 mg/kg **(Table 3.1)**.

***c) Moss***

Moss between the 3 sites were compared at Platbos, but showed no statistically significant differences between the sites in terms of Al (P=0.108), Fe (P=0.228) and Mn (P=0.069) concentrations (**Table 3.1)**.

Pairwise multiple comparisons showed statistically significant differences in Cu concentrations in moss samples (P<0.05) for the following comparisons: 1 vs 2 and 1 vs 3. There were no significant differences found between sites 2 and 3 (P>0.05). The mean Cu concentration at site 1 (2.88 ± 1.24 mg/kg) was the highest of the 3 sites **(Table 3.1)**.

Pairwise multiple comparisons between sites 1 vs 3 and 2 vs 3 (P<0.05) showed statistically significant differences between them in terms of Zn concentrations. No significant differences were found between sites 1 and 2 (P>0.05). Site 1 had the higher mean Zn concentration (16.14 ± 3.37 mg/kg) (**Table 3.1)**.

***d) Lichen***

No statistically significant differences were found between lichen samples collected from the three sites in terms of Al (P=0.085), Fe (P=0.145), Mn (P=0.093), Cu (P=0.139) or Zn concentrations (P=0.326) (**Table 3.1)**.

***e) Millipedes***

No millipedes were found at Platbos forest.

### *3.2.1.2) Comparisons of metal concentrations between soil and leaf litter at sites 1, 2 and 3*

Mean metal concentrations in soil and leaf litter at sites 1, 2 and 3 of Platbos forest for the sampling occasion in May 2014 are shown in **Figs 3.1 to 3.5**. Statistical signifcant differences (P<0.050) between soil and leaf litter are indicated with an asterisk above the graph bars.

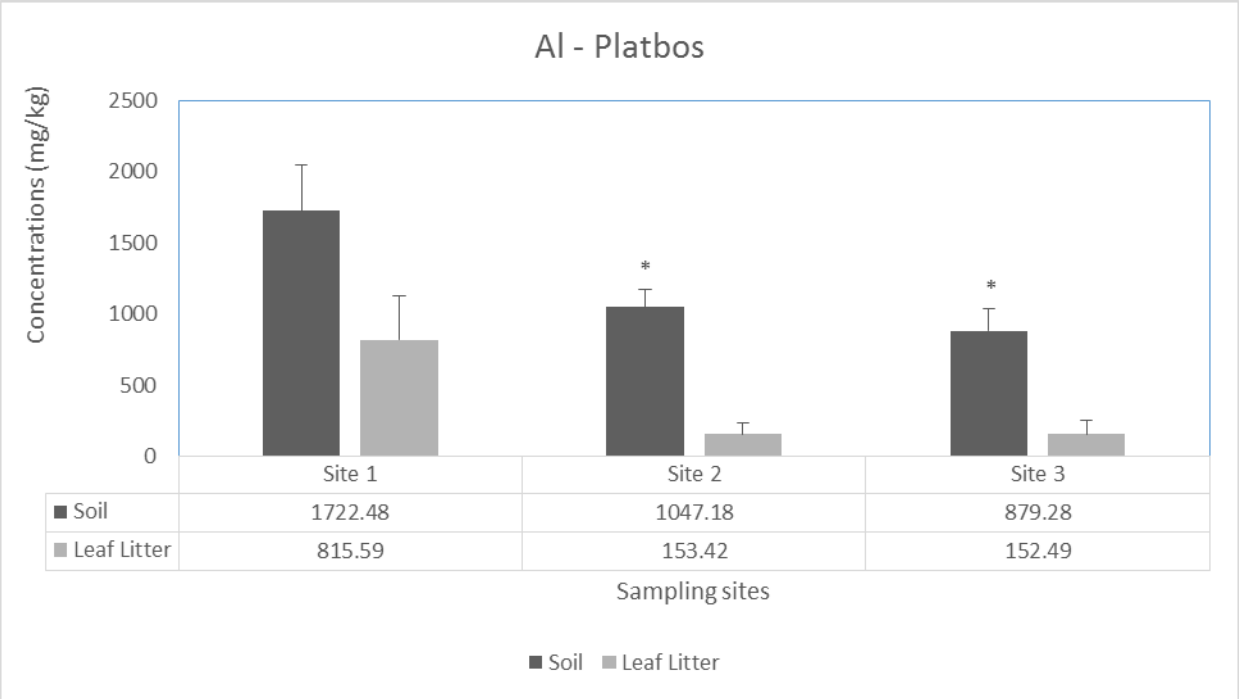


**Figure 3.1:** The mean Al concentrations (mg/kg) (± SD) in soil and leaf litter at sites 1, 2 and 3 of Platbos forest for the sampling occasion in May 2014. N=5.

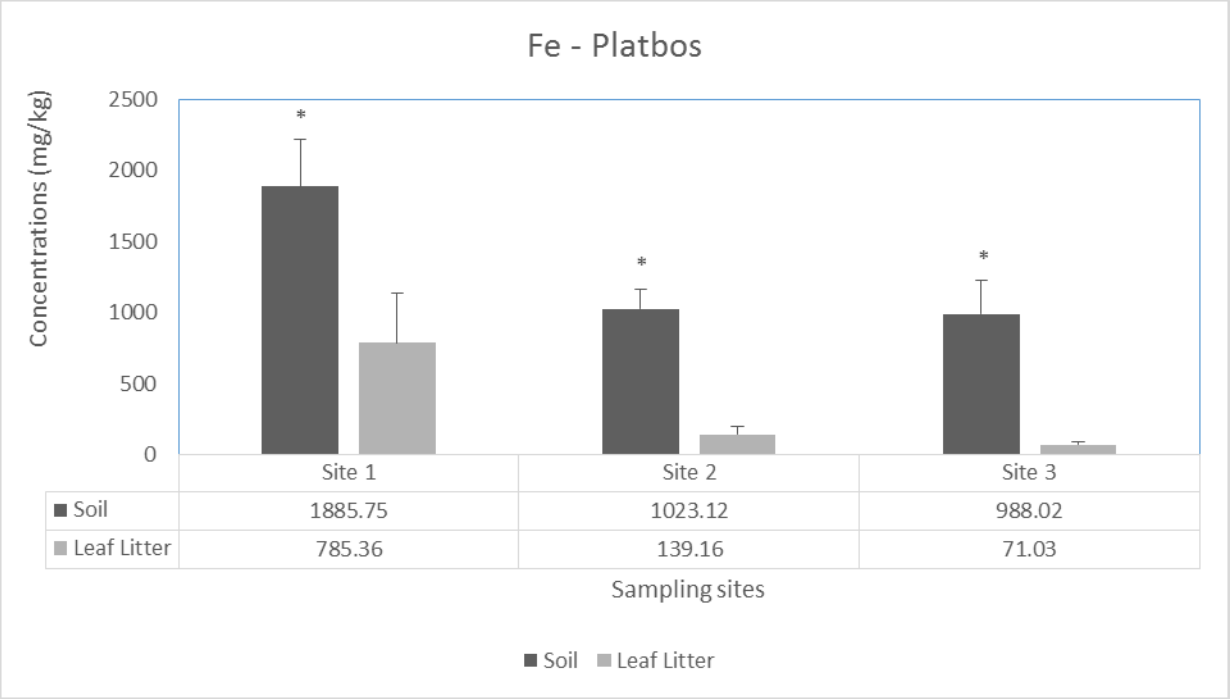


**Figure 3.2:** The mean Fe concentrations (mg/kg) (± SD) in soil and leaf litter at sites 1, 2 and 3 of Platbos forest for the sampling occasion in May 2014. N=5.

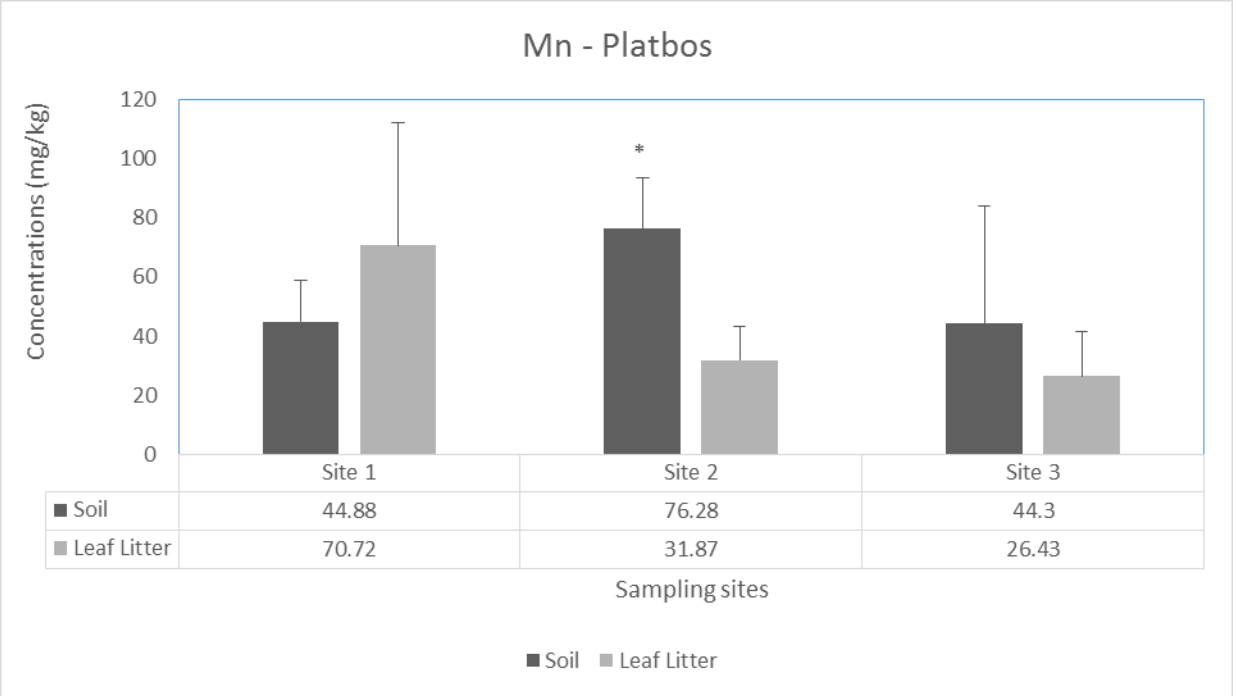


**Figure 3.3:** The mean Mn concentrations (mg/kg) (± SD) in soil and leaf litter at sites 1, 2 and 3 of Platbos forest for the sampling occasion in May 2014. N=5.

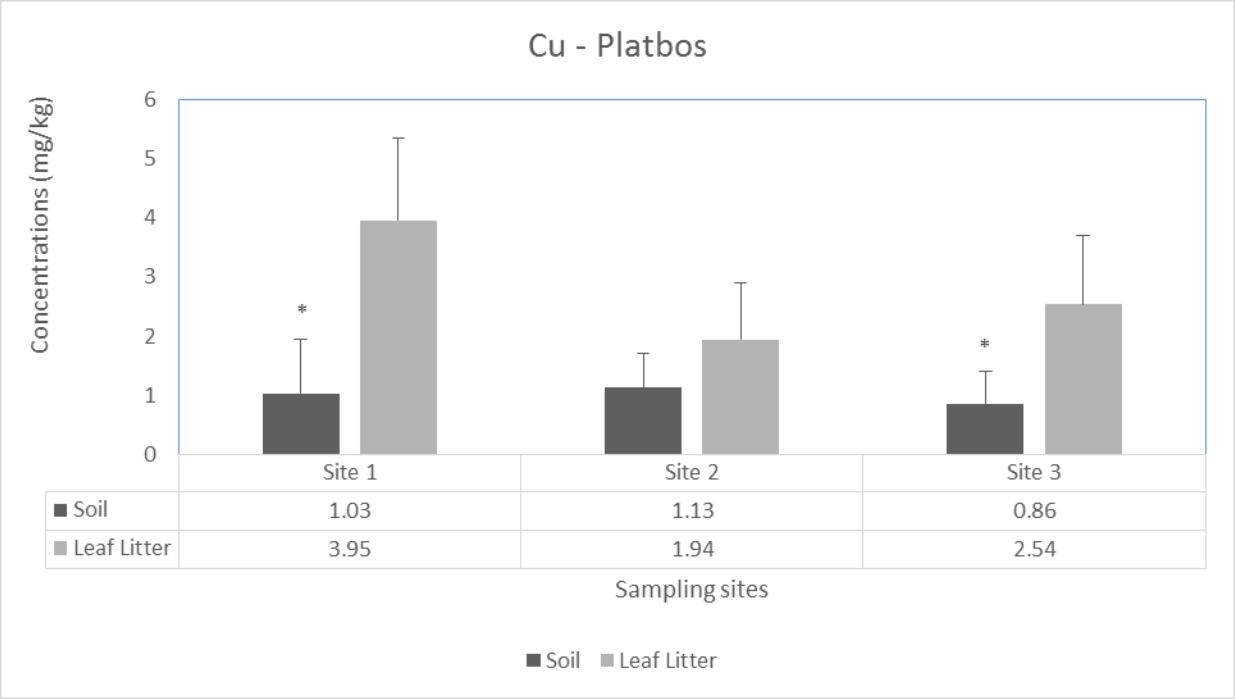


**Figure 3.4:** The mean Cu concentrations (mg/kg) (± SD) in soil and leaf litter at sites 1, 2 and 3 of Platbos forest for the sampling occasion in May 2014. N=5.

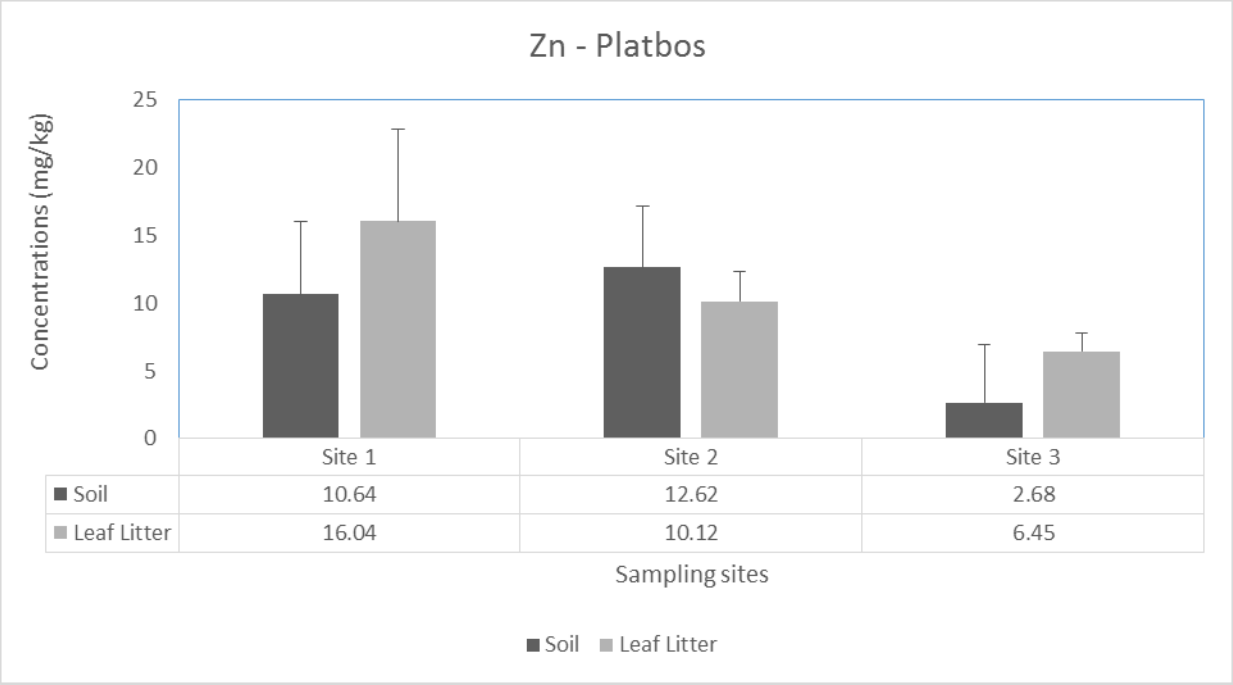


**Figure 3.5:** The mean Zn concentrations (mg/kg) (± SD) in soil and leaf litter at sites 1, 2 and 3 of Platbos forest for the sampling occasion in May 2014. N=5.

There were no statistically significant differences found in Al concentrations between soil and leaf litter at site 1 (P=0.564), but significant differences were, however found at site 2 (P=0.008) and site 3 (P=0.008). The mean concentrations at site 2 (1047.18 ± 126.54 mg/kg) and site 3 (879.28 ± 154.37 mg/kg) were significantly higher in the soil as opposed to leaf litter **(Fig 3.1)**.

Statistically significant differences between soil and leaf litter were found in Fe concentrations at site 1 (P=0.008), site 2 (P=0.008) and site 3 (P=0.008). The Fe concentrations were also found to be higher in the soil. Site 1 had the highest mean concentration (1885.75 ± 331.17 mg/kg) **(Fig 3.2)**.

Manganese concentrations between soil and leaf litter did not differ significantly from each other at site 1 (P=0.222) and site 3 (P=0.222), but significant differences were found at site 2 (P=0.008). The mean concentration (76.28 ± 17.29 mg/kg) was higher in the soil at this site **(Fig 3.3)**.

When Cu concentrations were compared between soil and leaf litter, statistically significant differences were found at site 1 (P=0.008) and site 3 (P=0.032). No significant differences were however found at site 2 (P=0.151). The mean concentration of 3.95 ± 1.39 mg/kg was the highest in leaf litter at site 1 **(Fig 3.4)**.

Leaf litter and soil showed no statistically significant differences between each other at sites: 1 (P=0.222), 2 (P=0.310) and 3 (P=0.151) when Zn concentrations were compared **(Fig 3.5)**.

### *3.2.1.3) Comparisons of metal concentrations between moss and lichen at sites 1, 2 and 3*

Mean metal concentrations in moss and lichen at sites 1, 2 and 3 of Platbos forest for the sampling occasion in May 2014 are shown in **Figs 3.6 to 3.10**. Statistical signifcant differences (P<0.050) between moss and lichen are indicated with an asterisk above the graph bars.

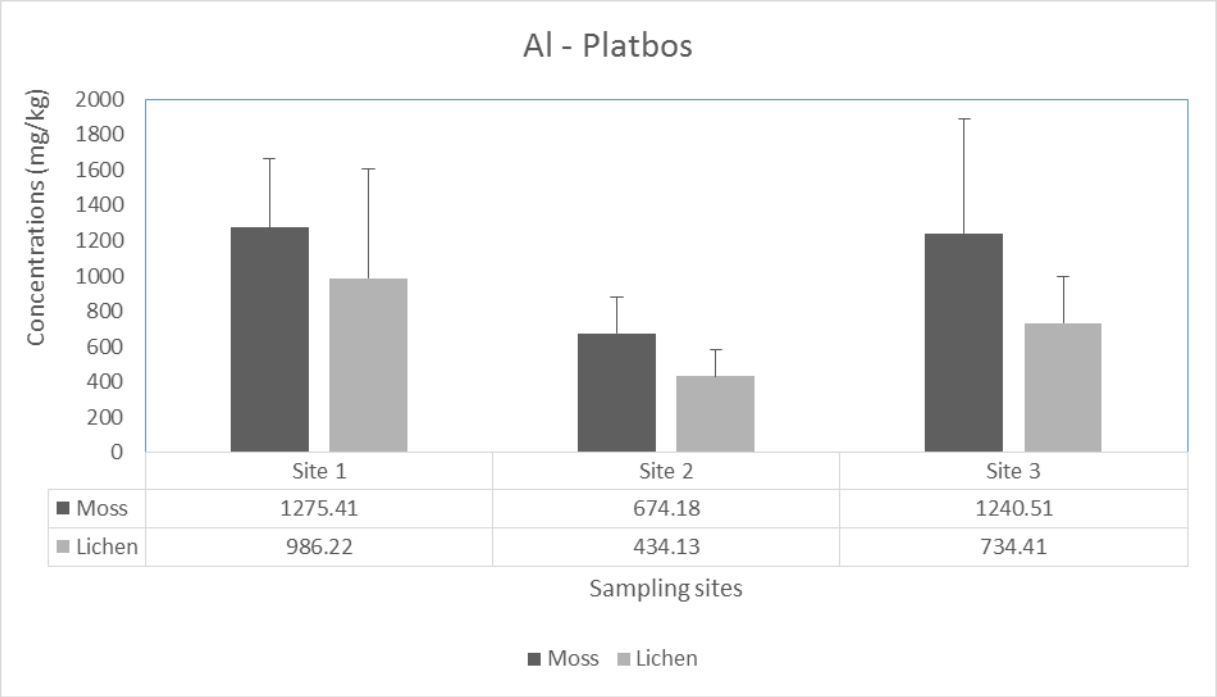


**Figure 3.6:** The mean Al concentrations (mg/kg) (± SD) in moss and lichen at sites 1, 2 and 3 of Platbos forest for the sampling occasion in May 2014. N=5.

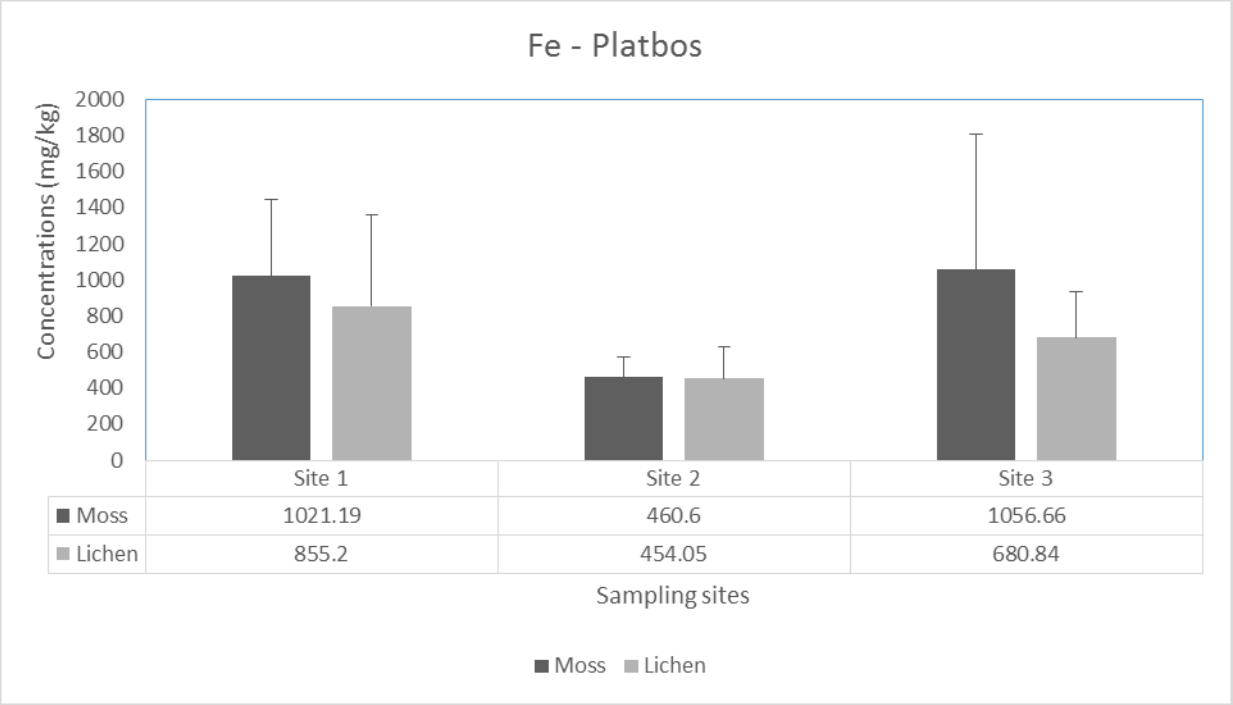


**Figure 3.7:** The mean Fe concentrations (mg/kg) (± SD) in moss and lichen at sites 1, 2 and 3 of Platbos forest for the sampling occasion in May 2014. N=5.

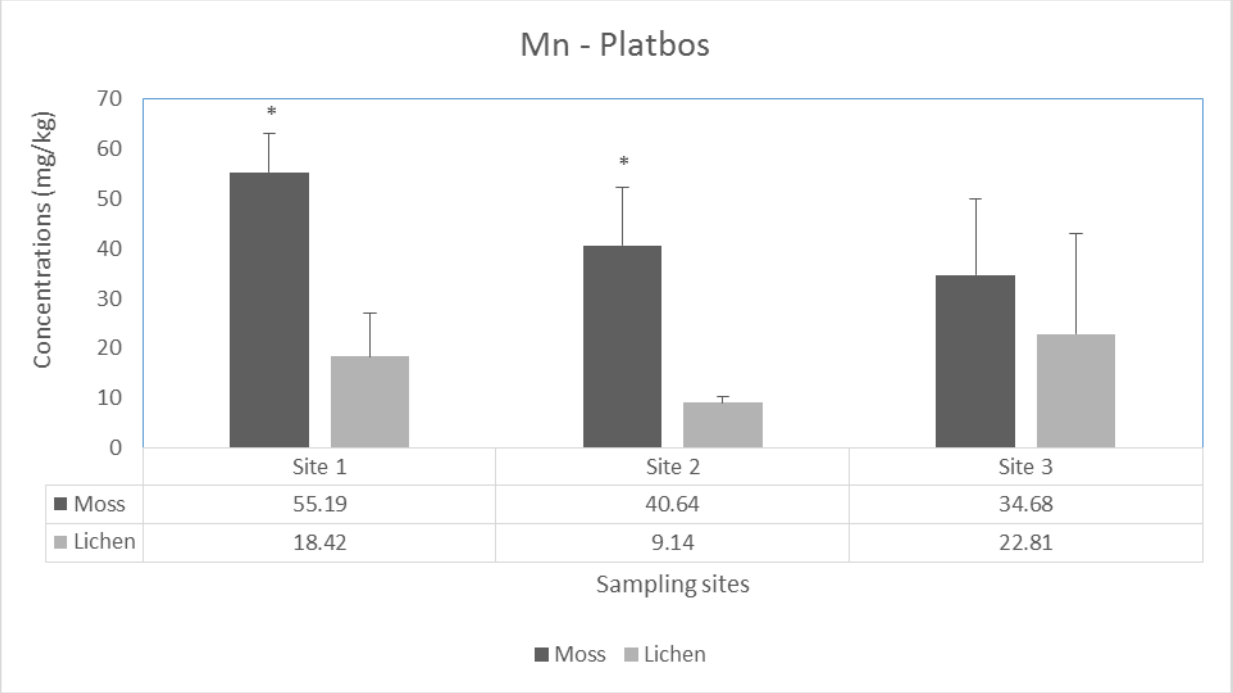


**Figure 3.8:** The mean Mn concentrations (mg/kg) (± SD) in moss and lichen at sites 1, 2 and 3 of Platbos forest for the sampling occasion in May 2014. N=5.

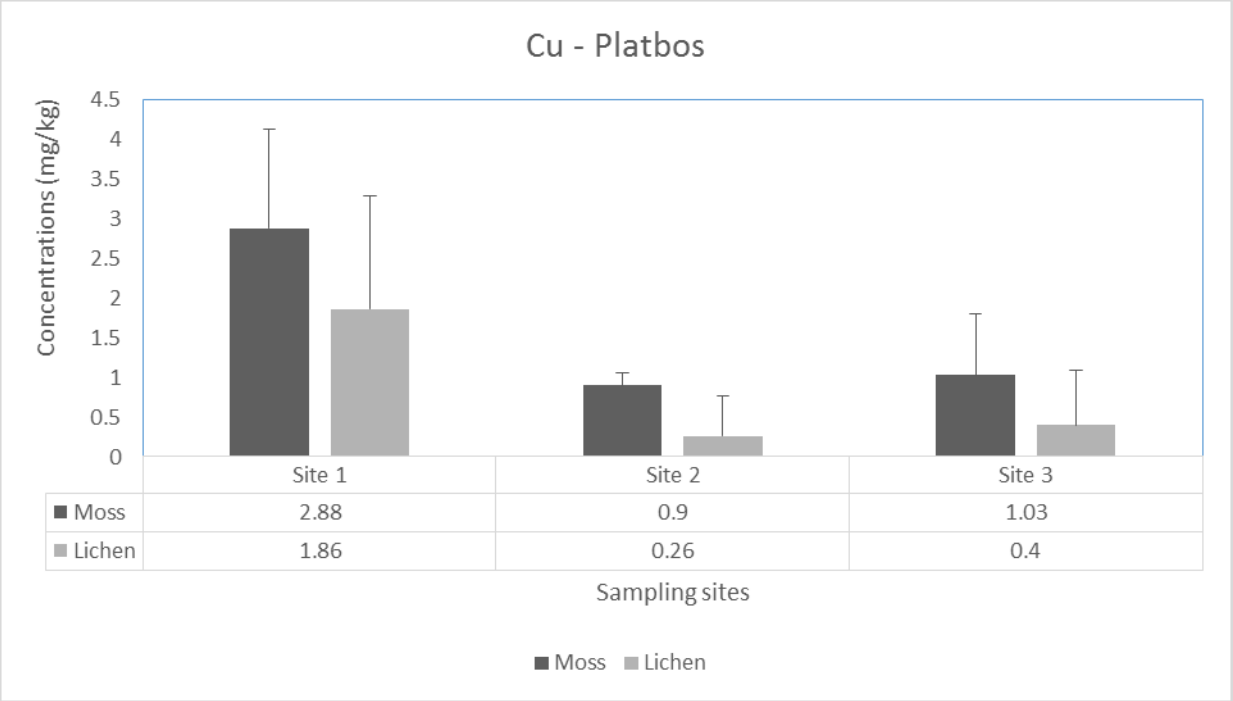


**Figure 3.9:** The mean Cu concentrations (mg/kg) (± SD) in moss and lichen at sites 1, 2 and 3 of Platbos forest for the sampling occasion in May 2014. N=5.

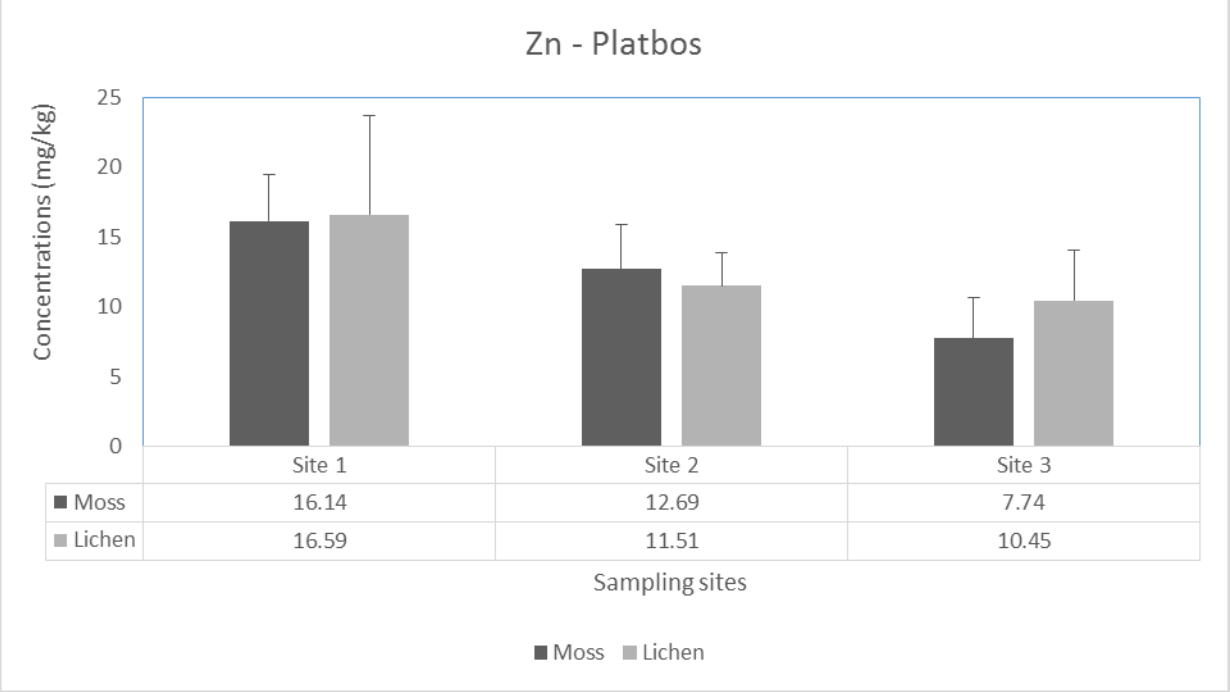


**Figure 3.10:** The mean Zn concentrations (mg/kg) (± SD) in moss and lichen at sites 1, 2 and 3 of Platbos forest for the sampling occasion in May 2014. N=5.

Aluminium concentrations found in moss and lichen samples did not differ significantly from each other at sites 1 (P=0.421), site 2 (P=0.095) and site 3 (P=0.310) **(Fig 3.6)**.

The Fe concentrations that were found, showed no statistically significant differences between moss and lichen at sites: 1 (P=0. 421), site 2 (P=0.690) and site 3 (P=0.421) **(Fig 3.7)**.

Statistically significant differences in Mn concentrations were found at Platbos sites 1 (P=0.008) and 2 (P=0.008). Moss and lichen samples revealed no significant differences between them at site 3 (P=0.310). The highest mean concentration of (55.19 ± 7.91 mg/kg) was found in moss at site 1 **(Fig 3.8)**.

Copper concentrations showed no statistically significant differences between the organisms at site 1 (P=0.421), 2 (P=0.151) and 3 (P=0.310) **(Fig 3.9)**.

When Zn concentrations in moss and lichen were compared, there were no statistically significant differences found between them at all the sites: 1: (P=0.690), 2: (P=0.690) and 3: (P=0.421) **(Fig 3.10)**.

### *3.2.1.4) pH and Moisture*

pH of the soil ranged from slightly acidic (6.48) to alkaline (7.28) and the moisture % of the soil ranged from a relatively dry 9.99 % to a dry 4.16 %

**Table 3.2:** pH and moisture % of soil of Platbos forest for the sampling occasion in May 2014.

| **PLATBOS FOREST** | **SOIL** | |
|---|---|---|
| Site 1 | **pH** | 7.28 |
| | *Moisture* | *4.16* |
| Site 2 | **pH** | 6.88 |
| | *Moisture* | *9.99* |
| Site 3 | **pH** | 6.48 |
| | *Moisture* | *9.99* |

Moisture % of the leaf litter ranged from a slightly moist 19.18 % to a moist 35.06 %.

**Table 3.3:** Moisture % of leaf litter of Platbos forest for the sampling occasion in May 2014.

| PLATBOS FOREST | % | LEAF LITTER |
|---|---|---|
| Site 1 | Moisture | 25.73 |
| Site 2 | Moisture | 35.06 |
| Site 3 | Moisture | 19.18 |

### *3.2.1.5) Weather data from the South African Weather Service in the vicinity of Gansbaai for Platbos forest*

**Table 3.4:** Weather data in the vicinity of Platbos forest for the sampling occasion in May 2014.

| SAMPLING SITES | SAMPLING OCCASION | | | |
|---|---|---|---|---|
| | DATE | DEGREES (°C) | PRECITATION (MM) | WIND SPEED (KM/H) |
| **PLATBOS FOREST** | | | | |
| Sites 1, 2, 3 | 25-May-14 | 18 | 0 | 28 |

### *3.2.1.6) Soil characterization for Platbos forest sites 1, 2 and 3*

**Table 3.5:** Characterization of soil for Platbos forest sites for the sampling occasion in May 2014.

| SAMPLING | (%) | | | | | | | WATER RETENTION | | |
|---|---|---|---|---|---|---|---|---|---|---|
| SITES | Clay | Silt | Fine sand | Medium sand | Coarse sand | Rock (v/v) | Classification | 10kPa | 100kPa | mm/m |
| PLATBOS FOREST | | | | | | | | | | |
| Site 1 | 5 | 2 | 65.4 | 21.6 | 6 | 1.4 | Sa | 22.35 | 9 | 133.5 |
| | | | | | | | | | | |
| Site 2 | 5 | 4 | 63.2 | 23.04 | 4.8 | 1.3 | Sa | 22.54 | 9.41 | 131.2 |
| | | | | | | | | | | |
| Site 3 | 3 | 2 | 72.4 | 19.44 | 3.2 | 1.0 | Sa | 23.16 | 8.64 | 145.2 |

### 3.2.2) ORANGE KLOOF FOREST

#### *3.2.2.1) Comparisons of metal concentrations in soil, leaf litter and sentinel organisms between sites 1, 2 and 3*

The mean metal concentrations in soil, leaf litter and sentinel organisms for sites 1, 2 and 3 of Orange Kloof forest for the sampling occasion in May 2014 are presented in **Table 3.6**. Metal concentrations are expressed in mg/kg.

**Table 3.6:** The mean metal concentrations (mg/kg) (± SD) in soil, leaf litter and sentinel organisms for sites 1, 2 and 3 of Orange Kloof forest for the sampling occasion in May 2014. N=5.

| METAL | | SOIL | | | LEAF LITTER | | | MOSS | | | LICHEN | | | MILLIPEDES | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Site 1 | Site 2 | Site 3 | Site 1 | Site 2 | Site 3 | Site 1 | Site 2 | Site 3 | Site 1 | Site 2 | Site 3 | Site 1 | Site 2 | Site 3 |
| Al | Mean | $^{a}$9086.99 | $^{b}$1943.97 | $^{a}$8019.59 | $^{a}$969.88 | $^{a}$368.10 | $^{a}$661.69 | $^{a}$1099.30 | $^{a}$1048.11 | $^{a}$2089.52 | $^{a}$1119.94 | $^{a}$1042.36 | $^{a}$564.37 | $^{a}$1358.76 | $^{b}$179.55 | $^{b}$310.35 |
| | *SD* | *1467.33* | *1083.29* | *4221* | *987.83* | *292.97* | *446.71* | *457.52* | *438.48* | *2542.12* | *563.44* | *472.89* | *218.51* | *147.92* | *153.67* | *157.19* |
| Fe | Mean | $^{a}$2783.03 | $^{b}$1573.83 | $^{a}$5713.17 | $^{a}$314.96 | $^{a}$346.99 | $^{a}$407.21 | $^{a}$713.51 | $^{a}$986.47 | $^{a}$1385.26 | $^{a}$937.41 | $^{a}$954.51 | $^{a}$616.57 | $^{a}$409.00 | $^{b}$186.09 | $^{b}$184.99 |
| | *SD* | *531.55* | *1002.11* | *3363.57* | *275.46* | *332.84* | *271.52* | *222.58* | *424.16* | *1198.83* | *533.38* | *368.14* | *248.06* | *31.6* | *120.85* | *69.62* |
| Mn | Mean | $^{a}$184.13 | $^{a}$258.26 | $^{a}$230.32 | $^{a}$305.26 | $^{b}$583.51 | $^{c}$293.70 | $^{a}$153.62 | $^{b}$633.32 | $^{a}$158.19 | $^{a}$179.06 | $^{a}$459.67 | $^{b}$65.01 | $^{a}$49.27 | $^{b}$133.21 | $^{c}$29.37 |
| | *SD* | *95.34* | *298.35* | *132.73* | *168.47* | *223.2* | *179.31* | *66.45* | *241.13* | *75.55* | *99.07* | *279.1* | *40.83* | *12.37* | *113.18* | *8.7* |
| Cu | Mean | $^{a}$34.30 | $^{a}$19.76 | $^{a}$1.79 | $^{a}$3.69 | $^{a}$5.48 | $^{a}$4.27 | $^{a}$3.67 | $^{b}$6.79 | $^{a}$3.92 | $^{a}$6.11 | $^{a}$4.66 | $^{a}$4.67 | $^{a}$4.17 | $^{b}$6.67 | $^{b}$10.84 |
| | *SD* | *62.56* | *34.78* | *1.15* | *1.3* | *1.64* | *0.37* | *1.36* | *1.74* | *1.14* | *1.29* | *1.03* | *4.09* | *1.75* | *1.3* | *6.63* |
| Zn | Mean | $^{a}$18.19 | $^{b}$6.51 | $^{b}$9.35 | $^{a}$30.34 | $^{b}$14.90 | $^{b}$12.00 | $^{a}$22.38 | $^{a}$21.52 | $^{a}$16.34 | $^{a}$41.46 | $^{b}$23.40 | $^{b}$18.96 | $^{a}$89.58 | $^{a}$102.47 | $^{a}$90.21 |
| | *SD* | *3.37* | *4.28* | *2.87* | *17.47* | *4.59* | *1.6* | *6.8* | *5.61* | *3.88* | *14.76* | *9.59* | *6.32* | *28.16* | *18.89* | *23.93* |

Statistical signifcant differences (P<0.050) between sites are indicated with different superscripted letters. Comparisons were done separately for the soil, leaf litter and sentinel organisms.

***a) Soil***

There were statistically significant differences found in Al concentrations ($P<0.05$) for the comparisons: sites 1 vs 2 and 2 vs 3, but no statistical differences were found between sites 1 and 3 ($P>0.05$). The mean concentration at site 1 (9086.99 ± 1467.33 mg/kg) was significantly higher than at site 2 (1943.97 ± 1083.29 mg/kg) and the mean concentration at site 3 (8019.59 ± 4221.00 mg/kg) was significantly higher than the concentration found at site 2 (1943.97 ± 1083.29 mg/kg) (**Table 3.6)**.

Pairwise multiple comparisons showed statistically significant differences in Fe concentrations in soil between sites 2 and 3 and sites 1 and 2 ($P<0.05$). No significant differences were, however found in soil between sites 1 and 3 ($P>0.05$). The mean concentration for site 3 (5713.17 ± 3363.57 mg/kg) was significantly higher than at sites 2 (1573.83 ± 1002.11 mg/kg) and 1 (2783.03 ± 531.55 mg/kg) **(Table 3.6)**.

The soil at Orange Kloof sites 1, 2 and 3 did not differ significantly from each other in terms of Mn ($P=0.827$) and Cu ($P=0.114$) concentrations when compared **(Table 3.6)**.

When Zn concentrations in soil were compared, statistically significant differences between sites 1 and 2 and 1 and 3 ($P<0.05$) were found. No significant differences were found between sites 2 and 3 ($P>0.05$). The mean concentration for site 1 (1819 ± 3.37 mg/kg) was significantly higher than at sites 2 (6.51 ± 4.28 mg/kg) and 3 (9.35 ± 2.87 mg/kg) **(Table 3.6)**.

***b) Leaf litter***

Leaf litter at Orange Kloof was compared at all the sites, but no significant differences were found in Al ($P=0.468$), Fe ($P=0.454$) and Cu ($P=0.065$) concentrations for this sampling occasion **(Table 3.6)**.

Pairwise multiple comparisons showed statistically significant differences in Mn concentrations between all of the sites ($P<0.05$) (**Table 3.6)**.

Pairwise comparisons of Zn in leaf litter between sites 1 and 2 and sites 1 and 3 ($P<0.05$) showed significant differences with the exception of the site 2 vs 3 comparison ($P>0.05$). The mean Zn concentration for site 1 (30.34 ± 17.47 mg/kg) was significantly higher than at sites 2 (14.90 ± 4.59 mg/kg) and 3 (12.00 ± 1.60) mg/kg **(Table 3.6)**.

***c) Moss***

Aluminium ($P=0.932$), Fe ($P=0.543$) and Zn ($P=0.340$) concentrations showed no no statistically significant differences between any of the sites **(Table 3.6)**.

Statistically significant differences in Mn moss concentrations were found between sites 1 and 2 and 2 and 3 ($P<0.05$), but no significant differences were found between sites 1 and 3 ($P>0.05$). Site 2 had the highest mean Mn concentration of 633.32 ± 241.13 mg/kg (**Table 3.6)**.

Site comparisons in terms of Cu concentrations in moss revealed statistically significant differences for the comparisons of sites 1 vs 2 and 2 vs 3 ($P<0.05$). No statistically differences were detected between sites 1 and 3 ($P>0.05$). The mean concentration at site 2 (6.79 ± 1.74 mg/kg) was higher than were found at the other sites **(Table 3.6)**.

***d) Lichen***

No statistically significant differences in Al ($P=0.147$), Fe ($P=0.468$) and Cu ($P=0.230$) concentrations between sites 1, 2 and 3 were found in May 2014 **(Table 3.6)**.

Pairwise multiple comparisons showed statistically significant differences in Mn concentrations for the comparisons of sites 2 vs 3 and 1 vs 3 ($P<0.05$) with the exception of sites 1 vs 2 ($P>0.05$). The mean concentration at site 2 (459.67 ± 279.10 mg/kg) was significantly higher than at site 3 (65.01 ± 40.83 mg/kg). The site 1 mean concentration (179.06 ± 99.07 mg/kg) was significantly higher than at site 3 (65.01 ± 40.83 mg/kg) **(Table 3.6)**.

Statistically significant differences in Zn concentrations between sites 1 and 3 and 1 and 2 ($P<0.05$) were found. No statistically significant differences were, however found between sites 2 and 3 ($P>0.05$). The mean concentration at site 1 (41.46 ± 14.76 mg/kg) was significantly higher than at site 3 (18.96 ± 6.32 mg/kg). Site 1 (41.46 ± 14.76 mg/kg) was significantly higher than site 2 (23.40 ± 9.59 mg/kg) **(Table 3.6)**.

***e) Millipedes***

Pairwise multiple comparisons showed statistically significant differences in Al concentrations in millipedes between sites 1 and 2 and sites 1 and 3 ($P<0.05$). No significant difference between sites 2 and 3 ($P>0.05$) was found. Site 1 had the highest mean Al concentration of 1358.76 ± 147.92 mg/kg **(Table 3.6)**.

For Fe, statistically significant differences in millipedes were found in site comparisons for 1 vs 2 and 1 vs 3 ($P<0.05$), but there were no significant difference found between sites 2 and 3 ($P>0.05$). The mean Fe concentration at site 1 (409.00 ± 31.60 mg/kg) was significantly higher than at site 2 (168.09 ± 120.85 mg/kg) and site 3 (184.99 ± 69.62 mg/kg) **(Table 3.6)**.

There were statistically significant differences found at Orange Kloof between sites 1, 2 and 3 ($P<0.05$) for Mn. Site 2 (133.21 ± 133.18 mg/kg) showed the highest mean concentration of Mn in the millipedes **(Table 3.6)**.

In terms of Cu concentrations at Orange Kloof, statistically significant differences were found between sites 1 and 2 and sites 1 and 3 ($P<0.05$). No significant differences were found between sites 2 and 3 ($P>0.05$). The mean concentration at site 1 (4.17 ± 1.75 mg/kg) was significantly lower than the concentrations found at sites 2 (6.67 ± 1.30 mg/kg) and 3 (10.84 ± 6.63 mg/kg) **(Table 3.6)**.

No statistically significant differences in millipede Zn concentrations between Orange Kloof sites 1, 2 and 3 ($P=0.395$) were found **(Table 3.6)**.

### *3.2.2.2) Comparisons of metal concentrations between soil and leaf litter at sites 1, 2 and 3*

Mean metal concentrations in soil and leaf litter at sites 1, 2 and 3 of Orange Kloof forest for the sampling occasion in May 2014 are shown in **Figs 3.11 to 3.15**. Statistical signifcant differences (P<0.050) between soil and leaf litter are indicated with an asterisk above the graph bars.

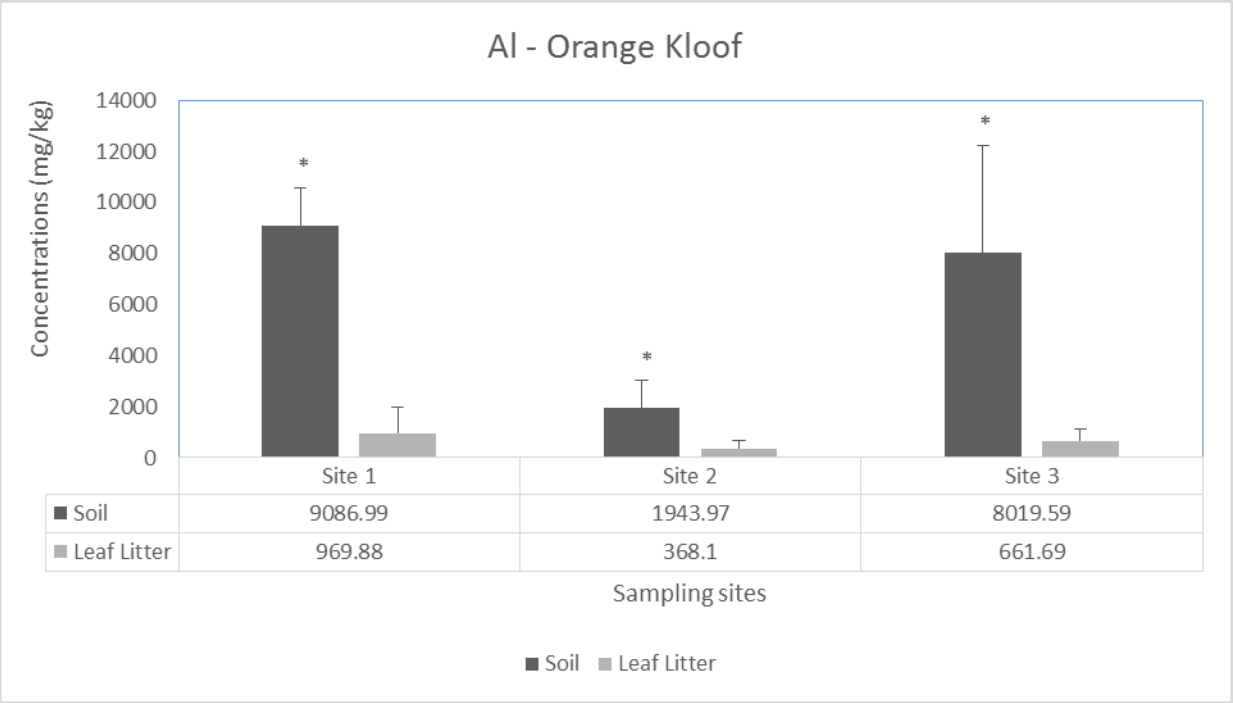


**Figure 3.11:** The mean Al concentrations (mg/kg) (± SD) in soil and leaf litter at sites 1, 2 and 3 of Orange Kloof forest for the sampling occasion in May 2014. N=5.

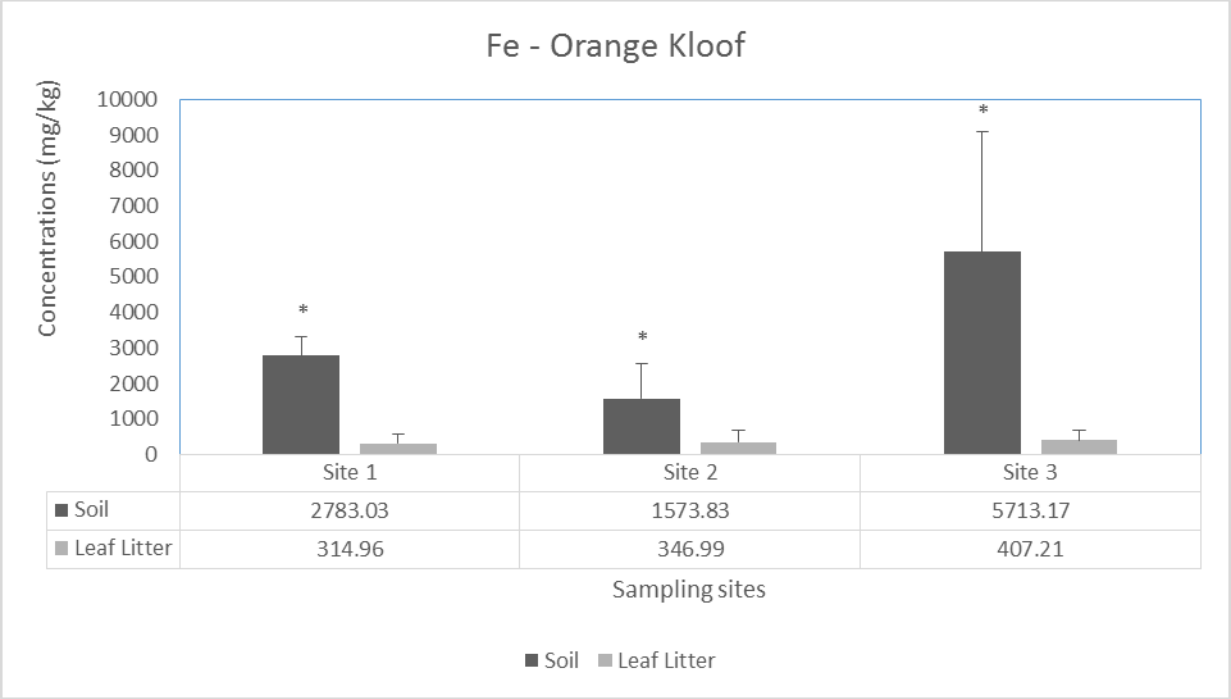


**Figure 3.12:** The mean Fe concentrations (mg/kg) (± SD) in soil and leaf litter at sites 1, 2 and 3 of Orange Kloof forest for the sampling occasion in May 2014. N=5.

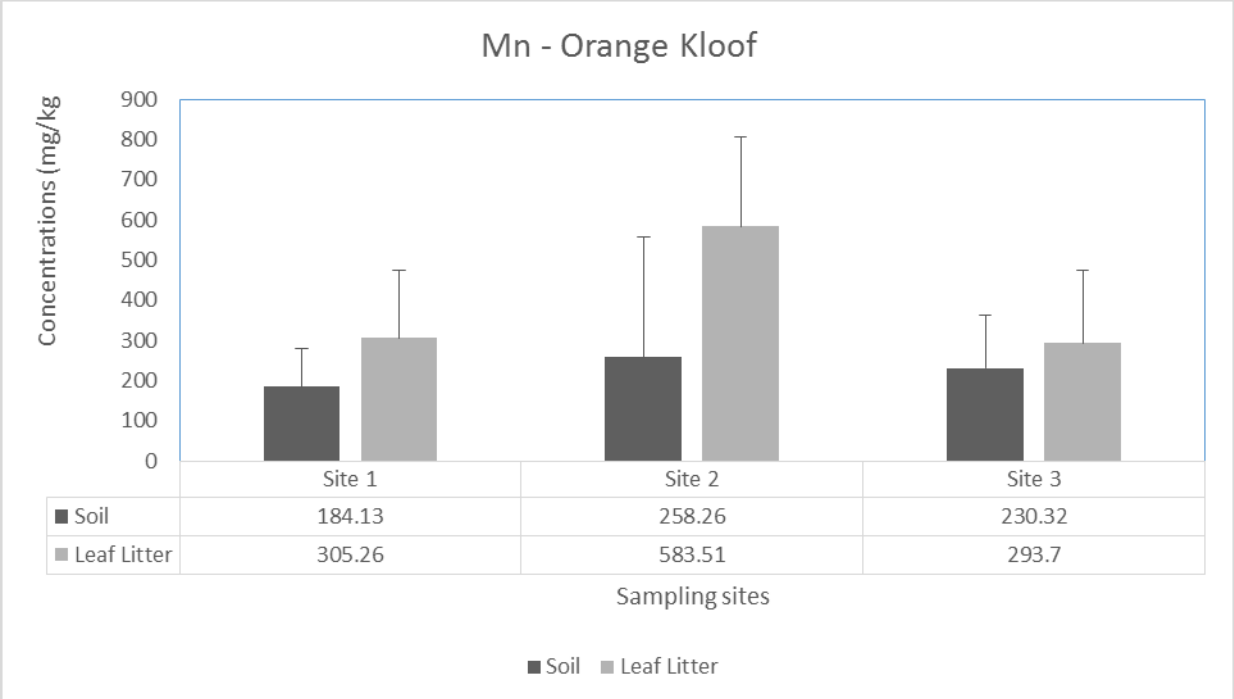


**Figure 3.13:** The mean Mn concentrations (mg/kg) (± SD) in soil and leaf litter at sites 1, 2 and 3 of Orange Kloof forest for the sampling occasion in May 2014. N=5.

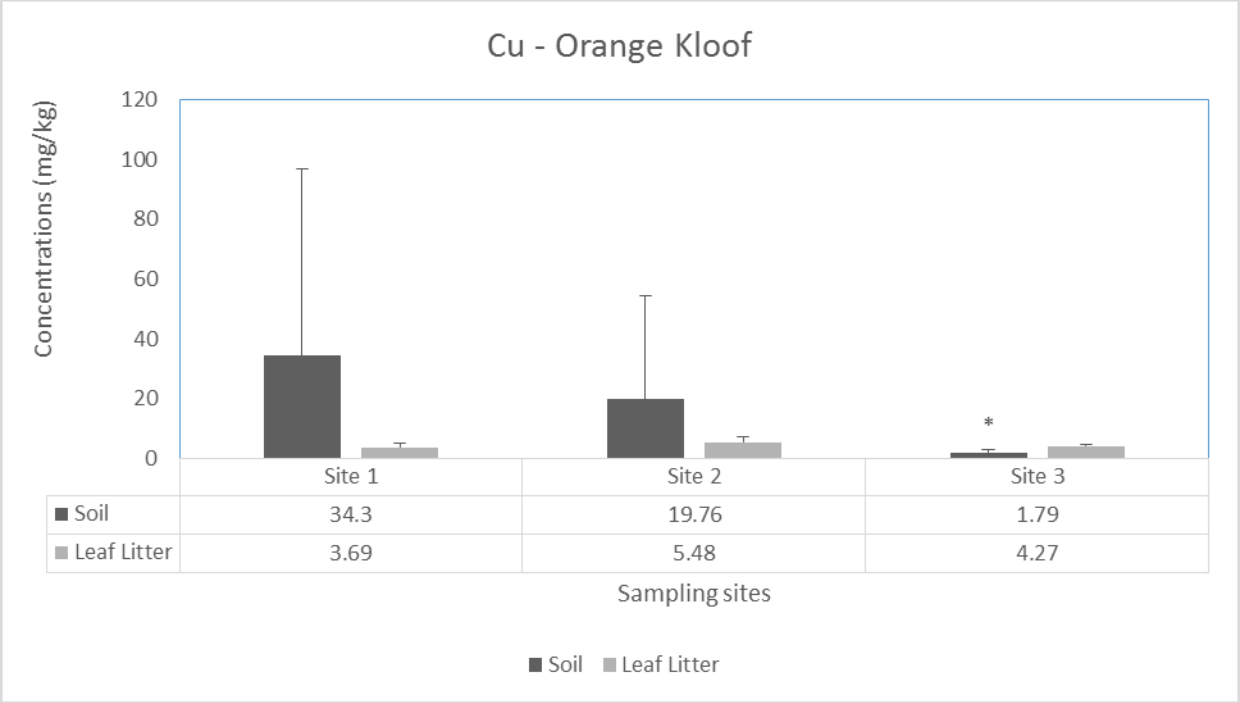


**Figure 3.14:** The mean Cu concentrations (mg/kg) (± SD) in soil and leaf litter at sites 1, 2 and 3 of Orange Kloof forest for the sampling occasion in May 2014. N=5.

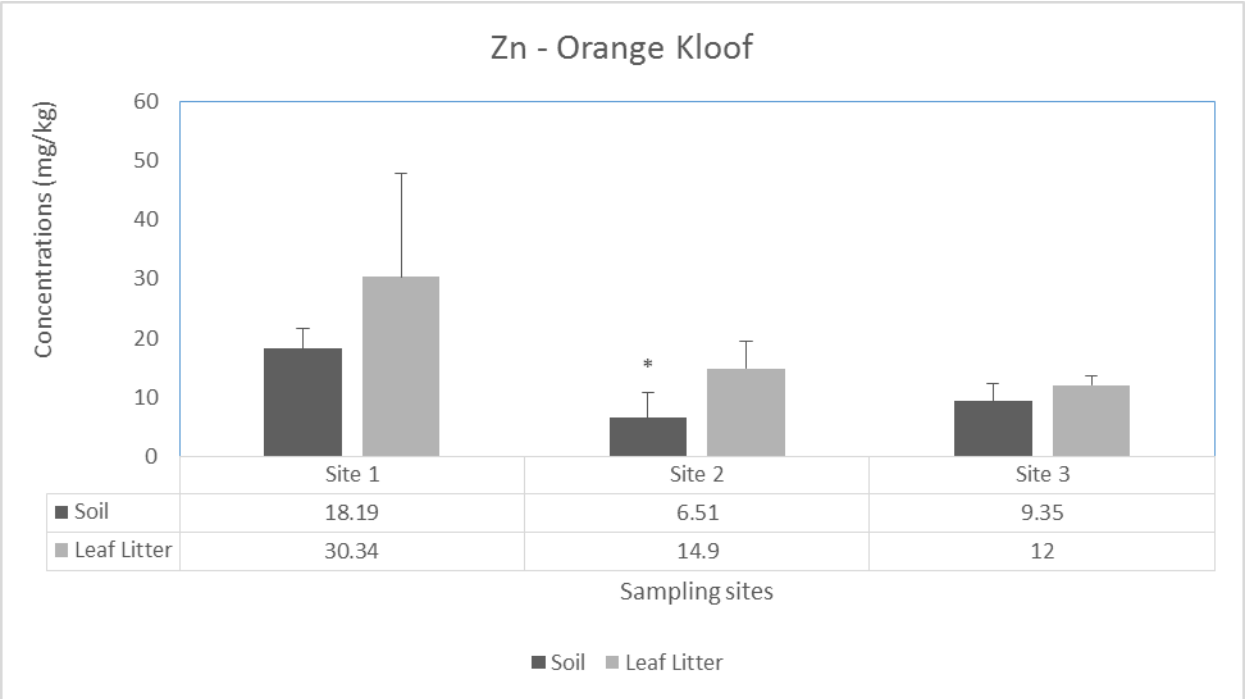


**Figure 3.15:** The mean Zn concentrations (mg/kg) (± SD) in soil and leaf litter at sites 1, 2 and 3 of Orange Kloof forest for the sampling occasion in May 2014. N=5.

When soil and leaf litter were compared in terms of Al concentrations, significant differences between each other at all the sites were found: site 1: (P=0.008), site 2: (P=0.016) and site 3: (P=0.008). The mean concentration at site 1 in soil (9086.99 ± 1467.33 mg/kg) was much higher than in leaf litter at the same site (969.88 ± 987.83 mg/kg) **(Fig 3.11)**.

Leaf litter and soil showed statistically significant differences between each other at Orange Kloof sites 1: (P=0.008), 2: (P=0.032) and 3: (P=0.008) in terms of Fe concentrations. Fe concentrations in the soil were found to be significantly higher than in leaf litter at site 3 (5713.17 ± 3363.57 mg/kg) **(Fig 3.12)**.

At Orange Kloof leaf litter and soil showed no statistically significant differences between each other: site 1: (P=0.222), site 2: (P<0.056) and site 3: (P=0.690) when Mn concentrations were compared **(Fig 3.13)**.

Comparisons between soil and leaf litter in terms of Cu concentrations did not differ significantly at site 1 (P=0.421) and site 2 (P=0.690). However, Cu concentrations in the leaf litter were found to be significantly higher than in soil at site 3 (P=0.008) **(Fig 3.14)**.

Soil and leaf litter Zinc concentrations at Orange Kloof, showed no statistically significant differences at site 1 (P=0.310) and site 3 (P=0.151), but significant differences were found at site 2 (P=0.032). Zinc concentrations in leaf litter were found to be significantly higher than in soil at site 2 (14.90 ± 4.59 mg/kg) **(Fig 3.15)**.

### *3.2.2.3) Comparisons of metal concentrations between moss and lichen at sites 1, 2 and 3*

Mean metal concentrations in moss and lichen at sites 1, 2 and 3 of Orange Kloof forest for the sampling occasion in May 2014 are shown in **Figs 3.16 to 3.20**. Statistical signifcant differences (P<0.050) between moss and lichen are indicated with an asterisk above the graph bars.

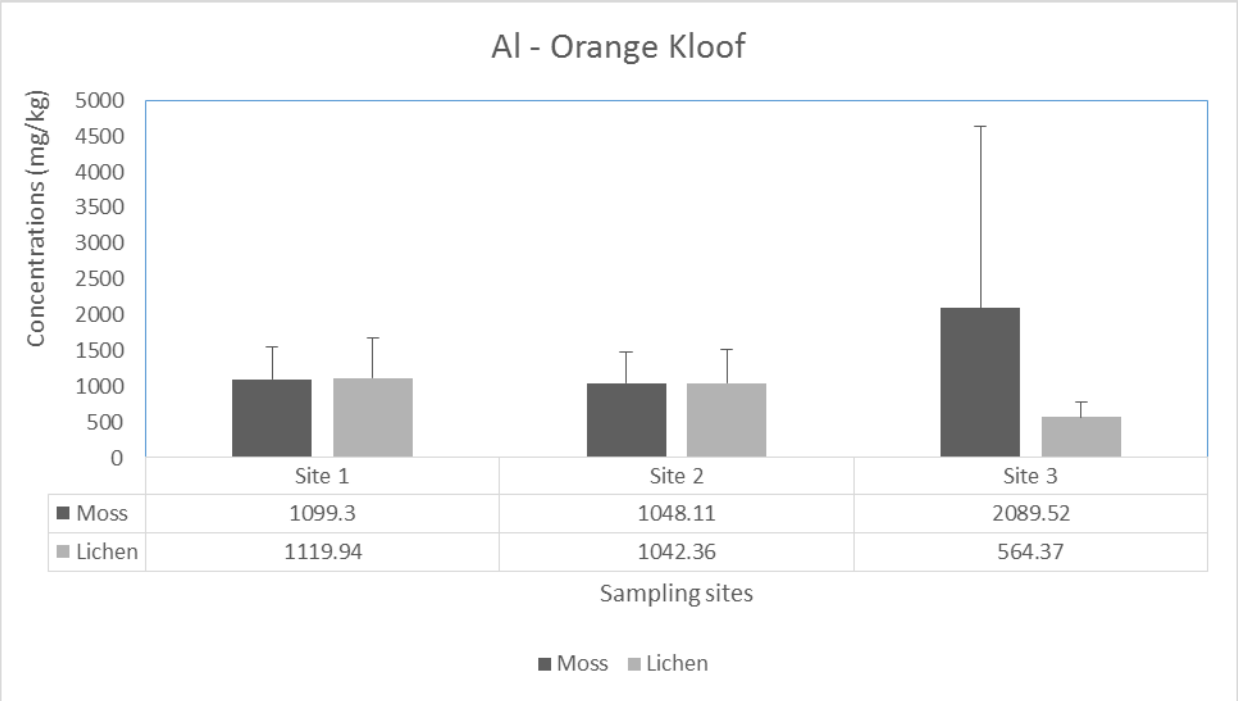


**Figure 3.16:** The mean Al concentrations (mg/kg) (± SD) in moss and lichen at sites 1, 2 and 3 of Orange Kloof forest for the sampling occasion in May 2014. N=5.

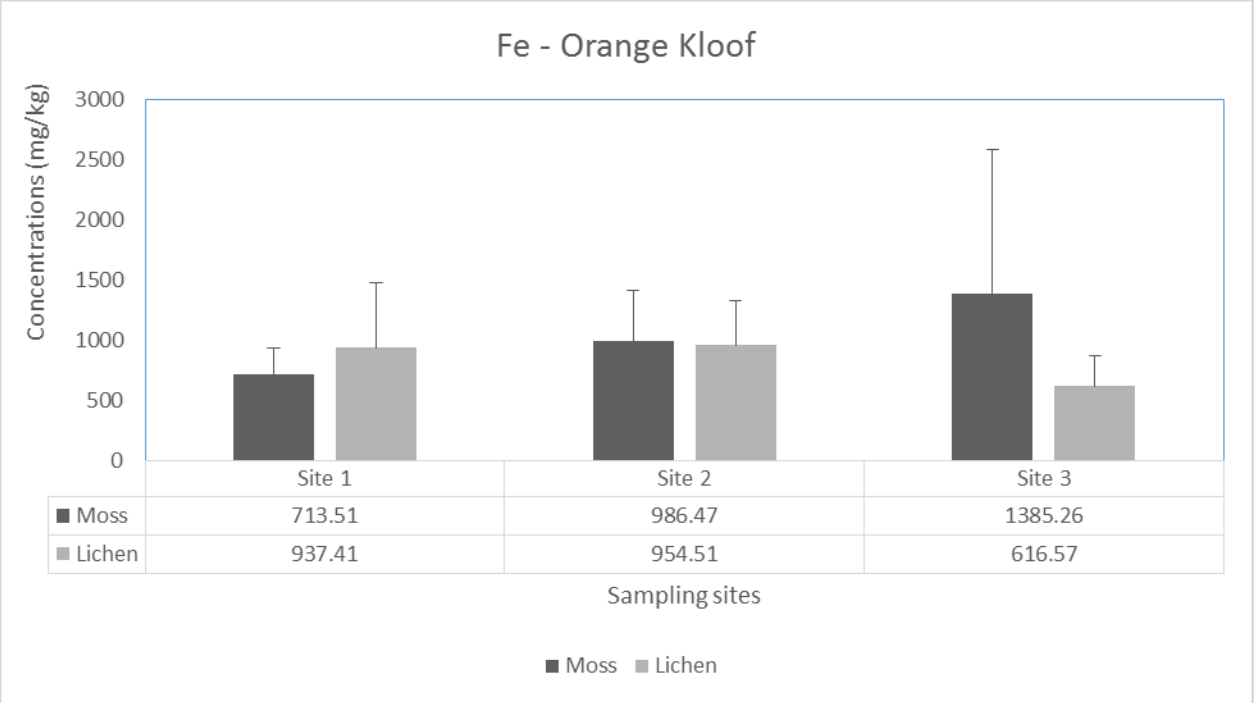


**Figure 3.17:** The mean Fe concentrations (mg/kg) (± SD) in moss and lichen at sites 1, 2 and 3 of Orange Kloof forest for the sampling occasion in May 2014. N=5.

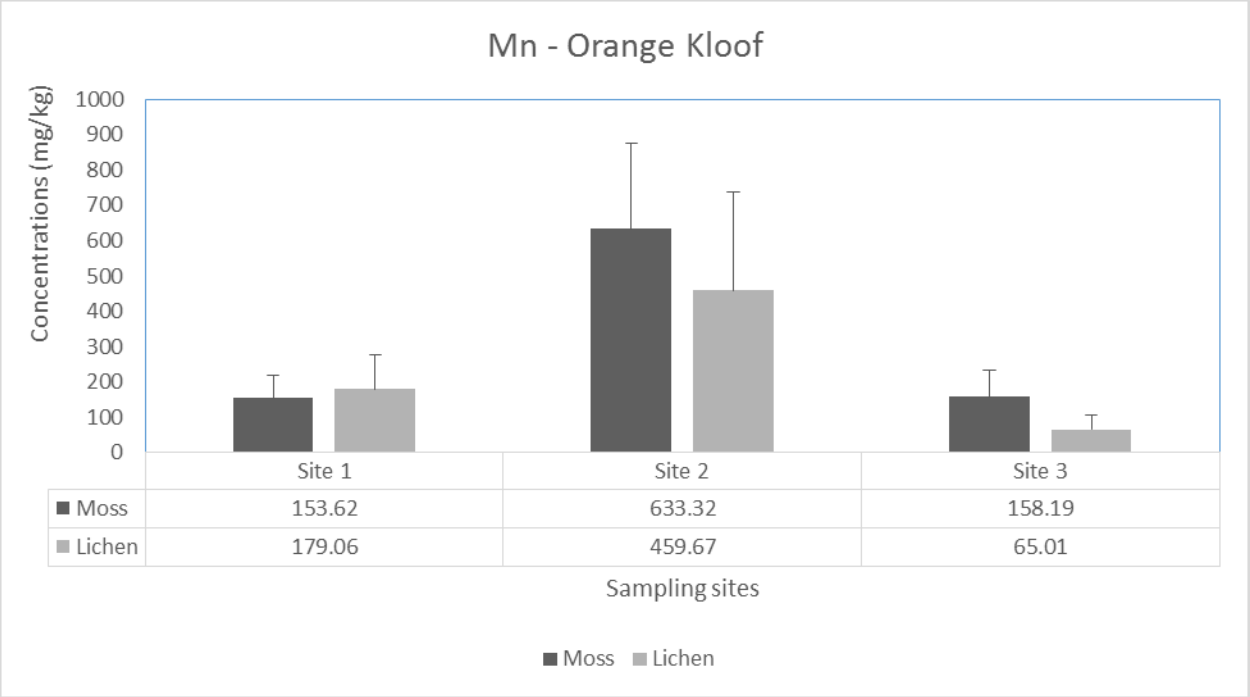


**Figure 3.18:** The mean Mn concentrations (mg/kg) (± SD) in moss and lichen at sites 1, 2 and 3 of Orange Kloof forest for the sampling occasion in May 2014. N=5.

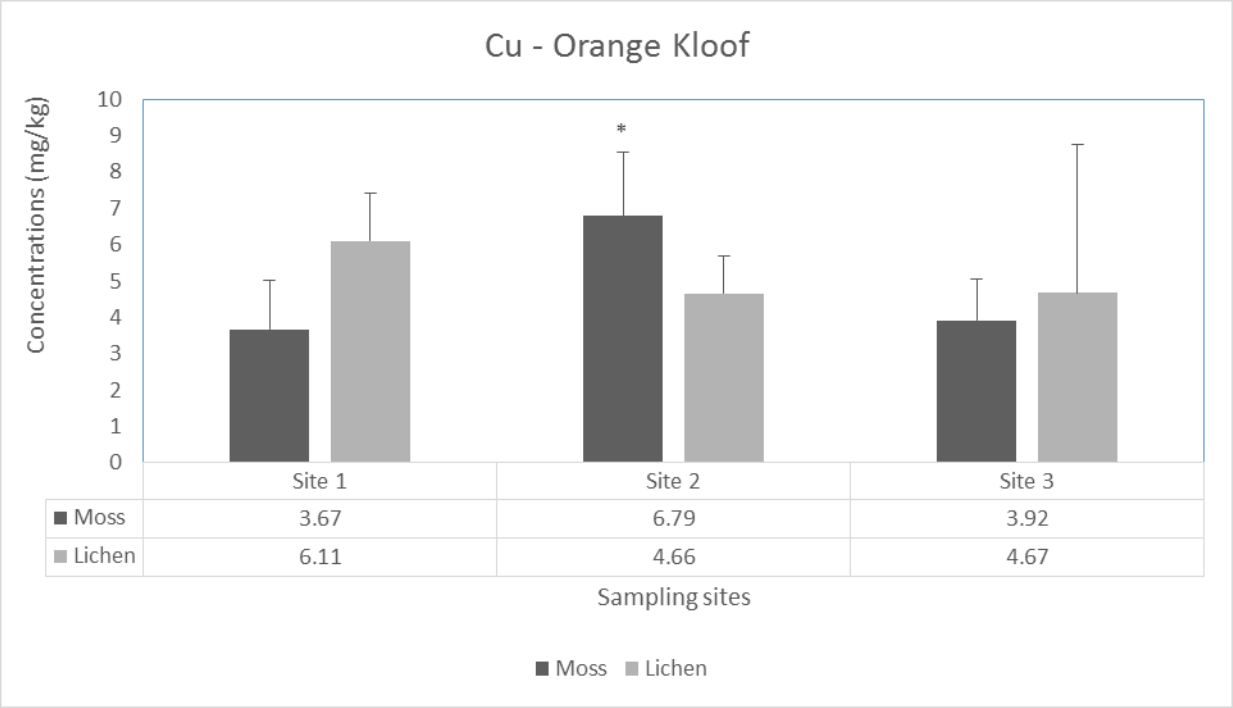


**Figure 3.19:** The mean Cu concentrations (mg/kg) (± SD) in moss and lichen at sites 1, 2 and 3 of Orange Kloof forest for the sampling occasion in May 2014. N=5.

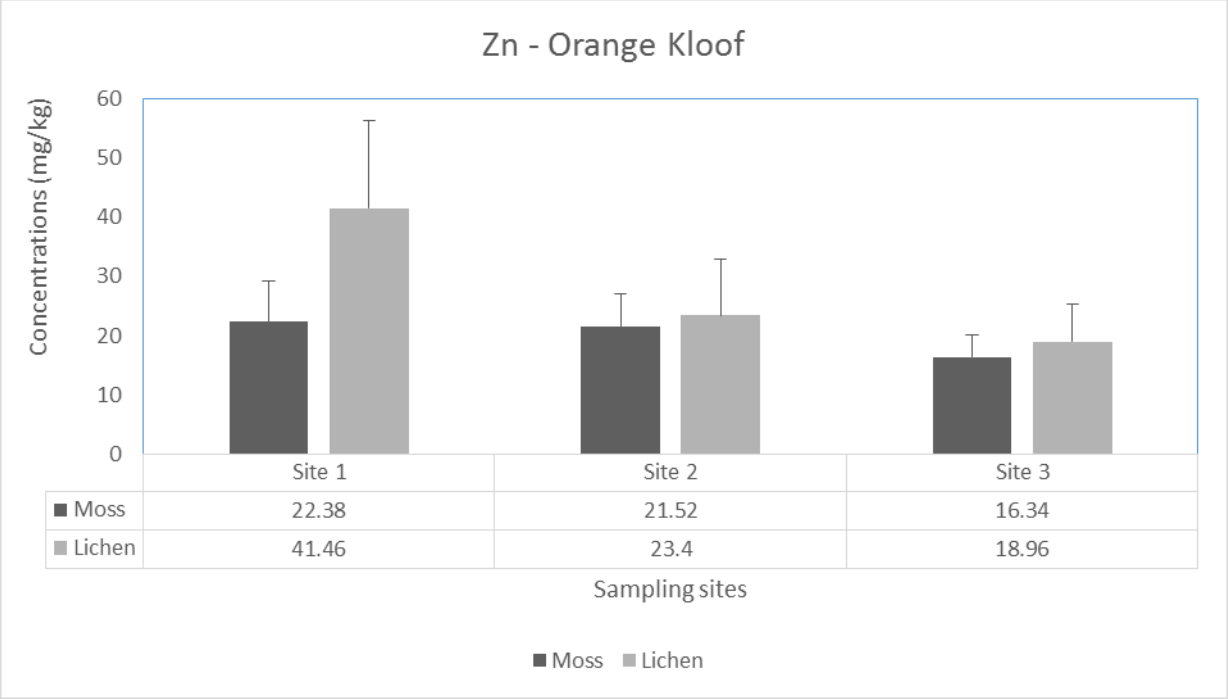


**Figure 3.20:** The mean Zn concentrations (mg/kg) (± SD) in moss and lichen at sites 1, 2 and 3 of Orange Kloof forest for the sampling occasion in May 2014. N=5.

Orange Kloof moss and lichen comparisons showed no statistically significant differences between each other at all the sites: 1 (P=1.000), 2 (P=1.000) and 3 (P=0.222) in terms of Al concentrations **(Fig 3.16)**.

Comparisons of Fe concentrations between moss and lichen did not differ significantly from each other at any of the sites (1: P=0.690; 2: P=1.000; 3: P=0.222) **(Fig 3.17)**.

Moss and lichen in this forest showed no statistically significant differences between each other at any of the sites: 1: (P=0.1000), 2: (P=0.421) and 3: (P=0.151) when Mn concentrations were compared **(Fig 3.18)**.

Site 1 (P=0.056) and 3 (P=1.000) moss and lichen did not differ significantly from each other, but there were significant differences found at site 2: (P=0.032) in terms of Cu concentrations. The mean concentration in moss (6.79 ± 1.74 mg/kg) was higher than in lichen at this site (4.66 ± 1.03 mg/kg) **(Fig 3.19)**.

The Zn comparisons between moss and lichen at Orange Kloof showed no statistically significant differences at site 1 (P=0.056), 2 (P=0.690) or 3 (P=0.690) **(Fig 3.20)**.

### *3.2.2.4) pH and Moisture*

pH of the soil of Orange Kloof ranged from slightly acidic (6.48) to alkaline (7.64) and the moisture % of the soil ranged from a relatively moist 20.96 % to a moist 36.77 %.

**Table 3.7:** pH and moisture % of soil of Orange Kloof forest for the sampling occasion in May 2014.

| **ORANGE KLOOF FOREST** | **SOIL** | |
|---|---|---|
| Site 1 | **pH** | 6.48 |
| | *Moisture* | *36.77* |
| Site 2 | **pH** | 7.64 |
| | *Moisture* | *20.96* |
| Site 3 | **pH** | 7.08 |
| | *Moisture* | *20.96* |

Moisture % of the leaf litter ranged from a relatively moist 50.77 % to a moist 64.38 %.

**Table 3.8:** Moisture % of the leaf litter of Orange Kloof forest for the sampling occasion in May 2014.

| ORANGE KLOOF FOREST | % | LEAF LITTER |
|---|---|---|
| Site 1 | Moisture | 64.25 |
| Site 2 | Moisture | 50.77 |
| Site 3 | Moisture | 64.38 |

### *3.2.2.5) Weather data from the South African Weather Service in the vicinity of Constantia' Cape Town for Orange Kloof forest*

**Table 3.9:** Weather data in the vicinity of Orange Kloof forest for the sampling occasion in May 2014.

| SAMPLING SITES | SAMPLING OCCASION | | | |
|---|---|---|---|---|
| | DATE | DEGREES (°C) | PRECITATION (MM) | WIND SPEED (KM/H) |
| **ORANGE KLOOF FOREST** | | | | |
| Site 1 | 22-May-14 | 26 | 0 | Light breeze |
| Sites 2, 3 | 27-May-14 | 14 | 0 | 26 |

### *3.2.2.6) Soil characterization for Orange Kloof forest sites 1, 2 and 3*

**Table 3.10:** Characterization of soil for Orange Kloof forest sites for the sampling occasion in May 2014.

| SAMPLING | (%) | | | | | | | WATER RETENTION | | |
|---|---|---|---|---|---|---|---|---|---|---|
| SITES | Clay | Silt | Fine sand | Medium sand | Coarse sand | Rock (v/v) | Classification | 10kPa | 100kPa | mm/m |
| ORANGE KLOOF FOREST | | | | | | | | | | |
| Site 1 | 5 | 6 | 46.8 | 28.6 | 13.6 | 8.0 | Sa | 19.08 | 9.13 | 99.5 |
| | | | | | | | | | | |
| Site 2 | 9 | 14 | 31.6 | 14 | 31.4 | 24.8 | LmSa | 20.22 | 12.19 | 80.4 |
| | | | | | | | | | | |
| Site 3 | 3 | 6 | 55.1 | 25.3 | 10.6 | 8.1 | Sa | 20.10 | 8.88 | 112.2 |

### 3.2.3) NEWLANDS FOREST

#### *3.2.3.1) Comparisons of metal concentrations in soil, leaf litter and sentinel organisms between sites 1, 2 and 3*

The mean metal concentrations in soil, leaf litter and sentinel organisms for sites 1, 2 and 3 of Newlands forest for the sampling occasion in May 2014 are presented in **Table 3.11.** Metal concentrations are expressed in mg/kg.

**Table 3.11:** The mean metal concentrations (mg/kg) (± SD) in soil, leaf litter and sentinel organisms for sites 1, 2 and 3 of Newlands forest for the sampling occasion in May 2014.

| METAL | | SOIL | | | LEAF LITTER | | | MOSS | | | LICHEN | | | MILLIPEDES | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Site 1 | Site 2 | Site 3 | Site 1 | Site 2 | Site 3 | Site 1 | Site 2 | Site 3 | Site 1 | Site 2 | Site 3 | Site 1 | Site 2 | Site 3 |
| Al | Mean | $^{a}$8309.51 | $^{a}$8679.72 | $^{b}$827.93 | $^{a}$2404.33 | $^{b}$535.06 | $^{c}$192.70 | $^{a}$3255.41 | $^{b}$1672.73 | $^{c}$644.14 | $^{a}$920.15 | $^{a}$835.40 | $^{a}$704.30 | $^{a}$819.80 | $^{a}$400.93 | $^{a}$216.51 |
| | *SD* | *4239.71* | *2002.8* | *180.79* | *1241.86* | *248.25* | *135.15* | *1373.22* | *764.77* | *157.63* | *655.29* | *446.31* | *405.59* | *462.64* | *205.08* | *205.17* |
| Fe | Mean | $^{a}$9482.26 | $^{a}$10593.39 | $^{b}$1720.43 | $^{a}$2387.36 | $^{b}$361.93 | $^{b}$310.38 | $^{a}$3811.87 | $^{b}$2067.48 | $^{c}$668.17 | $^{a}$1185.57 | $^{a}$1060.68 | $^{a}$940.89 | $^{a}$832.86 | $^{a}$485.01 | $^{a}$433.58 |
| | *SD* | *3717.25* | *2475.37* | *367.55* | *1229.81* | *249.1* | *255.36* | *1556.66* | *961.1* | *344.78* | *833.24* | *608.04* | *645.77* | *516.34* | *216.1* | *396.22* |
| Mn | Mean | $^{a}$454.51 | $^{a}$672.24 | $^{a}$997.06 | $^{a}$564.88 | $^{b}$855.75 | $^{b}$1142.39 | $^{a}$346.09 | $^{a}$341.69 | $^{a}$611.13 | $^{a}$94.64 | $^{a}$199.85 | $^{a}$348.45 | $^{a}$96.61 | $^{a}$903.95 | $^{a}$201.49 |
| | *SD* | *30.8* | *202.81* | *511.37* | *82.71* | *153.08* | *350.81* | *149.8* | *209.86* | *206.33* | *75.95* | *147.36* | *198.36* | *88.57* | *127.44* | *134.74* |
| Cu | Mean | $^{a}$7.43 | $^{a}$34.57 | $^{a}$10.06 | $^{a}$8.39 | $^{a}$6.41 | $^{a}$8.20 | $^{a}$8.82 | $^{b}$14.62 | $^{a}$8.43 | $^{a}$2.89 | $^{a}$8.15 | $^{a}$7.33 | $^{a}$10.16 | $^{a}$15.09 | $^{b}$5.57 |
| | *SD* | *3.57* | *46* | *5.71* | *3.35* | *2.22* | *1.76* | *2.01* | *3.76* | *2.36* | *1.43* | *5.69* | *4.69* | *4.74* | *5.55* | *2.19* |
| Zn | Mean | $^{a}$25.36 | $^{a}$24.46 | $^{a}$29.99 | $^{a}$27.66 | $^{a}$26.21 | $^{a}$33.77 | $^{a}$28.25 | $^{a}$33.04 | $^{a}$32.68 | $^{a}$37.32 | $^{a}$41.27 | $^{a}$40.71 | $^{a}$105.24 | $^{a}$148.41 | $^{a}$95.49 |
| | *SD* | *8.61* | *5.38* | *23.59* | *5.65* | *13.27* | *14.22* | *4.8* | *9.15* | *8.61* | *15.07* | *10.43* | *12.86* | *8.65* | *48.1* | *28* |

Statistical signifcant differences (P<0.050) between forests are indicated with different superscripted letters. Comparisons were done separately for the soil, leaf litter and sentinel organisms. N=5.

***a) Soil***

Site comparisons showed statistically significant differences in the soil Al concentrations ($P<0.05$) for the comparisons of sites 1 vs 3 and 2 vs 3 with the exception of sites 1 vs 2 ($P>0.05$). The concentrations found at sites 1 (8309.51 ± 4239.71 mg/kg) and 2 (8679.72 ± 2002.80 mg/kg) were significantly higher than at site 3 (827.93 ± 180.79 mg/kg) **(Table 3.11)**.

In terms of Fe concentrations at Newlands, statistically significant differences were found between site 1 and site 3 and site 2 and site 3 ($P<0.05$), but no significant differences were found in the soil between sites 1 and 2 ($P>0.05$). The mean concentration at site 3 (1720.43 ± 367.55 mg/kg) was significantly lower than the mean concentration found at site 2 (10593.39 ± 2475.37 mg/kg) and site 1 (9482.26 ± 3717.25 mg/kg) **(Table 3.11)**.

Newlands forest soil did not differ significantly in Mn ($P=0.137$), Cu ($P=0.151$) and Zn ($P<0.811$) concentrations between the three sites when compared **(Table 3.11)**.

***b) Leaf litter***

Pairwise multiple comparisons showed statistically significant differences in the leaf litter between all the sites ($P<0.05$). Site 1 had the highest mean Al concentration of 2404.33 ± 1241.86 mg/kg **(Table 3.11)**.

In terms of Fe concentrations, statistically significant differences were found between site 1 and site 3 and site 1 and site 2 ($P<0.05$). No significant differences were found between sites 2 and 3 ($P>0.05$). The mean concentration at site 1 (2387.36 ± 1229.81 mg/kg) was significantly higher than the concentration at site 2 (361.93 ± 249.10 mg/kg) and 3 (310.38 ± 255.36 mg/kg) **(Table 3.11)**.

Manganese concentrations differed significantly between sites 1 and 3 and sites 1 and 2 ($P<0.05$). No significant differences were found between sites 2 and 3 ($P>0.05$). The mean concentration at site 1 (564.88 ± 82.71 mg/kg) was significantly lower than the mean concentration at site 2 (855.75 ± 153.08 mg/kg) and 3 (1142.39 ± 350.81 mg/kg) **(Table 3.11)**.

The Cu (P=0.468) and Zn (P=0.445) concentrations found in leaf litter did not show any statistically significant differences between the three sites during this sampling occasion **(Table 3.11)**.

***c) Moss***

Comparisons of Al concentrations showed statistically significant differences in moss between all the sites (P<0.05). The mean Al concentration at site 1 (3255.41 ± 1373.22 mg/kg) was significantly higher than at the other two sites **(Table 3.11)**.

Significant differences in Fe concentrations in moss between all the sites (P<0.05) were found. The mean Fe concentration at site 1 (3811.87 ± 1556.66 mg/kg) was significantly higher than at site 2 (2067.48 ± 961.10 mg/kg) and site 3 (668.17 ± 344.78 mg/kg) **(Table 3.11)**.

This forest did not show any significant differences in Mn concentrations in moss samples between the three sites for this sampling session (P=0.112) **(Table 3.11)**.

Pairwise multiple comparisons showed statistically significant differences of Cu concentrations in moss for the comparisons of sites 2 and 3 and sites 1 and 2 (P<0.05). However, no differences between sites 1 and 3 (P>0.05) were found. Site 2 with a mean concentration of 14.62 ± 3.76 mg/kg was significantly higher than at sites 1 and 3 **(Table 3.11)**.

Moss samples did not differ significantly between any of the sites in terms of Zn concentration (P=0.468) at this forest **(Table 3.11)**.

***d) Lichen***

No significant differences in lichen Al (P=0.827); Fe (P=0.827); Mn (P=0.065); Cu (P=0.145) and Zn concentrations (P=0.961) were found between any of the sites **(Table 3.11)**.

***e) Millipedes***

There were no statistical differences found in millipede Al (P=0.056); Fe (P=0.357); Mn (P=0.145) and Zn concentrations (P=0.065) between the three sites **(Table 3.11)**.

Pairwise multiple comparisons showed statistically significant differences in millipedes in terms of Cu concentrations for the comparisons of sites 2 vs 3 and sites 1 vs 3 (P<0.05). No statistically differences between sites 1 vs 2 (P>0.05) were found. The mean Cu concentration at site 2 (15.09 ± 5.55. mg/kg) was significantly higher than at sites 1 and 3 **(Table 3.11)**.

### *3.2.3.2) Comparisons of metal concentrations between soil and leaf litter at sites 1, 2 and 3*

Mean metal concentrations in soil and leaf litter at sites 1, 2 and 3 of Newlands forest for the sampling occasion in May 2014 are shown in **Figs 3.21 to 3.25**. Statistical signifcant differences (P<0.050) between soil and leaf litter are indicated with an asterisk above the graph bars.

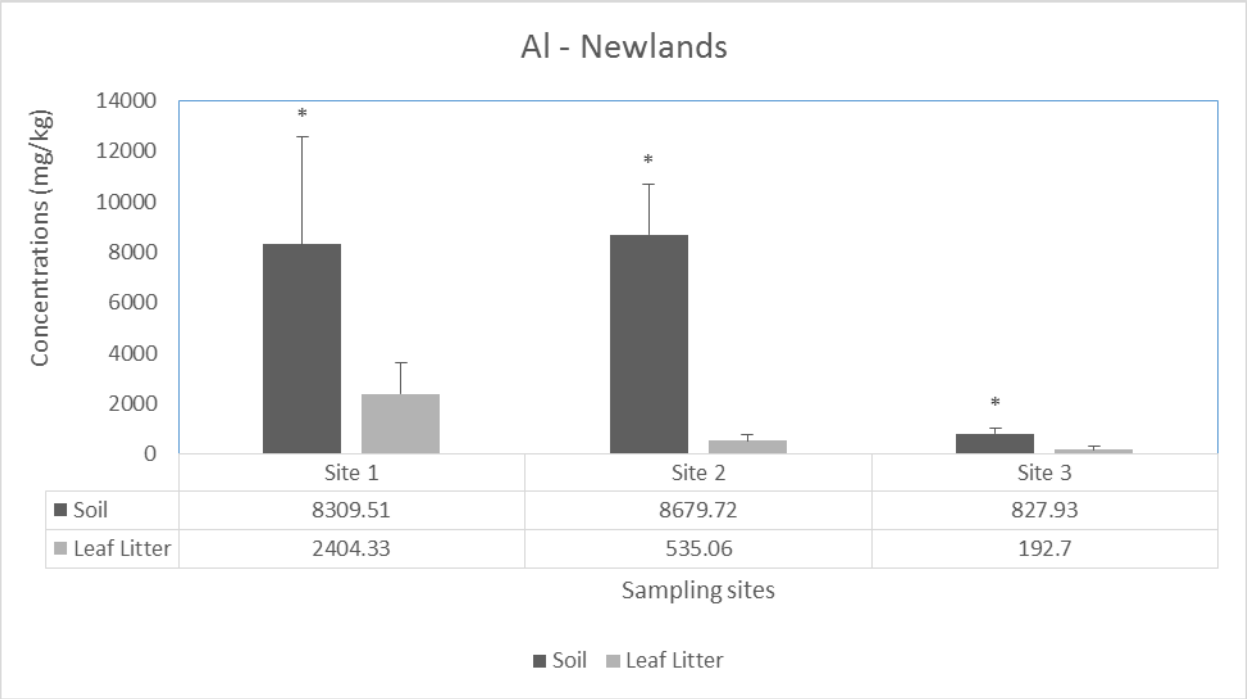


**Figure 3.21:** The mean Al concentrations (mg/kg) (± SD) in soil and leaf litter at sites 1, 2 and 3 of Newlands forest for the sampling occasion in May 2014. N=5.

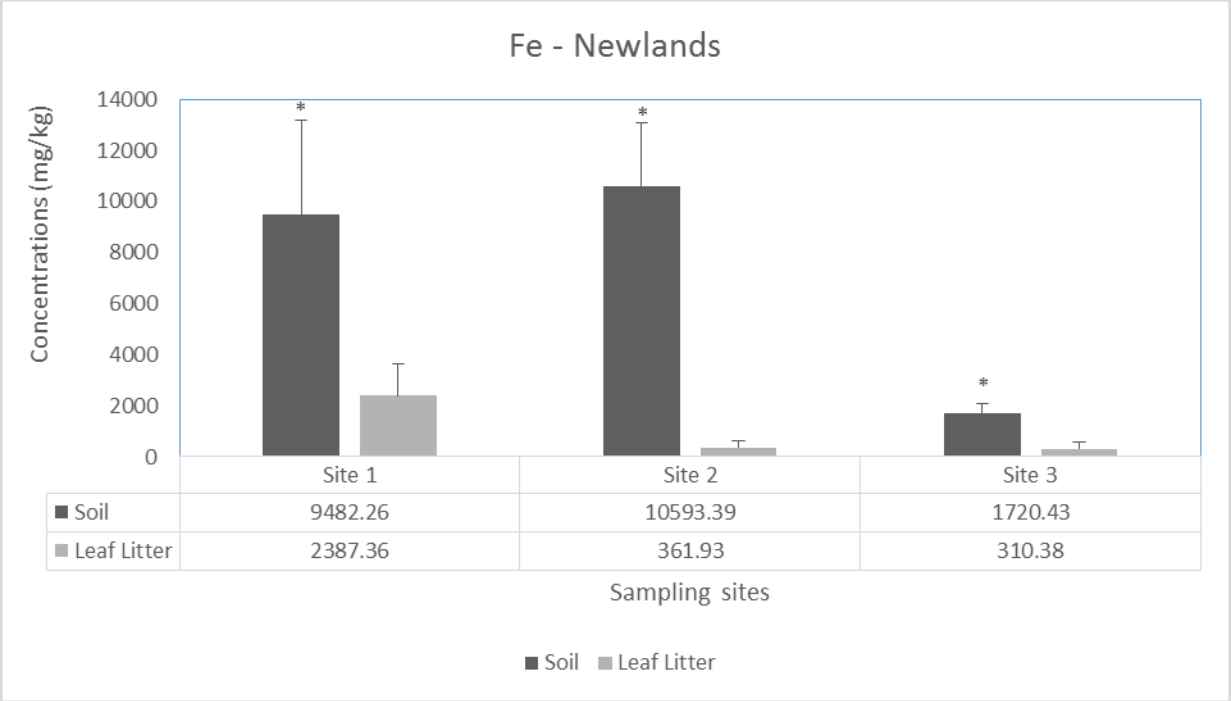


**Figure 3.22:** The mean Fe concentrations (mg/kg) (± SD) in soil and leaf litter at sites 1, 2 and 3 of Newlands forest for the sampling occasion in May 2014. N=5.

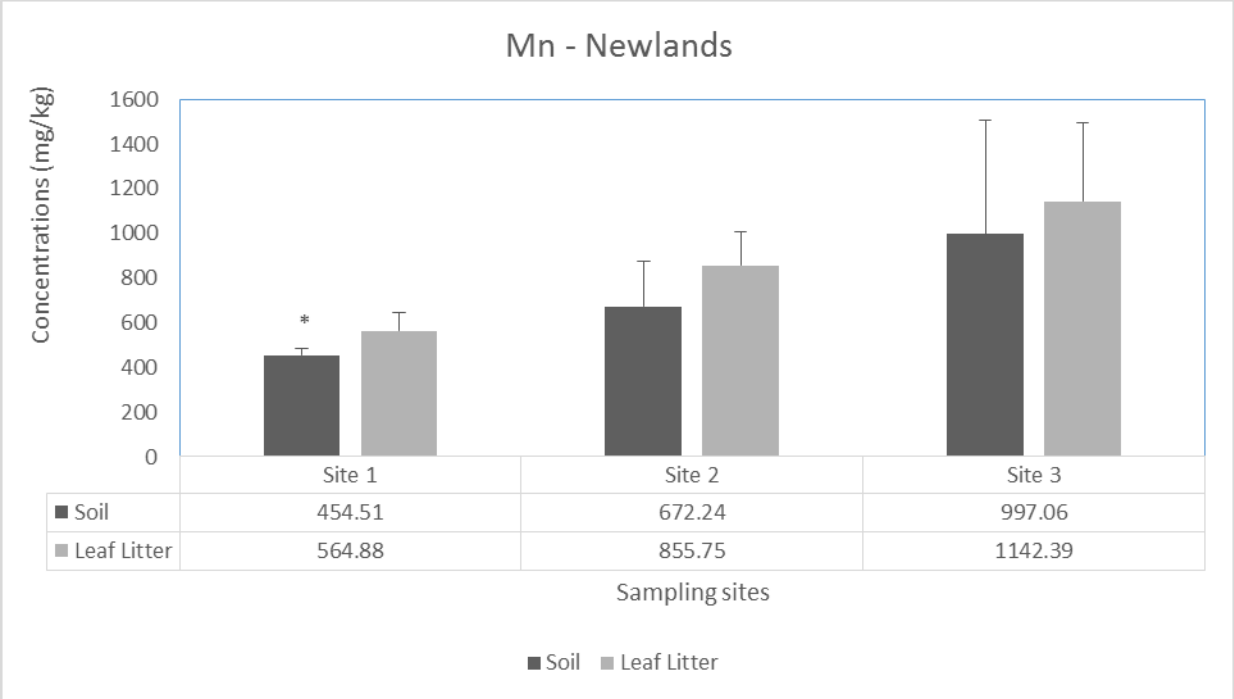


**Figure 3.23:** The mean Mn concentrations (mg/kg) (± SD) in soil and leaf litter at sites 1, 2 and 3 of Newlands forest for the sampling occasion in May 2014. N=5.

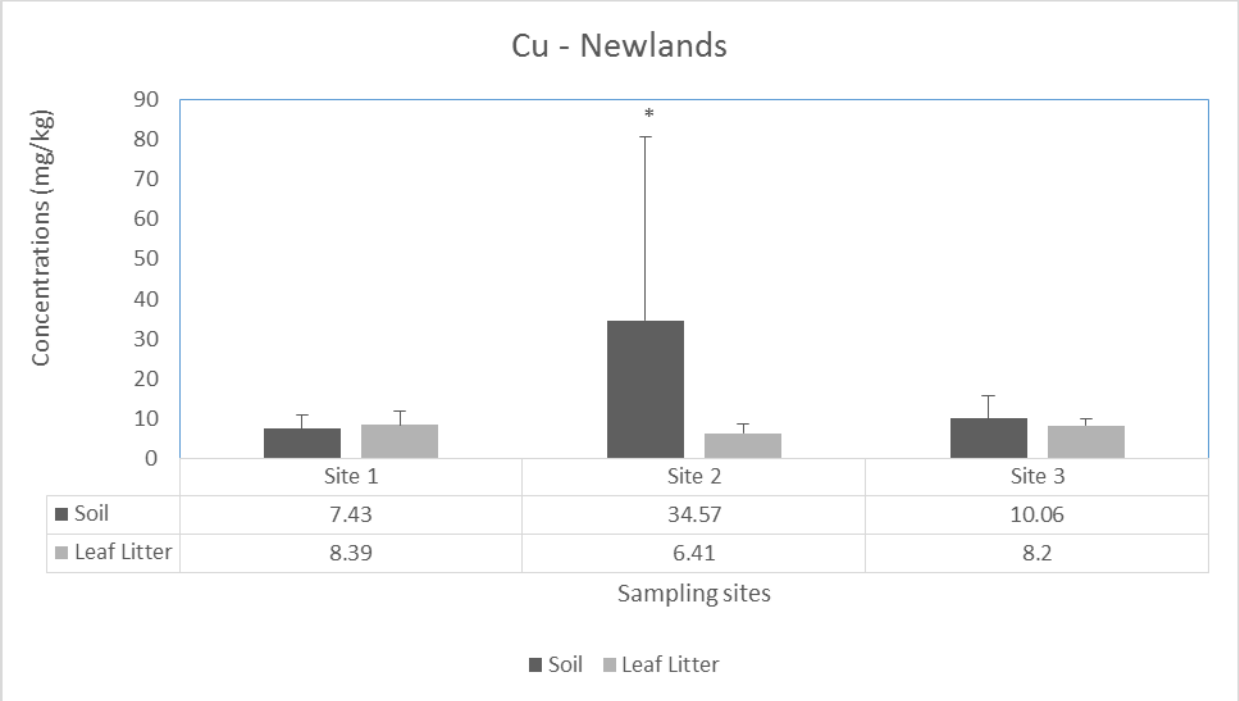


**Figure 3.24:** The mean Cu concentrations (mg/kg) (± SD) in soil and leaf litter at sites 1, 2 and 3 of Newlands forest for the sampling occasion in May 2014. N=5.

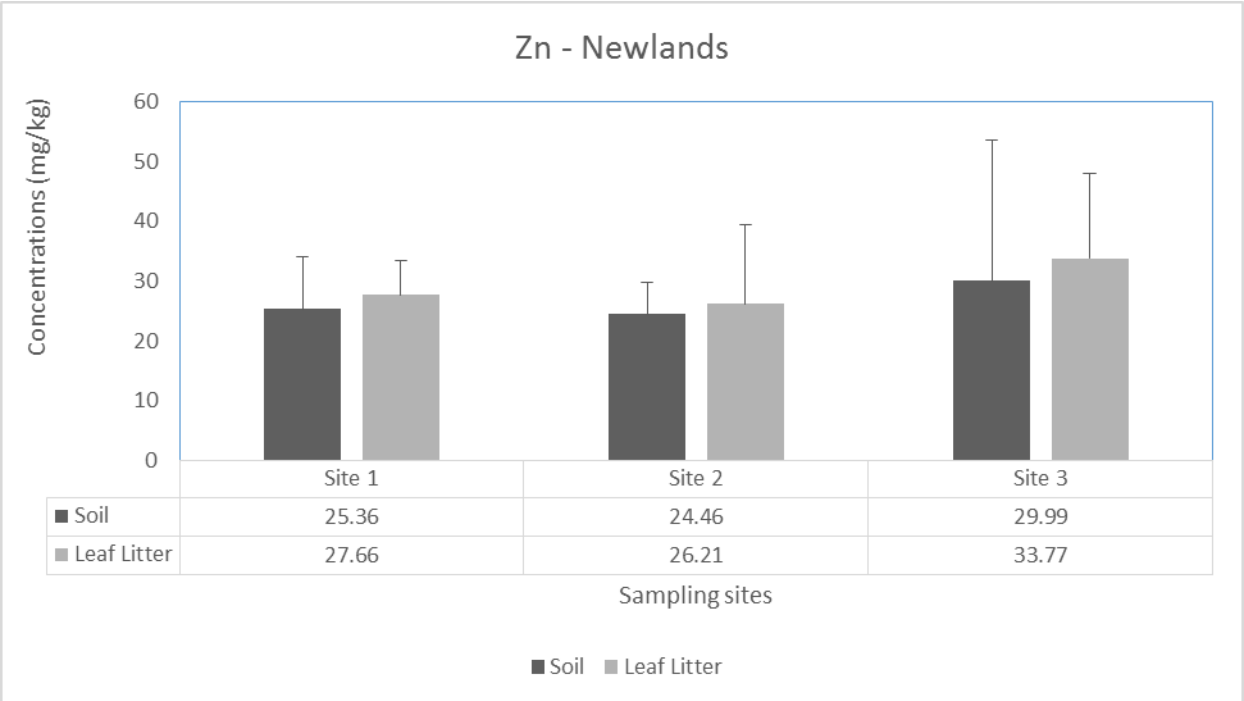


**Figure 3.25:** The mean Zn concentrations (mg/kg) (± SD) in soil and leaf litter at sites 1, 2 and 3 of Newlands forest for the sampling occasion in May 2014. N=5.

Aluminium concentrations at sites 1 (P=0.008), 2 (P=0.008) and site 3 (P=0.008) showed statistically significant differences between soil and leaf litter. The highest mean Al concentration was found in soil at site 2 (8679.51 ± 4239.71 mg/kg) **(Fig 3.21)**.

All the sites, 1 (P=0.008), 2 (P=0.008) and site 3 (P=0.008) at Newlands showed statistically significant differences in terms of Fe concentrations in soil vs leaf litter comparisons. The mean concentrations were higher in soil at all the sites **(Fig 3.22)**.

Site 1 (P=0.232) showed statistically significant differences between soil and leaf litter, but there were no statistically significant differences found at site 2 (P=0.151) and 3 (P=0.548) in terms of Mn concentrations. The highest mean concentration at site 1 (564.88 ± 82.71 mg/kg) was found in leaf litter **(Fig 3.23)**.

No significant differences in Cu concentrations between soil and leaf litter at site 1 (P=0.690) and 3 (P=1.000) were found. Significant differences were found at site 2 (P=0.032) and the mean concentration at site 2 (34.57 ± 46.00 mg/kg) was higher in the soil **(Fig 3.24)**.

Comparisons showed no significant differences at any of the sites: 1 (P=0.310) and 2 (P=0.690) and 3 (P=0.310) when Zn concentrations between soil and leaf litter were compared **(Fig 3.25)**.

### *3.2.3.3) Comparisons of metal concentrations between moss and lichen at sites 1, 2 and 3*

Mean metal concentrations in moss and lichen at sites 1, 2 and 3 of Newlands forest for the sampling occasion in May 2014 are shown in **Figs 3.26 to 3.30**. Statistical signifcant differences (P<0.050) between moss and lichen are indicated with an asterisk above the graph bars.

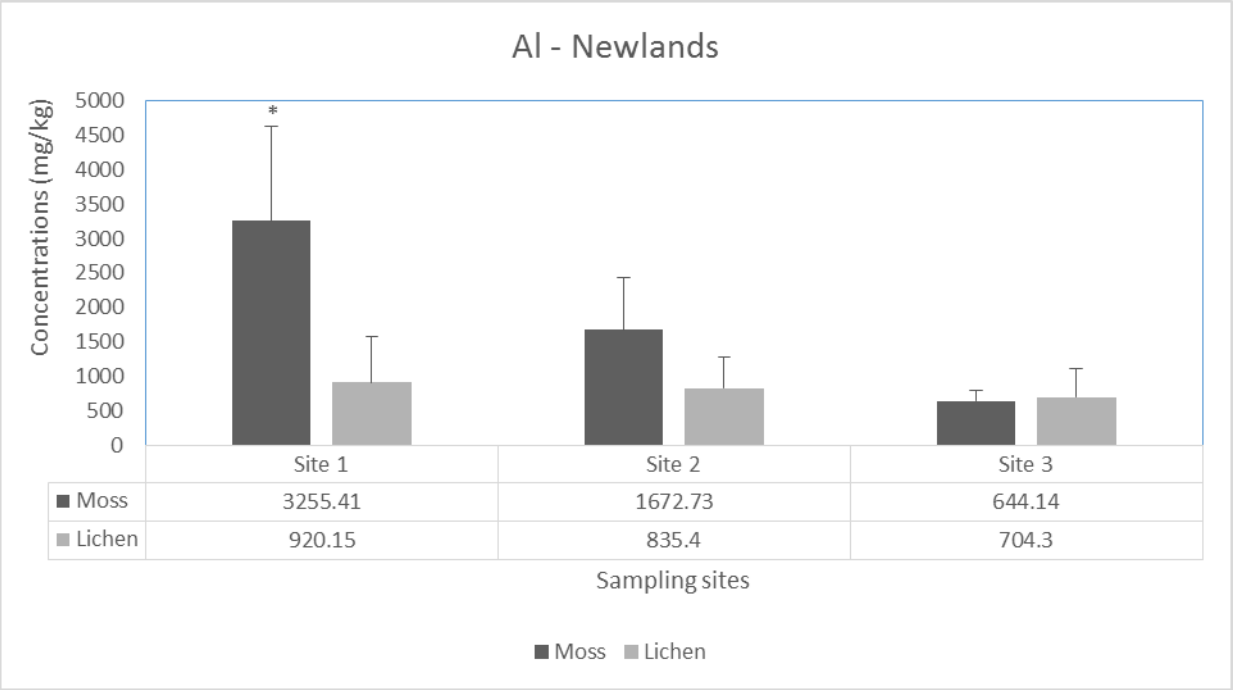


**Figure 3.26:** The mean Al concentrations (mg/kg) (± SD) in moss and lichen at sites 1, 2 and 3 of Newlands forest for the sampling occasion in May 2014. N=5.

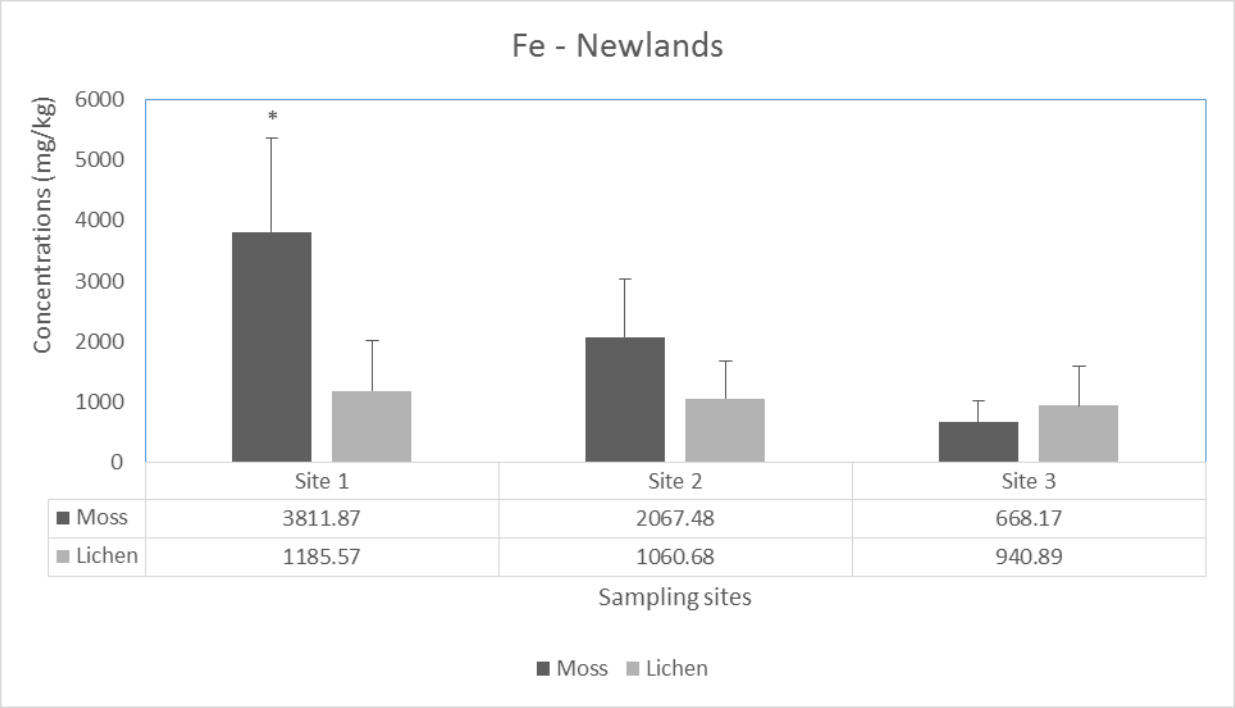


**Figure 3.27:** The mean Fe concentrations (mg/kg) (± SD) in moss and lichen at sites 1, 2 and 3 of Newlands forest for the sampling occasion in May 2014. N=5.

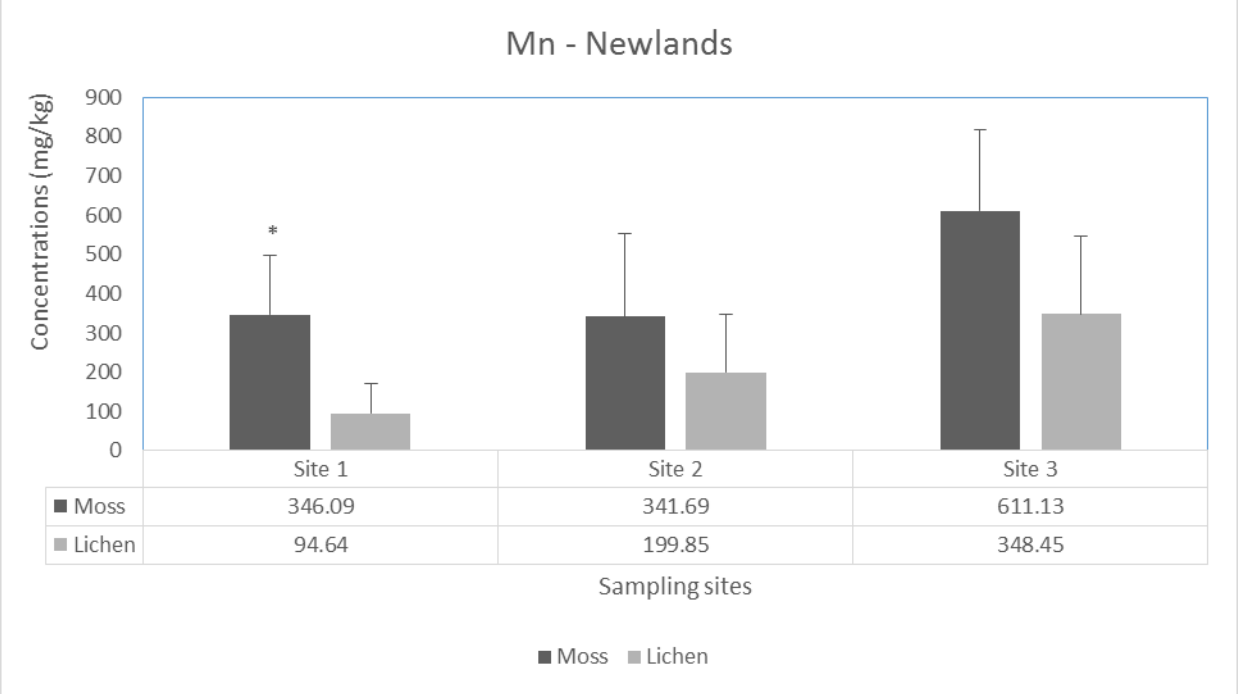


**Figure 3.28:** The mean Mn concentrations (mg/kg) (± SD) in moss and lichen at sites 1, 2 and 3 of Newlands forest for the sampling occasion in May 2014. N=5.

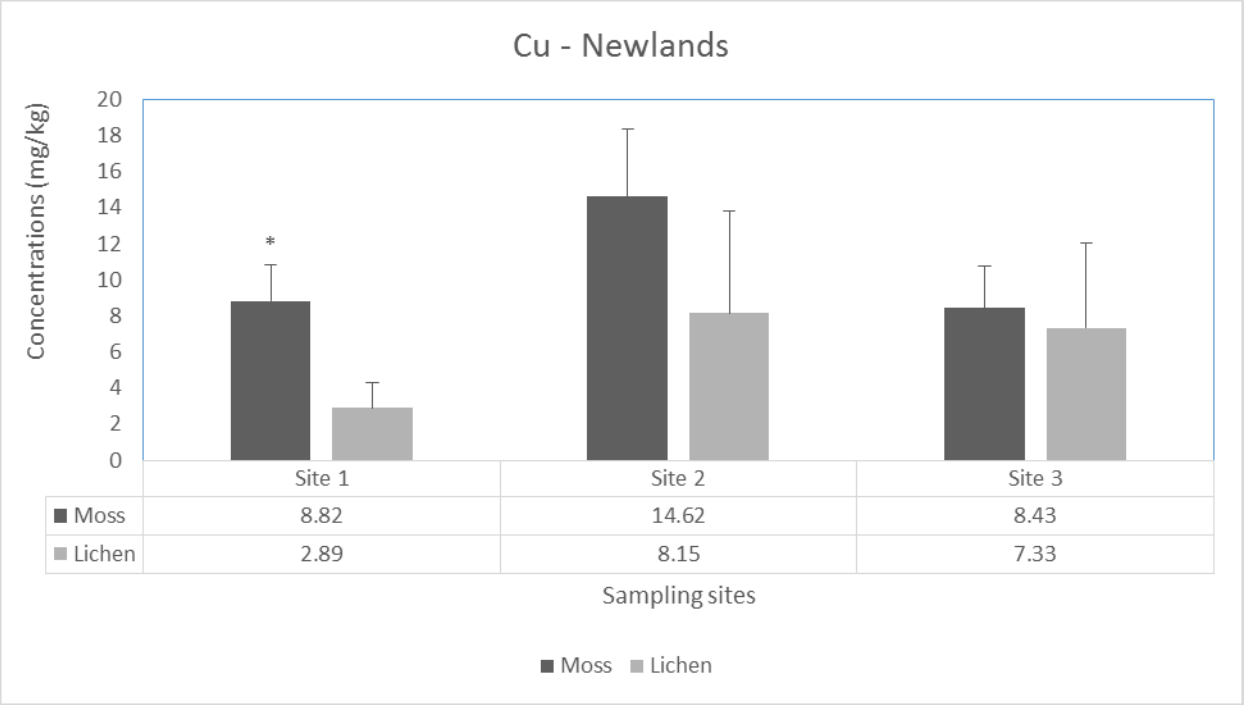


**Figure 3.29:** The mean Cu concentrations (mg/kg) (± SD) in moss and lichen at sites 1, 2 and 3 of Newlands forest for the sampling occasion in May 2014. N=5.

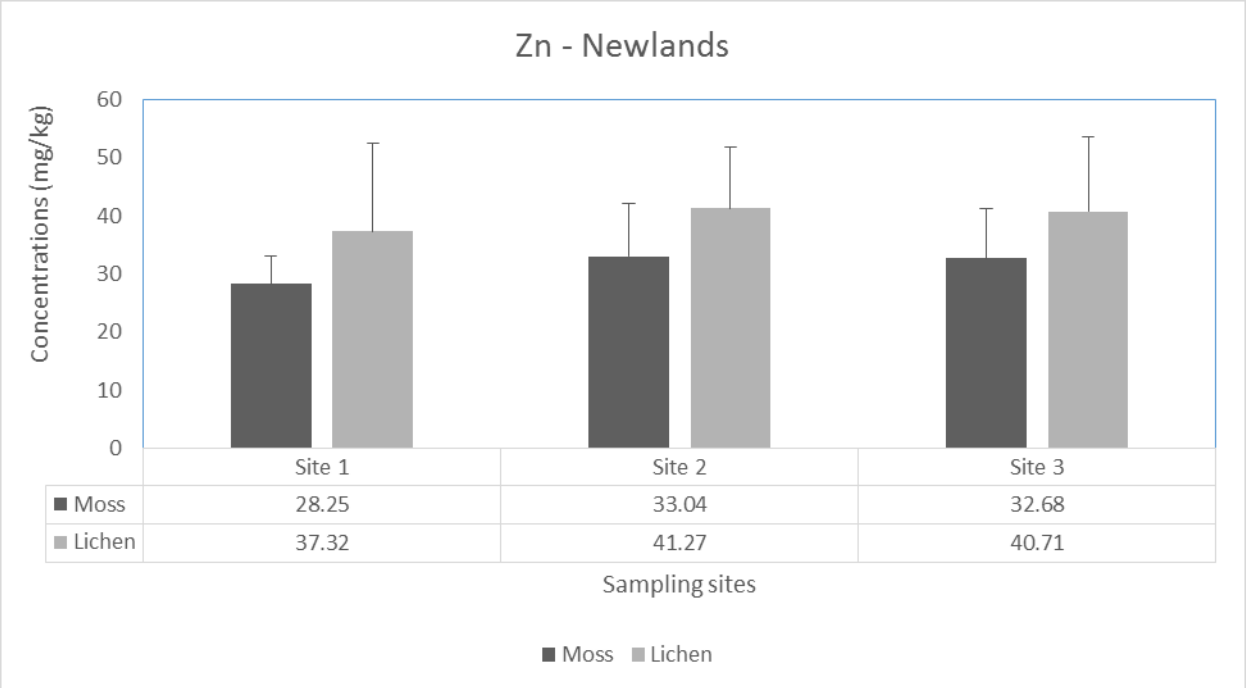


**Figure 3.30:** The mean Zn concentrations (mg/kg) (± SD) in moss and lichen at sites 1, 2 and 3 of Newlands forest for the sampling occasion in May 2014. N=5.

Site 1 (P=0.008) showed statistically significant differences between moss and lichen, but no significant differences were found between the organisms at site 2 (P<0.056) and site 3 (P=0.841) when Al concentrations were compared. The highest mean concentration at site 1 (3255.41 ± 1373.22 mg/kg) was found in moss **(Fig 3.26)**.

Statistically significant differences in Fe concentrations between moss and lichen were found at site 1 (P=0. 008), but not at site 2 (P=0.095) and 3 (P=0.548). The highest mean concentration at site 1 (3811.87 ± 1556.56 mg/kg) was found in moss **(Fig 3.27)**.

Manganese concentration comparisons between moss and lichen at site 1 (P=0.016) showed statistically significant differences. No significant differences were found at site 2 (P=0.310) and 3 (P=0.151). Once again the highest mean concentration at site 1 (346.09 ± 149.80 mg/kg) was found in moss **(Fig 3.28)**.

Site 1 (P=0.008) showed statistically significant differences between moss and lichen, of which the highest mean concentration at that site (8.82 ± 2.01 mg/kg) was found in moss. No significant differences were found at site 2 (P=0.095) and 3 (P=0.548) when mean Cu concentrations were compared **(Fig 3.29)**.

No statistically significant differences at any of the sites: 1 (P=0.548), 2 (P=0.310) and 3 (P=0.310) were found in Zn concentrations when moss and lichen was compared **(Fig 3.30)**.

### *3.2.3.4) pH and Moisture*

pH of the soil was slightly acidic (6.19) to (6.39) and the moisture % of the soil ranged from a relatively moist 21.57 % to a moist 40.33 %.

**Table 3.12:** pH and moisture % of soil of Newlands forest for the sampling occasion in May 2014.

| NEWLANDS FOREST | SOIL | |
|---|---|---|
| Site 1 | **pH** | 6.19 |
| | *Moisture* | *21.57* |
| Site 2 | **pH** | 6.28 |
| | *Moisture* | *23.5* |
| Site 3 | **pH** | 6.39 |
| | *Moisture* | *40.33* |

Moisture % of leaf litter ranged from a relatively moist 55.7 % to a moist 72.2 %.

**Table 3.13:** Moisture % of the leaf litter of Newlands forest for the sampling occasion in May 2014.

| NEWLANDS FOREST | % | LEAF LITTER |
|---|---|---|
| Site 1 | Moisture | 55.7 |
| Site 2 | Moisture | 72.23 |
| Site 3 | Moisture | 64.02 |

### *3.2.3.5) Weather data from the South African Weather Service in the vicinity of Newlands for Newlands forest*

**Table 3.14:** Weather data in the vicinity of Newlands forest for the sampling occasion in May 2014.

| SAMPLING SITES | SAMPLING OCCASION | | | |
|---|---|---|---|---|
| | DATE | DEGREES (°C) | PRECITATION (MM) | WIND SPEED (KM/H) |
| **NEWLANDS FOREST** | | | | |
| Site 1 | 15-May-14 | 14 | 0.3 | Light breeze |
| Site 2 | 16-May-14 | 13 | 0 | Light breeze |
| Site 3 | 17-May-14 | 24 | 0 | No wind |

### *3.2.3.6) Soil characterization for Newlands forest sites 1, 2 and 3*

**Table 3.15:** Characterization of soil for Newlands forest sites for the sampling occasion in May 2014.

| SAMPLING | (%) | | | | | | | WATER RETENTION | | |
|---|---|---|---|---|---|---|---|---|---|---|
| SITES | Clay | Silt | Fine sand | Medium sand | Coarse sand | Rock (v/v) | Classification | 10kPa | 100kPa | mm/m |
| NEWLANDS FOREST | | | | | | | | | | |
| Site 1 | 7 | 12 | 41.8 | 22 | 17.2 | 23.1 | LmSa | 18.93 | 10.30 | 86.2 |
| | | | | | | | | | | |
| Site 2 | 3 | 4 | 24.7 | 43.3 | 25 | 2.0 | Sa | 13.63 | 7.30 | 63.3 |
| | | | | | | | | | | |
| Site 3 | 9 | 18 | 44.8 | 18.24 | 10 | 18.6 | LmSa | 23.14 | 13.03 | 101.1 |

### 3.2.4) THE THREE FORESTS

#### *3.2.4.1) Comparisons of metal concentrations in soil, leaf litter and sentinel organisms between the three forests: Platbos, Orange Kloof and Newlands.*

The mean metal concentrations in soil, leaf litter and sentinel organisms for each forest for the sampling occasion (May 2014) are presented in **Tables 3.16 to 3.20**. No millipedes were found at Platbos forest. The metal concentrations for the sites within each forest were pooled to calculate the mean concentrations for each forest. Metal concentrations are expressed in mg/kg.

***a) Al***

**Table 3.16:** The mean Al concentrations (mg/kg) (± SD) in soil, leaf litter and sentinel organisms for forests for the sampling occasion in May 2014.

| FOREST | | SOIL | LEAF LITTER | MOSS | LICHEN | MILLIPEDES |
|---|---|---|---|---|---|---|
| Platbos | Mean | $^{a}$1216.31 | $^{a}$373.83 | $^{a}$1063.37 | $^{a}$718.25 | NA |
| | *SD* | *429.36* | *369.81* | *508.09* | *434.98* | *NA* |
| Orange Kloof | Mean | $^{b}$6350.19 | $^{a}$666.55 | $^{a}$1412.31 | $^{a}$908.89 | $^{a}$616.22 |
| | *SD* | *4079.85* | *651.94* | *1485.69* | *482.58* | *564.34* |
| Newlands | Mean | $^{b}$5939.05 | $^{a}$1044.03 | $^{a}$1857.43 | $^{a}$819.95 | $^{a}$479.08 |
| | *SD* | *4506.7* | *1214.77* | *1396.02* | *484.82* | *391.74* |

Statistical signifcant differences (P<0.050) between the three forests are indicated with different superscripted letters. Comparisons were done separately for the soil, leaf litter and sentinel organisms. NA=Not Available. N=5.

When soil was compared between forests, statistically significant differences were found in Al concentrations between Orange Kloof and Platbos and Newlands and Platbos (P=<0.001). The lowest mean concentration in soil was found at Platbos forest (1216.31 ± 429.36 mg/kg). No statistically significant differences were found between Orange Kloof and Newlands **(Table 3.16)**.

There were no statistically significant differences found in Al concentrations between the 3 forests in leaf litter (P=0.127); moss (P=0.245) and lichen respectively (P=0.433) **(Table 3.16)**.

Millipede comparisons between Orange Kloof and Newlands forests revealed no statistically significant differences (P=0.709) in Al concentrations **(Table 3.16)**.

***b) Fe***

**Table 3.17:** The mean Fe concentrations (mg/kg) (± SD) in soil, leaf litter and sentinel organisms for forests for the sampling occasion in May 2014.

| FOREST | | SOIL | LEAF LITTER | MOSS | LICHEN | MILLIPEDES |
|---|---|---|---|---|---|---|
| Platbos | Mean | $^{a}$1298.96 | $^{a}$331.85 | $^{a}$846.15 | $^{a}$663.37 | NA |
| | *SD* | *487.39* | *384.03* | *544.06* | *360.69* | *NA* |
| Orange Kloof | Mean | $^{b}$3356.68 | $^{b}$356.38 | $^{a}$1028.42 | $^{a}$836.16 | $^{a}$254.03 |
| | *SD* | *2614.58* | *275.61* | *746.8* | *404.32* | *136.96* |
| Newlands | Mean | $^{c}$7265.36 | $^{c}$1019.89 | $^{b}$2182.51 | $^{a}$1062.38 | $^{b}$583.82 |
| | *SD* | *4735.92* | *1212.73* | *1661.96* | *658.67* | *409.96* |

Statistical signifcant differences (P<0.050) between the three forests are indicated with different superscripted letters. Comparisons were done separately for the soil, leaf litter and sentinel organisms. NA=Not Available. N=5.

Pairwise multiple comparisons showed statistically significant differences in soil between all the forests (P<0.05) during the sampling occasion. The highest mean Fe concentration (7265.36 ± 4735.92 mg/kg) was found at Newlands **(Table 3.17)**.

Iron concentration comparisons of leaf litter showed statistically significant differences between Newlands and Platbos, Newlands and Orange Kloof, as well as between Orange Kloof and Platbos forests (P=0.048). A mean Fe concentration of 1019.89 ± 1212.73 mg/kg was found at Newlands, which was the highest of the three forests **(Table 3.17)**.

Statistically significant differences were found when moss was compared between Newlands and Platbos, as well as Newlands and Orange Kloof (P<0.05). No significant differences were, however found between Orange Kloof and Platbos in terms of Fe concentrations (P=0.015). Newlands forest, again had the highest mean concentration (2182.51 ± 1661.96 mg/kg). The lowest mean concentration was found at Platbos (846.15 ± 544.06 mg/kg) **(Table 3.17)**.

Lichen comparisons between forests showed no statistically significant differences between any of the forests (P=0.433) with regard to Fe concentrations **(Table 3.17)**.

Millipedes at Orange Kloof and Newlands forests were compared and statistically significant differences in Fe concentrations were found between them (P=0.016). The mean Fe concentration found at Newlands forest (583.82 ± 409.96 mg/kg) was higher than the mean concentration at Orange Kloof (254.03 ± 136.96 mg/kg) **(Table 3.17)**.

***c) Mn***

**Table 3.18:** The mean Mn concentrations (mg/kg) (± SD) in soil, leaf litter and sentinel organisms for forests for the sampling occasion in May 2014.

| FOREST | | SOIL | LEAF LITTER | MOSS | LICHEN | MILLIPEDES |
|---|---|---|---|---|---|---|
| **Platbos** | **Mean** | **$^{a}$55.15** | **$^{a}$43.01** | **$^{a}$43.5** | **$^{a}$16.79** | **NA** |
| | *SD* | *28.85* | *31.72* | *14.15* | *13.14* | *NA* |
| **Orange Kloof** | **Mean** | **$^{b}$224.24** | **$^{b}$394.16** | **$^{b}$315.04** | **$^{b}$234.58** | **$^{a}$70.62** |
| | *SD* | *184.56* | *225.3* | *271.61* | *234.52* | *76.78* |
| **Newlands** | **Mean** | **$^{c}$707.94** | **$^{c}$854.34** | **$^{c}$432.97** | **$^{b}$214.31** | **$^{b}$159.63** |
| | *SD* | *374.14* | *321.51* | *219.47* | *175.24* | *119.47* |

Statistical signifcant differences (P<0.050) between the three forests are indicated with different superscripted letters. Comparisons were done separately for the soil, leaf litter and sentinel organisms. N=5; NA=Not Available.

Pairwise multiple comparisons showed statistically significant differences in Mn concentrations in soil between all the forests (P<0.05). The mean Mn concentration (707.94 ± 376.14 mg/kg) was the highest at Newlands forest and the lowest mean concentration of (55.15 ± 28.85 mg/kg) was found at Platbos forest **(Table 3.18)**.

During this sampling session, comparisons of leaf litter showed statistically significant differences in Mn concentrations between all the forests (P<0.05). The mean Mn concentration of 43.01 ± 31.72 mg/kg at Platbos was lower than at the other forests. Newlands had the highest mean concentration of 854.34 ± 321.51 mg/kg **(Table 3.18)**.

Significant differences in Mn concentrations were found between all three forests in terms moss comparisons (P<0.05). Newlands forest, once again showed the highest mean concentration (432.97 ± 219.47 mg/kg) **(Table 3.18)**.

Lichen comparisons between forests showed statistically significant differences in Mn concentrations between the following: Newlands vs Platbos and Orange Kloof vs Platbos (P<0.05), with the exception of Newlands vs Orange Kloof forests (P>0.05). The lowest mean concentration was found at Platbos (16.79 ± 13.14 mg/kg) **(Table 3.18)**.

Millipedes between Orange Kloof and Newlands forests were compared and it was found that they differed significantly from each other (P=0.003) in terms of Mn concentrations. A higher mean concentration (159.63 ± 119.47 mg/kg) was found at Newlands forest **(Table 3.18)**.

***d) Cu***

**Table 3.19:** The mean Cu concentrations (mg/kg) (± SD) in soil, leaf litter and sentinel organisms for forests for the sampling occasion in May 2014.

| FOREST | | SOIL | LEAF LITTER | MOSS | LICHEN | MILLIPEDES |
|---|---|---|---|---|---|---|
| **Platbos** | **Mean** | **$^{a}$1.01** | **$^{a}$2.81** | **$^{a}$1.6** | **$^{a}$0.9** | **NA** |
| | *SD* | *0.65* | *1.4* | *1.22* | *1.14* | *NA* |
| **Orange Kloof** | **Mean** | **$^{b}$18.62** | **$^{b}$4.48** | **$^{b}$4.79** | **$^{b}$5.15** | **$^{a}$7.23** |
| | *SD* | *40.66* | *1.37* | *1.97* | *2.46* | *4.69* |
| **Newlands** | **Mean** | **$^{c}$17.35** | **$^{c}$7.67** | **$^{c}$10.62** | **$^{b}$6.13** | **$^{a}$10.27** |
| | *SD* | *27.88* | *2.52* | *3.92* | *4.67* | *5.73* |

Statistical signifcant differences (P<0.050) between the three forests are indicated with different superscripted letters. Comparisons were done separately for the soil, leaf litter and sentinel organisms. N=5; NA=Not Available.

Pairwise multiple comparisons showed statistically significant differences in Cu concentrations in soil between all the forests (P<0.05). Orange Kloof showed the highest mean concentration (18.62 ± 40.66 mg/kg) for this comparison **(Table 3.19)**.

Significant differences in Cu concentrations between all the forest sites (P<0.05) in terms of leaf litter comparisons were found during this sampling session. The lowest mean Cu concentration (2.81 ± 1.4 mg/kg) was found at Platbos **(Table 3.19)**.

In terms of Cu concentrations, statistically significant differences were found when moss was compared between all three forests (P<0.05). Platbos showed the lowest mean concentration (1.6 ± 1.22 mg/kg) and Newlands the highest (10.62 ± 3.92 mg/kg) **(Table 3.19)**.

Pairwise multiple comparisons between forests for Cu concentrations in lichens showed statistically significant differences between Newlands and Platbos and Orange Kloof and Platbos (P<0.05), but there were no statistically significant differences found when Newlands and Orange Kloof was compared (P>0.05). The mean Cu concentration at Newlands (6.13 ± 4.67 mg/kg) was higher than the mean concentration at Orange Kloof (5.15 ± 2.46 mg/kg) and Platbos (0.9 ± 1.14 mg/kg) **(Table 3.19)**.

Copper concentrations in millipedes between Orange Kloof and Newlands forests did not differ statistically significantly between the forests (P=0.147) **(Table 3.19)**.

***e) Zn***

**Table 3.20:** The mean Zn concentrations (mg/kg) (± SD) in soil, leaf litter and sentinel organisms for forests for the sampling occasion in May 2014.

| FOREST | | SOIL | LEAF LITTER | MOSS | LICHEN | MILLIPEDES |
|---|---|---|---|---|---|---|
| Platbos | Mean | $^{a}$8.65 | $^{a}$10.87 | $^{a}$12.19 | $^{a}$12.85 | NA |
| | *SD* | *6.25* | *5.63* | *4.62* | *5.24* | *NA* |
| Orange Kloof | Mean | $^{a}$11.35 | $^{b}$19.08 | $^{b}$20.08 | $^{b}$27.94 | $^{a}$94.09 |
| | *SD* | *6.11* | *12.78* | *5.84* | *14.19* | *23.02* |
| Newlands | Mean | $^{b}$25.92 | $^{c}$29.21 | $^{c}$31.32 | $^{c}$39.77 | $^{a}$116.38 |
| | *SD* | *14.05* | *11.34* | *7.54* | *12.1* | *38.38* |

Statistical signifcant differences (P<0.050) between the three forests are indicated with different superscripted letters. Comparisons were done separately for the soil, leaf litter and sentinel organisms. N=5; NA=Not Available.

Soil was compared between forests and statistically significant differences in Zn concentrations were found between Newlands vs Platbos and Newlands vs Orange Kloof ($P<0.05$). No significant differences were found between the Orange Kloof vs Platbos comparison ($P>0.05$). The highest Zn concentration was found at Newlands (25.92 ± 14.05 mg/kg) **(Table 3.20)**.

Comparisons of Zn concentrations in leaf litter showed statistically significant differences between all the forest sites ($P<0.05$). The Zn leaf litter concentration at Newlands (29.21 ± 11.34 mg/kg) was found to be higher than at Orange Kloof and Platbos **(Table 3.20)**.

In terms of Zn concentrations, statistically significant differences were found when moss was compared between all three forests ($P<0.05$). The mean concentration in moss was once again the highest at Newlands (31.32 ± 7.54 mg/kg) and the lowest at Platbos (12.19 ± 4.62 mg/kg) (**Table 3.20)**.

Comparisons of Zn concentrations in lichens between forests showed statistically significant differences between all the forests ($P<0.05$). The highest mean concentration (39.77 ± 12.1 mg/kg) was found at Newlands. Orange Kloof had a mean Zn concentration of 27.94 ± 14.19 mg/kg and the mean concentration at Platbos was 12.85 ± 5.24 mg/kg **(Table 3.20)**.

In May 2014 no statistically significant differences were found in millipedes in terms of Zn concentrations between Orange Kloof and Newlands forests ($P=0.056$) when compared (**Table 3.20)**.

## 3.3 DISCUSSION

### 3.3.1 Metal contamination found in Platbos, Orange Kloof and Newlands forests

It is a well studied fact that a major source of the metals, Al, Fe, Mn, Cu and Zn are vehicle related, whether it be from vehicle exhaust (Adachi and Tainoshob, 2004), petrol (Nogueira and Röllin, 2011), tires, brakes and parts wear, or even resuspended road dust (Peden, 2002). These metals are quite often observed in a polluted related pattern of decreasing metal concentrations with distance from roads and decreasing traffic volume (Dierkes and Geiger, 1999; Turer and Maynard, 2003; Li, 2006; Kluge et al., 2014) and similar patterns were also noticed in this current study at all three forests. From these patterns one may go as far as to say that the origin of these metals are highly likely from vehicle exhaust and non-exhaust emissions, emitted from the vehicle related activities within the vicinity of the sites, and may thus have contributed to the metal load (Forman, 2000; Lee et al., 2012).

At the Platbos forest, the mean concentrations of Al, Fe, Mn, Cu and Zn found in most of the sentinel organisms, as well as soil and leaf litter showed a tendency to be constantly higher at the first site, which is in close proximity of a gravel road, showing the pollution pattern mentioned above. The concentrations then become lower as one goes deeper into the forest to sites 2 and 3. There is, however a minor gravel road in the vicinity of site 2, as well as camp sites in the vicinity of site 3. Except for Al in soil and leaf litter and Mn in leaf litter, most of the concentrations of these metals found at Platbos did not exceed general background concentrations reported in soil or concentrations cited in the literature of studies done in unpolluted forests. This is discussed below under the headings for Platbos: metals in soil, leaf litter, moss and lichen respectively **(Table 3.1).**

Platbos forest is not located close to any major sources of industrial pollution. However, site 1 is located in the vicinity of the gravel road leading up to the parking area for hikers entering the forest, as well as for visitors to the wholesale nursery. Cars are generally parked right in front of this open entrance to the forest. This road extends further for visitors to the Old Olive cabin, private houses and makes a turn to go to the two camp sites. The road also functions as a service road and is therefore

used by heavy vehicles on a small scale. It is not a main road with high vehicle density or even high traffic volume, but rather a road that exerts a traffic type of behaviour, categorized by frequent braking and acceleration (Apeagyei et al., 2011) This type up traffic behaviour is associated with non-exhaust emissions of metals such as Fe, Cu and Zn from brake wear (Johansson et al., 2009).

A pollution pattern observed from the metals, Al, Fe, Mn, Cu and Zn measured at Orange Kloof forest displayed constant higher concentrations at site 1 in the vicinity of a couple of gravel roads in most of the soil, leaf litter and sentinel organisms. The metal concentrations, except for Al and Fe, became lower as one crossed the Disa River and moved up the mountain to sites 2 and 3 **(Table 3.6)**.

Orange Kloof is located approximately twenty five kilometres from the City of Cape Town, a major City with industrial sources of pollution that includes high vehicle traffic (Abdul-Wahab and Yaghi, 2004). The first site is more than 300 m away from the parking area for busses with regular tour groups, a starting point for hiking trails, as well as a major traffic circle and roads in Constantia. The gravel road leading up to site 1 is used by visitors to a tented camp and SANParks maintenance vehicles. Lawn mowers, weed eaters and chain saws are also frequently operated in this area. The vehicles, machinery and driving styles in the parking area, circle, as well as the road leading up to site 1 are generally associated traffic volumes and traffic behaviour (frequent braking and acceleration) (Apeagyei et al., 2011) and high concentrations of vehicle related metals are therefore found in road dust samples (Charlesworth et al., 2003).

Soil, leaf litter and sentinel organisms at Newlands forest showed a tendency to have higher concentrations of the metals Al, Fe, Mn, Cu and Zn at the first site, once again showing a pattern of pollution. Slightly higher concentrations of Al and Fe were observed at site 2 close to the contour path. Site 3, higher up the mountain displayed the lowest mean metal concentrations **(Table 3.11).**

Newlands forest, approximately 9 km away from the City of Cape Town is surrounded by air pollution related sources that include particulate emissions from industries, construction, exhaust and non-exhaust emissions from vehicles and

vehicle wear (Abdul-Wahab and Yaghi, 2004). Although site 1 is the closest to the parking area and major highway (De Waal Drive), the picnic area in the vicinity of site 2 is a high activity area, especially over weekends. Site 2 is also close to the contour path, which is used by SANParks maintenance vehicles. Newlands forest and specifically site 2 is much closer to and more open to wind and pollution arising from the City of Cape Town, which may explain some of the higher concentrations of metals found at this site. It is evident in this pattern that air pollution is not confined to the city, but remote areas like forest ecosystems can be reached via long range transport through the atmosphere (Steinnes and Friedland, 2006)

In addition to the metals from vehicles, road dust includes finely ground minerals of the road's parent material derived from natural and anthropogenic sources, which accumulate on the road surface as a result of road surface abrasion (Auerbach et al., 1997; Pant and Harrison, 2013). The road dust may be resuspended into the air by turbulence from passing vehicles, shearing stress of the tires or wind (Kupiainen, 2007), thereby increasing metal concentrations (Adachi and Tainoshob, 2004). Wind currents may carry mobilized dust for hundreds of meters (Auerbach et al., 1997), although maximum dust and pollution attenuation occurs within a 100 m (Tamm and Troedsson, 1955; Yin et al., 2011), which may explain the higher metal concentrations found at Platbos site 1 and the decreasing concentrations at sites 2 and 3, as well as the pattern of higher metal concentrations found at Orange Kloof site 1 and the decreasing concentrations at sites 2 and 3. It is also quite possibly the reason for the higher metal concentrations found at Newlands site 1 and the decreasing concentrations at site 3.

Aluminium and Fe concentrations may have been enhanced by soil parent material (Pierson and Brachaczek, 1983), which is evident in a clear pattern of the overall higher levels of these two metals found in the soil, leaf litter, moss and lichen samples. These concentrations were considerably higher in relation to the mean concentrations of Mn, Cu and Zn found in the same samples at the same sites. The Al and Fe concentrations were, however significantly higher in soil than in the leaf litter, moss and lichen samples. Similar patterns were found by other authors, who attributed this phenomenon to lithogenic elements such as Al and Fe being associated with soil minerals (Adachi and Tainoshob, 2004; Manno et al., 2006;

Brun et al., 2010; Amato et al., 2011). According to Lantzy and MacKenzie (1979) Al is such a prominent element in the earth's crust that the natural weathering processes far exceed the anthropogenic contribution of releases to air, water and land. Al is the third most abundant and Fe the fourth most abundant elements in the earth's crust (Dockery et al., 1993; Boudot et al., 1996). It has also been suggested that sparse forests with less dense vegetation better accumulate lithogenic elements brought by dust, as in the case with Platbos having many hiking paths with good visibility. Orange Kloof and Newlands forests have areas that are covered by dense tree canopies, but are in many areas relatively exposed, causing the forest to be susceptible to contamination from road dust via wind). (Heinrichs and Mayer, 1977; Lovett and Lindberg, 1984; Rea et al., 2001; Ukonmaanaho et al., 2001; Kupiainen, 2007; Gandois et al., 2010, 2014; Platbos, 2016).

Manganese concentrations on the other hand were significantly higher than the mean Cu and Zn concentrations found in all the samples and sites, which may also be due to Mn being from crustal origin and therefore further contributing to the Mn concentration levels (Pacheco et al., 2002). The presence of a number of small Mn deposits, especially on the Table Mountain slopes in Cape Town above Chapman's Peak Drive near Hout Bay may also have contributed to higher concentrations. The mine, however closed down many years ago (Jones, 2010). It should nevertheless be noted that Mn losses due to chemical weathering are minor and increased Mn concentrations in soil are rather due to increasing atmospheric inputs (Herndon et al., 2011).

### 3.3.2 Platbos forest

#### *3.3.2.1 Metals in the soil of Platbos forest*

The lithogenic elements, Al (1722.48 ± 327. 71 mg/kg) and Fe (1885.75 ± 331.17 mg/kg) showed elevated concentrations in soil at all three sites, which may have been enhanced by the soil parent material (Pierson and Brachaczek, 1983). However, site 1 in the vicinity of the gravel road displayed the overall highest concentrations, indicating contamination by pollution related sources (Dierkes and Geiger, 1999; Turer and Maynard, 2003; Li, 2006; Kluge et al., 2014).

The concentrations found at Platbos were significantly lower than background concentrations that are generally found in soil for Al ranging from approximately 7000 to over 100 000 mg/kg (Sorenson et al., 1974; USGS, 1984) and Fe ranging from 2945 to 9686 mg/kg (Yang et al., 2012).

Aluminium concentrations at site 1 and 2 did, however exceed the concentration of 991.96 mg/kg found by Drabek et al. (2003) when testing for possible methods of Al speciation in forest soils in Northern Bohemia. Aluminium causes soil acidity (Ma et al., 2001) and Al toxicity in soils with very low acidity may cause serious forest damage (Boudot et al., 1996). The pH in Platbos soil ranged from slightly acidic (6.48) to alkaline (7.28), which do not pose any threats to the organisms in the forest at this stage **(Table 3.2)**.

Iron concentrations of up to 9760 mg/kg were found in a forest in Poland surrounding a major city (Magiera et al., 2007), which is significantly higher than the concentrations found in this study.

Site 2 showed the highest mean concentrations of Mn (76.28 ± 17.29 mg/kg), Cu (1.13 ± 0.57 mg/kg) and Zn (12.6 ± 4.52 mg/kg) in soil. These concentrations were only slightly higher than the concentrations found at site 1. Site 2 is situated deeper into the forest, further away from the busier gravel road than site 1, but is close to a minor gravel road turning off to the camp sites. Road dust from this minor road may have increased the metal concentrations at site 2 arising from the vehicles going to the campsites (Adachi and Tainoshob, 2004; Kupiainen, 2007).

The metal concentrations found in Platbos soil were significantly lower when compared to the following general background concentrations for soil: Mn (40-900 mg/kg) (Nogueira and Röllin, 2011), Cu (5-70 mg/kg) (ATSDR, 2004) and Zn (10-300 mg/kg) (IZA, 2015), as well as concentrations found in unpolluted French forests of Mn (446.71-1648.91 mg/kg), Cu (17.70-19.95 mg/kg) and Zn (97.45-140.48 mg/kg) (Gandois et al., 2014) **(Table 3.1)**.

### *3.3.2.2 Metals in the leaf litter of Platbos forest*

The highest mean Al (815.59 ± 311.89 mg/kg) and Fe (785.36 ± 352.36 mg/kg) concentrations in leaf litter were found at site 1. The concentrations decreased at site 2 and even more at site 3, which correlate with the decreasing concentrations of these lithogenic elements, in the soil at site 1, 2 and 3 (Adachi and Tainoshob, 2004; Manno et al., 2006; Brun et al., 2010; Amato et al., 2011). However, the concentrations of Al and Fe at site 1 were significantly higher than the concentrations found at sites 2 and 3. The higher Al and Fe concentrations in the litter suggests vehicle related and enhanced metal deposition to the vegetation at site 1, which in all probability came from the frequent braking and acceleration from vehicles using this gravel road on a daily basis (Monaci et al., 2000; Peachey et al., 2009). Brun et al. (2010) attributed this occurrence to elements being released in and possibly lost from the litter, due to a slower rate than the decomposition.

Aluminium concentrations of 73 mg/kg in leaf litter, reported by Nikula et al. (2010) were found in rural forest litter in Helsinki, which are significantly lower than were found in this study. A mean Fe concentration of 1540 mg/kg, also found in an unpolluted forest in Helsinki exceeded the concentrations found at Platbos forest (Hristovskia et al., 2014).

Mean Mn (70.72 ± 41.34 mg/kg), Cu (3.95 ± 1.39 mg/kg) and Zn (16.04 ± 6.77 mg/kg) concentrations in leaf litter were the highest at site 1, which is the site that received the most vehicle related inputs (Adachi and Tainoshob, 2004; Kupiainen, 2007).

Hristovskia et al. (2014) in an unpolluted forest in Finland reported significantly lower Mn (0.94 mg/kg), comparable Cu (Cu 4.08 mg/kg) and significantly higher Zn (96.36 mg/kg) concentrations in their study **(Table 3.1)**.

### *3.3.2.3 Comparison of metal concentrations in soil and leaf litter*

Comparisons between soil and leaf litter showed that the mean metal concentrations of elements associated with soil minerals, (Al, Fe) were constantly higher in soil than in leaf litter at most of the sites, which according to Brun et al. (2010) is usually an indication of the large pools of elements located in soil minerals and organic matter.

Copper mean concentrations, however showed a tendency to be higher in leaf litter at most of the sites and may be explained by Cu's high affinity for organic matter (Nriagu, 1979; Adriano, 1986; Slooff et al., 1989; Alloway, 1990).

Zinc concentrations also showed a tendency to be higher in leaf litter at most of the sites. Metal enrichment in leaf litter, especially Zn may be due to polluted soil underneath the leaf litter (Scheid et al., 2009), moving upward via microbiota, thus enriching the leaf litter (Lomander and Johansson, 2001; Kaila et al., 2012) **(Figs 3.1 to 3.5 & Table 3.1)**.

#### *3.3.2.4 Metals in the moss of Platbos forest*

Aluminium (1275.41 ± 389.04 mg/kg) mean concentrations in moss were the highest at site 1 and Fe (1056.66 ± 753.12 mg/kg) concentrations the highest at site 3. These concentrations were significantly higher than the concentrations found at site 2. High levels of Al in moss may be as a result of the resuspension of soil particles in the ambient air, arising from the already high concentrations of the crustal elements at these sites (Jacques et al., 2008). The concentration levels may also have been higher due to metal inputs at site 1 from the vehicle traffic on the gravel road and site 3 from the vehicle traffic from the campsites (Apeagyei et al., 2011).

The concentrations of Al and Fe were generally comparable to the following concentrations found in unpolluted forests in France for: Al (386.13-1633.35 mg/kg) and Fe (280.13-989.07 mg/kg) (Gandois et al., 2014).

The highest mean Mn (55.19 ± 7.91 mg/kg), Cu (2.88 ± 1.24 mg/kg) and Zn (16.14 ± 3.37 mg/kg) concentrations in moss were found at site 1, the site receiving the most vehicle related pollution (Schauer et al., 2006; Apeagyei et al., 2011).

The mean concentrations in Platbos moss were lower than the Mn (97.40-172.81 mg/kg), Cu (3.78-8.20 mg/kg) and Zn (24.31-34.72 mg/kg) concentrations found in unpolluted French forests (Gandois et al., 2014) **(Table 3.1)**.

### *3.3.2.5 Metals in the lichen of Platbos forest*

Aluminium (986.22 ± 618.35 mg/kg) and Fe (855.20 ± 507.25 mg/kg) mean concentrations in lichen displayed the highest values at sites 1 and 3, which may be as a result of resuspension of soil particles in the surrounding air (Jacques et al., 2008). Metal inputs at site 1 from the vehicle traffic on the gravel road and site 3 from the vehicle traffic from the campsites may also have enhanced the concentrations (Apeagyei et al., 2011).

The concentrations were similar to the mean Al (988.32-2364.13 mg/kg) and Fe (759.13-1347.08 mg/kg) concentrations found by Gandois et al. (2014) in unpolluted forests in France.

Site 3 displayed the highest mean Mn (22.81 ± 20.16 mg/kg) concentrations, however site 1 showed only slightly lower concentrations. Copper (1.86 ± 1.43 mg/kg) and Zn (16.59 ± 7.1 mg/kg) concentrations in lichen were the highest at site 1. These sites receive metal inputs from the vehicle traffic on the gravel road and site 3 from the vehicle traffic from the campsites and may have contributed to the higher concentrations (Apeagyei et al., 2011).

Similar concentrations of Mn (25.14-29.29 mg/kg), Cu (4.66-6.88 mg/kg) and Zn (22.05-30 mg/kg) were found by Gandois et al. (2014) in relatively clean forests in France **(Table 3.1)**.

### *3.3.2.6 Comparison of metal oncentrations in moss and lichen*

Moss and lichen comparisons showed higher accumulation in moss than in lichen as were also found by other authors (Bargagli et al., 2002; Basile et al., 2008) and may be due to several factors. Lichens and mosses differ in trace metal accumulation capabilities (Nieboer et al., 1978; Beckett and Brown, 1984; Bargagli et al., 2002), even between different species of lichen and moss. The moss, *Hypnym cupressiforme* has a dense carpet, which is known to absorb pollutants, especially metals directly from atmospheric deposition (Sacharová and Suchara, 1998).

Zinc, however was enriched in lichen at most of the sites and may be due to biological recycling, via through fall (Gandois et al., 2014) **(Figs 3.6 to 3.10 & Table 3.1)**.

#### *3.3.2.7 Millipedes in Platbos forest*

There were no pill millipedes found at any of the sites during this sampling session, even though these diplopods have been seen the previous year during a rainy site visit. Pill millipedes are usually found in habitats with high moisture and dense leaf litter in a loam soil, but they can survive dry conditions by burrowing deeper into the soil and rolling into a ball (SANBI, 2016). The absence of pill millipedes during this sampling session could possibly be attributed to the low moisture content of 4.16 % to 9.99 % in soil, the sparse layer of organic material observed and absence of rain **(Table 3.4)** at the time of sampling **(Table 3.1)**.

### 3.3.3 Orange Kloof forest

#### *3.3.3.1 Metals in the soil of Orange Kloof forest*

The highest concentrations of the crustal elements, Al (9086.99 ± 1467.33 mg/kg) were found at site 1 and Fe (5713.17 ± 3363.57 mg/kg) at site 3, although all three sites displayed elevated concentrations. The natural crustal origin of these elements may have enhanced the Al and Fe concentrations (Pierson and Brachaczek, 1983), although the higher concentrations at site 1 and the more exposed site 3, may have resulted from pollution related sources (Dierkes and Geiger, 1999; Turer and Maynard, 2003; Li, 2006; Kluge et al., 2014).

The concentrations of Al and Fe were also unusually high at site 1 and site 3 in relation to site 2. Site 3 is situated higher up the mountain in the vicinity of a path leading to the edge of the forest. This path opens up to a fynbos area overlooking Hout Bay and the ocean. It is highly likely for wind to have created a tunnel effect through the forest that could quite easily have reached site 3. It is also possible that wind currents carrying air pollution that include particulate emissions from mineral soils, industries, construction, exhaust and non-exhaust emissions from vehicles and vehicle wear caused the higher Al and Fe concentrations at site 3 (Abdul-Wahab and Yaghi, 2004; Kupiainen, 2007).

Aluminium concentrations found at the sites were comparable to the lower concentration of background concentrations found in soil for Al ranging from about 7000 to over 100 000 mg/kg (Sorenson et al., 1974; USGS, 1984) and Fe ranging from 2945 to 9686 mg/kg (Yang et al., 2012).

An Al concentration of 991.96 mg/kg, significantly lower than in this study was found by Drabek et al. (2003) in forest soils in Bohemia. Low acidity and high Al levels in soil (Ma et al., 2001) may result in severe forest damage (Boudot et al., 1996). The pH in Orange Kloof soil ranged from slightly acidic (6.48) to alkaline (7.64) **(Table 3.7)**, which is not currently a cause for concern.

Iron concentrations did not exceed concentrations of up to 9760 mg/kg found in a forest surrounding a major city in the most urbanized region of Poland (Magiera et al., 2007) **(Table 3.6)**.

Site 2 showed the highest concentrations of Mn (258.26 ± 298.35 mg/kg) in soil. The higher concentrations may be due to its crustal origin and Mn deposits present on the mountain (Jones, 2010), which is a prime source of metals (Li et al., 2013).

Copper (34.30 ± 62.56 mg/kg) and Zn (18.19 ± 3.37 mg/kg) concentrations were the highest in soil at site 1. These metals are associated with traffic behaviour (Apeagyei et al., 2011), which is a prominent activity in the vicinity of site 1 and may have added to the higher concentrations of those metals.

The metal concentrations found at Orange Kloof were comparable to the following general background concentrations for soil: Mn (40-900 mg/kg) (Nogueira and Röllin, 2011), Cu (5-70 mg/kg) (ATSDR, 2004) and Zn (10-300mg/kg) (IZA, 2015). The concentrations were, however significantly lower than the Mn (446.71-1648.91 mg/kg) and Zn (97.45-140.48 mg/kg) concentrations, but comparable to the Cu (17.70-19.95 mg/kg) concentrations found in clean forests in France during the assessment of environmental influence of metals (Gandois et al., 2014) **(Table 3.6)**.

### *3.3.3.2 Metals in the leaf litter of Orange Kloof forest*

Mean concentrations of Al (969.88 ± 987.83 mg/kg) in leaf litter were the highest at site 1, which are most likely vehicle related, as a result of the high traffic in the vicinity of the site (Apeagyei et al., 2011). The concentrations at site 3 were, however significantly higher than at site 2. Fe (407.21 ± 271.52 mg/kg) mean concentrations were only slightly higher at site 3 than at site 1. Site 3 is more exposed and situated in the vicinity of the open area overlooking Hout Bay and most likely receives additional metal inputs from windblown dust (Abdul-Wahab and Yaghi, 2004; Kupiainen, 2007). Due to the lithogenic origin of Al and Fe, these concentrations correlated with the highest concentrations of these metals, also found in soil at site 1 for Al and site 3 for Fe (Adachi and Tainoshob, 2004; Manno et al., 2006; Brun et al., 2010; Amato et al., 2011).

A concentration of 73 mg/kg for Al found in rural forest litter in Southern Finland polluted by major emission sources such as traffic and power production (City of Helsinki Urban Facts, 2009) was reported by Nikula et al. (2010). This concentration was significantly lower than were found in this study. The Fe concentration in this study, however exceeded the concentration of 1540 mg/kg in reported in unpolluted Finnish forest litter by Hristovskia et al. (2014).

Manganese (583.51 ± 223.2 mg/kg) concentrations in leaf litter were significantly higher at the more secluded site 2 in comparison with the other two sites. The higher concentrations may be as a result of its crustal origin (Pacheco et al., 2002) and Mn deposits located naturally on the mountain (Jones, 2010).

Copper (5.48 ± 1.64 mg/kg) mean concentrations were also the highest at site 2 and may be explained by Cu's high affinity for organic matter (Nriagu, 1979; Adriano, 1986; Slooff et al., 1989; Alloway, 1990).

Zinc (30.34 ± 17.47 mg/kg) concentrations being the highest at site 1 suggests contamination from vehicle traffic in the vicinity of the site (Apeagyei et al., 2011).

Manganese concentrations in this study were comparable to Mn concentrations found in a forest ecosystem in Medvednica Nature Park, between 385-1070 mg/kg

(Jelaska et al., 2007). Concentrations of Cu (4.08 mg/kg) were comparable and Zn (69.63 mg/kg) significantly higher in the unpolluted Finnish forest (Hristovskia et al., 2014) **(Table 3.6)**.

#### *3.3.3.3 Comparison of metal concentrations in soil and leaf litter*

Metal concentrations of the elements associated with soil minerals, such as Al and Fe were constantly higher in soil than in leaf litter at most of the sites when compared. Brun et al. (2010) suggested that elements associated with soil minerals are usually related to the large pools of elements found naturally in soil minerals and organic matter.

Manganese concentrations were mostly higher in leaf litter than in soil and may be due to physico-chemical processes in the tree canopy (Avila and Rodrigo, 2004) or sun leaves containing higher Mn concentrations than shade leaves (McCain and Markley, 1989).

Copper mean concentrations showed a tendency to be higher in leaf litter at most of the sites and may be explained by Cu's high affinity for organic matter (Nriagu, 1979; Adriano, 1986; Slooff et al., 1989; Alloway, 1990).

Zinc mean concentrations were mostly higher in leaf litter at most of the sites, which may be due to polluted soil underneath the leaf litter (Scheid et al., 2009), made possible by upward movement via microbiota (Lomander and Johansson, 2001; Kaila et al., 2012) **(Figs 3.11 to 3.15 & Table 3.6)**.

#### *3.3.3.4 Metals in the moss of Orange Kloof forest*

Aluminium (2089.52 ± 2542.12 mg/kg) and Fe (1385.26 ± 1198.83 mg/kg) concentrations in moss were the highest at site 3, which is the site in the vicinity of the open fynbos area overlooking Hout Bay and the Atlantic Ocean. The higher concentrations may thus have resulted from industrial emissions and the geological origin of wind, soil and dust (Bekteshi et al., 2015). Iron is an essential plant micronutrient and background concentrations in mosses will be present as a result of internal cycling from old to new growing tissue (Harmens et al., 2015), which may have played a role in the elevated concentrations.

The concentrations of Al and Fe exceeded the following concentrations found in unpolluted forests in France for: Al (386.13-1633.35 mg/kg) and Fe (280.13-989.07 mg/kg) (Gandois et al., 2014).

The highest mean Mn (633.32 ± 241.13 mg/kg) and Cu (6.79 ± 1.74 mg/kg) concentrations were found at site 2, at the more secluded site. Mn enrichment of mosses is a reflection of Mn cycling in forest ecosystems and recretion by the canopy (Petty et Lindberg, 1990; Gandois et al., 2010), which accentuates the canopy influence on moss record (Schilling and Lehman, 2002).

The slightly enhanced Cu concentrations are most likely due to Cu being an essential plant micronutrient and therefore displaying background concentrations due to internal cycling from old to new growing tissue (Harmens et al., 2015),

Zinc (22.38 ± 6.8 mg/kg) concentrations in moss were the highest at site 1, which is the site in closest proximity of motor vehicle traffic (Schauer et al., 2006; Apeagyei et al., 2011).

The mean concentrations at Orange Kloof were significantly higher than the Mn (97.40-172.81 mg/kg) concentrations found in the unpolluted French forests, but comparable to the Cu (3.78-8.20 mg/kg) and Zn (24.31-34.72 mg/kg) concentrations found in the same forests (Gandois et al., 2014) **(Table 3.6)**.

#### *3.3.3.5 Metals in the lichen of Orange Kloof forest*

Aluminium (1119.94 ± 563.44 mg/kg) mean concentrations were the highest at site 1. Mean Fe (954.51 ± 368.14 mg/kg) concentrations in lichen were the highest at site 2, although these concentrations were only slightly higher than at site 1. Lichens have a long life span and older lichens may have had a longer accumulation time (Armstrong and Bradwell, 2010), which may be the reason for the slightly higher concentrations at site 2. Re-suspension of soil particles in the atmosphere (Jacques et al., 2008) or/and contributions of vehicle traffic in the vicinity of site 1 may have been the origin of the high Al and Fe concentrations (Apeagyei et al., 2011).

The concentrations found in this study were comparable to the mean Al (988.32-2364.13 mg/kg) and Fe (759.13-1347.08 mg/kg) concentrations found by Gandois et al. (2014) in forests that were deemed unpolluted.

The highest mean Mn (459.67 ± 279.1 mg/kg) concentration was found at site 2. Soil, leaf litter and moss also displayed elevated concentrations of Mn at this site and may be due to the elements' crustal origin (Pacheco et al., 2002) and Mn deposits on Table Mountain.

Copper (6.11 ± 1.29 mg/kg) and Zn (41.46 ± 14.76 mg/kg) concentrations in lichen were the highest at site 1, reflecting vehicle related pollution (Kupiainen, 2007).

The Mn concentrations in this study exceeded the Mn (25.14-29.29 mg/kg) concentrations found by Gandois et al. (2014) in forests in France by a significant amount. Copper (4.66-6.88 mg/kg) concentrations were comparable and Zn (22.05-30 mg/kg) concentrations significantly lower in this study **(Table 3.6)**.

#### *3.3.3.6 Comparison of metal concentrations in moss and lichen*

Moss and lichen comparisons determined that accumulation was higher in moss than in lichen. Similar accumulation behaviour was confirmed by (Bargagli et al., 2002; Basile et al., 2008). Mosses absorb pollutants, especially metals directly from atmospheric deposition and the species used in this study, *Hypnym cupressiforme* with its dense carpet is known to accumulate high concentrations of metals (Sacharová and Suchara, 1998).

Zinc however, was enriched in lichen at most of the sites and may be due to biological recycling, via through fall, which is a usual occurrence in forest lichens (Gandois et al., 2014) **(Figs 3.16 to 3 20 & Table 3.6).**

#### *3.3.3.7 Metals in the millipedes of Orange Kloof forest*

Millipedes showed unusually high mean Al concentrations of (1358.76 ± 147.92 mg/kg) mg/kg at site 1 in comparison with the millipedes at site 2 (179.55 ± 153.67 mg/kg) and (310.35 ± 157.19 mg/kg) site 3. The explanation for the higher metal content may be due to plants and organisms accumulating metals (Heikens et al.,

2001), which are taken up by higher trophic level consumers, thereby enhancing the metal exposure. The millipedes (Diplopoda) consuming the polluted litter, (Da Silva Souza et al., 2014) could therefore be responsible for the higher concentrations of Al found in them at site 1. Fe (409.00 ± 31.6 mg/kg) concentrations in millipedes were the highest at site 1, but significantly lower than Al concentrations, also found at site 1. There were no concentrations for comparison found for Al and Fe in millipedes in the literature.

Manganese (133.21. ± 113.18 mg/kg) and Zn (102.47. ± 18.89 mg/kg) mean concentrations were the highest at site 2. Copper (10.84. ± 6.63 mg/kg) mean concentrations were higher at site 3.

The following metal concentrations in different millipedes were found in unpolluted forests in the Netherlands for Cu: 306-585 mg/kg and Zn: 152-827 mg/kg. According to Hobbelen et al. (2004) the Cu concentrations were elevated and the Zn concentrations comparable to reference concentrations found in the literature. These concentrations exceeded the concentrations measured in millipedes in this study **(Table 3.6)**.

### 3.3.4 Newlands forest

#### *3.3.4.1 Metals in the soil of Newlands forest*

Elevated levels of the lithogenic elements, Al (8679.72 ± 2002.8 mg/kg) and Fe (10593.39 ± 2475.37 mg/kg) were found in soil at all three sites, although the highest concentrations were found at site 2. The natural geological origin may have enhanced the Al and Fe concentrations (Pierson and Brachaczek, 1983), although the higher concentrations at site 2 is most likely as a result of pollution (Dierkes and Geiger, 1999; Turer and Maynard, 2003; Li, 2006; Kluge et al., 2014). Site 2, being closer to the contour path and picnic area is relatively open to wind currents carrying air pollution from the City of Cape Town that includes, particulate emissions from industrial and urban activities (Abdul-Wahab and Yaghi, 2004; Kupiainen, 2007).

The Al concentrations were comparable to the lower concentration of background concentrations found in soil for Al ranging from about 7000 to over 100 000 mg/kg

(Sorenson et al., 1974; USGS, 1984) and Fe were comparable with the higher background concentration ranging from 2945 to 9686 mg/kg (Yang et al., 2012).

A forest in Poland, also used recreationally and surrounding a major urban and industrial area of Central Europe, have suffered chemical degradation due to anthropogenic activities arising from such a large urbanized city. Dust, containing metals from industrial and urban origin accumulated mostly in the top soil of these forests. Fe concentrations for example, of up to 9760 mg/kg was reported by Magiera et al. (2007), which is significantly lower than the concentrations found at site 2. It is thus highly likely that site 2 was contaminated through metal contaminated dust arising from the City of Cape Town, as were found in the Polish forest.

The Al concentrations found at sites 1 and 3 exceeded the concentration of 991.96 mg/kg found by Drabek et al. (2003) in forest soils in Northern Bohemia. Site 3, being more protected was, however more comparable. Low soil acidity and Al (Ma et al., 2001) may result in severe forest damage (Boudot et al., 1996). The pH in Newlands soil was, however only slightly acidic (6.19 to 6.39) **(Table 3.12)**, which at this stage is not low enough to cause concern.

Manganese (997.06 ± 511.37 mg/kg) and Zn (29.99 ± 23.59 mg/kg) concentrations in soil were significantly higher at site 3 compared to the other two sites. Site 3 is the more secluded site, higher up in the mountain and concentrations may have been enhanced by natural factors, such as soil minerals, which an important metal source (Li et al., 2013).

Copper (34.57 ± 46.00 mg/kg) concentrations were the highest at site 2, suggesting vehicle traffic pollution (Adachia and Tainoshob; 2004, Schauer et al., 2006) at this relatively exposed site (Kupiainen, 2007).

The metal concentrations found at Newlands were comparable to the following general background concentrations for soil: Mn (40-900 mg/kg) (Nogueira and Röllin, 2011), Cu (5-70 mg/kg) (ATSDR, 2004) and Zn (10-300mg/kg) (IZA, 2015). The concentrations were also comparable to the Mn (446.71-1648.91 mg/kg)

concentrations, lower than Zn (97.45-140.48 mg/kg) and higher than concentrations of Cu (17.70-19.95 mg/kg) found in unpolluted French forests during the assessment of environmental influence of metals (Gandois et al., 2014) **(Table 3.11)**.

### *3.3.4.2 Metals in the leaf litter of Newlands forest*

The lithogenic elements Al (2404.33 ± 1241.9 mg/kg) and Fe (2387.36 ± 1229.8 mg/kg) showed the highest concentrations in leaf litter at site 1. The concentrations also correlated with the higher concentrations of Al and Fe found in soil at site 1 (Adachi and Tainosho, 2004; Manno et al., 2006; Brun et al., 2010; Amato et al., 2011), which receives metal inputs, most likely from vehicle related emissions (Abdul-Wahab and Yaghi, 2004).

Nikula et al. (2010) reported a mean concentration of 73 mg/kg for Al found in rural forest litter in Southern Finland polluted by major emission sources such as traffic and power production (City of Helsinki Urban Facts, 2009). The Al concentration is this study exceeded the concentration found in the Finnish forest. A mean concentration for Fe of 1540 mg/kg was found in unpolluted Finnish forest litter by Hristovskia et al. (2014), when studying the decomposition of leaf litter and its relation to metals. This Fe concentration was significantly lower than the Newlands Fe concentration found in this study.

Manganese (1142.39 ± 350.81 mg/kg) concentrations were significantly higher at site 3 than at site 1 and 2 and correlates with the higher concentrations of Mn found in soil at the same site. The Mn concentrations were also relatively high at site 2 in the vicinity of the exposed contour path and open area overlooking the City of Cape Town, which receives major metal inputs from vehicle traffic pollution (Adachia and Tainoshob; 2004, Schauer et al., 2006) (Kupiainen, 2007).

Copper (8.39 ± 3.35 mg/kg) concentrations were the highest at site 1 and is most likely from exhaust and non-exhaust emissions from vehicles and vehicle wear arising from the major surrounding highways (Abdul-Wahab and Yaghi, 2004).

Zinc (33.77 ± 14.22 mg/kg) concentrations also displayed the highest concentrations in leaf litter at site 3, although it was not by a significant amount in comparison with

the other two sites. The higher concentrations at the more protected site may be as a result of Zn moving upward from the polluted soil into the leaf litter via microbiota (Lomander and Johansson, 2001; Kaila et al., 2012)

Manganese concentrations in this study were comparable to Mn concentrations found in a forest ecosystem in Medvednica Nature Park, between 385-1070 mg/kg (Jelaska et al., 2007). Concentrations of Cu (4.08 mg/kg) were comparable and Zn (69.63 mg/kg) significantly higher in the clean Finnish forest (Hristovskia et al., 2014) **(Table 3.11)**.

### *3.3.4.3 Comparison of metal concentrations in soil and leaf litter*

Comparisons between soil and leaf litter showed that metal concentrations of elements associated with soil minerals, such as Al and Fe were constantly higher in soil than in leaf litter at most of the sites. Brun et al. (2010) suggests that elements associated with soil minerals are usually related to the large pools of elements found naturally in soil minerals and organic matter. Soil receives major atmospheric inputs, thus acting as a sink for contaminants (Xing et al., 2004; Zhang et al., 2013). Lower metal concentrations in leaf litter as opposed to soil may also be caused by elements being released in and possibly lost from the litter, due to a slower rate than the decomposition (Brun et al., 2010).

The leaf litter at all the sites in this forest had a higher Mn content compared to the soil. Higher Mn content in leaves could be explained by physico-chemical processes in the tree canopy, (Avila and Rodrigo, 2004) or sun leaves possibly having higher Mn concentrations than shade leaves in some tree species (McCain and Markley, 1989).

Zinc content in leaf litter was also higher than were found in the soil, which may possibly be due to Zn moving upward from the polluted soil into the leaf litter via microbiota (Lomander and Johansson, 2001; Kaila et al., 2012) **(Figs 3.21 to 3.25)**.

### *3.3.4.4 Metals in the moss of Newlands forest*

Aluminium (3255.41 ± 1373.22 mg/kg) and Fe (3811.87 ± 1556.66 mg/kg) mean concentrations in moss were the highest at site 1 and is possibly as a result of

emissions from industrial and urban activities (Abdul-Wahab and Yaghi, 2004; Kupiainen, 2007). High levels of Al in moss may also have been enhanced by the resuspension of soil particles in the ambient air (Jacques et al., 2008).

The concentrations of Al and Fe exceeded the following concentrations found in unpolluted forests in France for: Al (386.13-1633.35 mg/kg) and Fe (280.13-989.07 mg/kg) (Gandois et al., 2014).

The highest mean Mn (611.13 ± 206.33 mg/kg) concentrations in mosses were found at the more secluded site 3. Mosses enriched by Mn is a reflection of Mn cycling in forest ecosystems and recretion by the canopy (Petty et Lindberg, 1990; Gandois et al., 2010). The more exposed site in the vicinity of the contour path, site 2 had the highest Cu (14.62 ± 3.76 mg/kg) and Zn (33.04 ± 9.15 mg/kg) mean concentrations, which may be attributed to air pollution related sources including particulate emissions from industries, construction, exhaust and non-exhaust emissions from vehicles and vehicle wear (Abdul-Wahab and Yaghi, 2004).

The Mn concentratiosn were significantly higher than the Mn (97.40-172.81 mg/kg) Cu (3.78-8.20 mg/kg) and Zn (24.31-34.72 mg/kg) concentrations found in the clean forests in France (Gandois et al., 2014) **(Table 3.11)**.

#### *3.3.4.5 Metals in the lichen of Newlands forest*

Aluminium (920.15 ± 655.29 mg/kg) and Fe (1185.57 ± 833.24 mg/kg) concentrations in lichen displayed the highest concentrations at site 2, which may be as a result of re-suspension of soil particles in the surrounding air (Jacques et al., 2008), as well particulate emissions from industries and vehicle traffic emissions arising from Cape Town (Abdul-Wahab and Yaghi, 2004).

The concentrations in this study were comparable to the mean Al (988.32-2364.13 mg/kg), but higher than Fe (759.13-1347.08 mg/kg) concentrations found in forests by Gandois et al. (2014).

Site 3 showed the highest mean Mn (348.45 ± 198.36 mg/kg) concentrations. Mn concentrations were the highest in soil, leaf litter and moss at this site and be

explained by the elements' crustal origin (Pacheco et al., 2002) and presence of Mn deposits on Table Mountain.

Copper (8.15 ± 5.69 mg/kg) and Zn (41.27 ± 10.43 mg/kg) concentrations in lichen were the highest at site 2.

The concentrations in this study exceeded the Mn (25.14-29.29 mg/kg), Cu (4.66-6.88 mg/kg) and Zn (22.05-30 mg/kg) concentrations found by Gandois et al. (2014) in unpolluted French forests **(Table 3.11)**.

### *3.3.4.6 Comparison of metal concentrations in moss and lichen*

Moss and lichen comparisons determined that accumulation was higher in moss than in lichen as were also found by (Bargagli et al., 2002; Basile et al., 2008). Pollutants, especially metals are absorbed by mosses directly from atmospheric deposition and the species used in this study, *Hypnym cupressiforme* with its dense carpet is known to accumulate high concentrations of metals (Sacharová and Suchara, 1998).

In a study done by Gandois et al., (2010a) in the Pyrenees, higher concentrations of Al and Fe were found in mosses and lichens compared to the other sites and were as a result of the high deposition of those elements in the areas, which concurs with our findings of higher metal Al and Fe concentrations at Newlands forest, which is the closest forest to the City of Cape.

Zinc, however was enriched in lichen at most of the sites and may be due to biological recycling, via through fall, which is common in lichens in forests (Gandois et al., 2014) **(Figs 3.25 to 3.30)**.

### *3.3.4.7 Metals in the millipedes of Newlands forest*

Mean Al (819.80 ± 462.64 mg/kg) and Fe (832.86 ± 516.34 mg/kg) concentrations in millipedes were unusually high at site 1 in comparison with the other two sites. The explanation for the higher metal content may be due to plants and organisms accumulating metals (Heikens et al., 2001), which are taken up by higher trophic level consumers, thereby enhancing the metal exposure. The millipedes (Diplopoda)

consuming the polluted litter, (Da Silva Souza et al., 2014) could therefore be attributed to the higher concentrations of Al found in them at site 1. There were no Al or Fe concentrations in millipedes cited in the literature for comparison.

Millipedes at site 2 had the highest concentrations of Mn (903.95 ± 127.44 mg/kg), Cu (15.09 ± 5.55 mg/kg) and Zn (148.41 ± 48.1 mg/kg).

Elevated concentrations of Cu (306-585 mg/kg) and comparable Zn (152-827 mg/kg) concentrations were found in millipedes in unpolluted forests in the Netherlands. These results were significantly higher than were found at the Newlands sites (Hobbelen et al., 2004) **(Table 3.11)**.

### 3.3.5 Comparison of the three forests in terms of metal concentrations in sentinel organisms

Platbos in Gansbaai, approximately a 170 km from Cape Town was the forest with the lowest mean metal concentrations in the soil, leaf litter and sentinel organisms in comparison with the two forests Orange Kloof and Newlands in close proximity of the City of Cape Town. Evidence in many studies supports findings of metals being transported over long distances, however the influence of emission hotspots like cities and factories decrease rapidly within kilometres (Cloquet et al., 2006; Aznar et al., 2008), explaining the reason for the much lower metal concentrations found at Platbos.

The same argument above is relevant for the opposite scenario and thus also explains the higher metal concentrations found at Orange Kloof and Newlands forests surrounded by a large City (Querol et al., 2004; Garimella and Deo, 2008) coupled with air pollution related sources (Abdul-Wahab and Yaghi, 2004). Newlands forest metal concentrations proved to have the highest values of the three forests and it was incidentally also the forest closest to the City of Cape Town. It is evident from this pattern that air pollution is not confined to the city, but supposedly remote areas like forest ecosystemsadjacent to cities are reachable via long range transport through the atmosphere (Steinnes and Friedland, 2006).

In industrialised regions, wooded areas such as Newlands forest are used recreationally, as well as a source of food such as edible mushrooms and fruits, which may pose an ecological risk to the food chain (Desaules et al. (2001). Newlands forest especially is popular both for recreation and mushrooms. Orange Kloof, being slightly further away from the city displayed the second highest metal concentrations.

Aluminium and Fe concentrations were consistently higher at all three forests in comparison with Mn, Cu and Zn concentrations. This phenomenon is an indication of its lithogenic origin and similar bioaccumulation behavior, which is strongly linked to the bedrock weathering process (Agnan et al., 2014). These metals both in fine and coarse particle fractions are major crustal components and consequently a major source of dusts and particulate matter (Kim et al., 2003; Wang et al., 2015). Windblown dust consists of re-suspension of mineral dust, which has a natural metal content that corresponds to the average metal concentration in the earth' crust. This fraction is generally high for metals such as Al and Fe associated with the earth' crust. The dust also contains historic atmospheric contributions of metals accumulated in soil and roadside dust (Ilyin et al., 2007).

Han et al. (2012) reported incidents of dust that were blown from deserts to populated cities in East Asia (Kwon et al., 2002; Kang et al., 2012). Such dust emissions from arid regions are known to be the main source of airborne Fe and particulate matter and the combustion of biomass and fuels are said to be a minor contributor (Luo et al., 2008; Mahowald et al., 2009). The Cape Flats in the Western Cape is open and characteristically sandy (Sheat, 1984), as well as prone to strong prevailing winds. The dust and particulate matter, arising from those areas, including historic atmospheric contributions of metals (Ilyin et al., 2007) could contain Al and Fe (Kim et al., 2003; Wang et al., 2015), which could quite possibly have been blown by the extremely strong prevailing winds in the direction of Table Mountain and settle in the forest ecosystems (Kwon et al., 2002; Kang et al., 2012), causing elevated Al and Fe concentrations.

#### *2.3.5.1 Comparison of the three forests in terms of metal concentrations in soil*

Al concentrations of 6350.19 ± 4079.85 mg/kg in soil were the highest at Orange Kloof forest and almost five times higher than the mean concentrations found in soil at Platbos forest, which served as control site for comparison. The concentrations were, however lower than background concentrations reported for soil ranging from approximately 7000 to over 100 000 mg/kg (Sorenson et al., 1974; USGS, 1984), but exceeded the concentration of 991.96 mg/kg found by Drabek et al. (2003) in Bohemian forest soils.

Newlands forest soil displayed the highest Fe concentrations (7265.36 ± 4735.92 mg/kg), which were almost six times higher than the concentrations found at Platbos forest. The background concentrations ranging from 2945 to 9686 mg/kg for Fe were comparable with the Fe concentrations found in at Newlands (Yang et al., 2012). Even higher Fe concentrations of up to 9760 mg/kg were reported by Magiera et al. (2007) in polluted forests surrounding a major urban and industrial area of Central Europe.

Orange Kloof and Newlands forests are surrounded by a major city and the pattern observed suggests contamination due to industrial pollution. The Al concentrations between Orange Kloof and Newlands were similar, but the Fe concentrations at Orange Kloof were significantly lower than Newlands concentrations, which is the closest forest to the City of Cape Town. The lithogenic elements may have played a role in the elevated concentrations (Agnan et al., 2014). It is also likely that particulate matter, containing metals were blown by the extremely strong prevailing winds in the direction of Table Mountain (Kwon et al., 2002; Kang et al., 2012) from the cape flats and the city, causing elevated Al and Fe concentrations

Manganese (707.94 ± 374.14 mg/kg) concentrations were twelve times higher than the concentrations found at Platbos. Even though concentrations of Mn may have been enhanced by its crustal origin (Pacheco et al., 2002), the pattern of increasing Mn concentrations closer to the city is a clear indication of vehicle related pollution (Thorpe and Harrison, 2008; Wik and Dave, 2009; Franco et al., 2013; Kumar et al., 2013; Pant and Harrison, 2013; Amato et al., 2014) that includes the gasoline additive methylcyclopentadienyl Mn tricarbonyl (MMT) (Nogueira and Röllin, 2011).

Soils enriched in Mn as a result of anthropogenic inputs, have the potential to negatively impact ecosystem function (Herndon et al., 2011). High Mn bioavailability in soils may cause Mn toxicity in plants and eventual dieback of trees (Horsley et al., 2000; Kogelmann and Sharpe, 2006).

The metal concentrations found at Newlands were almost as high as the general background concentrations of Mn for soil (40-900 mg/kg) (Nogueira and Röllin, 2011). Higher Mn concentrations of 446.71-1648.91 mg/kg were, however found by Gandois et al. (2014) in relatively clean forests.

Zinc (29.99 ± 23.59 mg/kg) concentrations were the highest at Newlands and the highest Cu concentrations (18.62 ± 40.66 mg/kg) were only slightly higher at Orange Kloof. These metals are emitted from traffic related sources (Thorpe and Harrison, 2008; Wik and Dave, 2009; Franco et al., 2013; Kumar et al., 2013; Pant and Harrison, 2013; Amato et al., 2014) and are most likely the route of contamination.

The concentrations were comparable to soil background concentrations for Cu (5-70 mg/kg) (ATSDR, 2004) and Zn (10-300 mg/kg) (IZA, 2015). Significantly higher Zn (97.45-140.48 mg/kg) and comparable Cu (17.70-19.95 mg/kg) concentrations were reported by Gandois et al. (2014) during the assessment of environmental influence of metals in clean forests **(Tables 3.16 to 3.20)**.

#### *3.3.5.2 Comparison of the three forests in terms of metal concentrations in leaf litter*

Aluminium (1044.03 ± 1214.77 mg/kg) and Fe (1019.89 ± 1212.73 mg/kg) mean concentrations in leaf litter were the highest at Newlands forest. Due to these metals' association with soil minerals, the concentrations correlated with the high Al and Fe concentrations found in the Newlands soil (Adachi and Tainosho, 2004; Manno et al., 2006; Brun et al., 2010; Amato et al., 2011). The concentrations being the highest at Newlands, the closest forest to Cape Town, however suggests contamination by industrial and vehicle related pollution (Abdul-Wahab and Yaghi, 2004).

Aluminium mean concentration of 73 mg/kg (Nikula et al., 2010) found in rural forest litter in Southern Finland polluted by major emission sources such as traffic and

power production (City of Helsinki Urban Facts, 2009) were significantly lower than were found in this study. Fe mean concentrations of 1540 mg/kg study found in clean Finnish forest litter exceeded the concentration found in this study (Hristovskia et al., 2014).

Manganese (854.34 ± 321.51 mg/kg) concentrations in leaf litter were the highest at Newlands and correlated with the high concentrations of Mn found in the soil. Some of the Mn concentrations may be from its natural origin (Pacheco et al., 2002). However, the concentrations elevate in closer proximity to Cape Town, which is an indication of vehicle related pollution (Thorpe and Harrison, 2008; Wik and Dave, 2009; Franco et al., 2013; Kumar et al., 2013; Pant and Harrison, 2013; Amato et al., 2014) that may include the gasoline additive methylcyclopentadienyl Mn tricarbonyl (MMT) (Nogueira and Röllin, 2011).

These concentrations were comparable to Mn concentrations found in a forest ecosystem in Medvednica Nature Park, between 385-1070 mg/kg (Jelaska et al., 2007).

Newlands also had the highest Cu (7.67± 2.52 mg/kg) and Zn (29.21± 11.34 mg/kg) concentrations in leaf litter, possibly due to exhaust and non-exhaust emissions from vehicles and vehicle wear (Abdul-Wahab and Yaghi, 2004).

Copper concentrations in this study were only slightly higher than were found in leaf litter in Finnish forests (4.08 mg/kg) (Hristovskia et al., 2014) and Zn (69.63 mg/kg) were lower than were found in the forests in Finland (Hristovskia et al., 2014) **(Tables 3.16 to 3.20)**.

#### *3.3.5.3 Comparison of the three forests in terms of metal concentrations in moss*

Aluminium (1857.43 ± 1396.02 mg/kg) and Fe (2182.51 ± 1661.96 mg/kg) mean concentrations in moss were the highest at Newlands, which might have been enhanced by the resuspension of soil particles in the atmosphere (Jacques et al., 2008). The concentrations being the highest at this forest in such close proximity of a

large city, is most likely due to industrial and vehicle related pollution (Abdul-Wahab and Yaghi, 2004).

The concentrations of Al and Fe were significantly higher than the following concentrations found in moss in forests in France that are deemed relatively clean: Al (386.13-1633.35 mg/kg) and Fe (280.13-989.07 mg/kg) (Gandois et al., 2014).

Manganese (432.97 ± 219.47 mg/kg), Cu (10.62 ± 3.92 mg/kg) and Zn (31.32 ± 7.54 mg/kg) concentrations in moss were the highest at Newlands. The pattern of pollution is clearly visible in the increasing concentrations of Mn, Cu and Zn with increasing traffic volume and industrial pollution in close proximity of a large city (Dierkes and Geiger, 1999; Turer and Maynard, 2003; Li, 2006; Kluge et al., 2014).

The mean concentrations at Newlands were significantly higher than the Mn (97.40-172.81 mg/kg) Cu (3.78-8.20 mg/kg) and Zn (24.31-34.72 mg/kg) concentrations found in the French forests (Gandois et al., 2014) **(Tables 3.16 to 3.20)**.

#### *3.3.5.4 Comparison of the three forests in terms of metal concentrations in lichen*

Aluminium (908.89 ± 482.58 mg/kg) concentrations in lichen were slightly higher at Orange Kloof, which correlates with the higher concentrations also found in soil at Orange Kloof. Fe (1062.38 ± 658.67 mg/kg) concentrations in lichen were the highest at Newlands and incidentally also correlates with the higher concentrations found in soil at Newlands. The resuspension of soil particles in the surrounding air in an area that already has elevated concentrations of these metals may have played a role in the higher concentrations of these metals found in lichen (Jacques et al., 2008).

The concentrations found in in this study were comparable to the mean Al (988.32-2364.13 mg/kg), but higher than Fe (759.13-1347.08 mg/kg) concentrations found in studies done in other forests (Gandois et al., 2014).

Manganese (234.58 ± 234.52 mg/kg) concentrations in lichen were slightly higher at Orange Kloof, which may be due to the Mn deposits on the mountain in Hout Bay in

close proximity of Orange Kloof forest. Copper (6.13 ± 4.67 mg/kg) and Zn (39.77 ± 12.1 mg/kg) concentrations were the highest at Newlands. Vehicle traffic is the most likely source of these metals found in high concentrations at Newlands (Thorpe and Harrison, 2008; Wik and Dave, 2009; Franco et al., 2013; Kumar et al., 2013; Pant and Harrison, 2013; Amato et al., 2014)

The concentrations in this study exceeded the Mn (25.14-29.29 mg/kg), Cu (4.66-6.88 mg/kg) and Zn (22.05-30 mg/kg) concentrations found by Gandois et al. (2014) in unpolluted French forests **(Tables 3.16 to, 3.20)**.

#### *3.3.5.5 Comparison of the three forests in terms of metal concentrations in millipedes*

Mean Al (616.22 ± 564.34 mg/kg) concentrations were the highest at Orange Kloof and Fe (583.82 ± 409.96 mg/kg) concentrations the highest at Newlands in millipedes. The explanation for the higher metal content may be due to plants and organisms accumulating metals (Heikens et al., 2001), which are taken up by higher trophic level consumers, thereby enhancing the metal exposure. The millipedes (Diplopoda) consuming the polluted litter, (Da Silva Souza et al., 2014) could therefore be attributed to the higher concentrations of Al found in them at site 1. There were no Al or Fe concentrations found for millipedes in the literature.

Millipedes at Newlands had the highest concentrations of Mn (159.63 ± 119.47 mg/kg), Cu (10.27 ± 5.73 mg/kg) and Zn (116.38 ± 38.38 mg/kg), which is the forest closest to Cape Town.

Concentrations of Cu (306-585 mg/kg) found in millipedes in unpolluted forests in the Netherlands were significantly higher in comparison with other reports and Zn (152-827 mg/kg) concentrations were comparable. These results were significantly higher than were found at the Newlands sites (Hobbelen et al., 2004) **(Tables 3.16, 3.20)**.

## 2.4 CONCLUSION

The results that were obtained from this study confirmed the occurrence of the metals Al, Fe, Mn, Cu and Zn at all three of the forests in the soil, leaf litter and sentinel organisms. The concentration levels in the current study were generally comparable to background concentrations and concentrations cited in the literature with some exceptions where those metal concentrations were exceeded, which was mostly at Newlands forest.

The two forests, which are closer to the City of Cape Town (Orange Kloof and Newlands), showed higher mean concentrations of metals than were found at Platbos forest, a 170 km away. Newlands, the closest forest to the City of Cape Town, received the most metal input of the three forests. Similarly, higher mean metal concentrations were found at the sites that were in the vicinity of higher traffic areas and lower concentrations at sites further away from vehicle related activities, which are located deeper into the forest. These metals are generally associated with soil minerals, vehicle traffic behaviour, exhaust and non-exhaust emissions and industries and clear patterns of metal concentrations becoming lower with distance from roads and traffic volume was observed.

The lithogenic elements, Al and Fe revealed high concentrations at all three forests at all the sites and were significantly higher than the concentrations of Mn, Cu and Zn, which is an indication of its crustal origin. However, the concentrations of these two metals were five to six times higher in the soil of Orange Kloof and Newlands forests, surrounded by a major city, compared to Platbos, suggesting contamination via air pollution. Al and Fe concentrations were also mostly higher at the first site, or at the sites, which were less dense in vegetation or more open to wind. Soil also generally displayed higher concentrations of Al and Fe in comparison with the leaf litter, moss, lichen and millipedes and is as a result of these metals association to soil minerals and its natural origin.

Manganese concentrations were also elevated, which may be due to its crustal origin and Mn deposits present on the mountain. However, the gasoline additive (MMT), emerging as a source of Mn pollution, may have played a role in enhancing

concentrations. Research also confirm that Mn losses due to chemical weathering are minor and that the increased Mn concentrations in soil are are as a result of increasing atmospheric inputs. Mn cycling in the forest were also observed in the high concentrations of this element found in mosses.

Higher accumulation by soil as opposed to leaf litter was observed, with the exception of Cu and Zn. This phenomenon, may be explained by Cu's high affinity for organic matter and Zn as a result of polluted soil underneath the leaf litter moving upward via microbiota, thus enriching the leaf litter. In some areas higher Mn content in leaves were observed and may be due to physico-chemical processes in the tree canopy or sun leaves possibly having higher Mn concentrations than shade leaves in some tree species.

Mosses showed higher accumulation than lichen. Their different different accumulation capabilities may have played a role, however the dense carpet of mosses offer a large area to absorb pollutants, especially metals directly from atmospheric deposition. High Al and Fe concentrations found in mosses and lichens were most likely as a result of the high deposition of those elements in the areas. Zinc, being enriched in lichen, however may be due to biological recycling, via through fall, which is common in lichens in forests.

High metal concentrations in millipedes correlated with the high metal concentrations in soil and leaf litter, which is possible when millipedes consume the polluted litter.

The sentinel organisms and especially the combined use of these organisms have proven to be reliable indicators of atmospheric pollution including the contaminations patterns that were observed.

The patterns of pollution found in this study are worth monitoring, especially Al levels in combination with pH and Mn, arising from motor vehicle traffic, considering Cape Towns' already high and increasing traffic volumes and economic activity, as well as the fact that Cape Town is already an area marked by distinction of species.

# CHAPTER FOUR

# DRY AND WET SEASON STUDY

# SEASONAL VARIATIONS OF METAL CONTAMINATION, INDUCED OXIDATIVE DAMAGE AND BIOACCUMULATION IN SOIL, LEAF LITTER AND SENTINEL ORGANISMS IN SOUTH AFRICAN FOREST POCKETS

## 4.1 INTRODUCTION

The health of forests as a whole, including their continuous growth and survival are under threat by air pollution, anthropogenic pressure and climate change. This can be seen by the pronounced effects recorded in many research studies done in forests, surrounded by rapidly expanding, urban areas and industrialization (Bytnerowicz et al., 2008). Air pollution behavior is influenced by a multitude of parameters, of which meteorological parameters are instrumental in the transport, diffusion and natural cleansing in the atmosphere. The different seasons with respect to temperature, amount of precipitation (Ambade, 2012, 2014), as well as wind speed and direction have been reported to significantly influence metal behaviour in fine and coarse particulate matter in the atmosphere (Harrison et al., 1997) and plays critical role in air pollution studies (Karar et al., 2006). Forest ecosystems may, furthermore be at risk from climate-related oxidative stress that amount from the different seasons that are found in Mediteranean climates, such as the drastic temperatures between summer and winter, drought and high irradiance (Tausz et al., 1998, 2007a,b; Bussotti, 2008).

Forest canopies capture aerosols and does so in even larger quantities per unit area than for example adjacent grasslands (Graustein and Armstrong, 1983; Belsky et al., 1989; Shaw et al., 1994; Fowler et al., 2004), which may cause more frequent enrichment of such soils via stemflow, throughfall and litter-fall (Mills et al., 2012). Evidence of such aerosol depositions have shown to contribute significantly to top soils of forests in the Amazon (Goudie and Middleton, 2001; Garrison et al., 2003) and southern Chile (Kennedy et al., 2002), which can magnify over the years, causing elevated concentrations in soil (Olowoyo et al., 2010).

Atmospheric deposition plays a major role in the build-up of metals in the top soil of forests (Bytnerowicz et al., 2008), even in supposedly pristine areas (De Vries et al.,

2002). Aerosols are highly enriched with metals (Pope, 2000; Saldiva et al., 2002; Shi et al., 2011), which are incidentally categorized as the most dangerous group of contaminants. Studying, therefore the interaction between metals and living organisms, the processes of their biogenic migration and consequently the effects thereof in ecosystems are thus of great significance (Weber and Karczewska, 2004).

The health of fauna and flora is directly affected by air pollution, but also indirectly through the contaminated soil and water that they are reliant on (DEADP, 2010). Such soils and plant surfaces become major sinks for these atmospheric pollutants and soil being the basis of the food chain, is the route by which biotoxic metals are transmitted to humans (Monaci et al., 2000). These pollutants are extremely dangerous to the health of humans and living organisms (Brunekreef and Holgate, 2002; Gurjar et al., 2010).

Forests are major sources of terrestrial biodiversity and climate mitigation is but one of the many important ecosystem services they provide (Venter and Venter, 2009). South African indigenous forests, covering only 0.56% of the land surface (Low and Rebelo, 1996) are of the most species-rich temperate forests in the world (Lawes et al., 2004). The afromontane forests in Table Mountain National Park are a good example of the many endemic species housed, which includes two moss species (Von Maltitz et al., 2003), arthropods and the critically endangered Table Mountain Ghost Frog (Pauw and Johnson, 1999). However, the Table Mountain Chain (Anderson and O'Farrell, 2012), which is internationally recognized for its extraordinary plant species diversity and endemism lies within the City of Cape Town, one of South Africa's largest urban areas (Helme and Trinder-Smith, 2006). With indigenous forests all over the world being under threat (Thompson et al., 2009), it is no wonder that the Table Mountain National Park forests are regarded as being of high conservation importance (Alston and Richardson, 2006).

The greater part of the population in the Western Cape resides in Cape Town and surrounding areas, which means that most of the emissions are generated in the Metro. This also means that the individual exposure to poor air quality is the highest in Cape Town. The main sources originate from industries and the transport sector,

but also from domestic fuels such as gas, wood and paraffin in low income township areas (StatsSA, 2013). The brown haze that is very often seen during the winter months in the Cape Town Metropolitan Area occurs when there is strong temperature inversions and limited wind dispersion, which contains gaseous and particulate pollution from these vehicular emissions (Wicking-Baird et al., 1997), industries and residential biomass burning (City of Cape Town, 2005). During the summer months the strong South Easterly wind blows dust, which exacerbates the problem by elevating the particulate matter levels and further contributes to $PM_{10}$ (DEADP, 2011).

Soils all over the world have deteriorated due to anthropogenic activities and when observing the state of air pollution in South Africa, this country's soils are not excluded (Papu-Zamxaka et al., 2010). Forty seven percent of the world's land is already moderately to very severely degraded, as are 22% of all forests, woodland, cropland and pasture (GLASOD, 2004). Soils and their innate, diverse communities are therefore in dire need of protection (Sijm et al., 2002; Römbke et al., 2005).

In Europe there is a growing awareness that nature can play a major role in combatting these environmental problems (EC, 2016) and are finding their solutions in the ecosystem services provided by urban and periurban forests, which can be instrumental in enhancing environmental quality (Costanza et al., 1998, De Groot et al., 2002, MA, 2005; Gómez-Baggethun and Barton, 2013). Urban and periurban forest vegetation is able to consistently reduce pollution levels by way of the uptake of gaseous pollutants and the adsorption of particulate matter on the surface of leaves (Nowak et al., 2014). However, the issue of the negative impacts of air pollution on forest health surrounded by urban areas and industrialization still remains (Bytnerowicz et al., 2008) and needs to be monitored.

Studies of atmospheric contamination are expensive due to the high cost of instruments and monitoring methods, notwithstanding the difficulties incurred by extensive spatial and temporal sampling (Anicic et al., 2011). Biological monitors can provide quantitative information on the atmospheric pollutant load in the environment (Sant'Ovaia et al., 2012) and for that reason biomonitoring methods based on using living organisms have become a viable option (Sujetoviene, 2015). Biological

indicators are then used for the detection, deposition, accumulation and distribution of these pollutants in the environment (Markert et al., 2000)**.**

The sentinel organisms, lichens and mosses are some of the biomonitors that are successfully used as indicators of contaminants in the air (Minger and Krahenbuhl, 1997; Conti and Cecchetti, 2001; Szczepaniak and Biziuk, 2003). Due to their different metal uptake and retention capabilities even better results are obtained when they are used in combination (Szczepaniak and Biziuk, 2003). Many researchers have, however found mosses to be the better choice after observing higher metal accumulation in mosses than in lichens (Coskun et al., 2009; State et al., 2012; Boltersdorf et al., 2014).

Mosses are universally used as bioindicators of atmospheric metal pollution (Mendil et al., 2009; Uyar et al., 2009; Sun et al., 2009a), as well as fore-warning elevated concentrations of metals (Bleuel et al., 2005; Sun et al., 2009b). The morphology and physiology of mosses enables them to accumulate copious amounts of metals through dry and wet deposition (Saxena et al., 2003). It should be noted that the desiccation of mosses during the dry season may alter the permeability of the plasma membrane, which may cause losses of intra-cellular nutrients (Bates, 1997; Beckett and Hoddinott, 1997), but is accentuated when rapid rehydration occurs in the wet season (Brown and Brumelis, 1996; Beckett and Hoddinott, 1997).

The moss *Hypnum cupressiforme* **(Fig 3.1)** with its dense carpet is found in the afromontane forests of Table Mountain, growing on tree trunks, logs, rocks and soil and are known to absorb pollutants, especially metals directly from atmospheric deposition (Sacharová and Suchara, 1998).

Many different lichen species have been used as biomonitors in air quality assessment research (Conti and Cecchetti, 2001; Garty, 2001; Conti et al., 2004), because they readily accumulate pollutants in their thallus. The entire surface of their thallus (Aznar et al., 2008; Conti et al., 2011) is used in the interception of allogeneic atmospheric materials dissolved in wet precipitation, dry depositions, and gaseous emissions (Nash, 2008). There is also a close correlation in accumulated pollutants with their atmospheric levels, which have proved the lichen's capability as an

effective biomonitor (Wolterbeek et al., 2003; Adamo et al., 2008; Godinho et al., 2009) *Parmotrema* **(Fig 3.2)** is a foliose lichen with short and broad, ciliate lobes and are commonly found on Table Mountain on trees, rocks, decaying wood and soil (Crespo et al*.,* 2010) and is also the genus used in this study.

Invertebrates such as millipedes have gained increased attention as bio-indicators to assess soil pollution (Hopkin et al., 1985; Godoy and Fontanetti, 2010; Nogarol and Fontanetti, 2010), as they are components of the edaphic fauna and are therefore constantly exposed to contaminants in the soil (Da Silva Souza et al., 2014). As decomposers in the trophic level they feed on detritus, fruits, organic matter and material of mineral origin. Millipedes are nocturnal, prefer humid environments and are generally found underneath fallen trunks and leaves (Schubart, 1942; Hoffmann et al., 2002; Ruppert and Barnes, 2005). In terrestrial ecosystems their role is significant with regard to soil aeration, decomposition of organic matter and nutrient recycling (Schubart, 1942; Hopkin and Read, 1992). Abundance and diversity of soil invertebrates in Mediterranean forests may differ depending on the season of sampling. This is due to the different climatic conditions, coupled with the various stages of the life cycles of organisms, which may affect the response of soil invertebrates to pollution. Variations in arthropod community structure in soils with different contamination levels have been reported. Despite that, information on the effects of climatic conditions on soil invertebrates in metal polluted soils are few and far between (Santorufo et al., 2012), which would make this current study valuable in that respect.

The bulky, pill shaped pill millipede *Sphaerotherium compressum* (Brandt, 1833) **(Fig 3.3)** used in this study is a diverse higher taxon of Diplopoda (Hoffman, 1980) and are also commonly found on Table Mountain in the afromontane forests (SANBI, 2016).

When monitoring the potential impact of a stressor in the environment on an ecosystem, the different levels of biological organization needs to be included (Moore et al., 2004), as metals play a significant role in the chemical, biological, biochemical, metabolic, catabolic and enzymatic reactions in living tissues (Hashmi et al., 2007). Using multiple biomarkers in ecotoxicological research (Lam, 2009) can

thoroughly assess exposure to contaminants and determine their impact on living organisms. Such indications give a more comprehensive and integrative analysis of biochemical and cellular effects, which are caused by environmental contaminants (Cazenave et al., 2009).

Oxidative stress biomarkers respond to a wide range of contaminants (Tsangaris et al., 2011), including metals. The toxic effect of metals in biological systems for example, may lead to the increased production of reactive oxygen species (ROS), affecting various cellular processes, primarily the functioning of the membrane system (Pinto et al., 2003; Valko et al., 2005). ROS plays a role in regulating cellular signalling via modulating redox status. Oxidative stress is defined as an imbalance between oxidants (like free radicals) and antioxidants in favour of oxidants, leading to a disruption of redox signalling and control and/or molecular damage (Sies, 1997). The impact of pollutants on organisms in field situations can thus be assessed using oxidative stress biomarkers (Regoli and Principato, 1995; Verlecar et al., 2008). It has, however been found that prolonged heat stress (Lushchak and Bagnyukova, 2006), freezing (Joanisse and Storey, 1996), physiological stress of anoxia (Hermes-Lima, 2004), estivation (Nowakowska et al., 2009) and seasonal variations (Verlecar et al., 2008) may also be conditions that changes the antioxidant defence systems in animals.

Living organisms developed an antioxidant defence system to balance the ROS that have formed naturally (Valavanidis et al., 2006). Enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POX), and ascorbate peroxidase, as well as non-enzymatic antioxidants with low molecular weights, such as proline, cysteine, non-protein thiol, ascorbic acid, and glutathione is able to reduce oxidative stress by scavenging ROS (Choudhury and Panda, 2004, 2005; Singh et al., 2006). Oxidative stresss causes modifications to important bio-molecules, such as proteins, lipids and genetic material (Sies and Cadenas, 1985). Membrane lipid peroxidation in the organisms can be estimated by measuring the content of malondialdehyde (MDA), which serves as an indicator of induced oxidative stress (Sujetovien and Galinyt, 2016).

Interaction between metals and constituents of the antioxidant defence systems plays a critical part in the ecotoxicological response of an organism to its environment (Regoli et al., 2006). Thus, such studies are imperative in the identification of biomarkers, which can serve as early warning systems for environmental monitoring. Seasonal fluctuations have been reported in such studies (Viarengo et al., 1991; Filho et al., 2001, Ramos-Vasconcelos et al., 2005).

South Africa has typically dry (annual average rainfall of 450 mm) soils (DEAT, 2006) and strong prevailing winds in which dust travels over enormous distances transporting high densities of soil-borne dust (Windfinder, 2014). Metals are blown in this manner to remote mountain areas via long-range atmospheric transport and accumulate in the soil and plants by wet or dry deposition (Gandois and Probst, 2012; Migon et al., 1997; Wu et al., 2011), which is the main source of pollution in natural forest areas (Gandois and Probst, 2012). Arthropod responses to abiotic properties have also been reported to differ significantly between seasons (Santorufo et al., 2014). The Cape Peninsula is characterized by a Mediterranean climate with dry summers and winter rainfall (Cowling et al., 1996) and seasonal variations in metal concentrations due to wind and rain have been reported (Keane et al., 2001). The information, thus derived from including seasonal differences in air pollution studies may be of utmost importance (Karar et al., 2006), with regard to metal behavior (Harrison et al., 1997) and climate-related induced oxidative stress in forest ecosystems (Tausz et al., 1998, 2007a,b; Bussotti, 2008).

The aims of this study were to compare the dry and wet season with regard to: (a) seasonal fluctuations of the concentrations of the metals Al, Fe and Mn; (b) the induced oxidative stress effect of metals, using two indicators, MDA - an oxidative lipid damage marker and tGSH level – an endogenous, non-ezymatic antioxidant associated with oxidative stress in the pill millipede, *Spaerotherium compressum*, the moss, *Hypnum cupressiforme* and the lichen, *Parmotrema sp.*

The sentinel organisms chosen for this study are due to their common presence in the forests under investigation: moss, *Hypnum cupressiforme* **(Fig 2.15)**, foliose lichen, *Parmotrema sp.* **(Fig 2.16)** and pill millipede, *Sphaerotherium compressum* **(Fig 2.18)**.

## 4.2 RESULTS

### 4.2.1 DRY SEASON: METAL CONTAMINATION AND BIOACCUMULATION

#### 4.2.1.1) ORANGE KLOOF FOREST

##### *4.2.1.1.1) Comparisons of metal concentrations in soil, leaf litter and sentinel organisms between sites C, 1, 2 and 3*

The mean metal concentrations in soil, leaf litter and sentinel organisms for sites C, 1, 2 and 3 of Orange Kloof forest for the dry sampling occasion in January 2015 are presented in **Table 4.1**. Metal concentrations are expressed in mg/kg.

**Table 4.1:** The mean metal concentrations (mg/kg) (± SD) in soil, leaf litter and sentinel organisms for sites C, 1, 2 and 3 of Orange Kloof forest for the dry sampling occasion in January 2015.

| METAL | | SOIL | | | | LEAF LITTER | | | | MOSS | | | | LICHEN | | | | MILLIPEDES | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Site C | Site 1 | Site 2 | Site 3 | Site C | Site 1 | Site 2 | Site 3 | Site C | Site 1 | Site 2 | Site 3 | Site C | Site 1 | Site 2 | Site 3 | Site C | Site 1 | Site 2 | Site 3 |
| Al | Mean | $^{cd}$13450.31 | $^{cd}$9990.24 | $^{ab}$1347.92 | $^{ab}$1030.06 | 220.42 | 357.25 | 303.50 | 136.93 | $^{bd}$1992.10 | $^{acd}$1566.82 | $^{bd}$1165.36 | $^{abc}$649.90 | 965.81 | 840.44 | 612.07 | 562.49 | $^{bcd}$3042.68 | $^{acd}$410.28 | $^{ab}$422.51 | $^{ab}$313.52 |
| | *SD* | *2851.26* | *2334.08* | *699.77* | *433.76* | *175.48* | *349.25* | *234.43* | *86.31* | *661.79* | *592.77* | *715.21* | *243.15* | *305.38* | *199.79* | *260.89* | *123.15* | *3935.36* | *179.13* | *193.24* | *302.70* |
| Fe | Mean | $^{bcd}$8016.54 | $^{acd}$3013.4 | $^{ab}$1081.87 | $^{ab}$1400.82 | 176.11 | 155.97 | 218.98 | 159.48 | 1694.47 | 2897.37 | 1219.81 | 723.81 | $^{bcd}$1115.87 | $^{ac}$636.42 | $^{abd}$538.46 | $^{ac}$524.75 | $^{bcd}$1556.14 | $^{ad}$210.89 | $^{ad}$280.57 | $^{abc}$485.61 |
| | *SD* | *1592.90* | *296.40* | *591.58* | *683.20* | *124.29* | *99.74* | *183.74* | *110.25* | *436.48* | *1813.88* | *682.10* | *392.78* | *417.65* | *153.39* | *178.36* | *111.80* | *2023.16* | *80.59* | *131.58* | *657.84* |
| Mn | Mean | 98.97 | 81.16 | 219.28 | 465.71 | $^{bcd}$64.97 | $^{acd}$226.12 | $^{abd}$457.22 | $^{abc}$1695.59 | $^{bcd}$77.51 | $^{acd}$181.16 | $^{ab}$527.39 | $^{ab}$673.74 | $^{bcd}$62.98 | $^{ac}$103.24 | $^{abd}$663.27 | $^{ac}$569.80 | $^{cd}$33.28 | $^{cd}$25.04 | $^{ab}$128.62 | $^{ab}$106.78 |
| | *SD* | *23.56* | *76.80* | *131.28* | *434.07* | *14.28* | *43.15* | *170.12* | *1041.97* | *21.77* | *96.75* | *190.86* | *402.20* | *16.77* | *27.88* | *987.71* | *532.93* | *26.22* | *6.76* | *74.22* | *61.17* |

Statistical signifcant differences (P<0.050) are indicated with different superscripted letters: site C=a, site 1=b, site 2=c, site 3=d. Comparisons were done separately for the soil, leaf litter and sentinel organisms. N=5.

***a) Soil***

There were statistically significant differences (P<0.05) found in Al concentrations for the comparisons: sites C (site C) vs 3; C vs 2; 1 vs 3 and 1 vs 2, but no statistical differences were found between sites C and 1 and 2 and 3 (P>0.05). The mean Al concentration at site C (13450.31 ± 2851.26 mg/kg) was significantly higher than at sites 1 (9990.24 ± 2334.08 mg/kg), 2 (1347.92 ± 699.77 mg/kg) and 3 (1030.06 ± 433.76 mg/kg) **(Table 4.1)**.

Pairwise multiple comparisons showed statistically significant differences in Fe concentrations between sites C and 1, 2 and 3; 1 and 2 and 1 and 3 (P<0.05). No significant differences were, however found in soil between sites 2 and 3 (P>0.05). The mean concentration for site C (8016.54 ± 1592.90 mg/kg) was significantly higher than at sites 1 (3013.40 ± 296.40 mg/kg), 2 (1081.87 ± 591.58 mg/kg) and 3 (1400.82 ± 683.20 mg/kg) **(Table 4.1)**.

The soil at sites C, 1, 2 and 3 did not differ significantly from each other in terms of Mn (P=0.123) concentrations when compared **(Table 4.1)**.

***b) Leaf litter***

Leaf litter at Orange Kloof was compared at all the sites, but no significant differences were found in Al (P=0.378) and Fe (P=0.904) concentrations for this sampling occasion **(Table 4.1)**.

Pairwise multiple comparisons showed statistically significant differences in Mn concentrations between all of the sites (P<0.05). The mean Mn concentration for site 3 (1695.59 ± 1041.97 mg/kg) was significantly higher than at the other three sites **(Table 4.1)**.

***c) Moss***

Site comparisons in terms of Al concentrations in moss revealed statistically significant differences for the comparisons of sites C vs 3, 1 vs 3 and 2 vs 3 (P<0.05). No statistically differences were detected between sites C and 1 and 2 and

sites 1 and 2 (P>0.05). The mean concentration at site C (1992.10 ± 661.79 mg/kg) was higher than were found at the other sites **(Table 4.1)**.

Iron (P=0.070) concentrations at Orange Kloof were not statistically different from each other when the four sites were compared during this sampling session **(Table 4.1)**.

Statistically significant differences in Mn concentrations were found between sites C and 1, 2 and 3, 1 and 3 and 1 and 2 (P<0.05), but no significant differences were found between sites 2 and 3 (P>0.05). Site 3 had the highest mean Mn concentration of 673.74 ± 402.20 mg/kg and site C the lowest mean concentration of 77.51 ± 21.77 mg/kg **(Table 4.1)**.

***d) Lichen***

No statistically significant differences in Al (P=0.066) concentrations between sites, C, 1, 2 and 3 were found in the dry season sampling **(Table 4.1)**.

Pairwise multiple comparisons showed statistically significant differences in Fe concentrations for the comparisons of sites C vs 1, 2 and 3 (P<0.05) with the exception of sites 1 vs 2 and 3 and 2 vs 3 (P>0.05). The mean concentration at site C (1115.87 ± 417.65 mg/kg) was significantly higher than at sites 1 (636.42 ± 153.39 mg/kg), 2 (538.46 ± 178.36 mg/kg) and 3 (524.75 ± 111.80 mg/kg) **(Table 4.1)**.

Statistically significant differences in Mn concentrations between sites C and 1, 2 and 3 (P<0.05) were found. No statistically significant differences were, however found between sites 1 and 3, 2 and 3 and 1 and 2 (P>0.05). The mean Mn concentration at site C (62.98 ± 16.77 mg/kg) was significantly lower than at sites 2 (663.27 ± 987.71 mg/kg) and 3 (569.80 ± 532.93 mg/kg) **(Table 4.1)**.

***e) Millipedes***

Pairwise multiple comparisons showed statistically significant differences in Al concentrations in millipedes between sites C and sites 1, 2 and 3 (P<0.05). No significant difference between sites 1 and 2, 1 and 3 and 2 and 3 (P>0.05) were

found. Site C had the highest mean Al concentration of 3042.68 ± 3935.36 mg/kg **(Table 4.1)**.

Statistically significant differences for Fe in millipedes were found in site comparisons for C vs 1, 2 and 3 ($P<0.05$), but there were no significant differences found between sites 1 vs 2, 1 vs 3 and 2 vs 3 ($P>0.05$). The mean Fe concentration at site C (1556.14 ± 2023.16 mg/kg) was significantly higher than at site 1 (210.89 ± 80.57 mg/kg), 2 (280.57 ± 131.58 mg/kg) and site 3 (485.61 ± 657.84 mg/kg) **(Table 4.1)**.

In terms of Mn concentrations at Orange Kloof, statistically significant differences were found between sites C vs 2; C vs 3; 1 vs 2 and 1 vs 3 ($P<0.05$). No significant differences were found between sites C vs 1 and 2 vs 3 ($P>0.05$). The mean concentration at site 1 (25.04 ± 6.76 mg/kg) was significantly lower than the concentrations found at sites 2 (128.62 ± 74.22 mg/kg) and 3 (106.78 ± 61.16 mg/kg) **(Table 4.1)**.

### *4.2.1.1.2) Comparisons of metal concentrations between soil and leaf litter at sites C, 1, 2 and 3*

Mean metal concentrations in soil and leaf litter at sites C, 1, 2 and 3 of Orange Kloof forest for the dry sampling occasion in January 2015 are shown in **Figs 4.1 to 4.3**. Statistical signifcant differences (P<0.050) between soil and leaf litter are indicated with an asterisk above the graph bars.

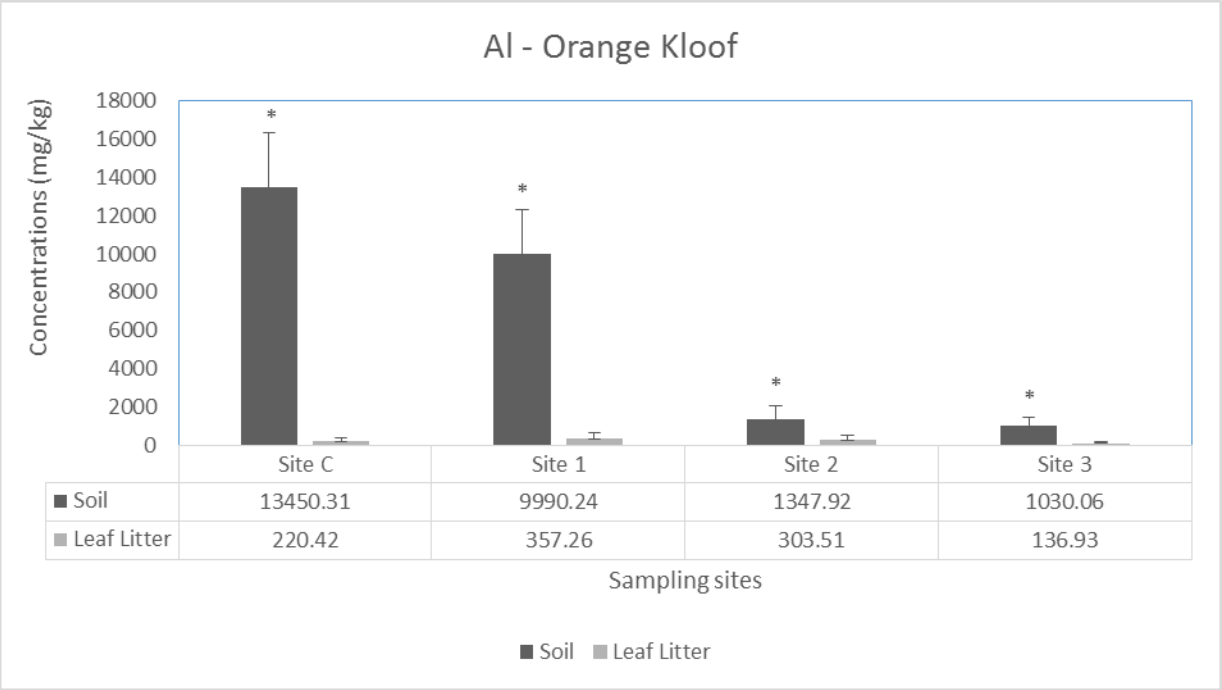


**Figure 4.1:** The mean Al concentrations (mg/kg) (± SD) in soil and leaf litter at sites C, 1, 2 and 3 of Orange Kloof forest for the dry sampling occasion in January 2015. N=5.

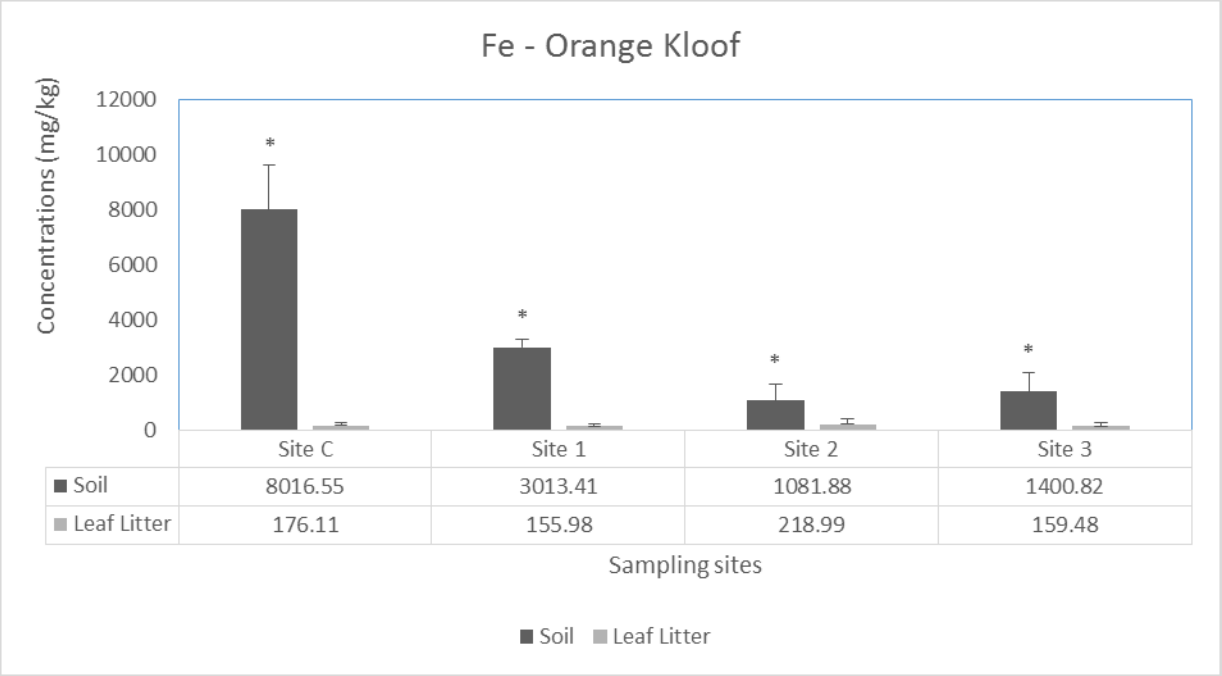


**Figure 4.2:** The mean Fe concentrations (mg/kg) (± SD) in soil and leaf litter at sites C, 1, 2 and 3 of Orange Kloof forest for the dry sampling occasion in January 2015. N=5.

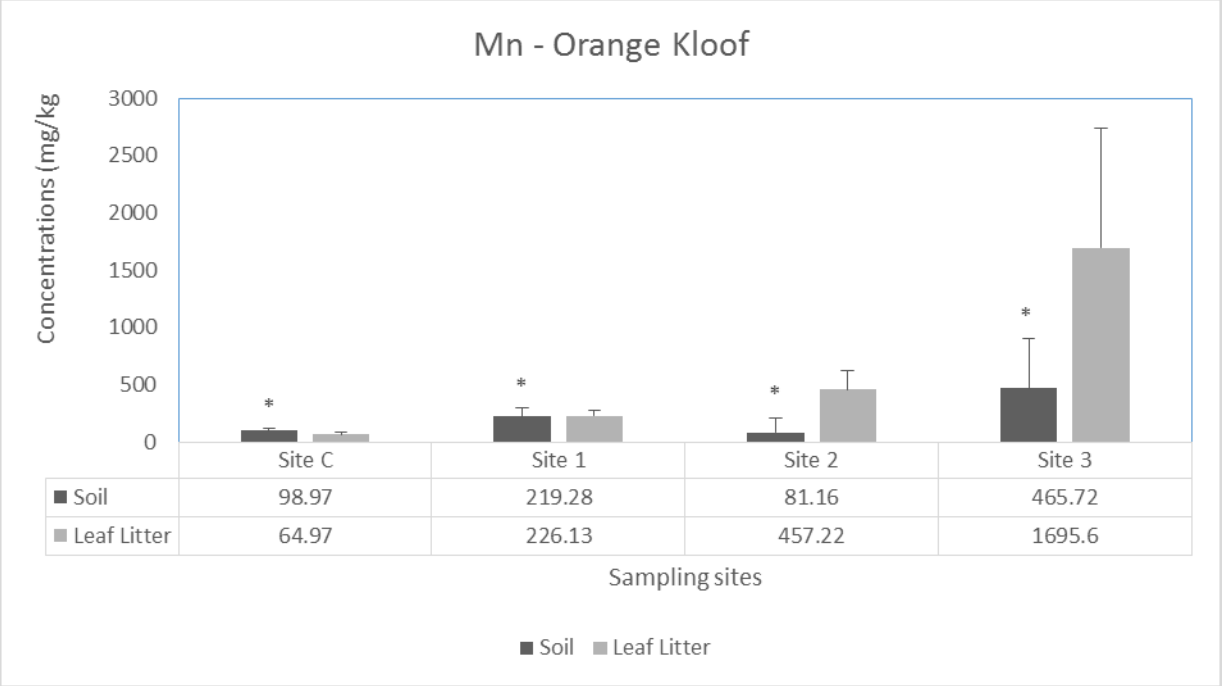


**Figure 4.3:** The mean Mn concentrations (mg/kg) (± SD) in soil and leaf litter at sites C, 1, 2 and 3 of Orange Kloof forest for the dry sampling occasion in January 2015. N=5.

When soil and leaf litter was compared in terms of Al concentrations, significant differences between each other at all the sites were found: site C (P=0.008), site 1 (P=0.008), site 2 (P=0.013) and site 3 (P=0.008). The mean concentrations were much higher in soil than in leaf litter at all the sites with site C showing the highest results (13450.31 ± 2851.26 mg/kg) **(Fig 4.1)**.

Leaf litter and soil showed statistically significant differences between each other at Orange Kloof sites C (P=0.008), 1 (P=0.008), 2 (P=0.014) and 3 (P=0.008) in terms of Fe concentrations. Fe concentrations were significantly higher in soil as opposed to leaf litter at all the sites. Site C showed the highest concentration of 8016.55 ± 1592.90 mg/kg **(Fig 4.2)**.

At Orange Kloof leaf litter and soil showed statistically significant differences between each other: sites C (P=0.025), 1 (P=0.032), 2 (P=0.038) and 3 (P=0.041) when Mn concentrations were compared. The concentrations in leaf litter at site 3 of 1695.60 ± 1041.97 mg/kg were significantly higher than in soil at the same site and was also the highest concentrations found **(Fig 4.3)**.

***4.2.1.1.3) Comparisons of metal concentrations between moss and lichen at sites C, 1, 2 and 3***

Mean metal concentrations in moss and lichen at sites C, 1, 2 and 3 of Orange Kloof forest for the dry sampling occasion January to March 2015 are shown in **Figs 4.4 to 4.6**. Statistical signifcant differences (P<0.050) between moss and lichen are indicated with an asterisk above the graph bars.

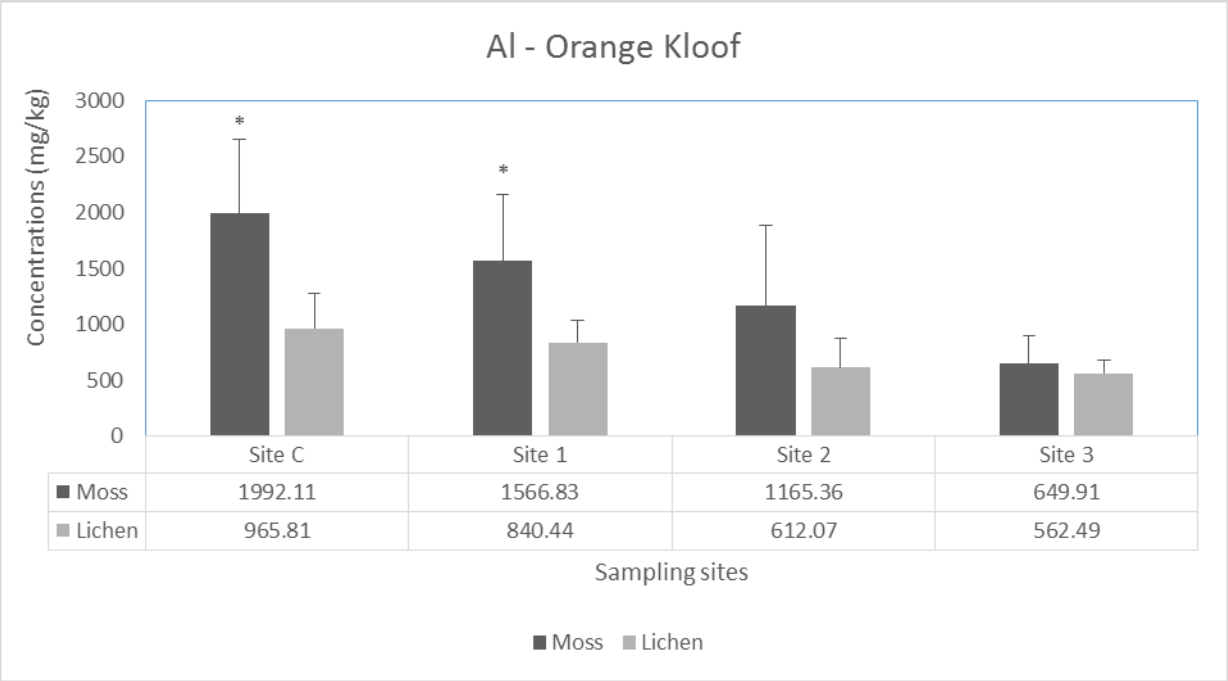


**Figure 4.4:** The mean Al concentrations (mg/kg) (± SD) in moss and lichen at sites C, 1, 2 and 3 of Orange Kloof forest for the dry sampling occasion in January 2015. N=5.

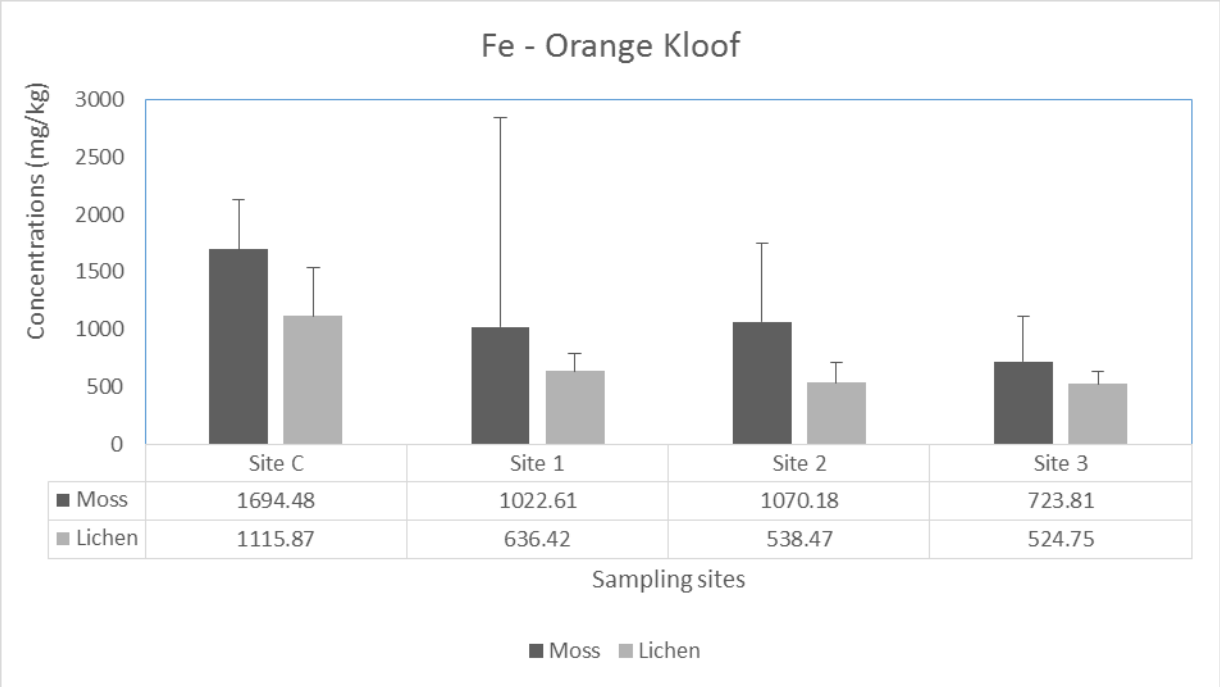


**Figure 4.5:** The mean Fe concentrations (mg/kg) (± SD) in moss and lichen at sites C, 1, 2 and 3 of Orange Kloof forest for the dry sampling occasion in January 2015. N=5.

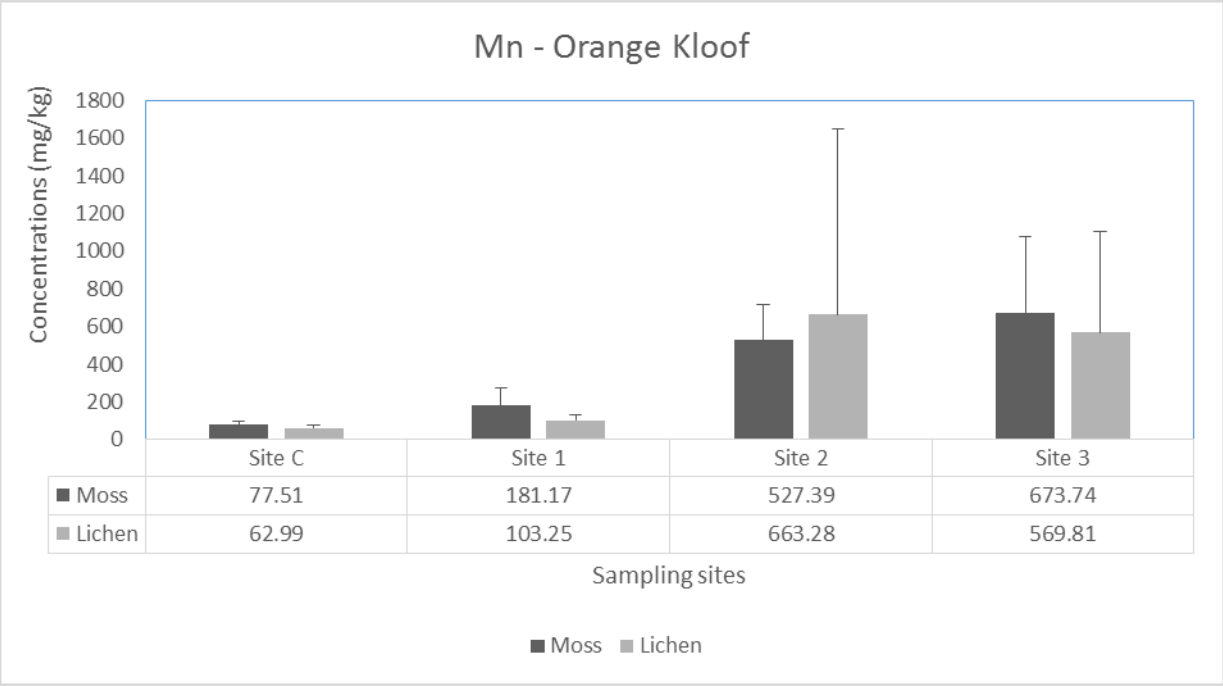


**Figure 4.6:** The mean Mn concentrations (mg/kg) (± SD) in moss and lichen at sites C, 1, 2 and 3 of Orange Kloof forest for the dry sampling occasion in January 2015. N=5.

Orange Kloof, moss and lichen comparisons showed statistically significant differences between each other at sites: C (P=0.014) and 1 (P=0.032). No statistically significant differences were, however found between each other at sites 2 (P=0.222) and 3 (P=0.494) in terms of Al concentrations. Moss samples had higher concentrations of Al compared to lichen with site C having the highest concentration of (1992.11 ± 661.78 mg/kg) **(Fig 4.4)**.

Comparisons of Fe concentrations between moss and lichen did not differ significantly from each other at any of the sites (C: P=0.065; 1: P=0.165; 2: P=0.421; 3: P=0.307) **(Fig 4.5)**.

Moss and lichen in this forest showed no statistically significant differences between each other at any of the sites: C (P=0.271), 1 (P=0.151), 2 (P=0.421) and 3: (P=0.548) when Mn concentrations were compared **(Fig 4.6)**.

#### *4.2.1.1.4) pH and Moisture*

pH of the soil of Orange Kloof ranged from slightly acidic (5.96 to 6.26) and the moisture % of the soil ranged from a dry 3.26 to 5.78 %.

**Table 4.2:** pH and moisture % of soil of Orange Kloof forest for the dry sampling occasion in January 2015.

| ORANGE KLOOF FOREST | SOIL | |
|---|---|---|
| Site C | **pH** | 6.1 |
| | *Moisture* | 5.51 |
| Site 1 | **pH** | 6.26 |
| | *Moisture* | *5.10* |
| Site 2 | **pH** | 6.14 |
| | *Moisture* | *5.78* |
| Site 3 | **pH** | 5.96 |
| | *Moisture* | *3.26* |

Moisture % of the leaf litter ranged from a relatively moist 17.56 to 23.23 %.

**Table 4.3:** Moisture % of the leaf litter of Orange Kloof forest for the dry sampling occasion in January 2015.

| ORANGE KLOOF FOREST | % | LEAF LITTER |
|---|---|---|
| Site C | Moisture | 17.56 |
| Site 1 | Moisture | 22.82 |
| Site 2 | Moisture | 23.23 |
| Site 3 | Moisture | 17.81 |

### *4.2.1.1.5) Weather data from the South African Weather Service in the vicinity of Constantia, Cape Town for Orange Kloof forest*

**Table 4.4:** Weather data in the vicinity of Orange Kloof forest for the dry sampling occasion in January 2015.

| SAMPLING SITES | SAMPLING OCCASION | | | |
|---|---|---|---|---|
| | DATE | DEGREES (°C) | PRECITATION (MM) | WIND SPEED (KM/H) |
| **ORANGE KLOOF FOREST** | | | | |
| Site C, 1, 2, 3 | 22-Jan-15 | 29 | 0 | 13 |
| Site 3 | 26-Jan-15 | 24 | 41 | 11 |
| Site C, 1, 3 | 19-Feb-15 | 26 | 0 | 37 |
| Site 3 | 25-Feb-15 | 26 | 0 | 28 |
| Site C, 1, 2, 3 | 19-Mar-15 | 23 | 0 | 17 |
| Site C, 1, 2, 3 | 31-Mar-15 | 26 | 0 | 19 |

### *4.2.1.1.6) Soil characterization for Orange Kloof forest sites C, 1, 2 and 3*

**Table 4.5:** Characterization of soil for Orange Kloof forest sites for the dry sampling occasion in January 2015.

| SAMPLING | (%) | | | | | | | WATER RETENTION | | |
|---|---|---|---|---|---|---|---|---|---|---|
| SITES | Clay | Silt | Fine sand | Medium sand | Coarse sand | Rock (v/v) | Classification | 10kPa | 100kPa | mm/m |
| ORANGE KLOOF FOREST | | | | | | | | | | |
| Site C | 9 | 12 | 31.6 | 26 | 21.4 | 21 | LmSa | 18.28 | 10.77 | 75.1 |
| | | | | | | | | | | |
| Site 1 | 5 | 6 | 46.8 | 28.6 | 13.6 | 8 | Sa | 19.08 | 9.13 | 99.5 |
| | | | | | | | | | | |
| Site 2 | 9 | 14 | 31.6 | 14 | 31.4 | 24.8 | LmSa | 20.22 | 12.19 | 80.4 |
| | | | | | | | | | | |
| Site 3 | 3 | 6 | 55.1 | 25.3 | 10.6 | 8.1 | Sa | 20.1 | 8.88 | 112.2 |

#### 4.2.1.2) NEWLANDS FOREST

##### *4.2.1.2.1) Comparisons of metal concentrations in soil, leaf litter and sentinel organisms between sites C, 1, 2 and 3*

The mean metal concentrations in soil, leaf litter and sentinel organisms for sites C, 1, 2 and 3 of Newlands forest for the dry sampling occasion in January 2015 are presented in **Table 4.6**. Metal concentrations are expressed in mg/kg.

**Table 4.6:** The mean metal concentrations (mg/kg) (± SD) in soil, leaf litter and sentinel organisms for sites C, 1, 2 and 3 of Newlands forest for the sampling occasion in January 2015.

| METAL | | SOIL | | | | LEAF LITTER | | | | MOSS | | | | LICHEN | | | | MILLIPEDES | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Site C | Site 1 | Site 2 | Site 3 | Site C | Site 1 | Site 2 | Site 3 | Site C | Site 1 | Site 2 | Site 3 | Site C | Site 1 | Site 2 | Site 3 | Site C | Site 1 | Site 2 | Site 3 |
| Al | Mean | $^{bcd}$13450.31 | $^{ac}$4925.34 | $^{abd}$8656.02 | $^{ac}$3957.51 | 220.42 | 488.89 | 529.29 | 323.15 | 1992.10 | 2439.71 | 1045.16 | 1822.75 | 965.81 | 1342.56 | 661.00 | 514.92 | $^{bcd}$3042.68 | $^{acd}$440.70 | $^{ab}$285.28 | $^{ab}$859.25 |
| | *SD* | *2851.26* | *749.14* | *1312.24* | *2353.78* | *175.48* | *176.35* | *521.53* | *374.95* | *661.79* | *1359.71* | *550.23* | *1454.52* | *305.38* | *945.87* | *169.65* | *226.29* | *3935.36* | *508.35* | *212.45* | *727.44* |
| Fe | Mean | $^{bd}$8016.54 | $^{acd}$6037.60 | $^{bd}$10225.38 | $^{abc}$10826.97 | 176.11 | 545.23 | 409.28 | 703.42 | 1694.47 | 2897.37 | 1219.81 | 3547.25 | 1115.87 | 1701.59 | 662.63 | 840.99 | 1556.14 | 555.32 | 392.72 | 1819.84 |
| | *SD* | *1592.90* | *740.32* | *1032.93* | *4286.19* | *124.29* | *205.53* | *551.00* | *764.89* | *436.48* | *1813.88* | *682.10* | *3079.44* | *417.65* | *1295.83* | *72.58* | *481.07* | *2023.16* | *541.19* | *230.26* | *1450.94* |
| Mn | Mean | $^{bcd}$98.97 | $^{ac}$232.37 | $^{abd}$489.96 | $^{ac}$340.12 | $^{bcd}$64.97 | $^{acd}$519.31 | $^{ab}$733.34 | $^{ab}$564.04 | $^{bcd}$77.51 | $^{acd}$346.42 | $^{ab}$437.66 | $^{ab}$197.86 | $^{cd}$62.98 | $^{cd}$115.05 | $^{ab}$234.52 | $^{ab}$195.27 | 33.28 | 39.77 | 72.72 | 62.45 |
| | *SD* | *23.56* | *71.76* | *107.44* | *134.17* | *14.28* | *108.33* | *153.96* | *470.51* | *21.77* | *186.68* | *247.35* | *42.58* | *16.77* | *117.27* | *79.82* | *94.87* | *26.22* | *28.54* | *17.79* | *55.42* |

Statistical signifcant differences (P<0.050) are indicated with different superscripted letters: site C=a, site 1=b, site 2=c, site 3=d. Comparisons were done separately for the soil, leaf litter and sentinel organisms. N=5.

***a) Soil***

Site comparisons showed statistically significant differences ($P<0.05$) in Al concentrations for the comparisons of sites C vs 1, 2 and 3 and sites 2 vs 3 and 1 vs 2, with the exception of sites 1 vs 3 ($P>0.05$). The concentrations found at sites C (13450.31 ± 2851.26 mg/kg) and 2 (8656.02 ± 1312.24 mg/kg) were significantly higher than at sites 1 (4925.34 ± 749.14 mg/kg) and 3 (3957.51 ± 2353.78 mg/kg) **(Table 4.6)**.

In terms of Fe concentrations at Newlands, statistically significant differences were found between sites C vs 1, 1 vs 2 and 1 vs 3 ($P<0.05$), but no significant differences were found between sites C vs 2 and 3 and sites 2 vs 3 ($P>0.05$). The mean concentration at site 1 (6037.60 ± 740.32 mg/kg) was significantly lower than the mean concentration found at sites C (8016.54 ± 1592.90 mg/kg), 2 (10225.38 ± 1032.93 mg/kg) and 3 (10826.97 ± 4286.19 mg/kg) **(Table 4.6)**.

Newlands forest soil differed significantly in Mn concentrations between sites C vs 1, 2 and 3, sites 1 vs 2 and 2 vs 3 ($P<0.05$). No significant differences were found between sites 1 vs 3 ($P>0.05$). Site C had the lowest mean concentration of (98.97 ± 23.56 mg/kg) when compared to the other sites **(Table 4.6)**.

***b) Leaf litter***

Pairwise multiple comparisons showed no statistically significant differences in leaf litter between any of the sites in terms of Al ($P=0.114$) and Fe ($P=0.059$) concentrations **(Table 4.6)**.

Manganese concentrations differed significantly between sites C and 1 and 2 and 3 ($P<0.05$). No significant differences were found between sites 1 and 2, 1 and 3 and 2 and 3 ($P>0.05$). The mean concentration at site C (64.97 ± 14.28 mg/kg) was significantly lower than the mean concentrations at sites 1 (519.31 ± 108.33 mg/kg), 2 (733.34 ± 153.96 mg/kg) and 3 (564.04 ± 470.51 mg/kg) **(Table 4.6)**.

***c) Moss***

This forest did not show any significant differences in Al (P=0.121) and Fe (P=0.212) concentrations in moss samples between the four sites for this sampling session **(Table 4.6)**.

Pairwise multiple comparisons showed statistically significant differences in Mn concentrations in moss for the comparisons of sites C and 1 and 2 and 3 (P<0.05). However, no differences between sites 1 vs 2, 1 vs 3 and 2 vs 3 (P>0.05) were found. Site C with a mean concentration of 77.51 ± 21.77 mg/kg was significantly lower than at sites 1, 2 and 3 **(Table 4.6)**.

***d) Lichen***

No significant differences in lichen Al (P=0.066) and Fe (P=0.235); concentrations were found between any of the sites **(Table 4.6)**.

Newlands forest lichen differed significantly in Mn concentrations between sites C, 2 and 3 and sites 1 and 2 and 1 and 3 (P<0.05). No significant differences were found between sites C and 1 and 2 and 3 (P>0.05). Site C had the lowest mean concentration of 62.99 ± 16.77 mg/kg when compared to the other site concentrations **(Table 4.6)**.

***e) Millipedes***

Pairwise multiple comparisons showed statistically significant differences in millipedes in terms of Al concentrations for the comparisons of sites C vs 1 and 2 and 3 (P<0.05). No statistically differences between sites 1 vs 2, 1 vs 3 and 2 vs 3 (P>0.05) were found. The mean Al concentration at site C (3042.68 ± 3935.36 mg/kg) was significantly higher than at sites 1, 2 and 3 **(Table 4.6)**.

There were no statistical differences found in Fe (P=0.056) and Mn (P=0.163) concentrations between the four sites **(Table 4.6)**.

***4.2.1.2.2) Comparisons of metal concentrations between soil and leaf litter at sites C, 1, 2 and 3***

Mean metal concentrations in soil and leaf litter at sites C, 1, 2 and 3 of Newlands forest for the dry sampling occasion in January 2015 are shown in **Figs 4.7 to 4.9**. Statistical signifcant differences (P<0.050) between soil and leaf litter are indicated with an asterisk above the graph bars.

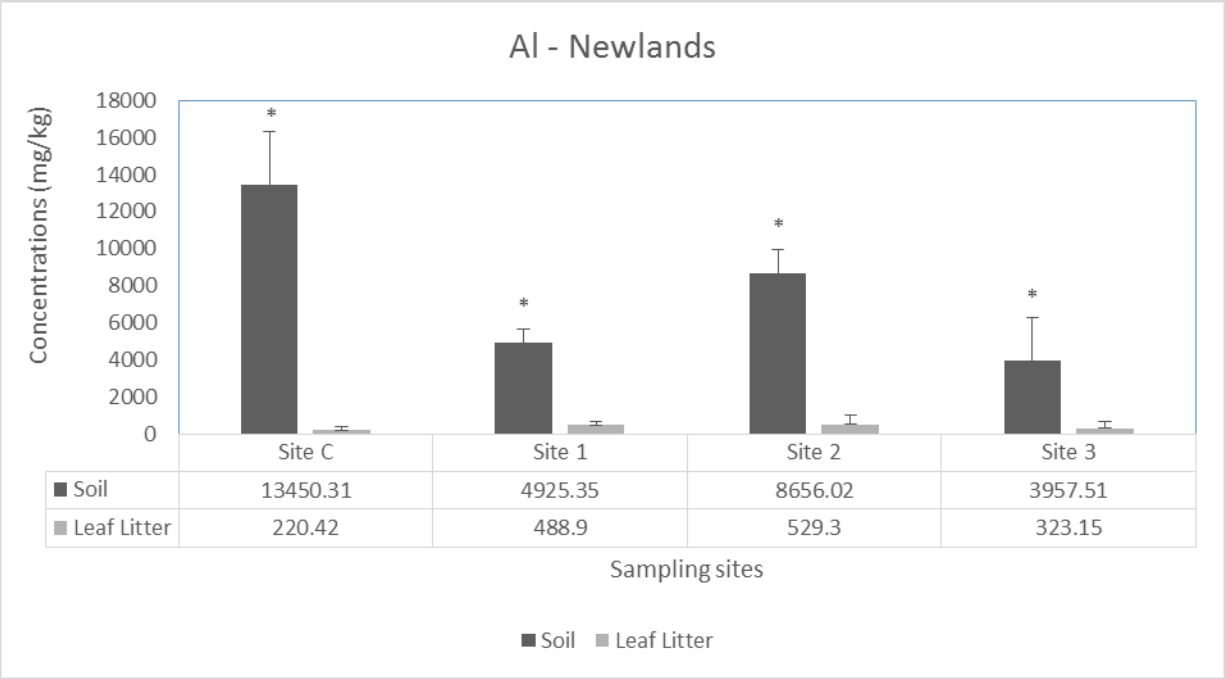


**Figure 4.7:** The mean Al concentrations (mg/kg) (± SD) in soil and leaf litter at sites C, 1, 2 and 3 of Newlands forest for the dry sampling occasion in January 2015. N=5.

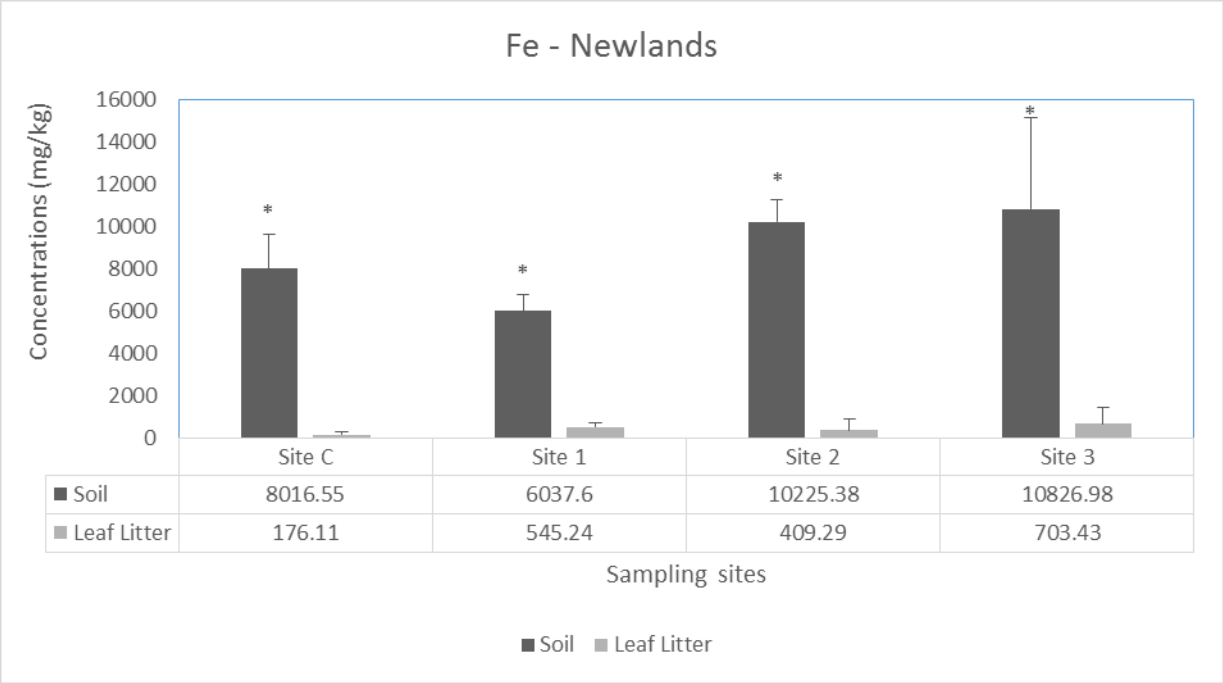


**Figure 4.8:** The mean Fe concentrations (mg/kg) (± SD) in soil and leaf litter at sites C, 1, 2 and 3 of Newlands forest for the dry sampling occasion in January 2015. N=5.

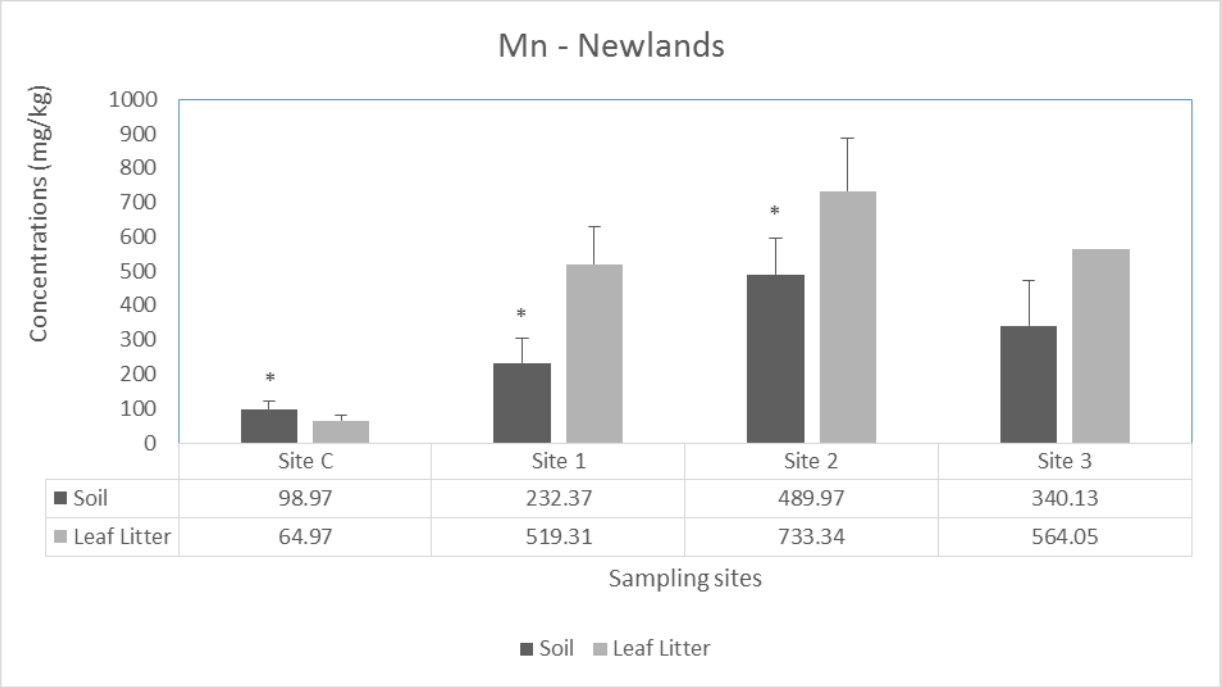


**Figure 4.9:** The mean Mn concentrations (mg/kg) (± SD) in soil and leaf litter at sites C, 1, 2 and 3 of Newlands forest for the dry sampling occasion in January 2015. N=5.

Aluminium concentrations at sites C (P=0.008), 1 (P<0.001), 2 (P=0.008) and 3 (P=0.009) showed statistically significant differences between soil and leaf litter. The highest Al concentrations were found in soil as opposed to leaf litter, with site C (13450.31 ± 2851.26 mg/kg) having the highest overall concentration **(Fig 4.7)**.

All the sites, C (P=0.008), 1 (P=<0.001), 2 (P=<0.001) and 3 (P=0.008) at Newlands showed statistically significant differences in terms of Fe concentrations in soil vs leaf litter comparisons. The mean concentrations were higher in soil at all the sites. Site 2 showed the highest mean concentration of (10225.38 ± 1032.92 mg/kg) **(Fig 4.8)**.

Site C (P=0.025), 1 (P=0.008) and 2 (P=0.020) showed statistically significant differences between soil and leaf litter, however there were no statistically significant differences found at site 3 (P=0.690) in terms of Mn concentrations. The highest mean concentration at site 2 (733.34 ± 153.96 mg/kg) was found in leaf litter **(Fig 4.9)**.

### *4.2.1.2.3) Comparisons of metal concentrations between moss and lichen at sites C, 1, 2 and 3*

Mean metal concentrations in moss and lichen at sites C, 1, 2 and 3 of Newlands forest for the sampling occasion in January 2015 are shown in **Figs 4.10 to 4.12**. Statistical signifcant differences (P<0.050) between moss and lichen are indicated with an asterisk above the graph bars.

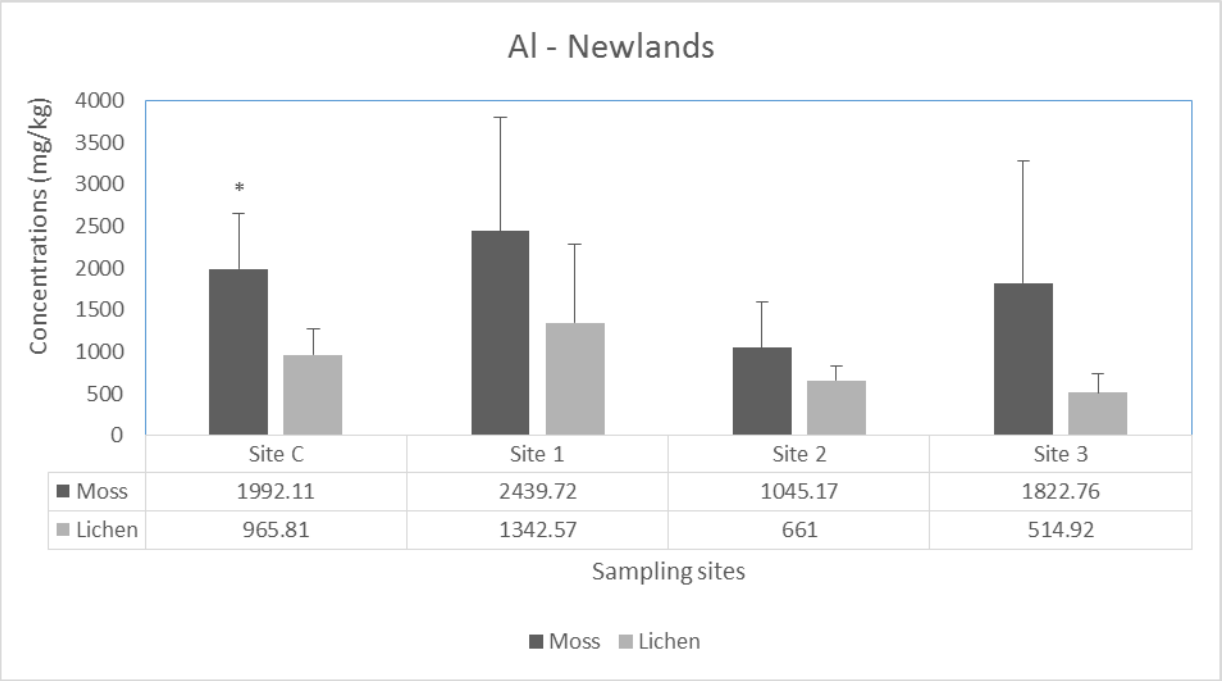


**Figure 4.10:** The mean Al concentrations (mg/kg) (± SD) in moss and lichen at sites C, 1, 2 and 3 of Newlands forest for the dry sampling occasion in January 2015. N=5.

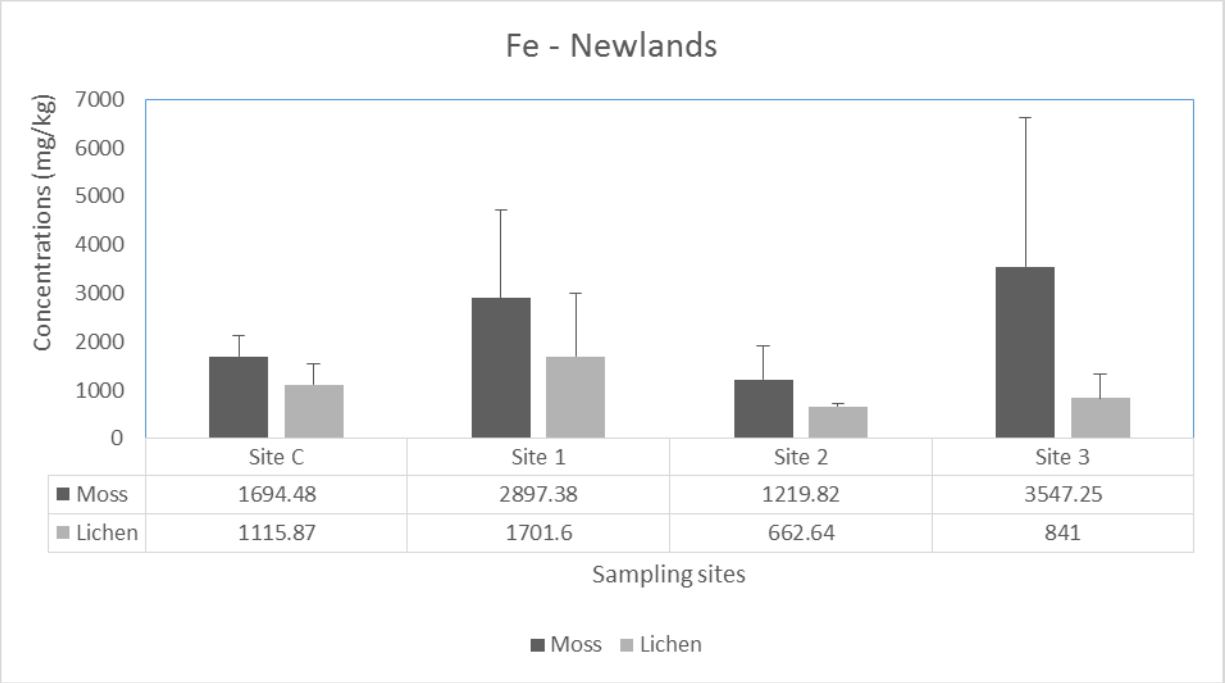


**Figure 4.11:** The mean Fe concentrations (mg/kg) (± SD) in moss and lichen at sites C, 1, 2 and 3 of Newlands forest for the dry sampling occasion in January 2015. N=5.

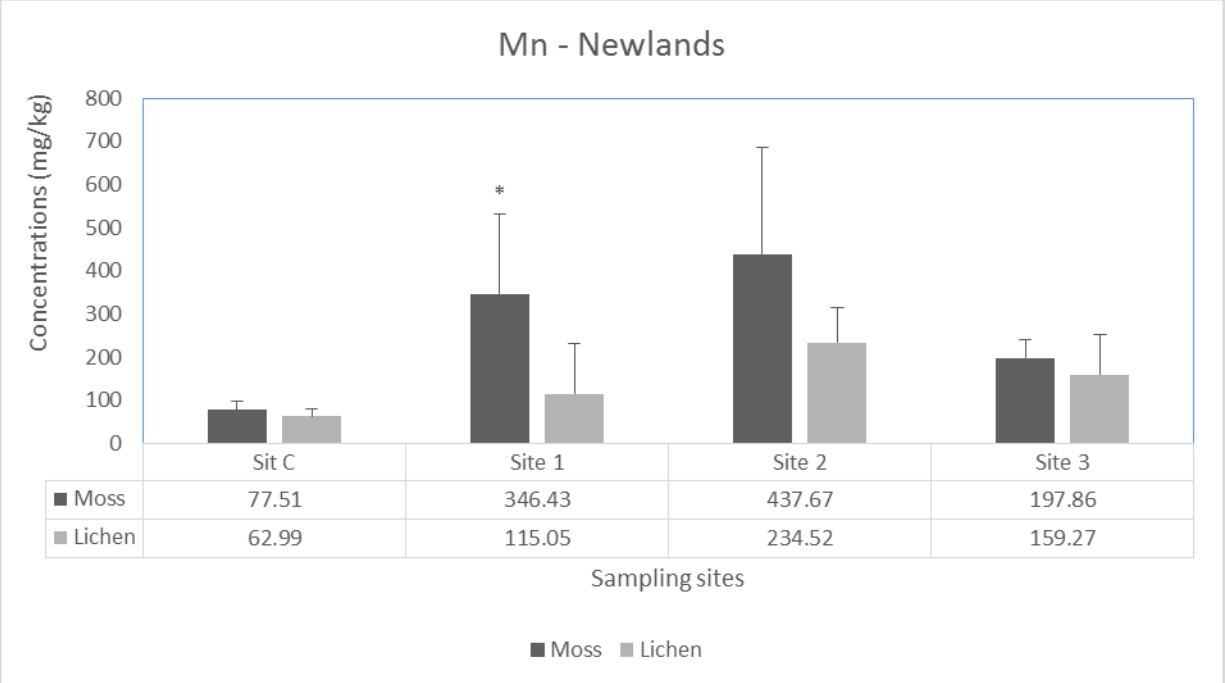


**Figure 4.12:** The mean Mn concentrations (mg/kg) (± SD) in moss and lichen at sites C, 1, 2 and 3 of Newlands forest for the dry sampling occasion in January 2015. N=5.

Site C (P=0.014) showed statistically significant differences between moss and lichen, but no significant differences were found between the organisms at sites 1, (P=0.177), 2 (P=0.174) and 3 (P=0.082) when Al concentrations were compared. The highest mean concentration at site 1 (2439.72 ± 1359.70 mg/kg) was found in moss. Moss showed higher concentrations of Al compared to lichen **(Fig 4.10)**.

No statistically significant differences in Fe concentrations were found at any of the sites: C (P=0. 065), 1 (P=0.265), 2 (P=0.222) and 3 (P=0.088). The highest mean concentration at site 3 (3547.25 ± 3079.43 mg/kg) was found in moss **(Fig 4.11)**.

Manganese concentration comparisons at site 1 (P=0.047) showed statistically significant differences. No significant differences were found at site C (P=0.271), 2 (P=0.0119) and 3 (P=0.957). The highest mean concentration was found at site 2 (437.67 ± 247.35 mg/kg) in moss **(Fig 4.12)**.

#### *4.2.1.2.4) pH and Moisture*

pH of the soil was slightly acidic (6.1) to alkaline (7.28) and the moisture % of the soil ranged from a dry 5.51 % to a moist 49.10 %.

**Table 4.7:** pH and moisture % of soil of Newlands forest for the dry sampling occasion in January 2015.

| **NEWLANDS FOREST** | **SOIL** | |
|---|---|---|
| Site C | **pH** | 6.1 |
| | *Moisture* | 5.51 |
| Site 1 | **pH** | 7.28 |
| | *Moisture* | *10.18* |
| Site 2 | **pH** | 7.26 |
| | *Moisture* | *8.76* |
| Site 3 | **pH** | 6.14 |
| | *Moisture* | *49.10* |

Moisture % of leaf litter ranged from a dry 17.56 % to a moist 56.10 %.

**Table 4.8:** Moisture % of the leaf litter of Newlands forest for the dry sampling occasion in January 2015.

| NEWLANDS FOREST | % | LEAF LITTER |
|---|---|---|
| Site C | Moisture | 17.56 |
| Site 1 | Moisture | 25.43 |
| Site 2 | Moisture | 23.41 |
| Site 3 | Moisture | 56.10 |

### *4.2.1.2.5) Weather data from the South African Weather Service in the vicinity of Newlands, Cape Town for Newlands forest*

**Table 4.9:** Weather data in the vicinity of Newlands forest for the sampling occasion in January 2015.

| SAMPLING SITES | SAMPLING OCCASION | | | |
|---|---|---|---|---|
| | DATE | DEGREES (°C) | PRECITATION (MM) | WIND SPEED (KM/H) |
| **NEWLANDS FOREST** | | | | |
| Site 1, 2 | 15-Jan-15 | 24 | 0 | 41 |
| Site 3 | 16-Jan-15 | 21 | 0 | 30 |
| Site 2, 3 | 14-Feb-15 | 23 | 0 | 32 |
| Site 1 | 16-Feb-15 | 27 | 0 | 9 |
| Site 2 | 17-Mar-15 | 26 | 0 | 20 |
| Site 3 | 30-Mar-15 | 26 | 0 | 13 |

### *4.2.1.2.6) Soil characterization for Newlands forest sites 1, 2 and 3*

**Table 4.10:** Characterization % of soil for Newlands forest sites for the dry sampling occasion in January 2015.

| SAMPLING | (%) | | | | | | | WATER RETENTION | | |
|---|---|---|---|---|---|---|---|---|---|---|
| SITES | Clay | Silt | Fine sand | Medium sand | Coarse sand | Rock (v/v) | Classification | 10kPa | 100kPa | mm/m |
| NEWLANDS FOREST | | | | | | | | | | |
| Site 1 | 7 | 12 | 41.8 | 22 | 17.2 | 23.1 | LmSa | 18.93 | 10.30 | 86.2 |
| | | | | | | | | | | |
| Site 2 | 3 | 4 | 24.7 | 43.3 | 25 | 2.0 | Sa | 13.63 | 7.30 | 63.3 |
| | | | | | | | | | | |
| Site 3 | 9 | 18 | 44.8 | 18.24 | 10 | 18.6 | LmSa | 23.14 | 13.03 | 101.1 |

### 4.2.1.3) FORESTS

#### *4.2.1.3.1) Comparisons of metal concentrations in soil, leaf litter and sentinel organisms between the forests: Site C, Orange Kloof and Newlands*

The mean metal concentrations in soil, leaf litter and sentinel organisms for each forest for the dry sampling occasion (January 2015) are presented in **Tables 4.11 to 4.13**. The metal concentrations for the sites within each forest were pooled to calculate the mean concentrations for each forest. Metal concentrations are expressed in mg/kg.

***a) Al***

**Table 4.11:** The mean Al concentrations (mg/kg) (± SD) in soil, leaf litter and sentinel organisms for forests for the dry sampling occasion in January 2015.

| FOREST | | SOIL | LEAF LITTER | MOSS | LICHEN | MILLIPEDES |
|---|---|---|---|---|---|---|
| **Site C** | **Mean** | **$^{bc}$13450.31** | **220.42** | **$^{bc}$1992.10** | **$^{bc}$965.81** | **$^{bc}$3042.68** |
| | *SD* | *2851.26* | *175.48* | *661.79* | *305.38* | *3935.36* |
| **Orange Kloof** | **Mean** | **$^{a}$4122.74** | **265.89** | **$^{a}$1127.36** | **$^{a}$671.66** | **$^{a}$382.10** |
| | *SD* | *4495.73* | *249.21* | *643.69* | *225.57* | *220.37* |
| **Newlands** | **Mean** | **$^{a}$5846.29** | **447.11** | **$^{a}$1769.21** | **$^{a}$839.49** | **$^{a}$528.41** |
| | *SD* | *2575.23* | *367.82* | *1252.20* | *646.42* | *548.51* |

Statistical signifcant differences (P<0.050) are indicated with different superscripted letters: site C=a, Orange Kloof=b, Newlands=c. Comparisons were done separately for the soil, leaf litter and sentinel organisms. N=5.

When soil was compared between forests, statistically significant differences were found between site C and Orange Kloof forest and site C and Newlands forest (P<0.050). No statistically significant differences were found between Orange Kloof and Newlands (P>0.050). The highest mean concentration in soil was found at site C (13450.31 ± 2851.26 mg/kg) **(Table 4.11)**.

There were no statistically differences found when the forests were compared in terms of leaf litter (P=0.063) **(Table 4.11)**.

In terms of Al concentration, statistically significant differences were found when moss was compared between site C and Orange Kloof, as well as site C and Newlands forests (P<0.05). There were, however no statistically significant differences found when Newlands and Orange Kloof was compared (P>0.05). Site C showed the highest mean concentration of 1992.10 ± 661.79 mg/kg **(Table 4.11)**.

Pairwise multiple comparisons between forests for Al concentrations in lichens showed statistically significant differences between site C and Orange Kloof and site C and Newlands forests (P<0.05), but there were no statistically significant differences found when Newlands and Orange Kloof was compared (P>0.05). The mean Al concentration at site C (965.81 ± 305.38 mg/kg) was once again the highest **(Table 4.11)**.

Millipede comparisons between Orange Kloof and Newlands forests revealed no statistically significant differences (P>0.050) in Al concentrations. Statistically significant differences were found between site C and Orange Kloof and site C and Newlands forests (P<0.050). The Newlands Al concentrations of 528.41 ± 548.51 mg/kg in millipedes were significantly higher than at the other two forests **(Table 4.11)**.

***b) Fe***

**Table 4.12:** The mean Fe concentrations (mg/kg) (± SD) in soil, leaf litter and sentinel organisms for forests for the dry sampling occasion in January 2015.

| FOREST | | SOIL | LEAF LITTER | MOSS | LICHEN | MILLIPEDES |
|---|---|---|---|---|---|---|
| **Site C** | **Mean** | **$^{b}$8016.54** | **$^{c}$176.11** | **$^{b}$1694.47** | **$^{bc}$1115.87** | **$^{b}$1556.14** |
| | *SD* | *1592.90* | *124.29* | *436.48* | *417.65* | *2023.16* |
| **Orange Kloof** | **Mean** | **$^{ac}$1832.03** | **$^{c}$178.14** | **$^{ac}$938.87** | **$^{ac}$566.54** | **$^{ac}$325.69** |
| | *SD* | *1012.07* | *129.83* | *538.29* | *148.43* | *380.81* |
| **Newlands** | **Mean** | **$^{b}$9029.98** | **$^{a\ b}$552.64** | **$^{b}$2554.81** | **$^{a\ b}$1068.40** | **$^{b}$922.63** |
| | *SD* | *3251.47* | *530.52* | *2193.75* | *876.27* | *1065.97* |

Statistical signifcant differences (P<0.050) are indicated with different superscripted letters: site C=a, Orange Kloof=b, Newlands=c. Comparisons were done separately for the soil, leaf litter and sentinel organisms. N=5.

Pairwise multiple comparisons showed statistically significant differences in soil between the forests site C vs Orange Kloof and Newlands vs Orange Kloof ($P<0.05$) during this sampling occasion. No statistically significant differences were found between site C and Newlands. The highest mean Fe concentration (9029.98 ± 3251.47 mg/kg) was found at Newlands forest **(Table 4.12)**.

Comparisons between leaf litter showed statistically significant differences between site C and Newlands and Orange and Newlands forests ($P<0.05$), but no statistically significant differences were found between site C and Orange Kloof ($P>0.050$). A mean concentration of 552.64 ± 530.52 mg/kg was found at Newlands, which was the highest of the three forests **(Table 4.12)**.

Statistically significant differences were found when moss was compared between site C and Orange Kloof and Newlands and Orange Kloof forests ($P<0.05$), however no significant differences were found between site C and Newlands forests in terms of Fe concentrations ($P>0.050$). Newlands, once again had the highest mean concentration (2554.81 ± 2193.75 mg/kg). The lowest mean concentration was found at Orange Kloof (938.87 ± 538.29 mg/kg) **(Table 4.12)**.

Lichen comparisons between forests showed statistically significant differences between all of the forests when mean Fe concentration was compared ($P<0.05$) **(Table 4.12)**.

Millipedes at site C and Orange Kloof and Newlands and Orange Kloof forests were compared and statistically significant differences were found between them ($P<0.05$). There were, however no statistically significant differences found when site C and Newlands was compared ($P>0.05$). The mean concentration found at site C forest (1556.14 ± 2023.16 mg/kg) was significantly higher than at the other forests **(Table 4.)**.

***c) Mn***

**Table 4.13:** The mean Mn concentrations (mg/kg) (± SD) in soil, leaf litter and sentinel organisms for forests for the dry sampling occasion in January 2015.

| FOREST | | SOIL | LEAF LITTER | MOSS | LICHEN | MILLIPEDES |
|---|---|---|---|---|---|---|
| Site C | Mean | $^{c}$98.97 | $^{bc}$64.97 | $^{bc}$77.51 | $^{bc}$62.98 | $^{bc}$33.28 |
| | *SD* | *23.56* | *14.28* | *21.77* | *16.77* | *26.22* |
| Orange Kloof | Mean | $^{c}$255.38 | $^{a}$792.98 | $^{a}$460.76 | $^{a}$445.44 | $^{a}$86.81 |
| | *SD* | *295.89* | *874.64* | *324.04* | *651.45* | *69.18* |
| Newlands | Mean | $^{a\ b}$354.15 | $^{a}$605.56 | $^{a}$327.31 | $^{a}$181.61 | $^{a}$58.31 |
| | *SD* | *147.88* | *287.19* | *196.01* | *104.74* | *37.46* |

Statistical signifcant differences (P<0.050) are indicated with different superscripted letters: site C=a, Orange Kloof=b, Newlands=c. Comparisons were done separately for the soil, leaf litter and sentinel organisms. N=5.

Pairwise multiple comparisons showed statistically significant differences in soil between the forests site C vs Newlands and Newlands vs Orange Kloof (P<0.05), but no statistically significant differences were found between site C and Orange Kloof forests (P>0.05). The mean Mn concentration (354.15 ± 147.88 mg/kg) was the highest at Newlands and the lowest mean concentration of (98.97 ± 23.56 mg/kg) was found at site C **(Table 4.13)**.

During this sampling session, comparisons between leaf litter showed statistically significant differences between site C and Orange Kloof and site C and Newlands forests (P<0.05), with the exception of the Newlands and Orange Kloof (P>0.05). The mean Mn concentration of 64.97 ± 14.28 mg/kg at site C was lower than at the other forests. Orange Kloof had the highest mean concentration of 792.98 ± 874.64 mg/kg **(Table 4.13)**.

Significant differences were found between the forests site C and Orange Kloof and site C and Newlands forests in terms of moss comparisons (P<0.05). There were no statistically significant differences found between Orange Kloof and Newlands (P>0.05). Orange Kloof, once again showed the highest mean concentration (460.76 ± 324.04 mg/kg) **(Table 4.13)**.

Lichen comparisons between forests showed statistically significant differences between the following: site C vs Orange Kloof and site C vs Newlands forests (P<0.05), with the exception of Orange Kloof vs Newlands forests (P>0.05). The lowest mean concentration was found at site C (62.98 ± 16.77 mg/kg) **(Table 4.13)**.

Millipedes between site C and Orange Kloof and site C and Newlands forests were compared and it was found that they differed significantly from each other ($P<0.050$). Comparisons between Orange Kloof and Newlands did, however not differ significantly from each other ($P>0.05$). The highest mean concentration (86.81 ± 69.18 mg/kg) was found at Orange Kloof **(Table 4.13)**.

### 4.2.2. WET SEASON: METAL CONTAMINATION AND BIOACCUMULATION

#### 4.2.2.1) ORANGE KLOOF FOREST

##### *4.2.2.1.1) Comparisons of metal concentrations in soil, leaf litter and sentinel organisms between sites C, 1, 2 and 3*

The mean metal concentrations in soil, leaf litter and sentinel organisms for sites C, 1, 2 and 3 of Orange Kloof forest for the wet sampling occasion in June 2015 are presented in **Table 4.14**. Metal concentrations are expressed in mg/kg.

**Table 4.14:** The mean metal concentrations (mg/kg) (± SD) in soil, leaf litter and sentinel organisms for sites C, 1, 2 and 3 of Orange Kloof forest for the wet sampling occasion in June 2015.

| METAL | | SOIL | | | | LEAF LITTER | | | | MOSS | | | | LICHEN | | | | MILLIPEDES | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Site C | Site 1 | Site 2 | Site 3 | Site C | Site 1 | Site 2 | Site 3 | Site C | Site 1 | Site 2 | Site 3 | Site C | Site 1 | Site 2 | Site 3 | Site C | Site 1 | Site 2 | Site 3 |
| Al | Mean | bd13940.73 | ac d8305.64 | bd22995.01 | a bc707.24 | bd1021.31 | ac319.99 | bd1050.96 | ac168.38 | bc d3341.43 | ac d1443.08 | a b 830.56 | a b 1105.48 | 768.84 | 1021.96 | 1792.10 | 994.16 | 1072.30 | 554.29 | 384.25 | 295.42 |
| | *SD* | *3263.24* | *2510.89* | *10084.25* | *119.01* | *263.66* | *207.16* | *683.28* | *80.96* | *2251.92* | *280.04* | *284.42* | *634.07* | *194.82* | *560.77* | *989.28* | *537.55* | *959.59* | *765.39* | *385.33* | *382.88* |
| Fe | Mean | bc d6503.83 | ac d3465 | a bd13427.4 | a bc874.29 | bc d683.95 | ad607.61 | ad1018.5 | a bc202.06 | bc d2379.91 | ad1012.5 | ad878.39 | a bc1204.2 | 885.15 | 761.00 | 1642.12 | 955.18 | 623.15 | 246.84 | 508.15 | 337.63 |
| | *SD* | *1225.00* | *936.08* | *5377.74* | *352.10* | *96.78* | *266.18* | *408.70* | *64.46* | *1288.60* | *170.33* | *408.33* | *752.82* | *448.32* | *339.63* | *769.20* | *599.81* | *497.07* | *242.49* | *351.42* | *384.59* |
| Mn | Mean | 186.68 | 110.10 | 213.91 | 289.03 | bc d177.24 | ad168.34 | ad248.17 | a bc968.2 | 150.27 | 174.48 | 259.86 | 425.39 | 69.77 | 57.35 | 144.50 | 212.36 | bd47.11 | ac d29.49 | bd78.06 | a bc199.66 |
| | *SD* | *47.58* | *31.31* | *79.33* | *245.47* | *35.28* | *37.91* | *125.65* | *438.24* | *59.78* | *56.26* | *171.50* | *308.52* | *39.44* | *33.85* | *110.53* | *120.59* | *12.46* | *12.43* | *33.64* | *99.97* |

Statistical signifcant differences (P<0.050) are indicated with different superscripted letters: site C=a, site 1=b, site 2=c, site 3=d. Comparisons were done separately for the soil, leaf litter and sentinel organisms. N=5.

***a) Soil***

Pairwise multiple comparisons showed statistically significant differences in Al concentrations between sites C vs 1, C vs 3, 1 vs 3, 1 vs 2 and 2 vs 3 ($P<0.05$). No significant differences were, however found in soil between sites C and 2 ($P>0.05$). The mean concentration for site 2 (22995.01 ± 10084.25 mg/kg) was significantly higher than at the other sites **(Table 4.14)**.

There were statistically significant differences ($P<0.05$) found in Fe concentrations for all the comparisons. The mean concentration at site 2 was once again the highest (13427.4 ± 5377.74 mg/kg). The lowest mean Fe concentration was found at site 3 (874.29 ± 352.10 mg/kg) **(Table 4.14)**.

The soil at Orange Kloof sites C, 1, 2 and 3 did not differ significantly from each other in terms of Mn ($P=0.257$) concentrations when compared **(Table 4.14)**.

***b) Leaf litter***

Site comparisons showed statistically significant differences in Al concentrations in leaf litter between the following sites: C vs 1, C vs 3, 2 vs 3 and 1 vs 2 ($P<0.05$), but no statistically significant differences were found for sites C and 2 and 1 and 3. The mean Al concentration for site 3 (168.38 ± 80.96 mg/kg) was significantly lower than at the other three sites **(Table 4.14)**.

Statistically significant differences in Fe concentrations between sites C and 3, 1 and 3 and 2 and 3 were found when leaf litter was compared ($P<0.05$). No statistically significant differences were, however found between sites C vs 1 and 2 and sites 1 vs 2 ($P>0.05$). The mean concentration at site 2 (1018.5 ± 408.70 mg/kg) was significantly higher than at sites C (683.95 ± 96.78 mg/kg), 1 (607.61 ± 266.18 mg/kg) and 3 (202.06 ± 64.46 mg/kg) (**Table 4.14)**.

Leaf litter at Orange Kloof was compared at all the sites in terms of Mn concentrations and statistically significant differences were found between sites C vs 3, 1 vs 3 and 2 vs 3. No significant differences were found between sites C and 1

and 2 and sites 1 and 2 (P>0.05). The highest mean Mn concentration of 968.2 ± 438.24 mg/kg was found at site 3 **(Table 4.14)**.

***c) Moss***

Pairwise multiple comparisons in terms of Al concentrations in moss revealed statistically significant differences for the comparisons of sites C vs 1, 2 and 3 and sites 1 vs 2 and 1 vs 3 (P<0.05). No statistically differences were detected between sites 2 vs 3 (P>0.05). The mean concentration at site C (3341.43 ± 2251.92 mg/kg) was higher than were found at the other sites **(Table 4.14)**.

Statistically significant differences in Fe moss concentrations were found between sites C and 1, 2 and 3 (P<0.05), but no significant differences were found between sites 1 and 2, 1 and 3 and 2 and 3 (P>0.05). Site C had the highest mean Fe concentration of 2379.91 ± 1288.60 mg/kg **(Table 4.14)**.

Manganese concentrations at Orange Kloof were not statistically different from each other when moss between the four sites were compared during this sampling session (P=0.196) **(Table 4.14)**.

***d) Lichen***

No statistically significant differences in Al (P=0.188), Fe (P=0.164) and Mn (P=0.071) concentrations between sites C, 1, 2 and 3 were found for this sampling occasion **(Table 4.14)**.

***e) Millipedes***

Site comparisons showed no statistically significant differences in Al (P=0.196) and Fe (P=0.284) concentrations in millipedes **(Table 4.14)**.

Statistically significant differences for Mn concentrations in millipedes were found in site comparisons: C vs 1 and 3, sites 1 vs 2, 1 vs 3 and 2 vs 3 (P<0.05), but there were no significant difference found between sites C and 2 (P>0.05). The mean Mn concentration at site 3 (199.66 ± 99.97 mg/kg) was significantly higher than at site 1 (29.49 ± 12.43 mg/kg), which was the lowest concentration of the four sites **(Table 4.14)**.

### *4.2.2.1.2) Comparisons of metal concentrations between soil and leaf litter at sites C, 1, 2 and 3*

Mean metal concentrations in soil and leaf litter at sites C, 1, 2 and 3 of Orange Kloof forest for the wet sampling occasion in June 2015 are shown in **Figs 4.13 to 4.15**. Statistical signifcant differences (P<0.050) between soil and leaf litter are indicated with an asterisk above the graph bars.

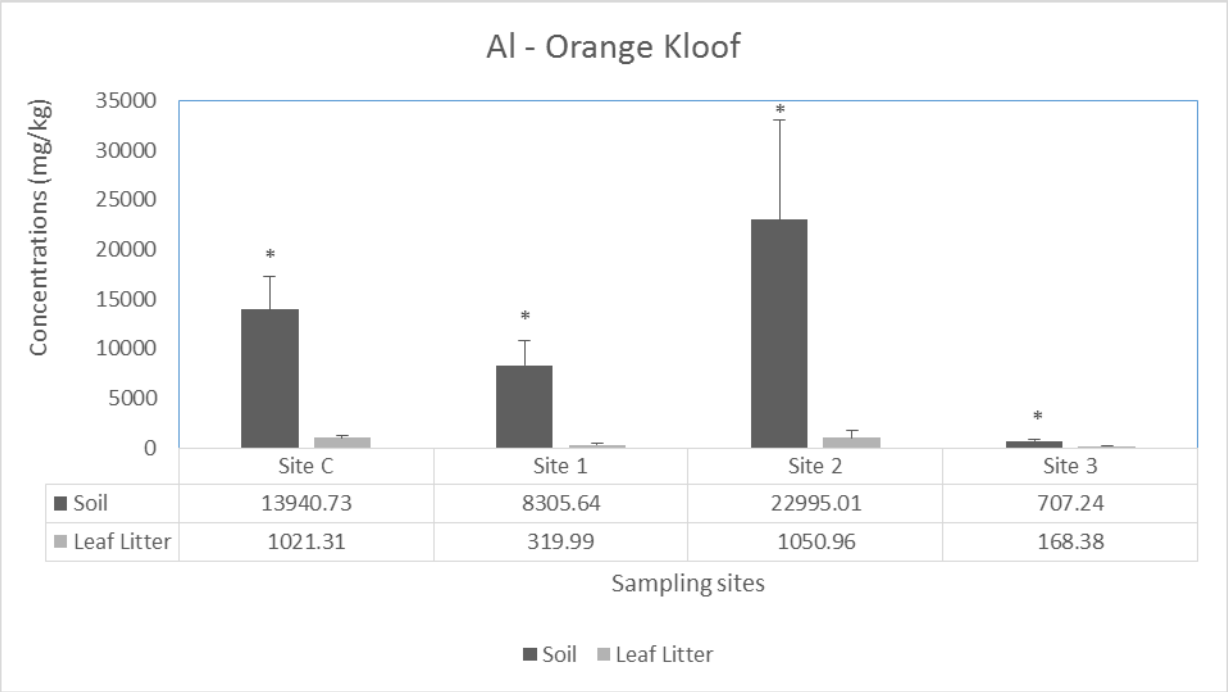


**Figure 4.13:** The mean Al concentrations (mg/kg) (± SD) in soil and leaf litter at sites C, 1, 2 and 3 of Orange Kloof forest for the wet sampling occasion in June 2015. N=5.

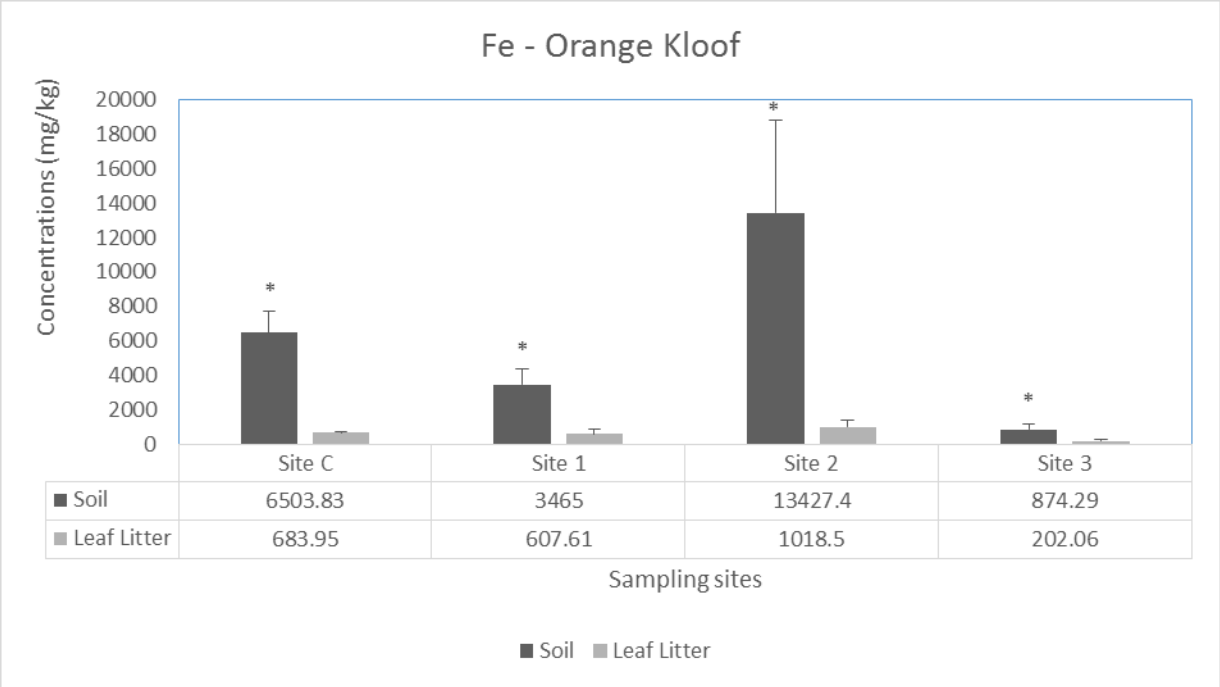


**Figure 4.14:** The mean Fe concentrations (mg/kg) (± SD) in soil and leaf litter at sites C, 1, 2 and 3 of Orange Kloof forest for the wet sampling occasion in June 2015. N=5.

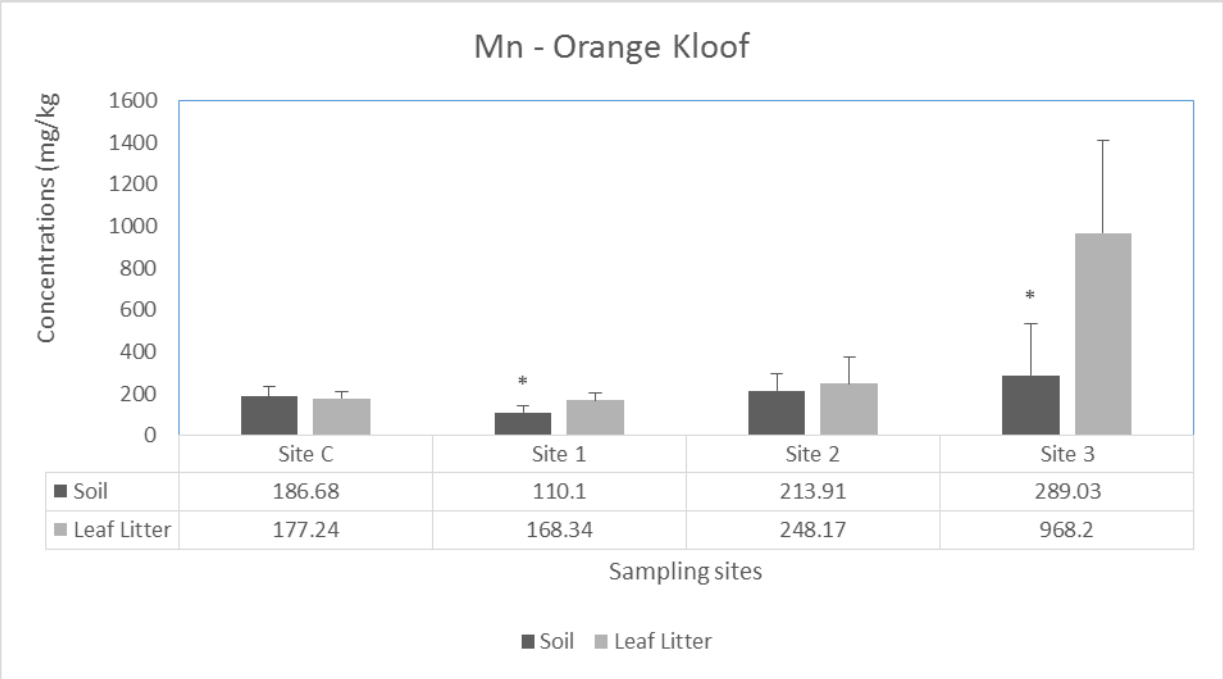


**Figure 4.15:** The mean Mn concentrations (mg/kg) (± SD) in soil and leaf litter at sites C, 1, 2 and 3 of Orange Kloof forest for the wet sampling occasion June 2015. N=5.

Leaf litter and soil showed statistically significant differences between each other at Orange Kloof sites C (P=<0.001), 1 (P=<0.001), 2 (P=0.001) and 3 (P=<0.001) in terms of Al concentrations. Al concentrations were significantly higher in soil at all the sites, whilst site 2 showed the highest concentration of 22995.01 ± 10084.25 mg/kg **(Fig 4.13)**.

When soil and leaf litter were compared in terms of Fe concentrations, significant differences between each other at all the sites were found: site C (P=<0.001), 1 (P=<0.001), 2 (P=<0.001) and 3 (P=0.003). As were found with Al, the mean Fe concentrations were much higher in soil than in leaf litter at all the sites with site 2 showing the highest concentration of 13427.04 ± 5377.74 mg/kg **(Fig 4.14)**.

At Orange Kloof, leaf litter and soil comparisons showed statistically significant differences between each other at sites 1 (P=0.029) and 3 (P=0.016), but site C (P=0.731) and site 2 (P=0.620) did not differ significantly between each when Mn concentrations were compared. The concentrations in leaf litter at site 3 of 968.2 ± 438.24 mg/kg were significantly higher than in soil at the same site **(Fig 4.15)**.

### *4.2.2.1.3) Comparisons of metal concentrations between moss and lichen at sites C, 1, 2 and 3*

Mean metal concentrations in moss and lichen at sites C, 1, 2 and 3 of Orange Kloof forest for the wet sampling occasion in June 2015 are shown in **Figs 4.16 to 4.18**. Statistical signifcant differences (P<0.050) between moss and lichen are indicated with an asterisk above the graph bars.

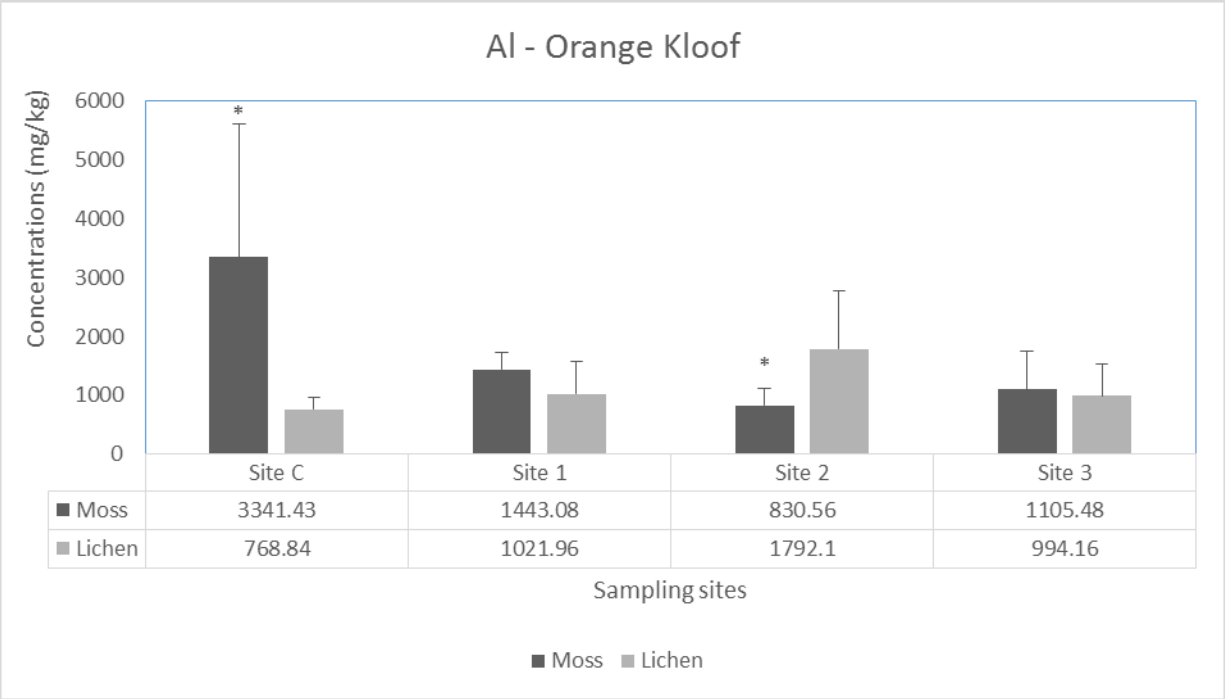


**Figure 4.16:** The mean Al concentrations (mg/kg) (± SD) in moss and lichen at sites C, 1, 2 and 3 of Orange Kloof forest for the wet sampling occasion in June 2015. N=5.

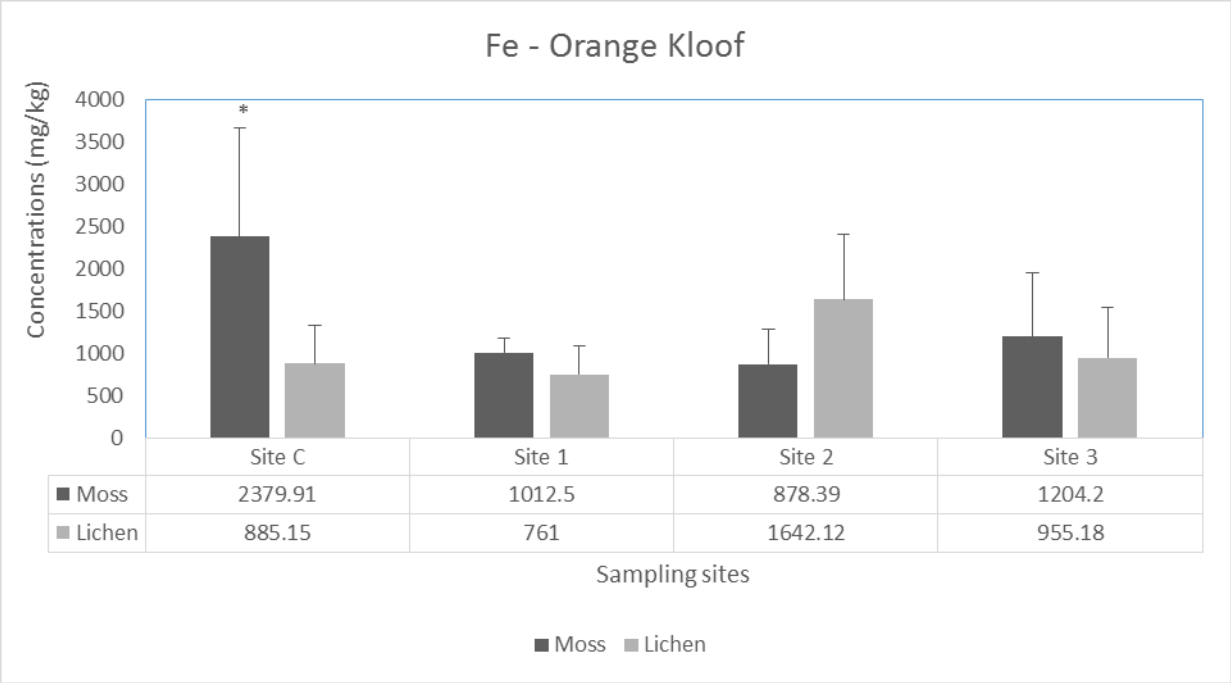


**Figure 4.17:** The mean Fe concentrations (mg/kg) (± SD) in moss and lichen at sites C, 1, 2 and 3 of Orange Kloof forest for the wet sampling occasion in June 2015. N=5.

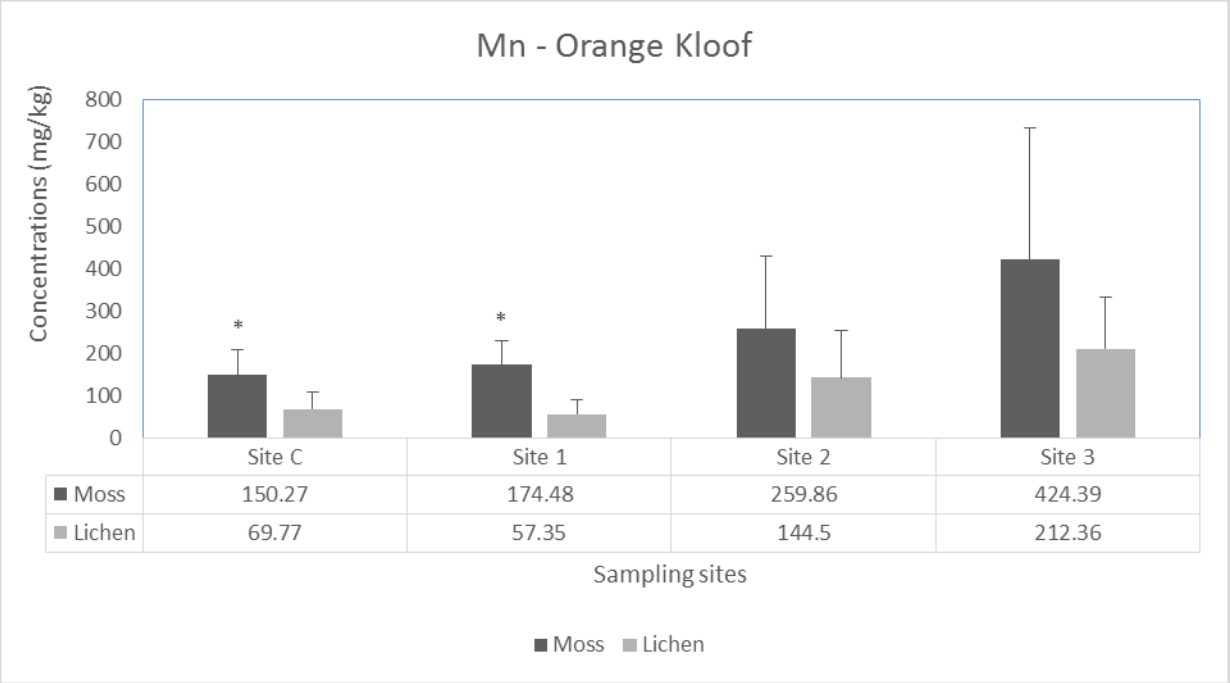


**Figure 4.18:** The mean Mn concentrations (mg/kg) (± SD) in moss and lichen at sites C, 1, 2 and 3 of Orange Kloof forest for the wet sampling occasion in June 2015. N=5.

Orange Kloof moss and lichen comparisons showed statistically significant differences between each other at sites: C (P=0.034) and 2 (P=0.032). There were, however no statistically significant differences found between each other at sites 1 (P=0.171) and 3 (P=0.421) in terms of Al concentrations. Moss samples were significantly higher in mean Al concentrations compared to lichen with site C having the highest concentration of 3341.43 ± 2251.92 mg/kg **(Fig 4.16)**.

Iron concentrations between moss and lichen differed significantly from each other at site C (P=0.040), but no significant differences were found at sites 1 (P=0.177), 2: (P=0.086) and 3 (P=0.579) **(Fig 4.17)**.

Statistically significant differences between moss and lichen in this forest were found at sites C (P=0.036) and 1 (P=0.151), but not at sites 2 (P=0.242) and 3 (P=0.188) when Mn concentrations were compared **(Fig 4.18)**.

#### *4.2.2.1.4) pH and Moisture*

pH of the soil of Orange Kloof ranged from alkaline (7.3 to 7.28) and the moisture % of the soil ranged from a moist 17.54 to 24.54 %.

**Table 4.15:** pH and moisture % of soil of Orange Kloof forest for the wet sampling occasion in June 2015.

| ORANGE KLOOF FOREST | SOIL | |
|---|---|---|
| Site C | **pH** | 7.28 |
| | *Moisture* | 21.72 |
| Site 1 | **pH** | 7.3 |
| | *Moisture* | *24.12* |
| Site 2 | **pH** | 7.14 |
| | *Moisture* | *24.54* |
| Site 3 | **pH** | 7.8 |
| | *Moisture* | *17.54* |

Moisture % of the leaf litter ranged from a moist 59.30 to 74.39 %.

**Table 4.16:** Moisture % of the leaf litter of Orange Kloof forest for the wet sampling occasion in June 2015.

| ORANGE KLOOF FOREST | % | LEAF LITTER |
|---|---|---|
| Site C | Moisture | 55.50 |
| Site 1 | Moisture | 74.39 |
| Site 2 | Moisture | 59.30 |
| Site 3 | Moisture | 67.63 |

### *4.2.2.1.5) Weather data from the South African Weather Service in the vicinity of Constantia, Cape Town*

**Table 4.17:** Weather data in the vicinity of Orange Kloof forest for the wet sampling occasion in June 2015.

| SAMPLING SITES | SAMPLING OCCASION | | | |
|---|---|---|---|---|
| | DATE | DEGREES (°C) | PRECITATION (MM) | WIND SPEED (KM/H) |
| **ORANGE KLOOF FOREST** | | | | |
| Site 1, 3 | 03-Jun-15 | 16 | 20 | 35 |
| Site 1, 2 | 05-Jun-15 | 13 | 0 | 11 |
| Site C | 08-Jun-15 | 14 | 0 | 16 |
| Site C | 25-Jun-15 | 11 | 0 | 11 |

### *4.2.2.1.6) Soil characterization for the forest, Orange Kloof at sites C, 1, 2 and 3*

**Table 4.18:** Characterization of soil for Orange Kloof forest sites for wet sampling occasion in June 2015.

| SAMPLING | (%) | | | | | | | WATER RETENTION | | |
|---|---|---|---|---|---|---|---|---|---|---|
| SITES | Clay | Silt | Fine sand | Medium sand | Coarse sand | Rock (v/v) | Classification | 10kPa | 100kPa | mm/m |
| ORANGE KLOOF FOREST | | | | | | | | | | |
| Site C | 9 | 12 | 31.6 | 26 | 21.4 | 21 | LmSa | 18.28 | 10.77 | 75.1 |
| | | | | | | | | | | |
| Site 1 | 5 | 6 | 46.8 | 28.6 | 13.6 | 8 | Sa | 19.08 | 9.13 | 99.5 |
| | | | | | | | | | | |
| Site 2 | 9 | 14 | 31.6 | 14 | 31.4 | 24.8 | LmSa | 20.22 | 12.19 | 80.4 |
| | | | | | | | | | | |
| Site 3 | 3 | 6 | 55.1 | 25.3 | 10.6 | 8.1 | Sa | 20.1 | 8.88 | 112.2 |

### 4.2.2.2) NEWLANDS FOREST

#### *4.2.2.2.1) Comparisons of metal concentrations in soil, leaf litter and sentinel organisms between sites C, 1, 2 and 3*

The mean metal concentrations in soil, leaf litter and sentinel organisms for sites C, 1, 2 and 3 of Newlands forest for the wet sampling occasion in June 2015 are presented in **Table 4.19.** Metal concentrations are expressed in mg/kg.

**Table 4.19:** The mean metal concentrations (mg/kg) (± SD) in soil, leaf litter and sentinel organisms for sites C, 1, 2 and 3 of Newlands forest for the wet sampling occasion in June 2015.

| METAL | | SOIL | | | | LEAF LITTER | | | | MOSS | | | | LICHEN | | | | MILLIPEDES | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Site C | Site 1 | Site 2 | Site 3 | Site C | Site 1 | Site 2 | Site 3 | Site C | Site 1 | Site 2 | Site 3 | Site C | Site 1 | Site 2 | Site 3 | Site C | Site 1 | Site 2 | Site 3 |
| Al | Mean | $^{bcd}$13940.73 | $^{ad}$ 6711.84 | $^{ad}$10395.26 | $^{abc}$7042.52 | 1021.31 | 965.78 | 907.12 | 1067.13 | $^{cd}$3341.43 | $^{cd}$2811.9 | $^{ab}$1141.67 | $^{ab}$6364.45 | $^{b}$768.84 | $^{acd}$2377.85 | $^{b}$966.45 | $^{b}$506.28 | 1072.30 | 3007.07 | 1717.00 | 1581.90 |
| | *SD* | *3263.24* | *1408.56* | *4201.44* | *2730.78* | *263.66* | *384.35* | *831.49* | *467.50* | *2251.92* | *1346.04* | *228.70* | *3120.95* | *194.82* | *901.34* | *336.42* | *280.86* | *959.59* | *1496.38* | *1833.08* | *638.54* |
| Fe | Mean | $^{bcd}$6503.83 | $^{acd}$7739.18 | $^{ab}$11587.74 | $^{ab}$16243.42 | $^{bcd}$683.95 | $^{ad}$1049.17 | $^{ad}$1007.01 | $^{abc}$2344.68 | 2379.91 | 3292.09 | 1361.78 | 7683.84 | $^{bcd}$885.15 | $^{acd}$3105.6 | $^{ab}$1116.85 | $^{ab}$758.49 | 623.15 | 2735.58 | 2550.90 | 3384.41 |
| | *SD* | *1225.00* | *1225.06* | *2729.20* | *4549.94* | *96.78* | *419.59* | *1093.64* | *1245.40* | *1288.60* | *1652.16* | *288.13* | *3500.28* | *448.32* | *1083.71* | *405.30* | *363.80* | *497.07* | *1300.32* | *2620.86* | *1573.51* |
| Mn | Mean | $^{bcd}$186.68 | $^{ac}$365.37 | $^{abd}$486.45 | $^{ac}$496.66 | $^{bcd}$177.24 | $^{ac}$421.69 | $^{abd}$987.63 | $^{ac}$532.74 | 150.27 | 444.22 | 515.10 | 389.00 | 69.77 | 234.18 | 156.16 | 77.84 | $^{cd}$47.11 | $^{cd}$71.03 | $^{ab}$173.13 | $^{ab}$143.05 |
| | *SD* | *47.58* | *172.52* | *124.64* | *135.99* | *35.28* | *146.43* | *265.82* | *136.97* | *59.78* | *296.09* | *286.35* | *144.10* | *39.44* | *354.52* | *111.43* | *25.02* | *12.46* | *29.89* | *75.90* | *45.41* |

Statistical signifcant differences (P<0.050) are indicated with different superscripted letters: site C=a, site 1=b, site 2=c, site 3=d. Comparisons were done separately for the soil, leaf litter and sentinel organisms. N=5.

***a) Soil***

Pairwise multiple comparisons showed statistically significant differences (P<0.05) in the soil Al concentrations for the comparisons of sites C vs 1, 2 and 3 with the exception of sites 1 vs 2, 1 vs 3 and 2 vs 3 (P>0.05). The concentrations found at sites C (13940.73 ± 3263.24 mg/kg) and 2 (10395.26 ± 4201.44 mg/kg) were significantly higher than at sites 1 (6711.84 ± 1408.56 mg/kg) and 3 (7042.52 ± 2730.78 mg/kg) **(Table 4.19)**.

Newlands forest soil differed significantly in Fe concentrations between sites C and 1, 2 and 3 and sites 1 vs 2 and 1 vs 3 (P<0.05). No significant differences were found between sites 2 and 3 (P>0.05). Site C had the lowest mean concentration of (6503.83 ± 1225.00 mg/kg) and site 3 the highest (16243.42 ± 4549.94 mg/kg) when compared **(Table 4.19)**.

In terms of Mn concentrations at Newlands, statistically significant differences were found between site C and sites 1, 2 and 3 (P<0.05), but no significant differences were found in the soil between sites 1 vs 2, 1 vs 3 and 2 vs 3 (P>0.05). The mean concentration at site C (186.68 ± 47.58 mg/kg) was significantly lower than the mean concentrations found at the other sites **(Table 4.19)**.

***b) Leaf litter***

Site comparisons of leaf litter showed no statistically significant differences between any of the sites in terms of Al concentrations (P=0.548) **(Table 4.19)**.

Iron concentrations in leaf litter differed significantly between sites: C vs 3, 1 vs 3 and 2 vs 3 (P<0.05). No significant differences were found between sites C vs 1, C vs 2 and 1 vs 2 (P>0.05). The mean concentration at site 3 (2344.68 ± 1245.40 mg/kg) was significantly higher than the mean concentration at site C (683.95 ± 96.78 mg/kg) **(Table 4.19)**.

Leaf litter in Newlands forest differed significantly in Mn concentrations between sites C and 1, 2 and 3 and sites 1 and 2 and 2 and 3 (P<0.05). No significant differences

were found between sites 1 vs 3 (P>0.05). Site C had the lowest mean concentration of 177.24 ± 35.28 mg/kg in this comparison **(Table 4.19)**.

***c) Moss***

Site comparisons showed statistically significant differences in Al concentrations in moss for the comparisons of sites C vs 2 and 3 and sites 1 vs 2 and 1 vs 3 (P<0.05). However, no differences between sites C vs 1 and 2 vs 3 (P>0.05) were found. Site 3 with a mean concentration of 6364.45 ± 3120.95 mg/kg was significantly higher than at sites C, 1, and 2 **(Table 4.19)**.

Newlands forest did not show any significant differences in Fe (P=0.089) and Mn (P=0.054) concentrations in moss samples between the four sites for this sampling session **(Table 4.19)**.

***d) Lichen***

Newlands forest lichen differed significantly in Al concentrations between sites C and 1, 1 and 2, 1 and 3 and 2 and 3 (P<0.05). No significant differences were found between sites C vs 2; and C vs 3 (P>0.05). Site 1 showed the highest mean concentration of (2377.85 ± 901.34 mg/kg) when compared to the other sites **(Table 4.19)**.

Statistically significant differences in Fe concentrations in lichen were found between sites C and 1, 1 and 2 and 1 and 3 (P<0.05), but no significant differences were found between sites C vs 2, C vs 3 and 2 vs 3 (P>0.05). Site 1 had the highest mean Fe concentration of 3105.6 ± 1083.71 mg/kg **(Table 4.19)**.

No significant differences in lichen Mn (P=0.244) concentrations were found between any of the sites **(Table 4.19)**.

***e) Millipedes***

There were no statistical differences found in millipede Al (P=0.198) and Fe (P=0.063) concentrations between the four sites **(Table 4.19)**.

Pairwise multiple comparisons showed statistically significant differences in millipedes in terms of Al concentrations for the comparisons of sites C vs 2 and 3, sites 1 vs 2 and 1 vs 3 ($P<0.05$). No statistically differences between sites C vs 1 and 2 vs 3 ($P>0.05$) were found. The mean Mn concentration at site C (47.11 ± 12.46 mg/kg) was significantly lower than at sites 1, 2 and 3 **(Table 4.19)**.

### *4.2.2.2.2) Comparisons of metal concentrations between soil and leaf litter at sites C, 1, 2 and 3*

Mean metal concentrations in soil and leaf litter at sites C, 1, 2 and 3 of Newlands forest for the wet sampling occasion in June 2015 are shown in **Figs 4.19 to 4.21**. Statistical signifcant differences (P<0.050) between soil and leaf litter are indicated with an asterisk above the graph bars.

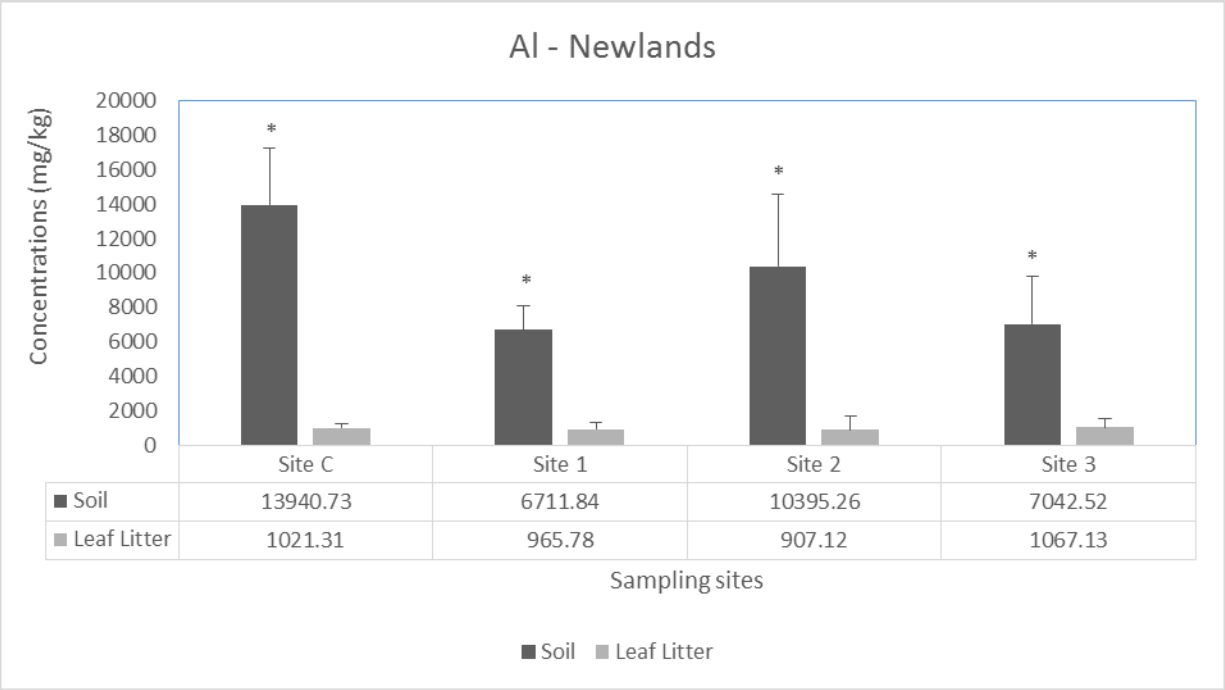


**Figure 4.19:** The mean Al concentrations (mg/kg) (± SD) in soil and leaf litter at sites C, 1, 2 and 3 of Newlands forest for the wet sampling occasion in June 2015. N=5.

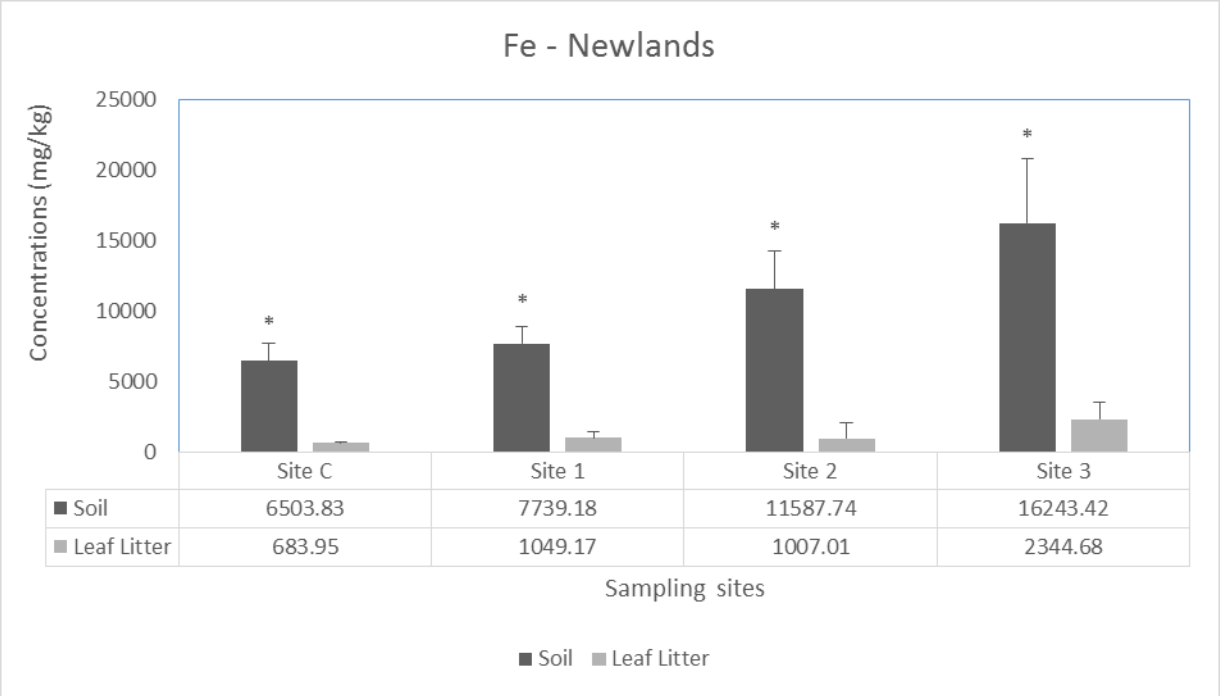


**Figure 4.20:** The mean Fe concentrations (mg/kg) (± SD) in soil and leaf litter at sites C, 1, 2 and 3 of Newlands forest for the wet sampling occasion in June 2015. N=5.

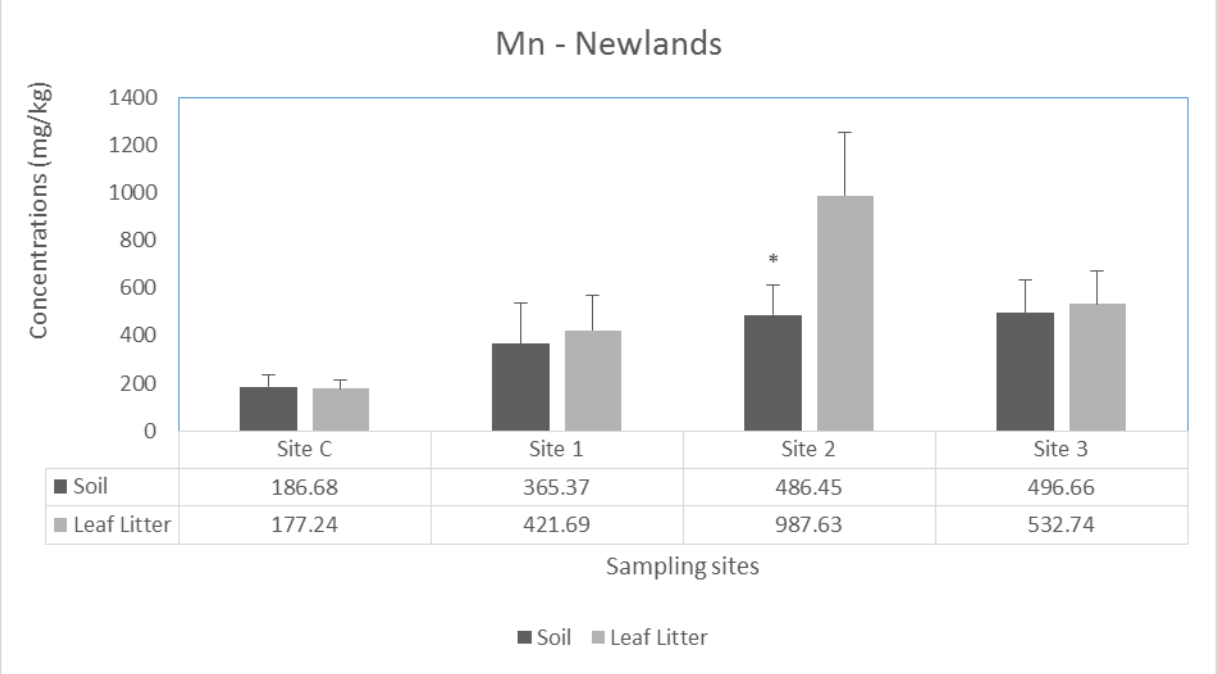


**Figure 4.21:** The mean Mn concentrations (mg/kg) (± SD) in soil and leaf litter at sites C, 1, 2 and 3 of Newlands forest for the wet sampling occasion in June 2015. N=5.

Aluminium mean concentrations at sites C (P=<0.001), 1 (P=0.001), 2 (P=0.001) and site 3 (P=0.001) showed statistically significant differences between soil and leaf litter. The highest mean Al concentrations were found in soil with site C (13940.73 ± 3263.24 mg/kg) showing the highest concentration **(Fig 4.19)**.

Sites, C (P=<0.001), 1 (P=<0.001), 2 (P=<0.001) and 3 (P=<0.001) at Newlands showed statistically significant differences in terms of Fe concentrations in soil vs leaf litter comparisons. The mean concentrations were higher in soil at all the sites with site 3 showing the highest mean concentration of (16243.42 ± 4549.94 mg/kg) **(Fig 4.20)**.

Site C, (P=0.731), 1 (P=0.593) and 3 (P=0.687) showed no statistically significant differences between soil and leaf litter, however there were statistically significant differences found at site 2 (P=0.005) in terms of Mn concentrations. The highest mean concentration at site 2 (987.63 ± 265.82 mg/kg) was found in leaf litter **(Fig 4.21)**.

***4.2.2.2.3) Comparisons of metal concentrations between moss and lichen at sites C, 1, 2 and 3***

Mean metal concentrations in moss and lichen at sites C, 1, 2 and 3 of Newlands forest for the wet sampling occasion in June 2015 are shown in **Figs 4.22 to 4.24**. Statistical signifcant differences (P<0.050) between moss and lichen are indicated with an asterisk above the graph bars.

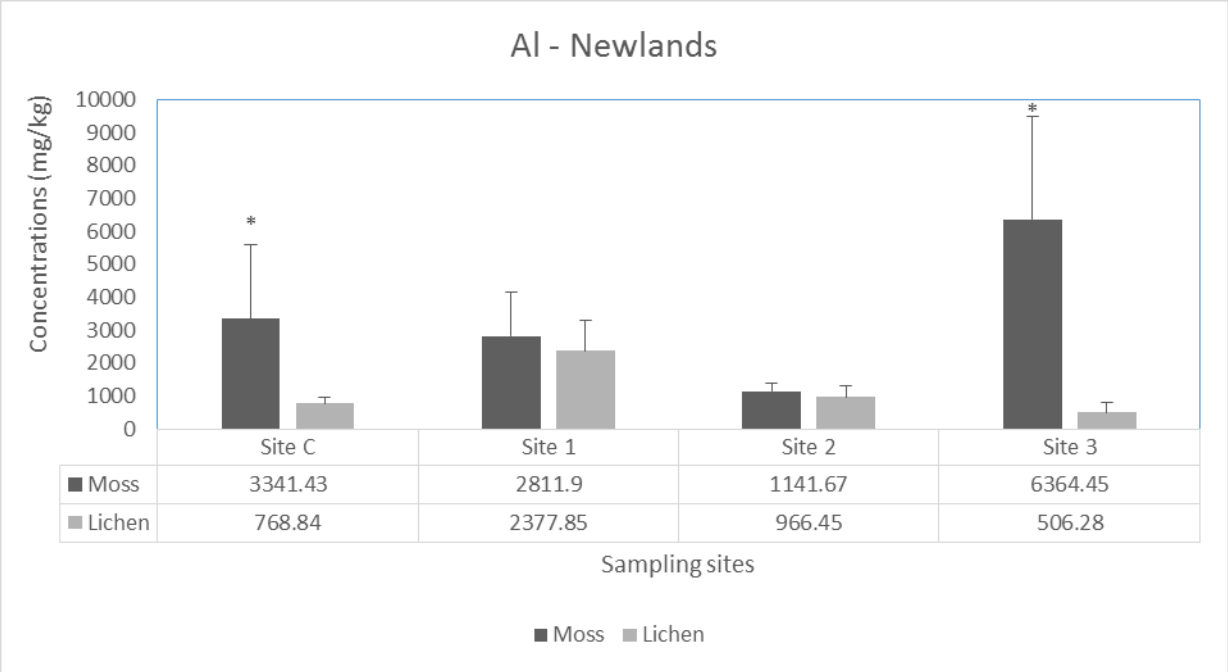


**Figure 4.22:** The mean Al concentrations (mg/kg) (± SD) in moss and lichen at sites C, 1, 2 and 3 of Newlands forest for the wet sampling occasion in June 2015. N=5.

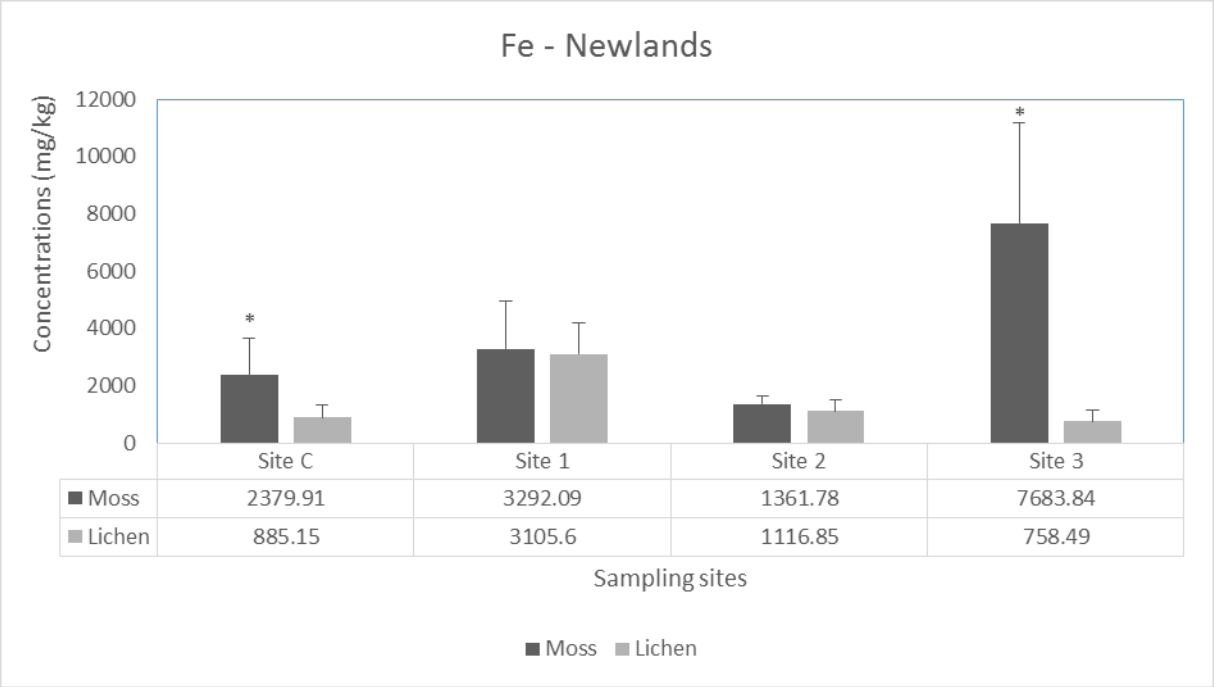


**Figure 4.23:** The mean Fe concentrations (mg/kg) (± SD) in moss and lichen at sites C, 1, 2 and 3 of Newlands forest for the wet sampling occasion in June 2015. N=5.

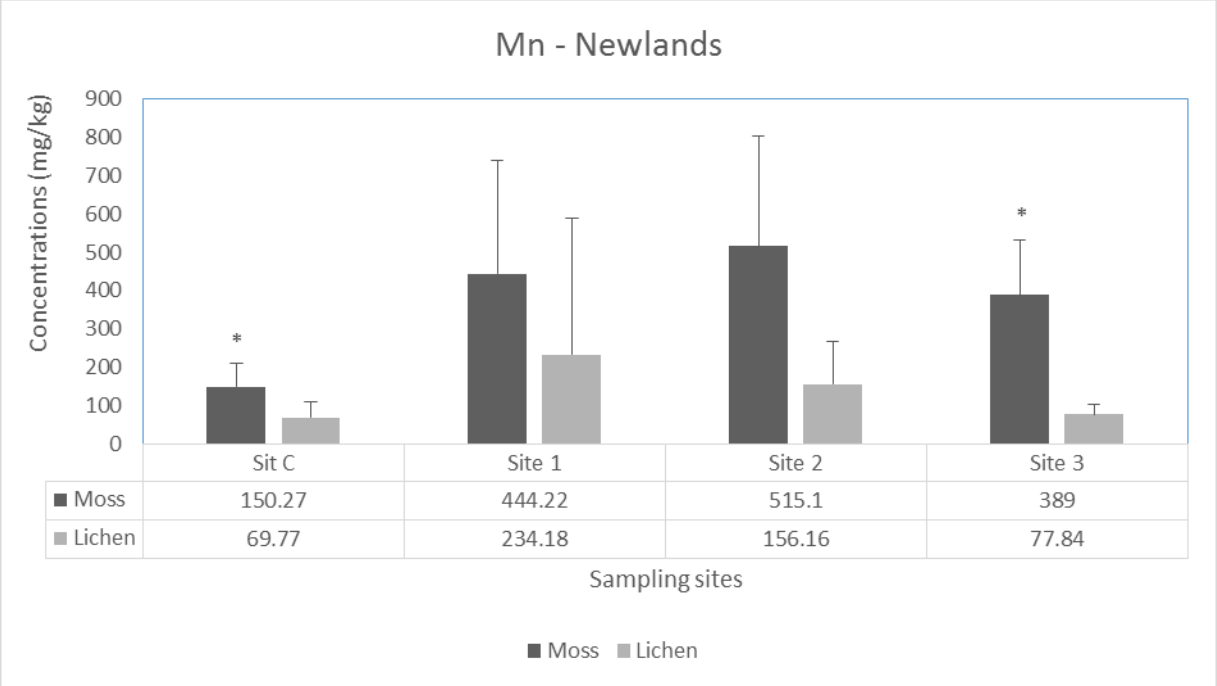


**Figure 4.24:** The mean Mn concentrations (mg/kg) (± SD) in moss and lichen at sites C, 1, 2 and 3 of Newlands forest for the wet sampling occasion in June 2015. N=5.

Sites C (P=0.034) and 3 (P=0.016) showed statistically significant differences between moss and lichen, but no significant differences were found between the organisms at sites 1 (P=0.566) and 2 (P=0.364) when Al concentrations were compared. The highest mean concentration at site 3 (6364.45 ± 3120.95 mg/kg) was found in moss **(Fig 4.22)**.

Statistically significant differences were found between moss and lichen at sites C (P=0.040) and 3 (P=0.015). No statistically significant differences in Fe concentrations were found at sites 1 (P=0.838) and 2 (P=0.303). Moss at site 3 had the highest mean concentration (7683.84 ± 758.49 mg/kg). Mean concentrations in moss were also higher than were found in lichen **(Fig 4.23)**.

Manganese concentration comparisons at sites C (P=0.036) and 3 (P=0.032) showed statistically significant differences. No significant differences were found at sites 1 (P=0.095) and 2 (P=0.056). The lowest mean concentration was found in lichen at site C (69.77 ± 39.44 mg/kg) and the highest mean concentration at site 2 (515.1 ± 286.35 mg/kg) in moss and **(Fig 4.24)**.

#### *4.2.2.2.4) pH and Moisture*

pH of the soil was slightly alkaline (7.5 to 7.38) and the moisture % of the soil ranged from a relatively moist 12.50 % to 21.72 %.

**Table 4.20:** pH and moisture % of soil of Newlands forest for the wet sampling occasion in June 2015.

| **NEWLANDS FOREST** | **SOIL** | |
|---|---|---|
| Site C | **pH** | 7.28 |
| | *Moisture* | 21.72 |
| Site 1 | **pH** | 7.5 |
| | *Moisture* | *12.50* |
| Site 2 | **pH** | 7.38 |
| | *Moisture* | *7.73* |
| Site 3 | **pH** | 7.18 |
| | *Moisture* | *16.39* |

Moisture % of leaf litter ranged from a moist 54.28 % to 67.86 %.

**Table 4.21:** Moisture % of the leaf litter of Newlands forest for the wet sampling occasion in June 2015.

| NEWLANDS FOREST | % | LEAF LITTER |
|---|---|---|
| Site C | Moisture | 55.50 |
| Site 1 | Moisture | 67.86 |
| Site 2 | Moisture | 54.28 |
| Site 3 | Moisture | 61.51 |

### *4.2.2.2.5) Weather data from the South African Weather Service in the vicinity of Newlands, Cape Town*

**Table 4.22:** Weather data in the vicinity of Newlands forest for the wet sampling occasion in June 2015.

| SAMPLING SITES | SAMPLING OCCASION | | | |
|---|---|---|---|---|
| | DATE | DEGREES (°C) | PRECITATION (MM) | WIND SPEED (KM/H) |
| NEWLANDS FOREST | | | | |
| Site 1 | 04-Jun-15 | 13 | 0 | 23 |
| Site 2, 3 | 06-Jun-15 | 16 | 0 | 19 |

### *4.2.2.2.6) Soil characterization for the forest, Newlands at sites 1, 2 and 3*

**Table 4.23:** Characterization % of soil for Newlands forest sites for the wet sampling occasion in June 2015.

| SAMPLING | (%) | | | | | | | WATER RETENTION | | |
|---|---|---|---|---|---|---|---|---|---|---|
| SITES | Clay | Silt | Fine sand | Medium sand | Coarse sand | Rock (v/v) | Classification | 10kPa | 100kPa | mm/m |
| NEWLANDS FOREST | | | | | | | | | | |
| Site 1 | 7 | 12 | 41.8 | 22 | 17.2 | 23.1 | LmSa | 18.93 | 10.30 | 86.2 |
| | | | | | | | | | | |
| Site 2 | 3 | 4 | 24.7 | 43.3 | 25 | 2.0 | Sa | 13.63 | 7.30 | 63.3 |
| | | | | | | | | | | |
| Site 3 | 9 | 18 | 44.8 | 18.24 | 10 | 18.6 | LmSa | 23.14 | 13.03 | 101.1 |

### 4.2.2.3) FORESTS

#### *4.2.2.3.1) Comparisons of metal concentrations in soil, leaf litter and sentinel organisms between the forests: Site C, Orange Kloof and Newlands*

The mean metal concentrations in soil, leaf litter and sentinel organisms for each forest for the wet sampling occasion (June 2015) are presented in **Tables 4.24 to 4.26**. The metal concentrations for the sites within each forest was pooled to calculate the mean concentrations for each forest. Metal concentrations are expressed in mg/kg.

***a) Al***

**Table 4.24:** The mean Al concentrations (mg/kg) (± SD) in soil, leaf litter and sentinel organisms for forests for the wet sampling occasion in June 2015.

| FOREST | | SOIL | LEAF LITTER | MOSS | LICHEN | MILLIPEDES |
|---|---|---|---|---|---|---|
| Site C | Mean | $^{bc}$13940.73 | $^{b}$1021.3 | $^{bc}$3341.43 | 768.84 | $^{bc}$1072.29 |
| | *SD* | *3263.24* | *263.66* | *2251.93* | *194.82* | *959.60* |
| Orange Kloof | Mean | $^{a}$10669.29 | $^{ac}$513.1 | $^{a}$1126.37 | 1269.40 | $^{ac}$411.31 |
| | *SD* | *11070.58* | *553.72* | *477.10* | *773.65* | *513.85* |
| Newlands | Mean | $^{a}$8049.87 | $^{b}$980 | $^{a}$1667.44 | 1283.52 | $^{a\ b}$2101.99 |
| | *SD* | *3272.21* | *553.96* | *1121.64* | *983.03* | *1469.16* |

Statistical signifcant differences (P<0.050) are indicated with different superscripted letters: site C=a, site 1=b, site 2=c, site 3=d. Comparisons were done separately for the soil, leaf litter and sentinel organisms. N=5.

Soil was compared between forests and statistically significant differences were found between site C and Orange Kloof and site C and Newlands forests (P<0.050) when Al concentrations were compared. No significant differences were found between the Orange Kloof and Newlands forest comparison (P>0.050). Soil showed the highest mean concentration of 13940.73 ± 3263.24 mg/kg at site C and the lowest concentration of 8049.87 ± 3272.21 mg/kg was found at Newlands **(Table 4.24)**.

Statistically significant differences were found between site C and Orange Kloof and Orange Kloof and Newlands when the forests were compared in terms of Al concentrations in leaf litter (P<0.05). There were, however no significant differences found between the site C and Newlands comparisons (P>0.05). Site C mean concentrations (1021.3 ± 263.66 mg/kg) were significantly higher than at the other two forests **(Table 4.24)**.

Aluminium concentrations in moss showed statistically significant differences between the site C and Orange and site C and Newlands forest comparisons (P<0.05), but no statistically significant differences were found when Newlands and Orange Kloof was compared (P>0.05). Site C showed the highest mean concentration of 3341.43 ± 2251.93 mg/kg and Orange Kloof displayed the lowest mean concentration of 1126.37 ± 477.10 mg/kg **(Table 4.24)**.

Lichen comparisons between forests for Al concentrations showed no statistically significant differences between the forests (P=0.123) **(Table 4.24)**.

Pairwise multiple comparisons between forests in terms of Al concentrations revealed statistically significant differences between all the forests when millipedes were compared (P<0.05) **(Table 4.24)**.

***b) Fe***

**Table 4.25:** The mean Fe concentrations (mg/kg) (± SD) in soil, leaf litter and sentinel organisms for forests for the wet sampling occasion in June 2015.

| FOREST | | SOIL | LEAF LITTER | MOSS | LICHEN | MILLIPEDES |
|---|---|---|---|---|---|---|
| **Site C** | **Mean** | **$^{c}$6503.82** | **$^{c}$683.95** | **$^{b}$2379.9** | **885.15** | **$^{bc}$623.14** |
| | *SD* | *1225.01* | *96.78* | *1288.61* | *448.33* | *497.07* |
| **Orange Kloof** | **Mean** | **$^{c}$5922.22** | **$^{c}$609.39** | **$^{ac}$1031.69** | **1119.43** | **$^{ac}$364.2** |
| | *SD* | *6318.44* | *433.81* | *486.84* | *676.68* | *326.99* |
| **Newlands** | **Mean** | **$^{a\ b}$11856.77** | **$^{a\ b}$1466.95** | **$^{b}$2035.24** | **1660.31** | **$^{a\ b}$2890.29** |
| | *SD* | *4628.74* | *1117.24* | *1297.96* | *1249.90* | *1813.82* |

Statistical signifcant differences (P<0.050) are indicated with different superscripted letters: site C=a, site 1=b, site 2=c, site 3=d. Comparisons were done separately for the soil, leaf litter and sentinel organisms. N=5.

Statistically significant differences in soil were found between the forests, Orange and Newlands and site C and Newlands ($P<0.05$) in this sampling occasion. No significant differences were found in Fe concentrations between site C and Orange Kloof forests ($P>0.05$). Newlands showed the highest mean Fe concentration (11856.77 ± 4628.74 mg/kg) **(Table 4.25)**.

Comparisons between leaf litter showed statistically significant differences between site C and Newlands and Orange and Newlands forests ($P<0.05$). There were, however no statistically significant differences found between site C and Orange Kloof ($P>0.050$). The Newlands mean Fe concentration of 1466.95 ± 1117.24 mg/kg was the highest of the three forests **(Table 4.25)**.

In terms of Fe concentrations in moss, statistically significant differences were found between site C and Orange Kloof forest, as well as Newlands and Orange Kloof forests ($P<0.05$), but no significant differences were found between site C and Newlands forest ($P>0.05$). In this comparison, site C showed the highest mean concentration (2379.9 ± 1288.61 mg/kg) as opposed to Orange Kloof that displayed the lowest mean concentration for this sampling occasion (1031.69 ± 486.84 mg/kg) **(Table 4.25)**.

Pairwise multiple comparisons between lichen showed no statistically significant differences between all of the forests when Fe concentrations were compared ($P=0.219$) **(Table 4.25)**.

Millipede comparisons determined that that were statistically significant differences in Fe concentrations between all three forests ($P<0.05$) **(Table 4.25)**.

***c) Mn***

**Table 4.26:** The mean Mn concentrations (mg/kg) (± SD) in soil, leaf litter and sentinel organisms for forests for the wet sampling occasion in June 2015.

| FOREST | | SOIL | LEAF LITTER | MOSS | LICHEN | MILLIPEDES |
|---|---|---|---|---|---|---|
| Site C | Mean | $^{c}$186.67 | $^{bc}$177.24 | $^{c}$150.27 | 69.77 | $^{b}$47.1 |
| | *SD* | *47.59* | *35.29* | *59.78* | *39.45* | *12.46* |
| Orange Kloof | Mean | $^{c}$204.34 | $^{ac}$461.57 | $^{c}$286.57 | 138.07 | $^{ac}$102.4 |
| | *SD* | *158.31* | *445.46* | *219.38* | *110.84* | *93.34* |
| Newlands | Mean | $^{a\,b}$449.49 | $^{a\,b}$ 647.35 | $^{a\,b}$403.6 | 156.06 | $^{a\,b}$129.07 |
| | *SD* | *148.45* | *309.69* | *257.68* | *209.77* | *66.76* |

Statistical signifcant differences (P<0.050) are indicated with different superscripted letters: site C=a, site 1=b, site 2=c, site 3=d. Comparisons were done separately for the soil, leaf litter and sentinel organisms. N=5.

Soil comparisons showed statistically significant differences between the forests: site C and Newlands and Orange Kloof and Newlands (P<0.05), but no statistically significant differences were found between site C and Orange Kloof (P>0.05). The Mn mean concentration (449.49 ± 148.45 mg/kg) was the highest at Newlands and the lowest mean concentration of 186.67 ± 47.59 mg/kg was found at site C **(Table 4.26)**.

In June 2015 significant differences were found in leaf litter between all the forests when Mn mean concentrations were compared (P<0.05). The mean Mn concentration of 647.35 ± 309.69 mg/kg at Newlands were higher than at the other forests **(Table 4.26)**.

Comparisons of Mn concentrations in moss showed statistically significant differences between all three forests (P<0.05) **(Table 4.26)**.

Lichen between forests were compared and no statistically significant differences were detected between any of the forests in terms of Mn concentrations (P=0.210) **(Table 4.26)**.

Pairwise multiple comparisons of millipedes between forests showed that there were statistically significant differences in Mn concentrations between all the forests (P<0.05). Newlands forest millipedes yielded the highest mean concentrations (129.07 ± 66.76 mg/kg) **(Table 4.26)**.

### 4.2.3 DRY AND WET SEASON: METAL CONTAMINATION AND BIOACCUMULATION

#### 4.2.3.1) ORANGE KLOOF FOREST

##### *4.2.3.1.1) Comparisons of metal concentrations of soil between dry and wet seasons at sites C, 1, 2 and 3*

Mean metal concentrations in soil at sites C, 1, 2 and 3 of Orange Kloof forest for the dry and wet sampling occasions in January and June 2015 are shown in **Figs 4.25 to 4.27**. Statistical signifcant differences (P<0.050) between the dry and wet seasons are indicated with an asterisk above the graph bars.

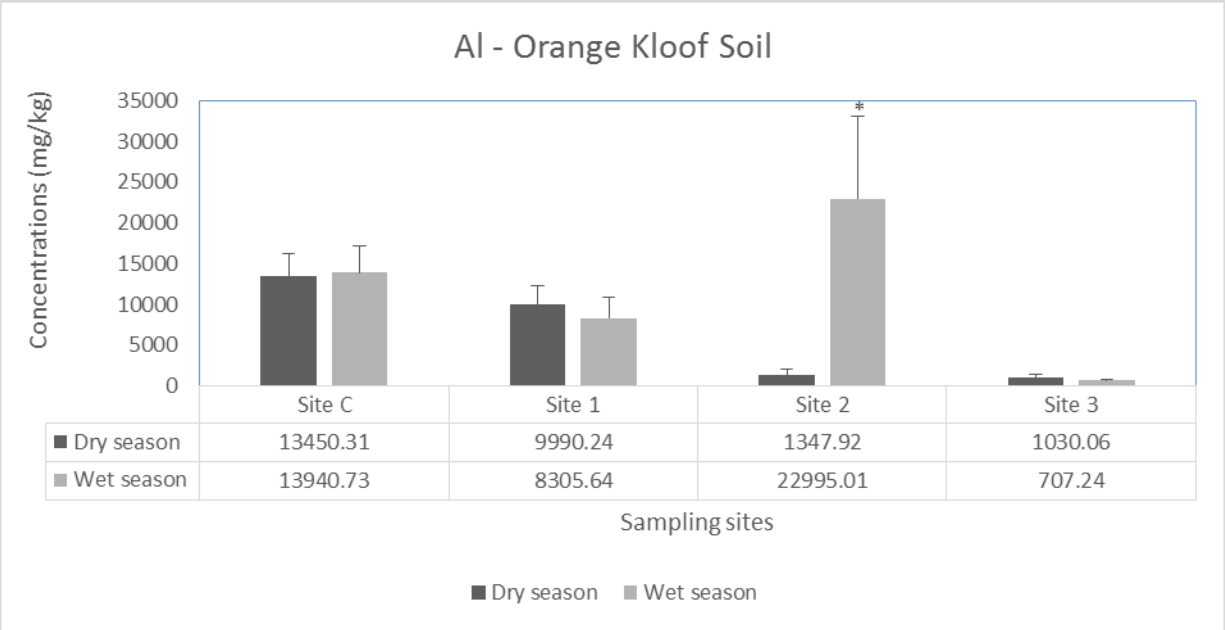


**Figure 4.25:** The mean Al concentrations (mg/kg) (± SD) in soil at sites C, 1, 2 and 3 of Orange Kloof forest for the dry and wet sampling occasions in January and June 2015. N=5.

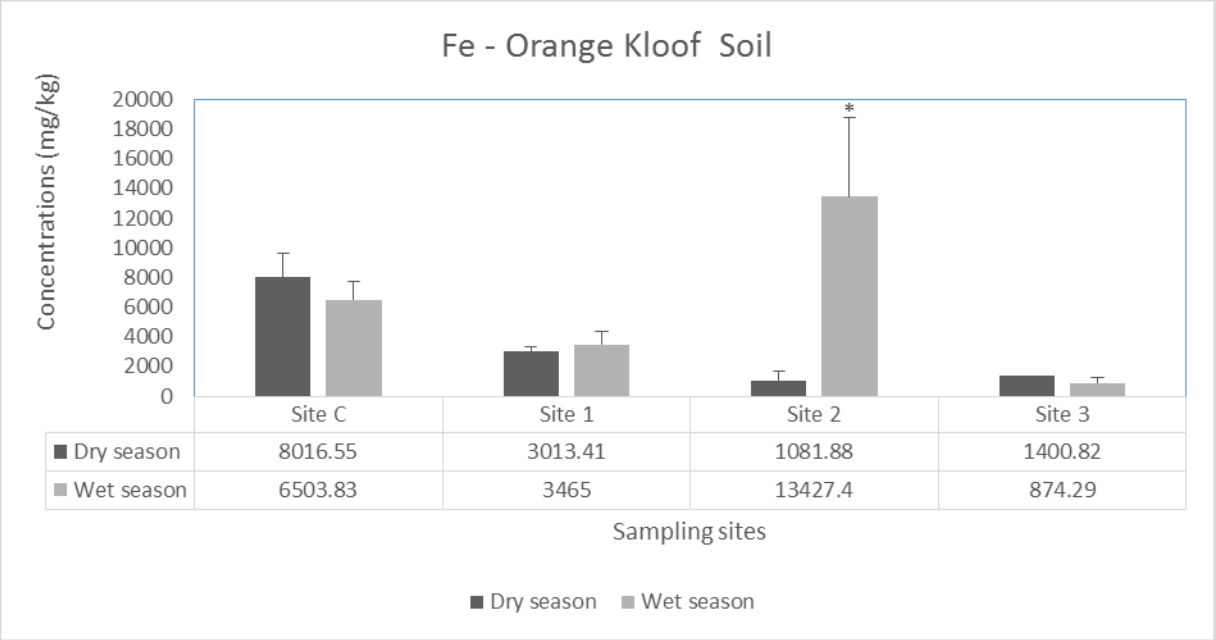


**Figure 4.26:** The mean Fe concentrations (mg/kg) (± SD) in soil at sites C, 1, 2 and 3 of Orange Kloof forest for the dry and wet sampling occasions in January and June 2015. N=5.

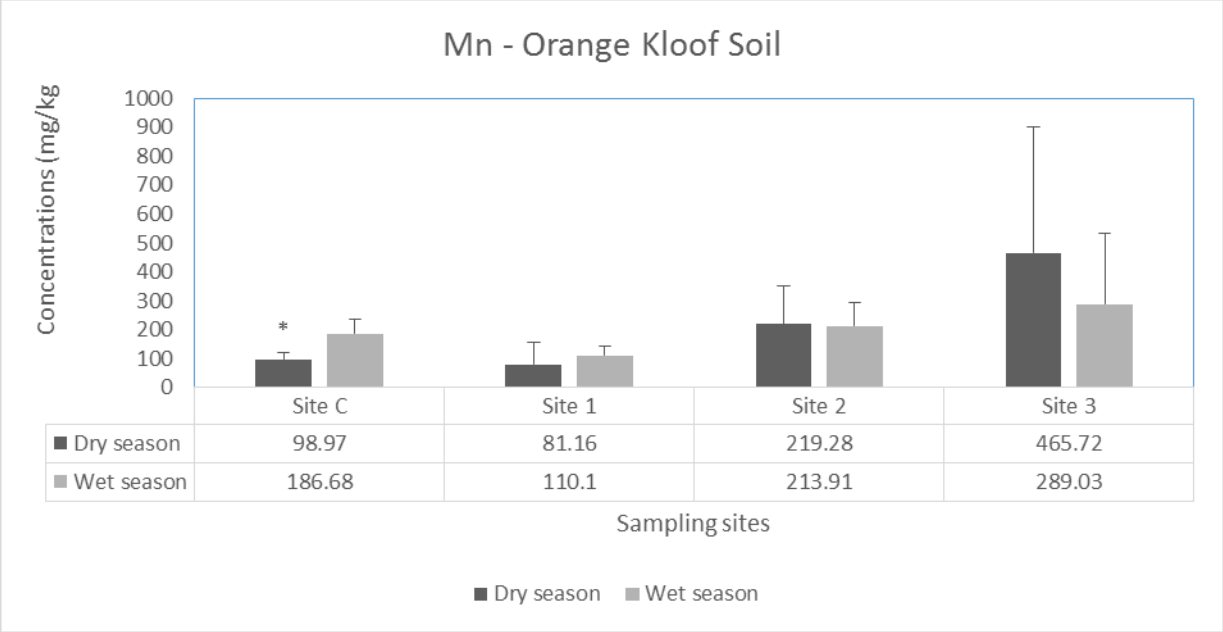


**Figure 4.27:** The mean Mn concentrations (mg/kg) (± SD) in soil at sites C, 1, 2 and 3 of Orange Kloof forest for the dry and wet sampling occasions in January and June 2015. N=5.

When soil was compared between the dry and wet seasons in terms of Al concentrations, significant differences between seasons at site 2 (P=0.008) were observed. There were no statistically significant differences found between the seasons at sites C (P=0.807), 1 (P=0.304) and 3 (P=0.310). The mean Al concentrations at site 2 (22995.01 ± 10084.25 mg/kg) in the wet season was significantly higher than at site 2 (1347.92 ± 699.77 mg/kg) in the dry season **(Fig 4.25)**.

The dry and wet seasons showed statistically significant differences between each other at site 2 (P=0.008) in terms of Fe concentrations. No statistically significant differences were, however found between seasons at sites C (P=0.131), 1 (P=0.334) and 3 (P=0.164). Iron concentrations calculated in soil at site 2 (13427.4 ± 5377.74 mg/kg) were once again significantly higher in the wet season as opposed to the dry season (1081.88 ± 591.57 mg/kg) **(Fig 4.26)**.

At Orange Kloof, the dry and wet seasons showed statistically significant differences between each other at site C (P=0.016), but not at sites 1 (P=0.151), 2 (P=0.939) and 3 (P=0.451) when Mn concentrations were compared. The lowest Mn concentrations were found in the dry season at site 1 (81.16 ± 76.79 mg/kg) **(Fig 4.27)**.

### *4.2.3.1.2) Comparisons of metal concentrations of leaf litter between dry and wet seasons at sites C, 1, 2 and 3*

Mean metal concentrations in leaf litter at sites C, 1, 2 and 3 of Orange Kloof forest for the dry and wet sampling occasions in January and June 2015 are shown in **Figs 4.28 to 4.30**. Statistical signifcant differences (P<0.050) between the dry and wet seasons are indicated with an asterisk above the graph bars.

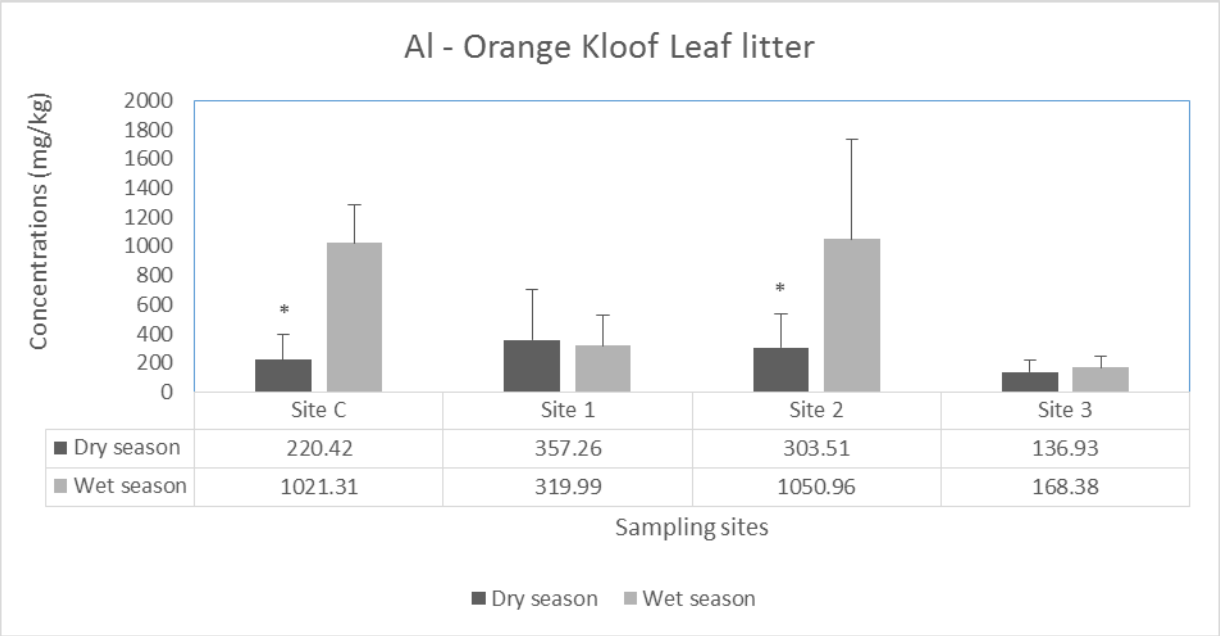


**Figure 4.28:** The mean Al concentrations (mg/kg) (± SD) in leaf litter at sites C, 1, 2 and 3 of Orange Kloof forest for the dry and wet sampling occasions in January and June 2015. N=5.

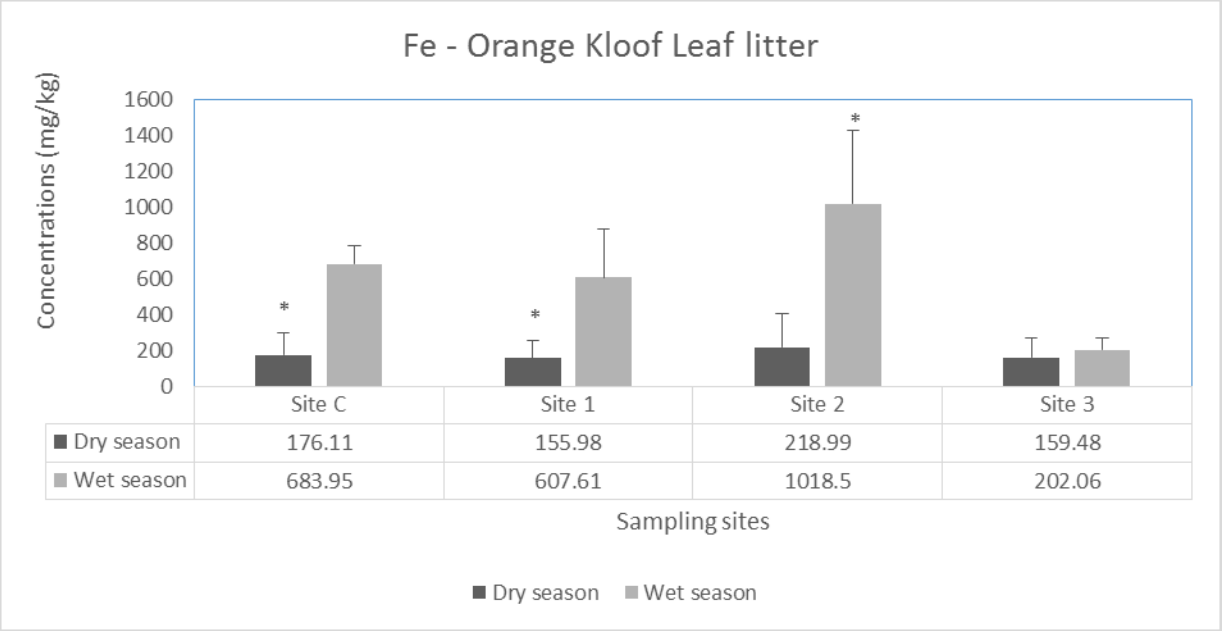


**Figure 4.29:** The mean Fe concentrations (mg/kg) (± SD) in leaf litter at sites C, 1, 2 and 3 of Orange Kloof forest for the dry and wet sampling occasions in January and June 2015. N=5.

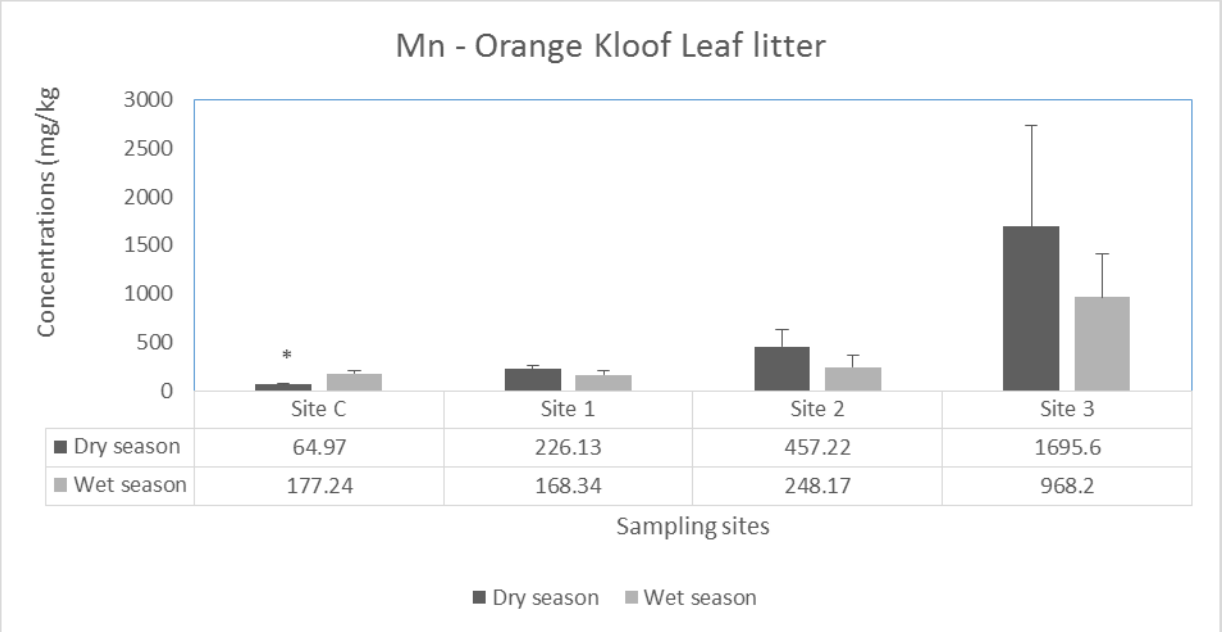


**Figure 4.30:** The mean Mn concentrations (mg/kg) (± SD) in leaf litter at sites C, 1, 2 and 3 of Orange Kloof forest for the dry and wet sampling occasions in January and June 2015. N=5.

Orange Kloof, dry and wet season comparisons showed statistically significant differences between each other at sites C (P=<0.001) and 2 (P=0.049) in terms of Al concentrations in leaf litter. No statistically significant differences were found between the seasons at sites 1 (P=0.843) and 3 (P=0.569). The wet season samples showed significantly higher mean concentrations of Al compared to the dry season samples at the same sites: C (1021.31 ± 263.66 mg/kg) and 2 (1050.96 ± 683.28 mg/kg) **(Fig 4.28)**.

Comparisons of Fe concentrations in leaf litter between the dry and wet sampling occasions differed significantly from each other at sites C (P=<0.001), 1 (P=0.016) and 2 (P=0.004) with the exception of site 3 (P=0.477). The highest Fe concentration was recorded at site 2 (1018.5 ± 408.70 mg/kg) in the wet season sampling occasion **(Fig 4.29)**.

Dry and wet season comparisons of Mn concentrations in leaf litter showed statistically significant differences between each other at site C (P=<0.001), but not at sites 1 (P=0.055), 2 (P=0.058) and 3 (P=0.188). A significantly lower Mn concentration was found at site C (64.97 ± 14.27 mg/kg) in the dry season **(Fig 4.30)**.

### *4.2.3.1.3) Comparisons of metal concentrations of moss between dry and wet seasons at sites C, 1, 2 and 3*

Mean metal concentrations in moss at sites C, 1, 2 and 3 of Orange Kloof forest for the dry and wet sampling occasions in January and June 2015 are shown **in Figs 4.31 to 4.33**. Statistical signifcant differences (P<0.050) between the dry and wet seasons are indicated with an asterisk above the graph bars.

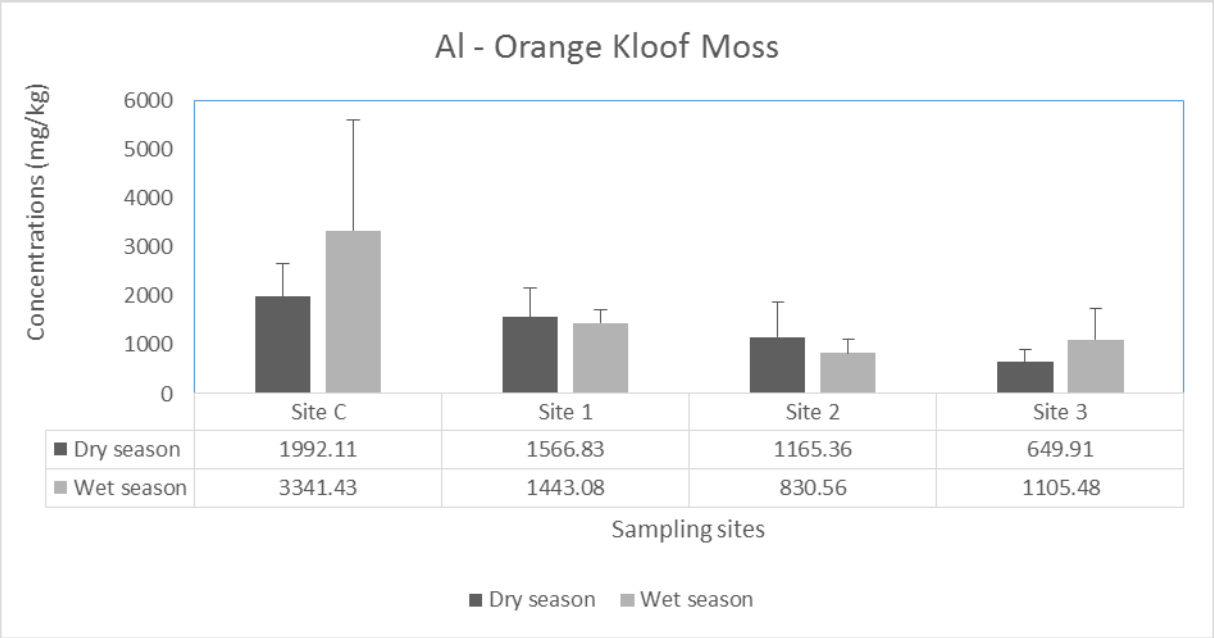


**Figure 4.31:** The mean Al concentrations (mg/kg) (± SD) in moss at sites C, 1, 2 and 3 of Orange Kloof forest for the dry and wet sampling occasions in January and June 2015. N=5.

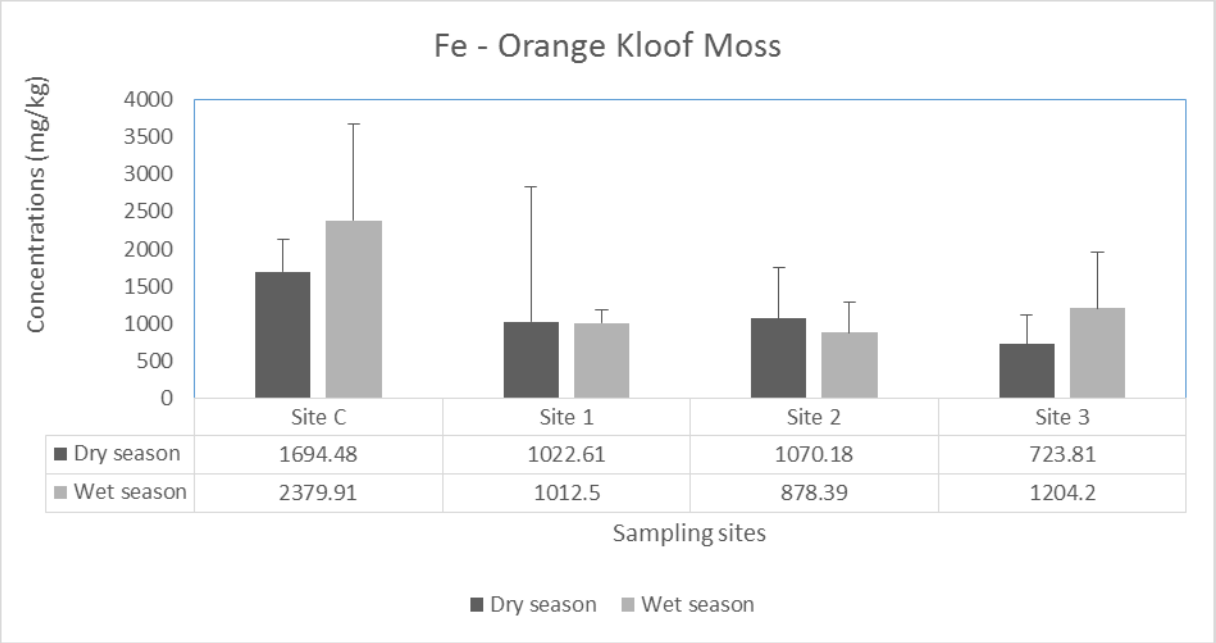


**Figure 4.32:** The mean Fe concentrations (mg/kg) (± SD) in moss at sites C, 1, 2 and 3 of Orange Kloof forest for the dry and wet sampling occasions in January and June 2015. N=5.

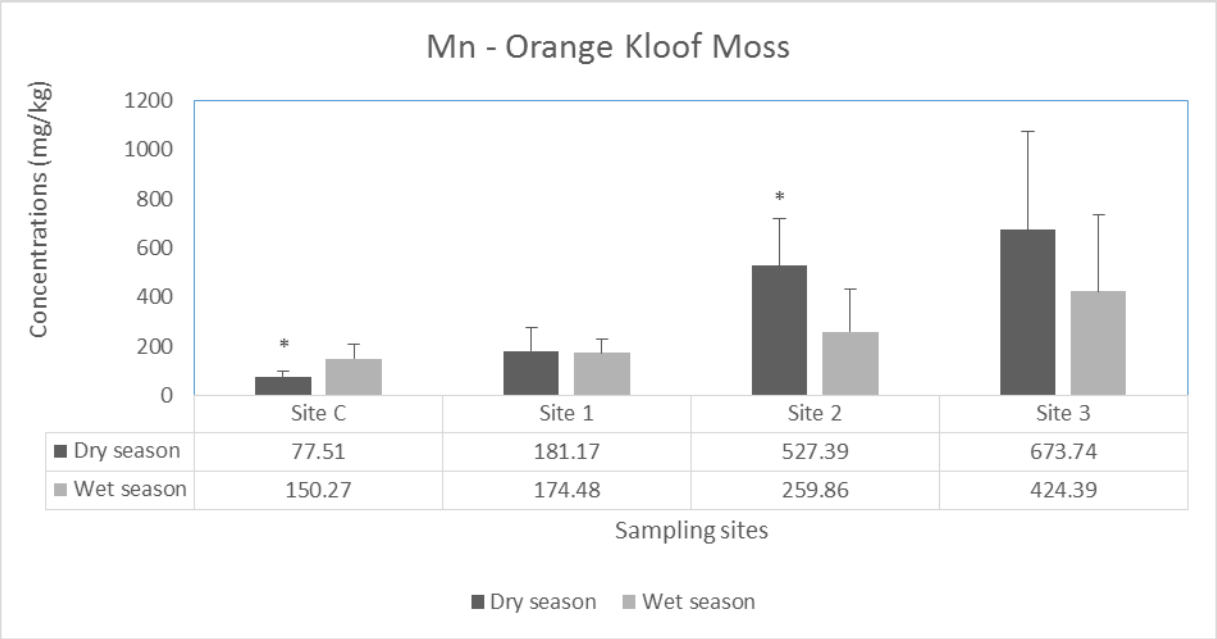


**Figure 4.33:** The mean Mn concentrations (mg/kg) (± SD) in moss at sites C, 1, 2 and 3 of Orange Kloof forest for the dry and wet sampling occasions in January and June 2015. N=5.

Aluminium concentrations in moss between the seasons at sites C (P=0.235), 1 (P=0.841), 2 (P=0.841) and 3 (P=0.310) did not differ significantly **(Fig 4.31)**.

No significant differences were found between seasons in moss at any of the sites [C (P=0.293), 1 (P=0.969), 2 (P=0.607) and 3 (P=0.151)] at Orange Kloof forest in terms of Fe concentrations **(Fig 4.32)**.

Sites C (P=0.034) and 2 (P=0.048) showed statistically significant differences between the dry and wet seasons in moss with regard to Mn concentrations. There were, however no statistically significant differences found between the seasons at sites 1 (P=0.897) and 3 (P=0.305). The highest mean Mn concentration was found at site 3 (673.74 ± 402.19 mg/kg) in the dry season **(Fig 4.33)**.

### *4.2.3.1.4) Comparisons of metal concentrations of lichen between dry and wet seasons at sites C, 1, 2 and 3*

Mean metal concentrations in lichen at sites C, 1, 2 and 3 of Orange Kloof forest for the dry and wet sampling occasions in January and June 2015 are shown in **Figs 4.34 to 4.35**. Statistical signifcant differences (P<0.050) between the dry and wet seasons are indicated with an asterisk above the graph bars.

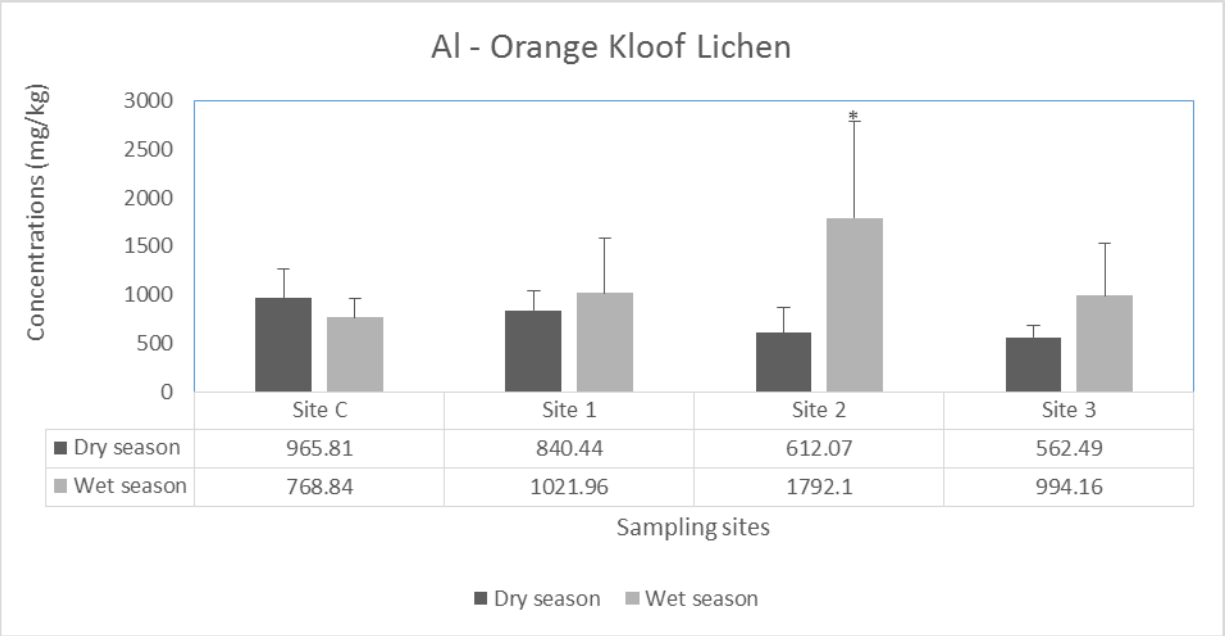


**Figure 4.34:** The mean Al concentrations (mg/kg) (± SD) in lichen at sites C, 1, 2 and 3 of Orange Kloof forest for the dry and wet sampling occasions in January and June 2015. N=5.

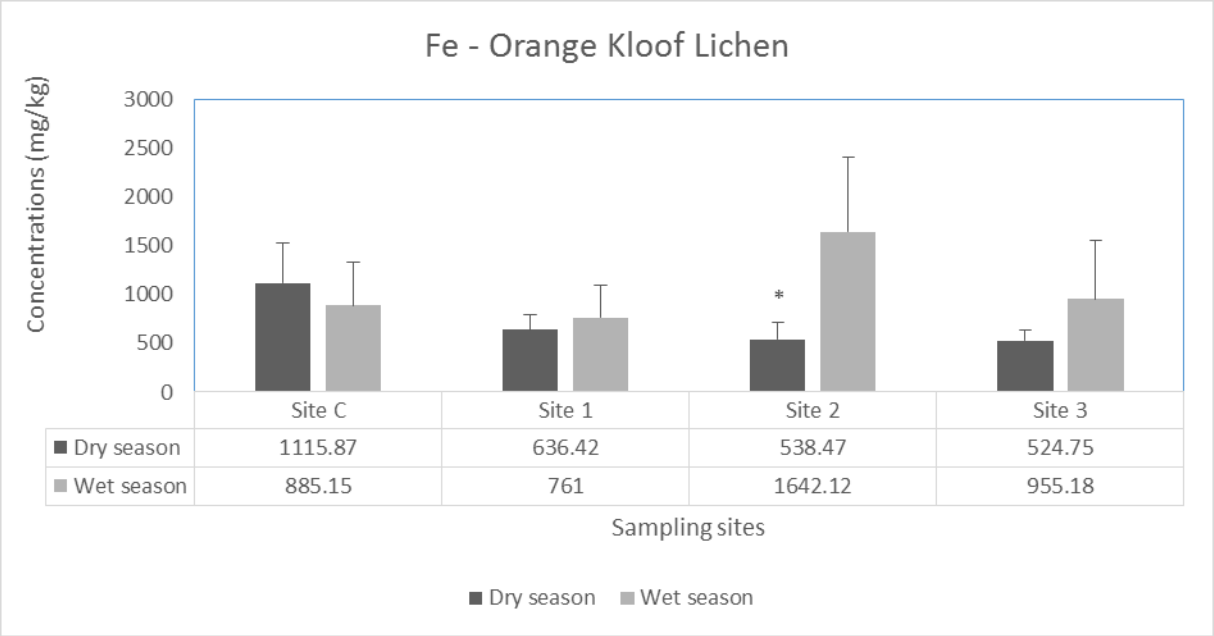


**Figure 4.35:** The mean Fe concentrations (mg/kg) (± SD) in lichen at sites C, 1, 2 and 3 of Orange Kloof forest for the dry and wet sampling occasions in January and June 2015. N=5.

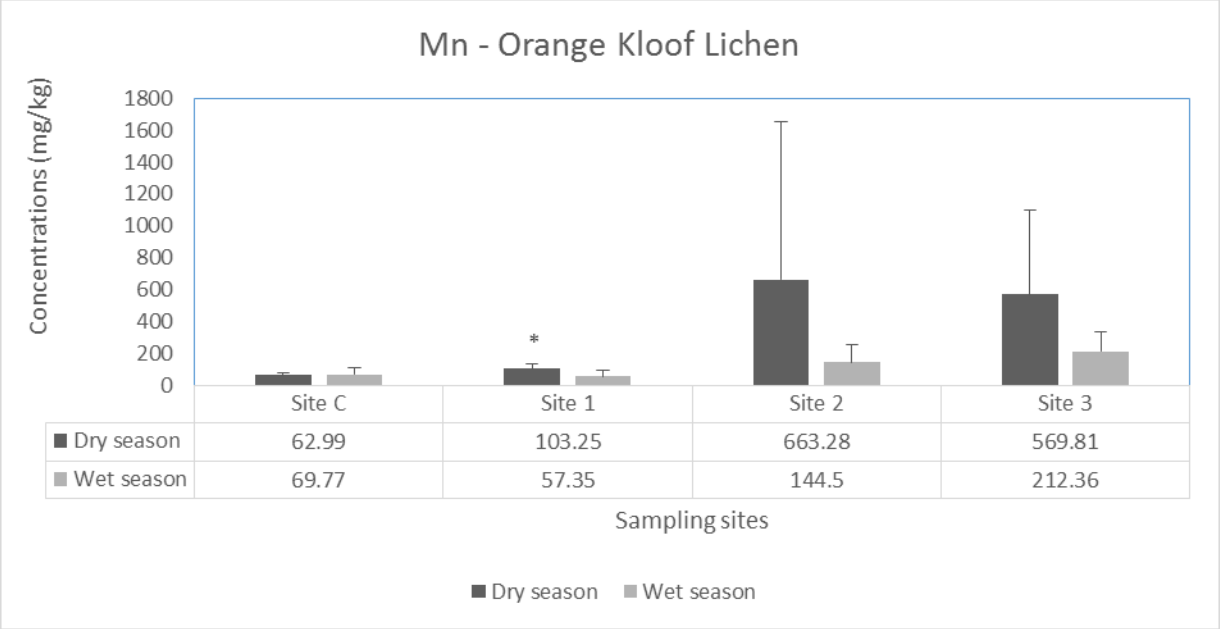


**Figure 4.36:** The mean Mn concentrations (mg/kg) (± SD) in lichen at sites C, 1, 2 and 3 of Orange Kloof forest for the dry and wet sampling occasions in January and June 2015. N=5.

No statistically significant differences in Al concentrations were found between the seasons in lichen at sites C (P=0.259), 1 (P=0.515) and 3 (P=0.118), but statistically significant differences were found between the seasons at site 2 (P=0.016). Site 2 displayed the highest mean Al concentration of 1792.1 ± 989.28 mg/kg in the wet season **(Fig 4.34)**.

Site C (P=0.424), site 1 (P=0.476) and site 3 (P=0.153) did not display any statistically significant differences between the dry and wet season sampling occasions in lichen with regard to Fe concentrations, but significant differences were found between the seasons at site 2 (P=0.008). The highest mean Fe concentration was measured at site 2 (1642.12 ± 769.20 mg/kg) in the wet season **(Fig 4.35)**.

Manganese concentration comparisons between the seasons in lichen at sites C (P=0.732), 2 (P=0.310) and 3 (P=0.095) showed no statistically significant differences, but significant differences were found between the seasons at site 1 (P=0.047). Lichen at sites 2 (663.28 ± 987.70 mg/kg) and 3 (569.81 ± 532.93 mg/kg) in the dry season showed significantly higher Mn concentrations than at the other sites **(Fig 4.36)**.

### *4.2.3.1.5) Comparisons of metal concentrations of millipedes between dry and wet seasons at sites C, 1, 2 and 3*

Mean metal concentrations in millipedes at sites C, 1, 2 and 3 of Orange Kloof forest for the dry and wet sampling occasions in January and June 2015 are shown **in Figs 4.37 to 4.39**. Statistical signifcant differences (P<0.050) between the dry and wet seasons are indicated with an asterisk above the graph bars.

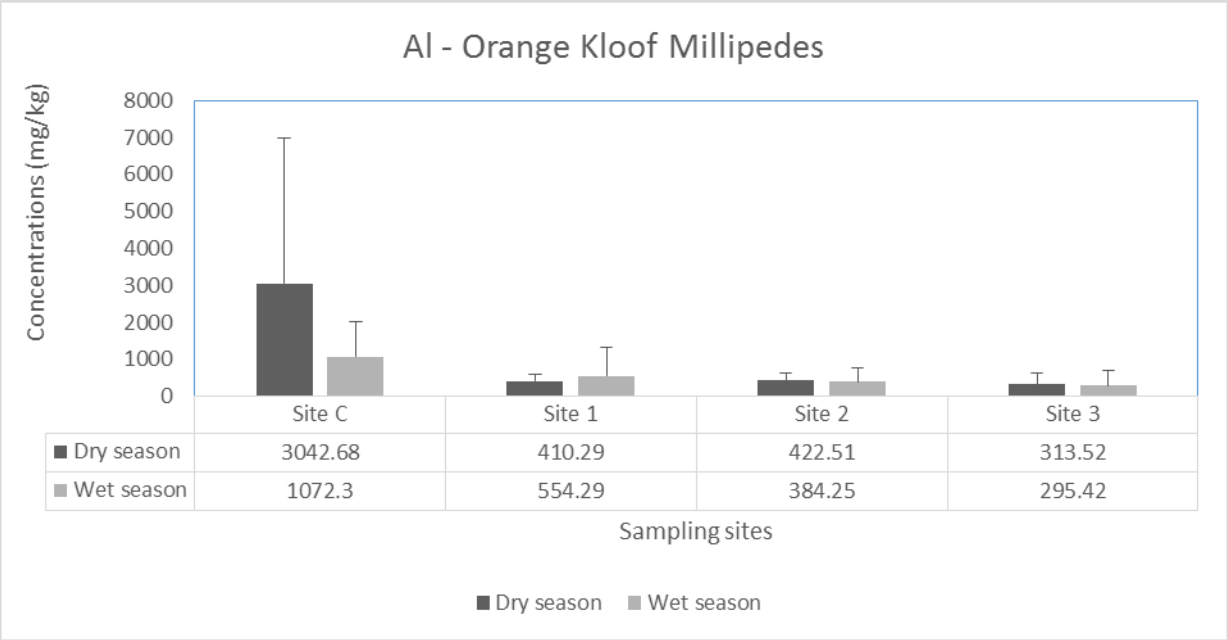


**Figure 4.37:** The mean Al concentrations (mg/kg) (± SD) in millipedes at sites C, 1, 2 and 3 of Orange Kloof forest for the dry and wet sampling occasions in January and June 2015. N=5.

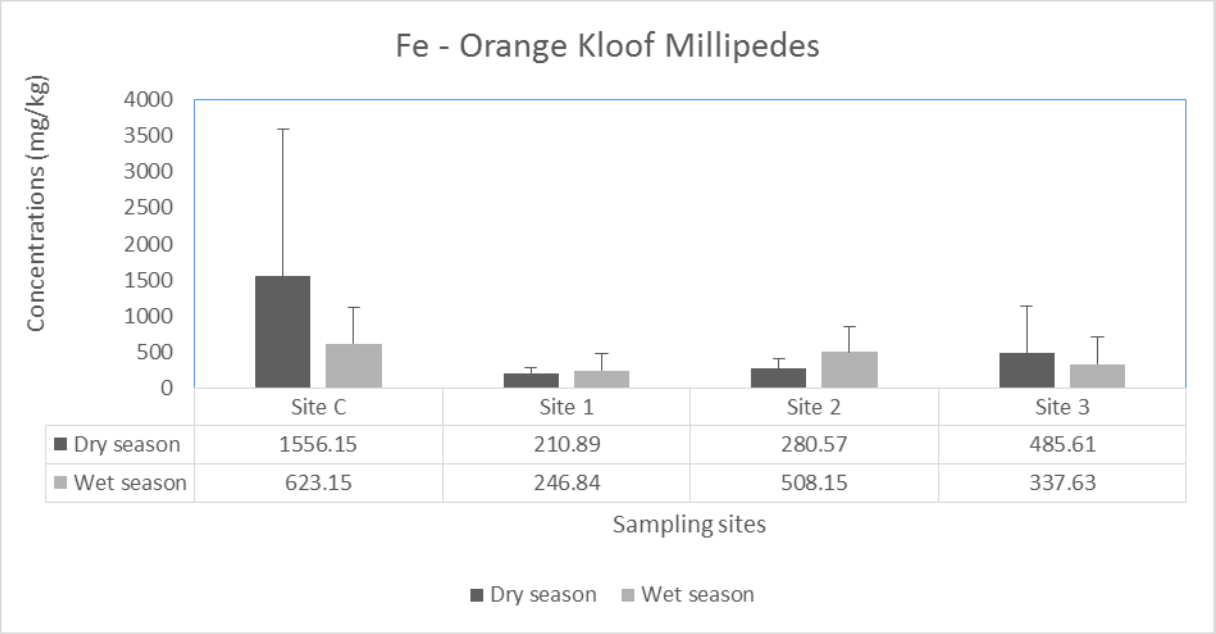


**Figure 4.38:** The mean Fe concentrations (mg/kg) (± SD) in millipedes at sites C, 1, 2 and 3 of Orange Kloof forest for the dry and wet sampling occasions in January and June 2015. N=5.

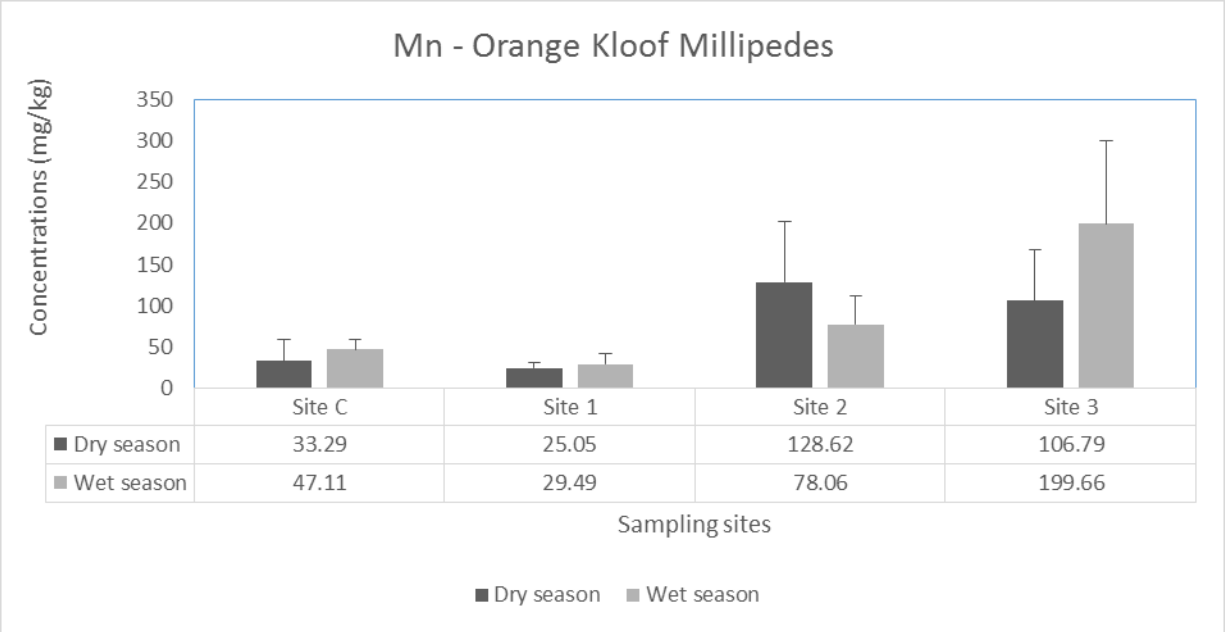


**Figure 4.39:** The mean Mn concentrations (mg/kg) (± SD) in millipedes at sites C, 1, 2 and 3 of Orange Kloof forest for the dry and wet sampling occasions in January and June 2015. N=5.

Aluminium concentrations between the dry and wet seasons measured in millipedes did not differ significantly at any of the sites C (P=0.222), 1 (P=0.548), 2 (P=0.848) and 3 (P=0.548) **(Fig 4.37)**.

Orange Kloof dry and wet season comparisons of Fe concentrations in millipedes showed no statistically significant differences at sites C (P=0.421), 1 (P=0.548), 2 (P=0.212) and 3 (P=0.841) **(Fig 4.38)**.

No statistically significant differences in Mn concentrations between seasons in millipedes were found at sites C (P=0.151), 1 (P=0.503), 2 (P=0.222) and 3 (P=0.114) **(Fig 4.39)**.

### 4.2.3.2) NEWLANDS FOREST

#### *4.2.3.2.1) Comparisons of metal concentrations of soil between dry and wet seasons at sites C, 1, 2 and 3*

Mean metal concentrations in soil at sites C, 1, 2 and 3 of Newlands forest for the dry and wet sampling occasions in January and June 2015 are shown in **Figs 4.40 to 4.42**. Statistical signifcant differences (P<0.050) between the dry and wet seasons are indicated with an asterisk above the graph bars.

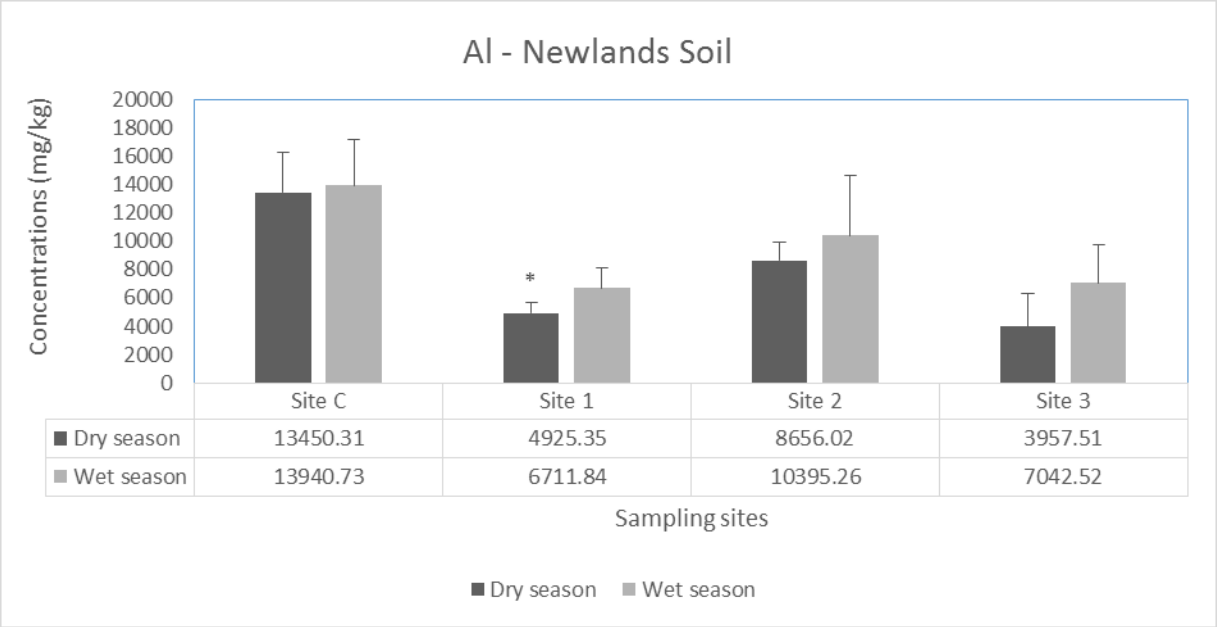


**Figure 4.40:** The mean Al concentrations (mg/kg) (± SD) in soil at sites C, 1, 2 and 3 of Newlands forest for the dry and wet sampling occasions in January and June 2015. N=5.

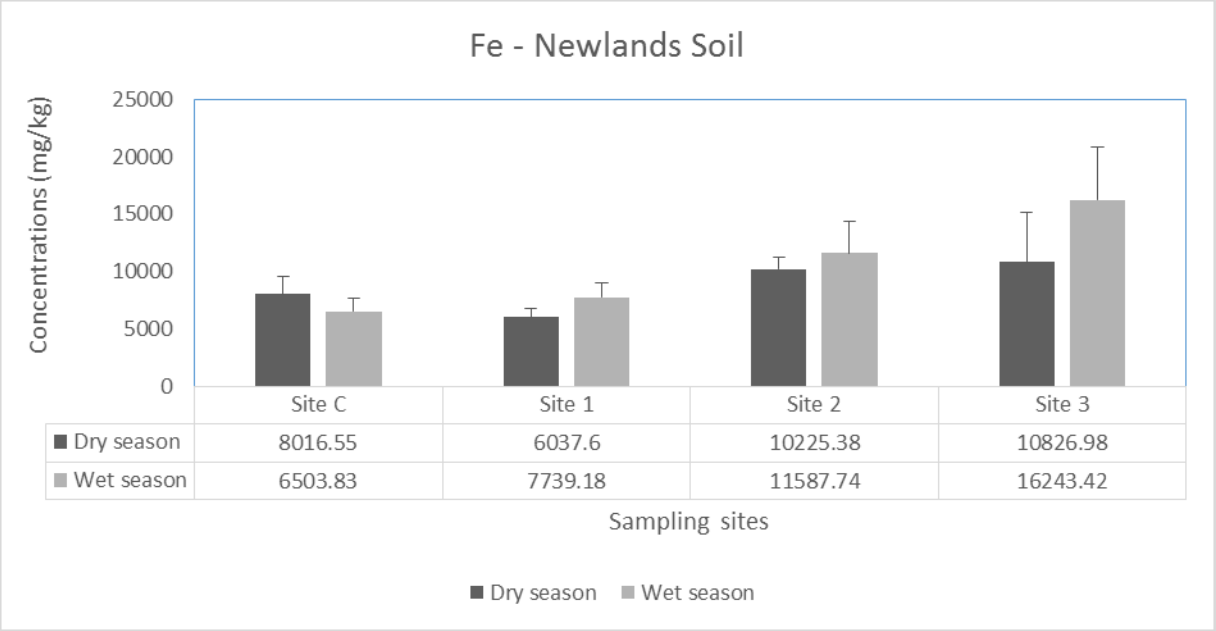


**Figure 4.41:** The mean Fe concentrations (mg/kg) (± SD) in soil at sites C, 1, 2 and 3 of Newlands forest for the dry and wet sampling occasions in January and June 2015. N=5.

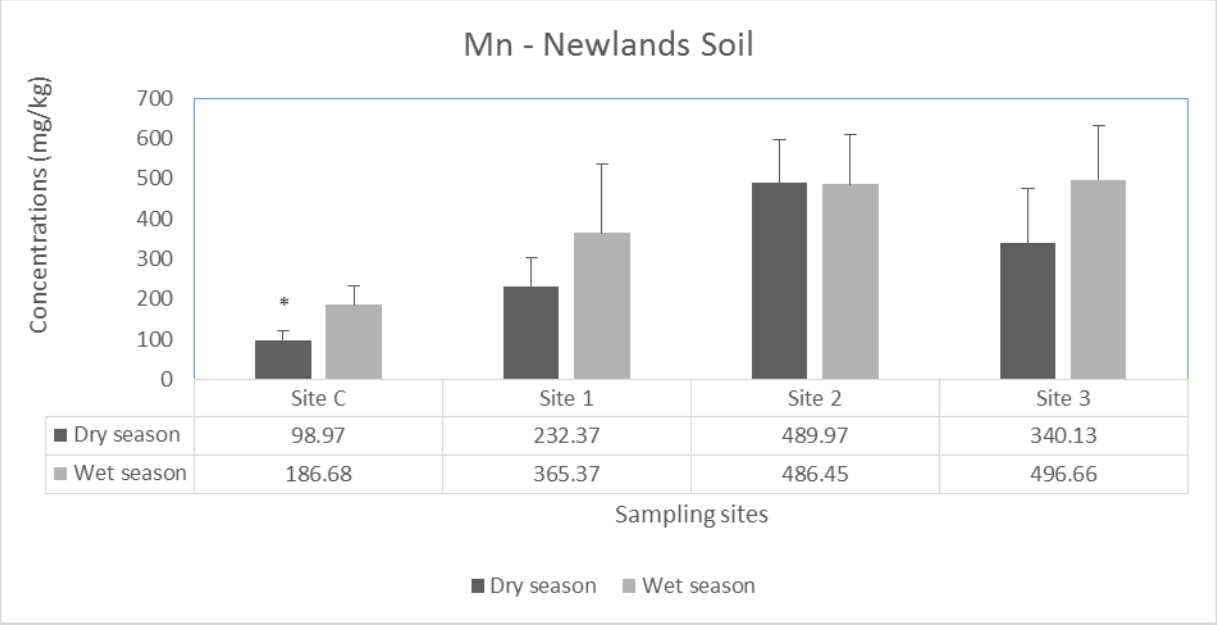


**Figure 4.42:** The mean Mn concentrations (mg/kg) (± SD) in soil at sites C, 1, 2 and 3 of Newlands forest for the dry and wet sampling occasions in January and June 2015. N=5.

Comparisons of Al concentrations in soil between the dry and wet seasons showed statistically significant differences between seasons at site 1 (P=0.037). No statistically significant differences were, however found between seasons at sites C (P=0.807), 2 (P=0.403) and 3 (P=0.092). Al concentrations calculated in soil at site C in the dry season (13450.31 ± 2851.26 mg/kg) and wet season (13940.73± 3263.24 mg/kg) were significantly higher than at the other sites **(Fig 4.40)**.

Soil between the dry and wet seasons were compared in terms of Fe concentrations and no significant differences between seasons were found at site C (P=0.131), 1 (P=0.056), 2 (P=0.327), and 3 (P=0.089) **(Fig 4.41)**.

At Newlands forest, the dry and wet seasons showed no statistically significant differences with regard to Mn concentrations in soil between seasons at sites 1 (P=0.421), 2 (P=0.963) and 3 (P=0.095). Site C (P=0.016), however differed significantly between seasons. The lowest concentration at this site was measured in the dry season (98.97 ± 23.56 mg/kg) **(Fig 4.42)**.

***4.2.3.2.2) Comparisons of metal concentrations of leaf litter between dry and wet seasons at sites C, 1, 2 and 3***

Mean metal concentrations in leaf litter at sites C, 1, 2 and 3 of Newlands forest for the dry and wet sampling occasions in January and June 2015 are shown in **Figs 4.43 to 4.45**. Statistical signifcant differences (P<0.050) between the dry and wet seasons are indicated with an asterisk above the graph bars.

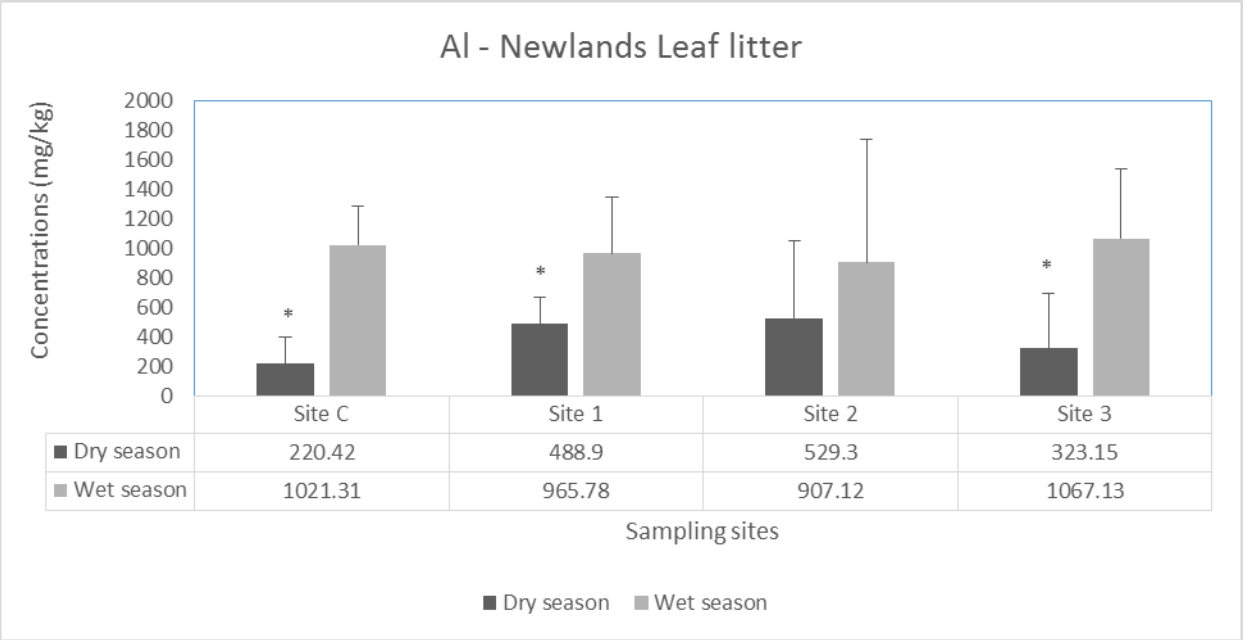


**Figure 4.43:** The mean Al concentrations (mg/kg) (± SD) in leaf litter at sites C, 1, 2 and 3 of Newlands forest for the dry and wet sampling occasions in January and June 2015. N=5.

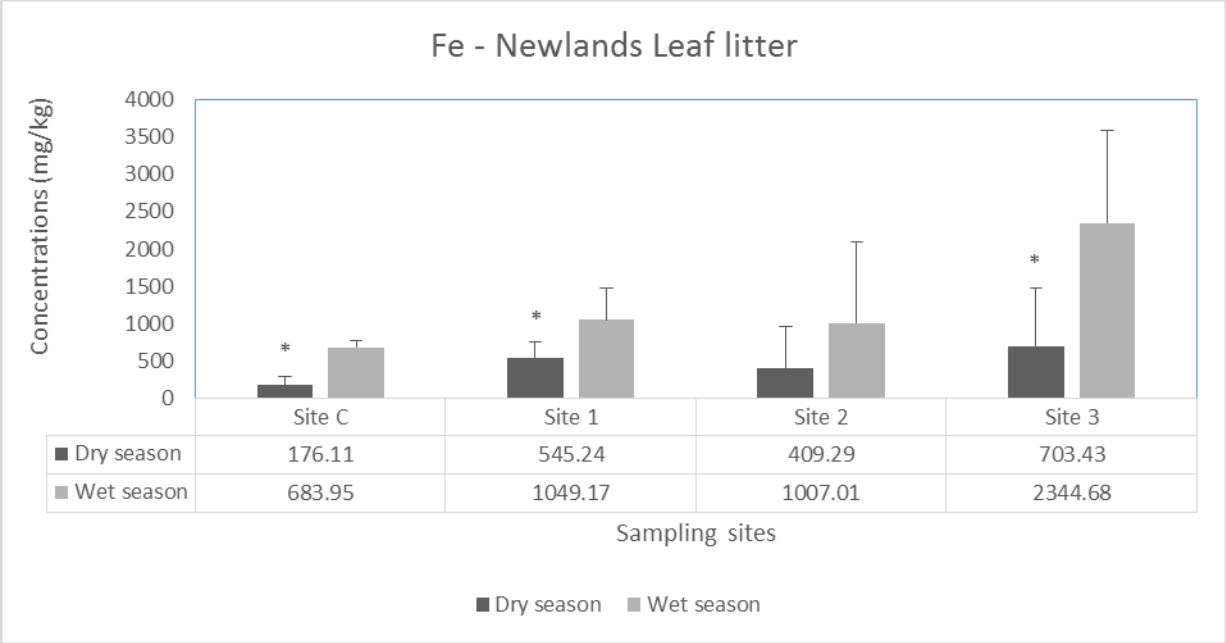


**Figure 4.44:** The mean Fe concentrations (mg/kg) (± SD) in leaf litter at sites C, 1, 2 and 3 of Newlands forest for the dry and wet sampling occasions in January and June 2015. N=5.

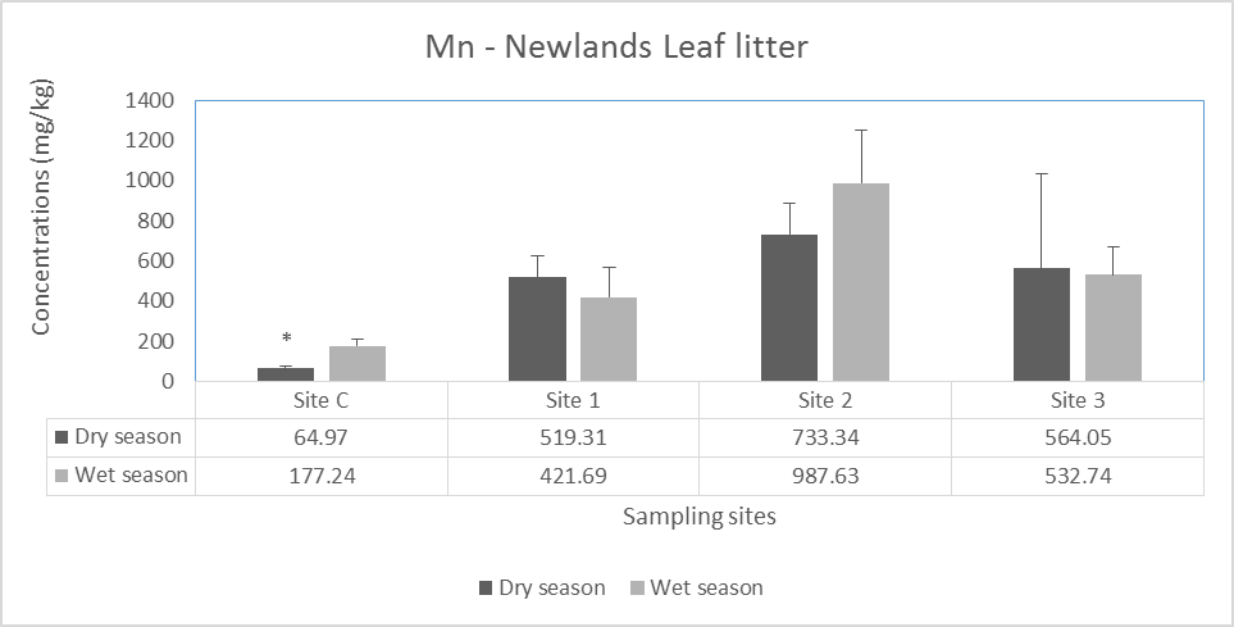


**Figure 4.45**: The mean Mn concentrations (mg/kg) (± SD) in leaf litter at sites C, 1, 2 and 3 of Newlands forest for the dry and wet sampling occasions in January and June 2015. N=5.

Newlands forest, dry and wet season comparisons of Al concentrations in leaf litter showed statistically significant differences between seasons at sites C (P=<0.001), 1 (P=0.036) and 3 (P=0.024) with the exception of site 2 (P=0.151). Significantly higher mean Al concentrations were observed in the wet season as opposed to the dry season in this study **(Fig 4.43)**.

Comparisons of Fe concentrations between the two seasons in leaf litter differed significantly between seasons at sites C (P=<0.001), 1 (P=0.042) and 3 (P=0.036), but not at site 2 (P=0.095). The wet season at site 3 yielded the highest mean Fe concentrations of 2344.68 ± 1245.40 mg/kg **(Fig 4.44)**.

Manganese concentrations in the dry and wet seasons showed statistically significant differences between them in leaf litter at site C (P=<0.001). However, no statistically significant differences were found between seasons at sites 1 (P=0.265), 2 (P=0.101) and 3 (P=0.548). Mean Mn concentrations at site 2 (987.63 ± 265.82 tmg/kg) in the wet season was significantly higher than in the dry season **(Fig 4.45)**.

### *4.2.3.2.3) Comparisons of metal concentrations of moss between dry and wet seasons at sites C, 1, 2 and 3*

Mean metal concentrations in moss at sites C, 1, 2 and 3 of Newlands forest for the dry and wet sampling occasions in January and June 2015 are shown in **Figs 4.46 to 4.48**. Statistical signifcant differences (P<0.050) between the dry and wet seasons are indicated with an asterisk above the graph bars.

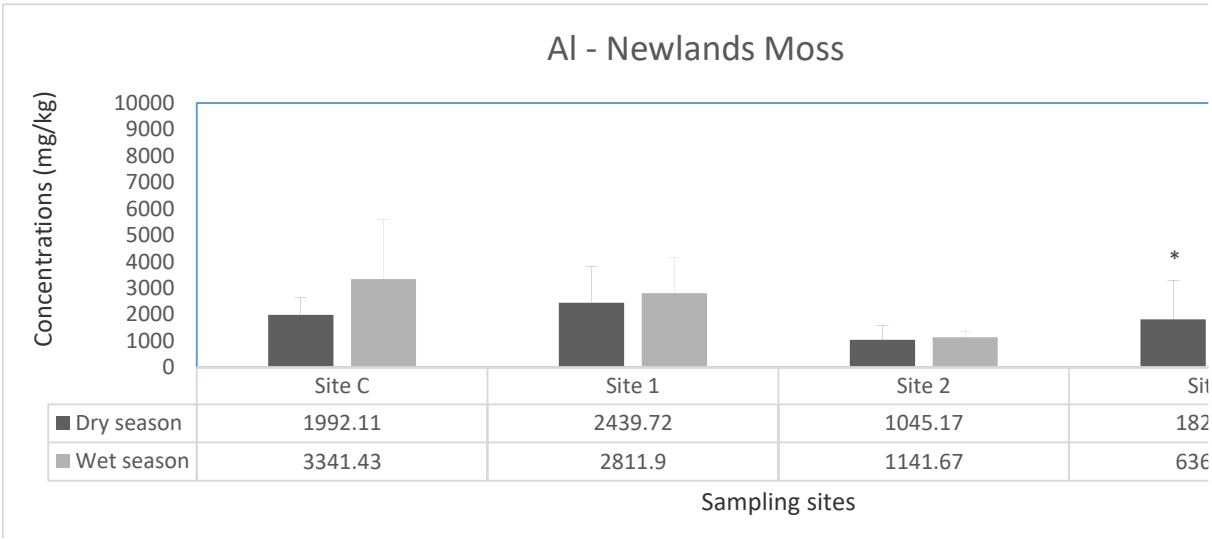


**Figure 4.46:** The mean Al concentrations (mg/kg) (± SD) in moss at sites C, 1, 2 and 3 of Newlands forest for the dry and wet sampling occasions in January and June 2015. N=5.

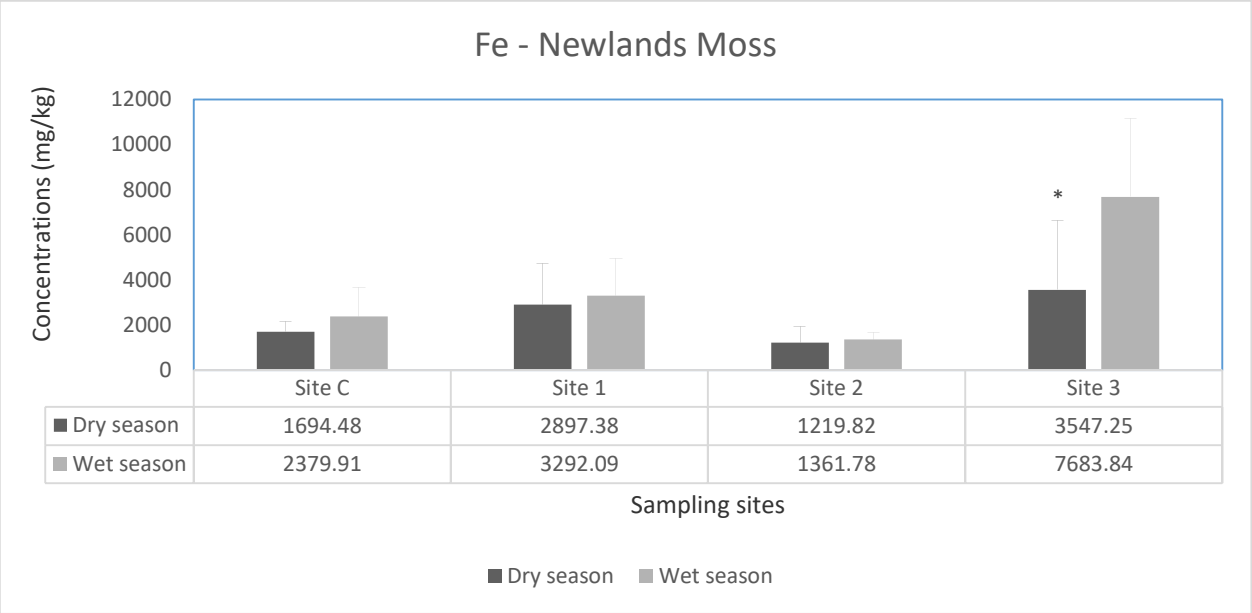


**Figure 4.47:** The mean Fe concentrations (mg/kg) (± SD) in moss at sites C, 1, 2 and 3 of Newlands forest for the dry and wet sampling occasions in January and June 2015. N=5.

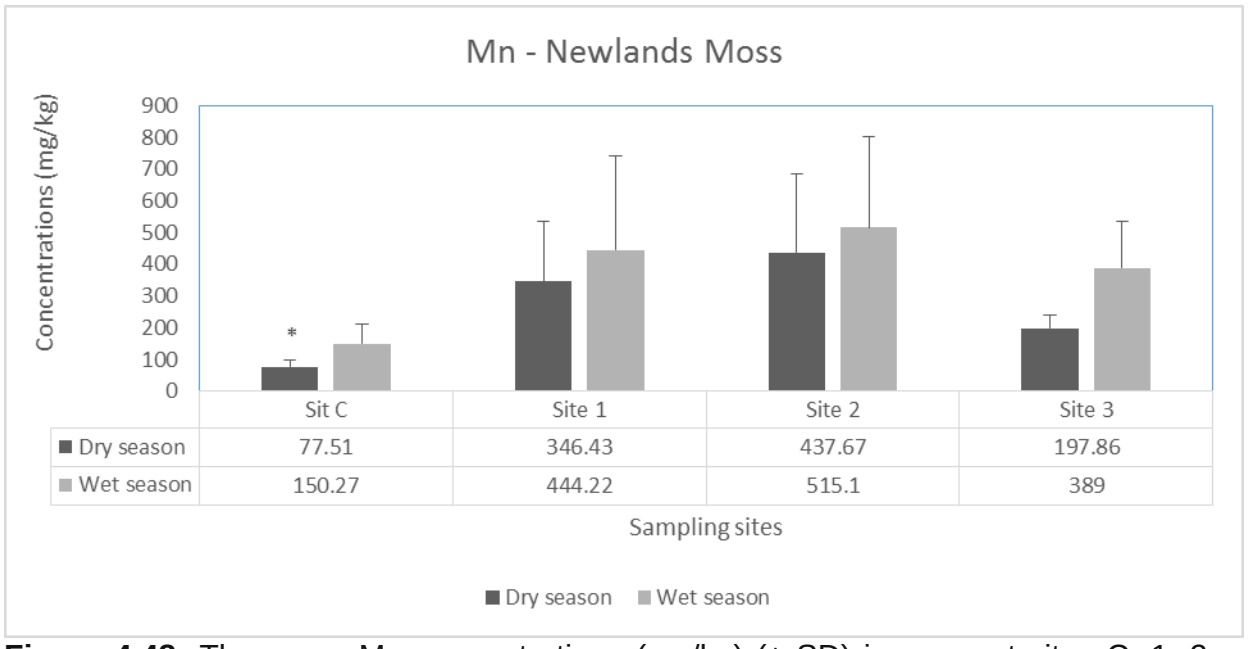


**Figure 4.48:** The mean Mn concentrations (mg/kg) (± SD) in moss at sites C, 1, 2 and 3 of Newlands forest for the dry and wet sampling occasions in January and June 2015. N=5.

No statistically significant differences were found Al concentrations between the dry and wet seasons at sites C (P=0.235), 1 (P=0.675) and 2 (P=0.727), but statistically significant differences were found between seasons at site 3 (P=0.138) in terms of moss **(Fig 4.46)**.

Iron concentrations at sites C (P=0.239), 1 (P=0.728) and 2 (P=0.679) in moss did not differ significantly between the dry and wet seasons, but significant differences were found between seasons at site 3 (P=0.084) **(Fig 4.47)**.

There were statistically significant differences found between seasons with regard to Mn concentrations in moss at site C (P=0.034). Sites 1 (P=0.550), 2 (P=0.659) and 3 (P=0.310) did not show any statistically significant differences between seasons. Site C in the dry season yielded the lowest mean concentration of 77.51 ± 21.77 mg/kg **(Fig 4.48)**.

### *4.2.3.2.4) Comparisons of metal concentrations of lichen between dry and wet seasons at sites C, 1, 2 and 3*

Mean metal concentrations in lichen at sites C, 1, 2 and 3 of Newlands forest for the dry and wet sampling occasions in January and June 2015 are shown in **Figs 4.49 to 4.51**. Statistical signifcant differences (P<0.050) between the dry and wet seasons are indicated with an asterisk above the graph bars.

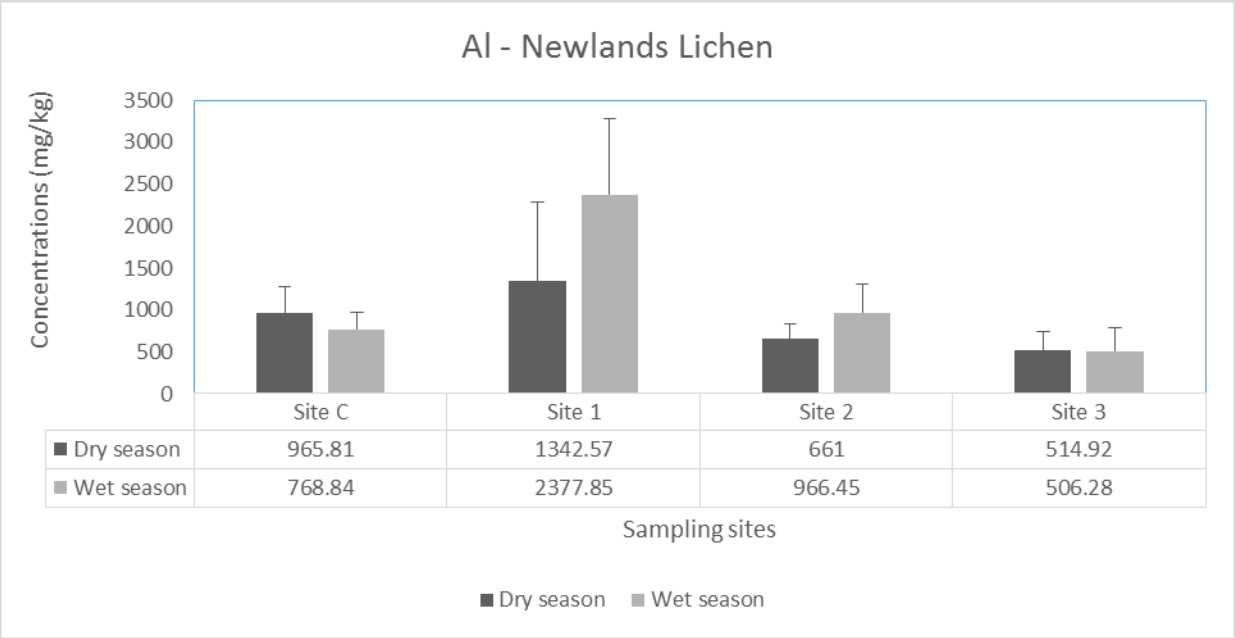


**Figure 4.49:** The mean Al concentrations (mg/kg) (± SD) in lichen at sites C, 1, 2 and 3 of Newlands forest for the dry and wet sampling occasions in January and June 2015. N=5.

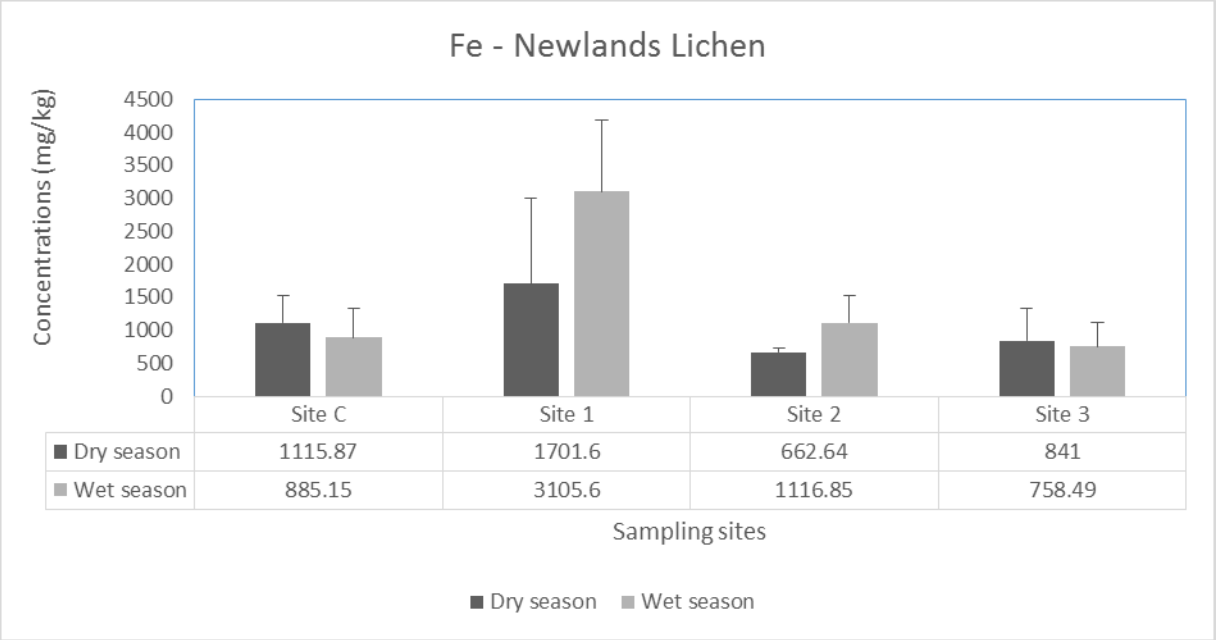


**Figure 4.50:** The mean Fe concentrations (mg/kg) (± SD) in lichen at sites C, 1, 2 and 3 of Newlands forest for the dry and wet sampling occasions in January and June 2015. N=5.

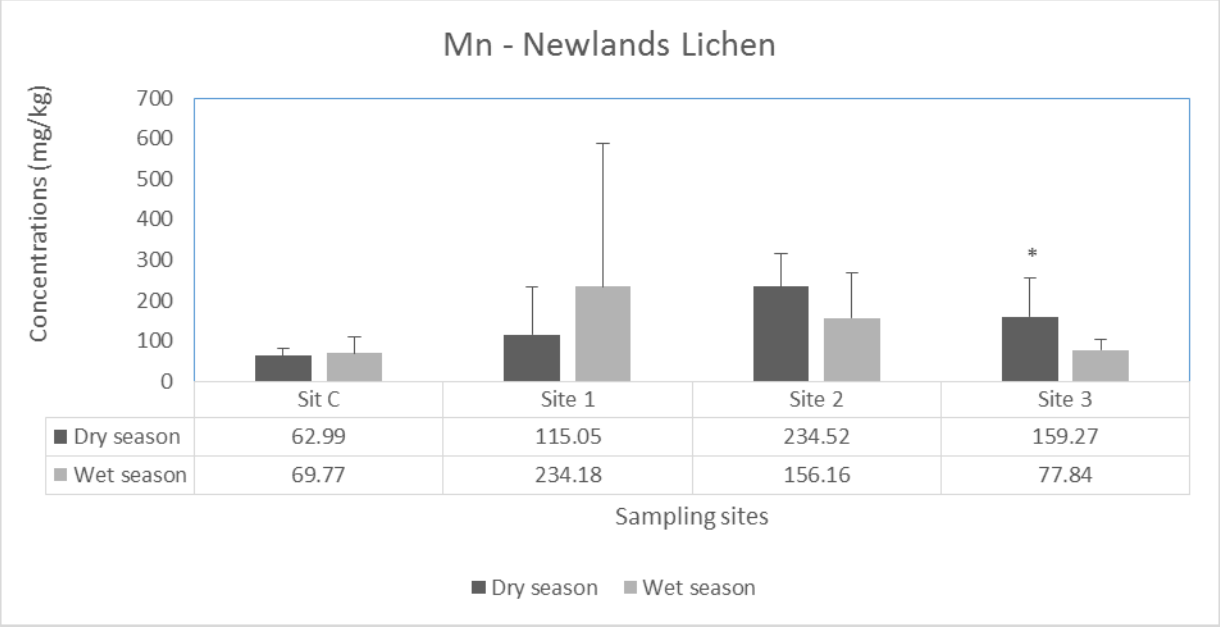


**Figure 4.51:** The mean Mn concentrations (mg/kg) (± SD) in lichen at sites C, 1, 2 and 3 of Newlands forest for the dry and wet sampling occasions in January and June 2015. N=5.

There were no statistically significant differences found between seasons in terms of Al concentrations in lichen at sites C (P=0.259), 1 (P=0.114), 2 (P=0.151) and 3 (P=0.959) **(Fig 4.49)**.

Lichen at the following sites: C (P=0.424), 1 (P=0.100), 2 (P=0.151) and 3 (P=0.768) did not display any statistically significant differences in Fe concentrations between dry and wet season samplings **(Fig 4.51)**.

Comparisons of Mn concentrations in lichen between seasons at sites C (P=0.732), 1 (P=0.548) and 2 (P=0.237) showed no statistically significant differences, but significant differences were found between seasons at site 3 (P=0.032). The lowest mean Mn concentrations were observed at site C in the dry (62.99 ± 16.77 mg/kg) and wet (69.77 ± 39.44 mg/kg) seasons **(Fig 4.52)**.

### *4.2.3.2.5) Comparisons of metal concentrations of millipedes between dry and wet seasons at sites C, 1, 2 and 3*

Mean metal concentrations in millipedes at sites C, 1, 2 and 3 of Newlands forest for the dry and wet sampling occasions in January and June 2015 are shown in **Figs 4.52 to 4.53**. Statistical signifcant differences (P<0.050) between the dry and wet seasons are indicated with an asterisk above the graph bars.

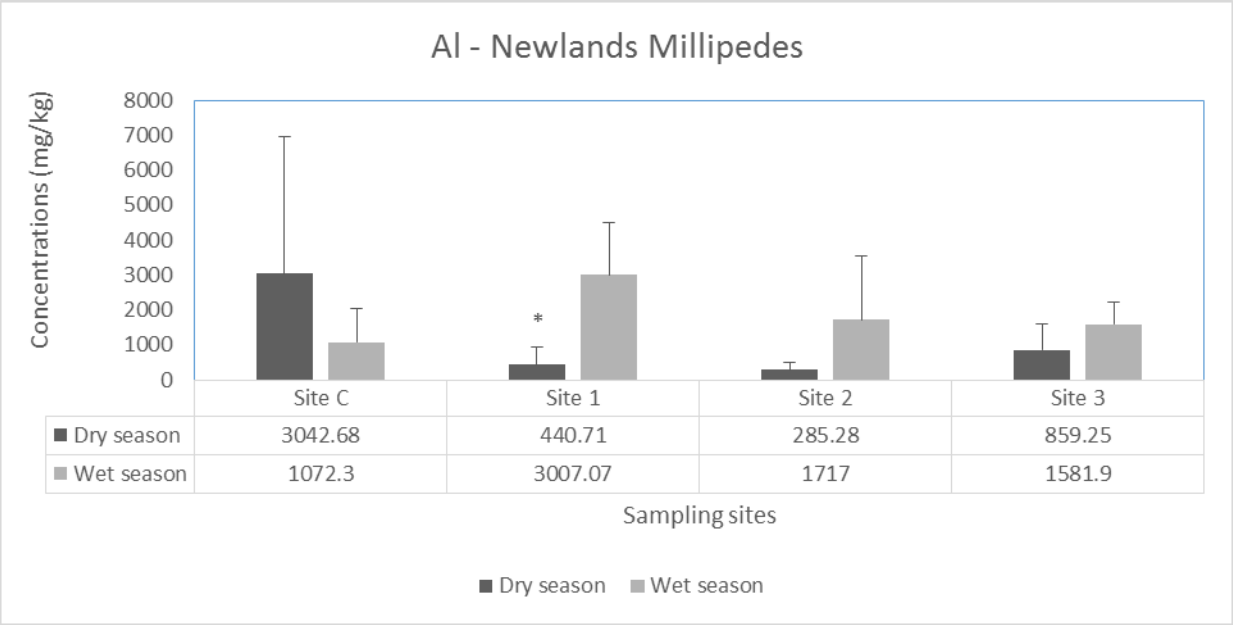


**Figure 4.52:** The mean Al concentrations (mg/kg) (± SD) in millipedes at sites C, 1, 2 and 3 of Newlands forest for the dry and wet sampling occasions in January and June 2015. N=5.

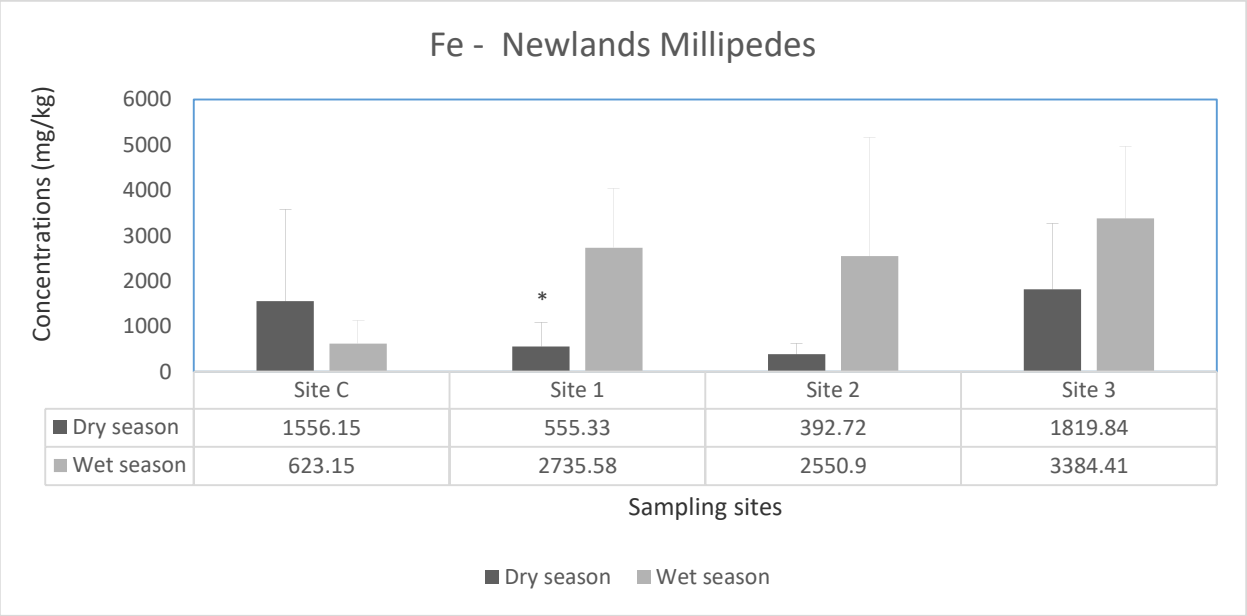


**Figure 4.53:** The mean Fe concentrations (mg/kg) (± SD) in millipedes at sites C, 1, 2 and 3 of Newlands forest for the dry and wet sampling occasions in January and June 2015. N=5.

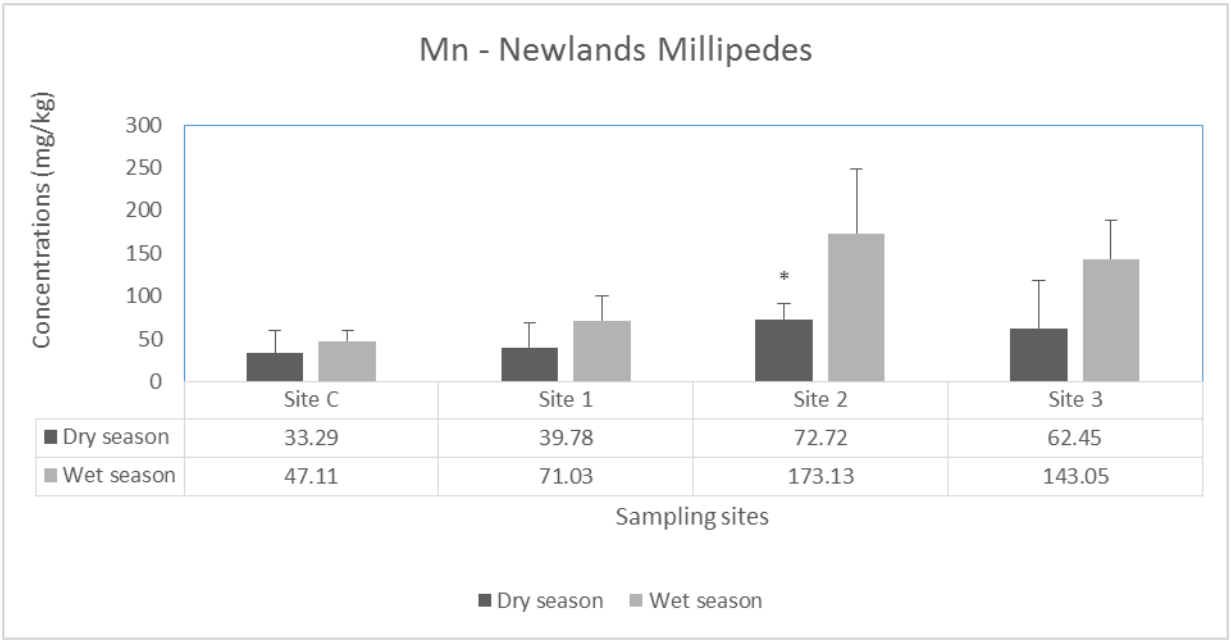


**Figure 4.54:** The mean Mn concentrations (mg/kg) (± SD) in millipedes at sites C, 1, 2 and 3 of Newlands forest for the dry and wet sampling occasions in January and June 2015. N=5.

Comparisons of Al concentrations in millipedes between the seasons showed significant differences at site 1 (P=0.007), but no statistically significant differences were found between seasons at sites C (P=0.222), 2 (P=0.121) and 3 (P=0.056). Sites C (3042.68 ± 3935.36 mg/kg) in the dry season and 1 (3007.07 ± 1496.38 mg/kg) in the wet season yielded significantly higher concentrations than at the other sites **(Fig 4.52)**.

Iron concentrations in millipedes were compared between the dry and wet seasons and statistically significant differences were detected between seasons at site 1 (P=0.009). At the following sites the wet and dry seasons did not differ significantly from each other: C (P=0.421), 2 (P=0.052) and 3 (P=0.056). The highest mean Fe concentrations were found at sites 1 (2735.58 ± 1300.32 mg/kg), 2 (2550.9 ± 2620.86 mg/kg) and 3 (3384.41 ± 1573.51 mg/kg) in the wet season **(Fig 4.53)**.

Statistically significant differences between seasons in Mn concentrations in terms of millipedes were found between seasons at site 2 (P=0.021). However, there were no statistically significant differences found between seasons at sites C (P=0.151), 1 (P=0.129) and 3 (P=0.095). Mn concentrations were at their lowest at site C (33.29 ± 26.21 mg/kg) in the dry season **(Fig 4.54)**.

### 4.2.3.3) FORESTS

#### *4.2.3.3.1) Comparisons of metal concentrations in soil, leaf litter and sentinel organisms between the forests: Site C, Orange Kloof and Newlands*

The mean metal concentrations in soil, leaf litter and sentinel organisms for each forest for the dry (January 2015) and wet (June 2015) sampling occasions are presented in **Tables 4.27 to 4.29**. The metal concentrations for the sites within each forest were pooled to calculate the mean concentrations for each forest. Metal concentrations are expressed in mg/kg.

***a) Al***

**Table 4.27:** The mean Al concentrations (mg/kg) (± SD) in soil, leaf litter and sentinel organisms for forests for the dry and wet sampling occasions in January and June 2015.

| FOREST | SEASON | SOIL | LEAF LITTER | MOSS | LICHEN | MILLIPEDES |
|---|---|---|---|---|---|---|
| Site C | Dry | 13450.31 ± 2851.26 | *220.42 ± 175.48 | 1992.10 ± 661.79 | 965.81 ± 305.38 | *3042.68 ± 3935.36 |
| | Wet | 13940.73 ± 3263.24 | 1021.3 ± 263.66 | 3341.43 ± 2251.93 | 768.84 ± 194.82 | 1072.29 ± 959.60 |
| Orange Kloof | Dry | 4122.74 ± 4495.73 | 265.89 ± 249.21 | 1127.36 ± 643.69 | *671.66 ± 225.57 | 382.10 ± 220.37 |
| | Wet | 10669.29 ± 11070.58 | 513.1 ± 553.72 | 1126.37 ± 477.10 | 1269.4 ± 773.65 | 411.31 ± 513.85 |
| Newlands | Dry | *5846.29 ± 2575.23 | *447.11 ± 367.82 | 1769.21 ± 1252.20 | 839.49 ± 646.42 | *528.41 ± 548.51 |
| | Wet | 8049.87 ± 3272.21 | 980.0 ± 553.96 | 1667.44 ± 1121.64 | 1283.52 ± 983.03 | 2101.99 ± 1469.16 |

Statistical signifcant differences (P<0.050) between dry and wet seasons are indicated with an asterisk. Comparisons were done separately for soil, leaf litter and sentinel organisms. N=5.

Dry and wet seasons were compared in terms of Al concentrations in soil and significant differences were found between seasons at Newlands forest (P=0.050). There were no statistically significant differences found between seasons at the site C (P=0.359) and Orange Kloof forest (P=0.281). Higher mean concentrations were observed in the wet season with site C (13940.73 ± 3263.24mg/kg) yielding the highest concentrations **(Fig 4.27)**.

The two seasons showed statistically significant differences in Al concentrations between them in leaf litter at site C (P=<0.001) and Newlands forest (P=0.002). No statistically significant differences were, however found between seasons at Orange Kloof (P=0.199). The wet season showed consistent higher mean Al concentrations in leaf litter at all three forests **(Fig 4.27)**.

Aluminium concentrations in moss at all three forests showed no statistically significant differences between seasons: site C (P=0.359), Orange Kloof (P=0.934) and Newlands (P=1.000) **(Fig 4.27)**.

No statistically significant differences in Al concentrations with regard to lichen were detected between the dry and wet seasons at the following forests: site C (P=0.096) and Newlands (P=0.158). There were, however significant differences found between seasons at Orange Kloof (P=0.005). The mean Al concentration at Newlands (1283.52 ± 983.03 mg/kg) in the wet season was the highest of the three forests **(Fig 4.27)**.

Comparisons of Al concentrations in millipedes between seasons showed statistically significant differences at site C (P=0.016) and Newlands (P=<0.001), with the exception of Orange Kloof (P=0.184). The highest mean Al concentrations were found at site C (3042.68 ± 3935.36 mg/kg) in the dry season and Newlands forest (2101.99 ± 1469.16 mg/kg) in the wet season **(Fig 4.27)**.

***b) Fe***

**Table 4.28:** The mean Fe concentrations (mg/kg) (± SD) in soil, leaf litter and sentinel organisms for forests for the dry and wet sampling occasions in January and June 2015.

| FOREST | SEASON | SOIL | LEAF LITTER | MOSS | LICHEN | MILLIPEDES |
|---|---|---|---|---|---|---|
| Site C | Dry | *8016.54 ± 1592.90 | *176.11 ± 124.29 | 1694.47 ± 436.48 | 1115.87 ± 417.65 | 1556.14 ± 2023.16 |
| | Wet | 6503.82 ± 1225.01 | 683.95 ± 96.78 | 2379.9 ± 1288.61 | 885.15 ± 448.33 | 623.14 ± 497.07 |
| Orange Kloof | Dry | 1832.03 ± 1012.07 | *178.14 ± 129.83 | 938.87 ± 538.29 | *566.54 ± 148.43 | 325.69 ± 380.81 |
| | Wet | 5922.22 ± 6318.44 | 609.39 ± 433.81 | 1031.69 ± 486.84 | 1119.43 ± 676.68 | 364.2 ± 326.99 |
| Newlands | Dry | 9029.98 ± 3251.47 | *552.64 ± 530.52 | 2554.81 ± 2193.75 | 1068.40 ± 876.27 | *922.63 ± 1065.97 |
| | Wet | 11856.77 ± 4628.74 | 1466.95 ± 1117.24 | 2035.24 ± 1297.96 | 1660.31 ± 1249.90 | 2890.29 ± 1813.82 |

Statistical signifcant differences (P<0.050) between dry and wet seasons are indicated with an asterisk. Comparisons were done separately for soil, leaf litter and sentinel organisms. N=5.

The following forests did not show statistically significant differences in Fe concentrations between the dry and wet seasons in terms of soil: Orange Kloof (P=0.062) and Newlands (P=0.063). Statistically significant differences were found between seasons at site C (P=0.005). A mean Fe concentration of 11856.77 ± 4628.74 mg/kg at Newlands in the wet season was significantly higher than at the other forests **(Fig 4.28)**.

Comparisons of Fe concentrations in leaf litter between the two seasons showed statistically significant differences between seasons at all the forests: site C (P=<0.001), Orange Kloof (P=<0.001) and Newlands (P=0.005). Higher mean Fe concentrations were observed in the wet season **(Fig 4.28)**.

Moss at all three forests were compared between the dry and wet seasons in terms of Fe concentrations and no statistically significant differences were detected between seasons at site C (P=0.359), Orange Kloof (P=0.407) and Newlands (P=0.803) **(Fig 4.28)**.

Iron concentrations in lichen between seasons were compared and showed statistically significant differences between seasons at Orange Kloof forest (P=0.014). Site C (P=0.197) and Newlands (P=0.135) did not differ significantly between seasons. The mean Fe concentration at Newlands (1660.31 ± 1249.90 mg/kg) in the wet season was the highest of the three forests **(Fig 4.28)**.

No statistically significant differences in millipedes between seasons were found when Fe concentrations were compared at site C (P=0.096) and Orange Kloof forest (P=1.000), with the exception of Newlands forest (P=0.002) where statistically significant differences between seasons were found. Site C (2890.29 ± 1813.82 mg/kg) in the wet season showed the highest mean Fe concentrations **(Fig 4.28)**.

***b) Mn***

**Table 4.29:** The mean Mn concentrations (mg/kg) (± SD) in soil, leaf litter and sentinel organisms for forests for the dry and wet sampling occasions in January and June 2015.

| FOREST | SEASON | SOIL | LEAF LITTER | MOSS | LICHEN | MILLIPEDES |
|---|---|---|---|---|---|---|
| Site C | Dry | *98.97 ± 23.56 | *64.97 ± 14.28 | *77.51 ± 21.77 | 62.98 ± 16.77 | *33.28 ± 26.22 |
| | Wet | 186.67 ± 47.59 | 177.24 ± 35.29 | 150.27 ± 59.78 | 69.77 ± 39.45 | 47.1 ± 12.46 |
| Orange Kloof | Dry | 255.38 ± 295.89 | 792.98 ± 874.64 | 460.76 ± 324.04 | 445.44 ± 651.45 | 86.81 ± 69.18 |
| | Wet | 204.34 ± 158.31 | 461.57 ± 445.46 | 286.57 ± 219.38 | 138.07 ± 110.84 | 102.4 ± 93.34 |
| Newlands | Dry | 354.15 ± 147.88 | 605.56 ± 287.19 | 327.31 ± 196.01 | 181.61 ± 104.74 | *58.31 ± 37.46 |
| | Wet | 449.49 ± 148.45 | 647.35 ± 309.69 | 403.6 ± 257.68 | 156.06 ± 209.77 | 129.07 ± 66.76 |

Statistical signifcant differences (P<0.050) between dry and wet seasons are indicated with an asterisk. Comparisons were done separately for soil, leaf litter and sentinel organisms. N=5.

Season comparisons of Mn concentrations in soil at the forests showed the following results: statistically significant differences between seasons at site C (P=<0.001) and no statistically significant differences between seasons at Orange Kloof (P=0.772) and Newlands (P=0.089). Mean Mn concentrations were the lowest at site C (98.97 ± 23.56 mg/kg) in the dry season **(Fig 4.29)**.

Manganese concentrations in leaf litter between the two seasons showed statistically significant differences between seasons at site C (P=<0.001), but not at Orange Kloof (P=0.089) and Newlands forests (P=0.772). The lowest mean Mn concentrations were observed in the dry season at site C (64.97 ± 14.28 mg/kg) **(Fig 4.29)**.

Moss at site C (P=0.005) displayed statistically significant differences between seasons with regard to Mn concentrations, but there were no statistically significant differences found between seasons at Orange Kloof (P=0.068) and Newlands forests (P=0.384). Once again the Mn concentrations at site C (77.51 ± 21.77 mg/kg) yielded the lowest results in the dry season **(Fig 4.29)**.

Manganese concentrations in lichen showed no statistically significant differences between the seasons at any of the forests: site C (P=0.868), Orange Kloof (P=0.081) and Newlands (P=0.158) **(Fig 4.29)**.

Comparisons of Mn concentrations in millipedes between seasons revealed statistically significant differences between the seasons at site C (P=0.005) and Newlands forests (P=<0.001), but no significant differences were found between seasons at Orange Kloof forest (P=0.803). Site C in the dry season showed the lowest mean Mn concentrations of 33.28 ± 26.22 mg/kg **(Fig 4.29)**.

### 4.2.4 DRY SEASON: BIOCHEMISTRY MARKERS OF LIPID PEROXIDATION AND TOTAL GLUTATHIONE LEVEL

#### 4.2.4.1) ORANGE KLOOF FOREST

##### *4.2.4.1.1) Comparisons of tGSH and MDA levels in moss, lichen and millipedes between sites C, 1, 2 and 3*

The mean tGSH and MDA (measured as TBARS) concentrations in moss, lichen and millipedes for sites C, 1, 2 and 3 of Orange Kloof forest for the dry sampling occasion in January 2015 are presented in **Table 4.30**. Concentrations are expressed in µmol/g.

**Table 4.30:** The mean tGSH and MDA levels (µmol/g) (± SD) in moss, lichen and millipedes for sites C, 1, 2 and 3 of Orange Kloof forest for the dry sampling occasion in January 2015.

| REDOX MARKER | | MOSS | | | | LICHEN | | | | MILLIPEDES | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Site C | Site 1 | Site 2 | Site 3 | Site C | Site 1 | Site 2 | Site 3 | Site C | Site 1 | Site 2 | Site 3 |
| **tGSH** | **Mean** | **14.36** | **50.42** | **90.21** | **15.28** | **ND** | **ND** | **ND** | **ND** | **542.81** | **461.25** | **495.50** | **401.26** |
| | *SD* | *11.44* | *53.28* | *100.56* | *2.68* | *ND* | *ND* | *ND* | *ND* | *142.90* | *96.39* | *68.75* | *201.55* |
| **MDA** | **Mean** | **0.49** | **0.76** | **0.65** | **0.27** | **0.10** | **0.06** | **0.73** | **0.10** | **0.58** | **2.97** | **0.74** | **0.34** |
| | *SD* | *0.26* | *0.41* | *0.29* | *0.05* | *0.03* | *0.05* | *0.26* | *0.03* | *0.28* | *2.51* | *0.63* | *0.16* |

Statistical signifcant differences (P<0.050) are indicated with different superscripted letters, where differences from: site C=a, site 1=b, site 2=c, site 3=d. Comparisons were done separately for the moss, lichen and millipedes. MDA (expressed as µmol TBARS per gram material). ND=Not Detected; SD=Standard Deviation; N=5.

***a) Moss***

Pairwise multiple comparisons showed no statistically significant differences in mean tGSH (P=0.347) or MDA (P=0.077) concentrations in moss between any of the sites C, 1, 2 and 3 **(Table 4.30)**.

***b) Lichen***

Lichen at Orange Kloof forest was compared between all the sites in terms of mean tGSH (P=0.530) and MDA (P=0.066) concentrations, but no significant differences were found between the sites for this sampling occasion **(Table 4.30)**.

***c) Millipedes***

Mean tGSH (P=0.875) and MDA (P=0.123) concentrations measured in millipedes at all four sites did not provide any statistically significant differences between any of those sites **(Table 4.30)**.

***4.2.4.1.2) Comparisons of tGSH and MDA levels between moss and lichen at sites C, 1, 2 and 3***

Moss and lichen were compared to determine the more appropriate indicator for this specific environment, due their different accumulation abilities.

Mean tGSH and MDA (measured as TBARS) concentrations in moss and lichen at sites C, 1, 2 and 3 of Orange Kloof forest for the dry sampling occasion in January 2015 are shown in **Figs 4.55 to 4.56**. Statistical signifcant differences (P<0.050) between moss and lichen are indicated with an asterisk above the graph bars.

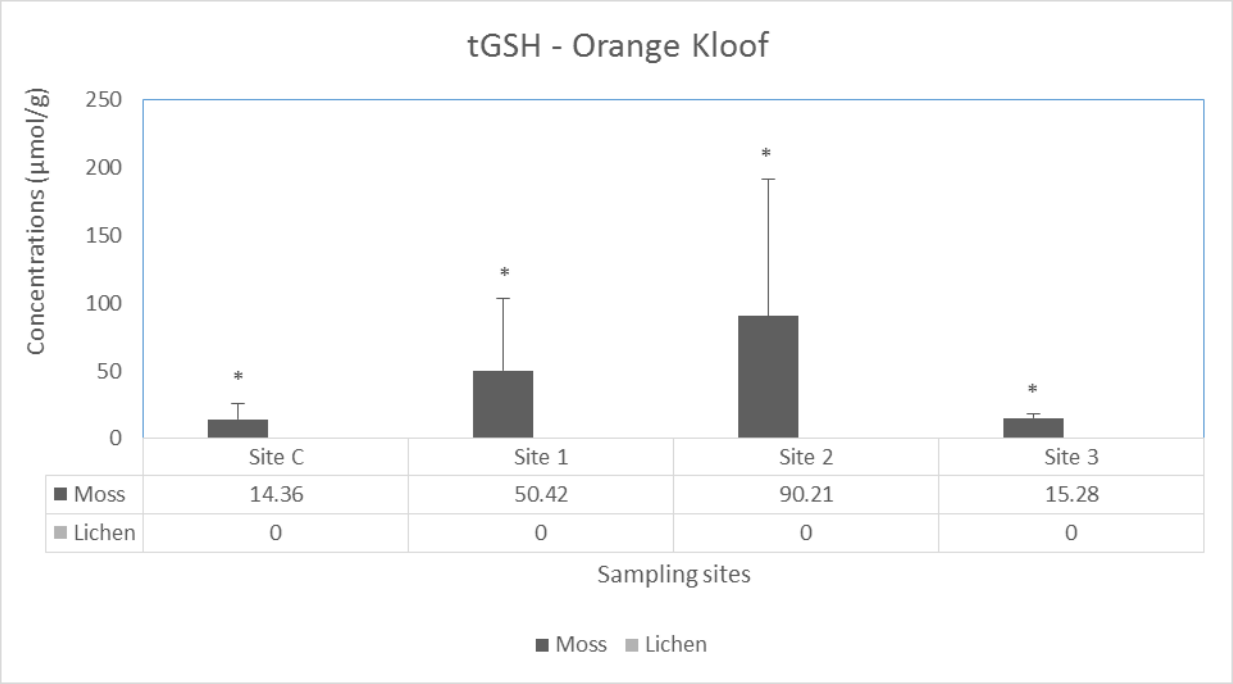


**Figure 4.55:** The mean tGSH concentrations (µmol/g) (± SD) in moss and lichen at sites C, 1, 2 and 3 of Orange Kloof forest for the dry sampling occasion in January 2015. SD=Standard Deviation; 0=Not Detected; N=5.

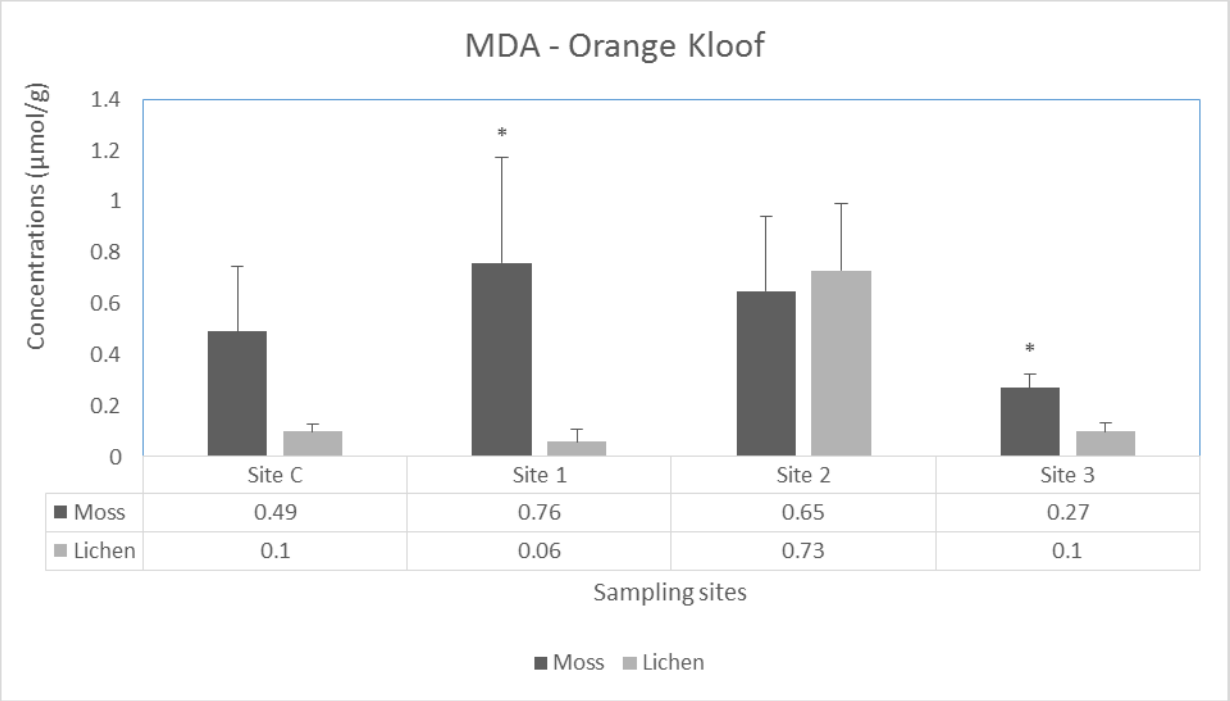


**Figure 4.56:** The mean MDA concentrations (µmol/g) (± SD) in moss and lichen at sites C, 1, 2 and 3 of Orange Kloof forest for the dry sampling occasion in January 2015. MDA (expressed as µmol TBARS per gram material); SD=Standard Deviation; N=5.

Moss and lichen showed statistically significant differences between each other at Orange Kloof sites: C (P=0.048), 1 (P=0.088), 2 (P=0.166) and 3 (P=0.277) in terms of mean tGSH concentrations **(Fig 4.55)**.

Orange Kloof moss and lichen showed statistically significant differences between each other at sites 1 (P=0.043) and 3 (P=0.011) when mean MDA concentrations were compared, but no statistically significant differences in MDA concentrations were found between moss and lichen at sites C (P=0.185) and 2 (P=0.752). Moss showed higher concentrations of MDA, with the exception at site 2, whereas lichen showed a slightly higher concentration of 0.73 ± 0.26 µmol/g **(Fig 4.56)**.

#### *4.2.4.1.3) Moisture percentage*

Moisture % of the moss ranged from a relatively moist 45.23 to 53 %. Lichen moisture % ranged from a relatively dry 19.95 to a moist 35.37 % and millipede moisture % was a relatively dry 6.80 to 10.51 %.

**Table 4.31:** Moisture % of moss, lichen and millipedes of Orange Kloof forest for the dry sampling occasion in January 2015.

| ORANGE KLOOF FOREST | % | MOSS | LICHEN | MILLIPEDES |
|---|---|---|---|---|
| Site C | Moisture | 45.23 | 19.95 | 6.74 |
| Site 1 | Moisture | 49.76 | 20.13 | 6.80 |
| Site 2 | Moisture | 53 | 33.51 | 10.51 |
| Site 3 | Moisture | 52.23 | 35.37 | 8.20 |

### 4.2.4.2) NEWLANDS FOREST

#### *4.2.4.2.1) Comparisons of tGSH and MDA levels in moss, lichen and millipedes between sites C, 1, 2 and 3*

The mean tGSH and MDA (measured as TBARS) concentrations in moss, lichen and millipedes for sites C, 1, 2 and 3 of Newlands forest for the dry sampling occasion in January 2015 are presented in **Table 32**. Concentrations are expressed in µmol/g.

**Table 4.32:** The mean tGSH and MDA levels (µmol/g) (± SD) in moss, lichen and millipedes for sites C, 1, 2 and 3 of Newlands forest for the dry sampling occasion in January 2015.

| REDOX MARKER | | MOSS | | | | LICHEN | | | | MILLIPEDES | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Site C | Site 1 | Site 2 | Site 3 | Site C | Site 1 | Site 2 | Site 3 | Site C | Site 1 | Site 2 | Site 3 |
| tGSH | Mean | 14.36 | 68.60 | 78.48 | 92.27 | ND | ND | ND | ND | 542.81 | 482.79 | 589.40 | 538.03 |
| | *SD* | *11.44* | *51.50* | *56.51* | *75.34* | *ND* | *ND* | *ND* | *ND* | *142.90* | *82.76* | *197.85* | *157.29* |
| MDA | Mean | $^{bcd}$0.49 | 0.04 | 0.05 | 0.21 | 0.10 | 0.29 | 0.55 | 0.05 | 0.58 | 0.43 | 0.62 | 0.95 |
| | *SD* | *0.26* | *0.04* | *0.03* | *0.09* | *0.03* | *0.41* | *0.45* | *0.03* | *0.28* | *0.35* | *0.19* | *0.83* |

Statistical signifcant differences (P<0.050) are indicated with different superscripted letters, where differences from: site C=a, site 1=b, site 2=c, site 3=d. Comparisons were done separately for the moss, lichen and millipedes. MDA (expressed as µmol TBARS per gram material). ND=Not Detected; SD=Standard Deviation; N=5.

***a) Moss***

No significant differences in moss in terms of mean tGSH (P=0.147) concentrations were found between any of the sites **(Table 4.32)**.

Statistically significant differences were, however found in mean MDA (P<0.05) concentrations between the sites: C vs 1, C vs 2 and C vs 3, but no significant differences were found between sites 1 vs 3, 1 vs 2 and 2 vs 3 (P>0.05). The highest concentration of 0.49 ± 0.26 µmol/g was measured at site C **(Table 4.32)**.

***b) Lichen***

Lichen comparisons showed no statistically significant differences in mean tGSH (P=1.000) or MDA (P=0.133) concentrations between any of the sites (**Table 4.32)**.

***c) Millipedes***

Newlands forest did not show any significant differences in mean tGSH (P=0.789) and MDA (P=0.764) concentrations in millipede samples between the four sites for this sampling session **(Table 4.32)**.

***4.2.4.2.2) Comparisons of tGSH and MDA levels between moss and lichen at sites C, 1, 2 and 3***

Moss and lichen were compared to determine the more appropriate indicator for this specific environment, due their different accumulation abilities.

Mean tGSH and MDA (measured as TBARS) concentrations in moss and lichen at sites C, 1, 2 and 3 of Newlands forest for the dry sampling occasion in January 2015 are shown in **Figs 4.57 to 4.58**. Statistical signifcant differences (P<0.050) between moss and lichen are indicated with an asterisk above the graph bars.

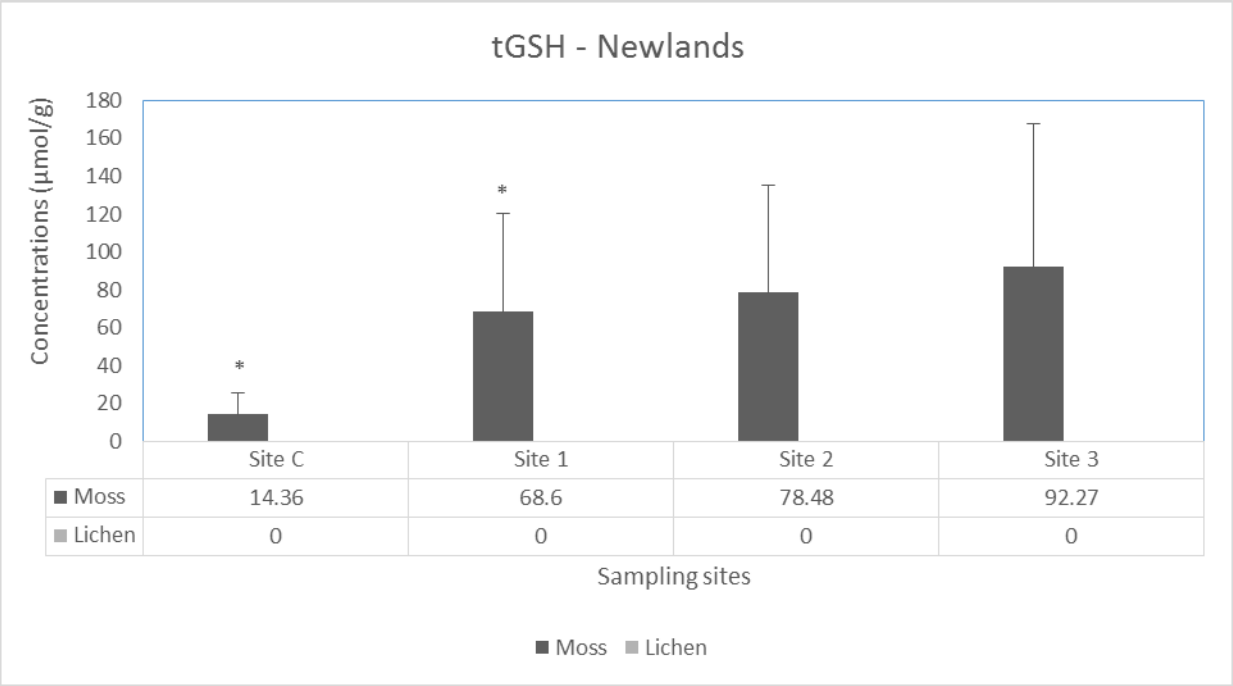


**Figure 4.57:** The mean tGSH concentrations (µmol/g) (± SD) in moss and lichen at sites C, 1, 2 and 3 of Newlands forest for the dry sampling occasion in January 2015. SD=Standard Deviation; 0=Not Detected; N=5.

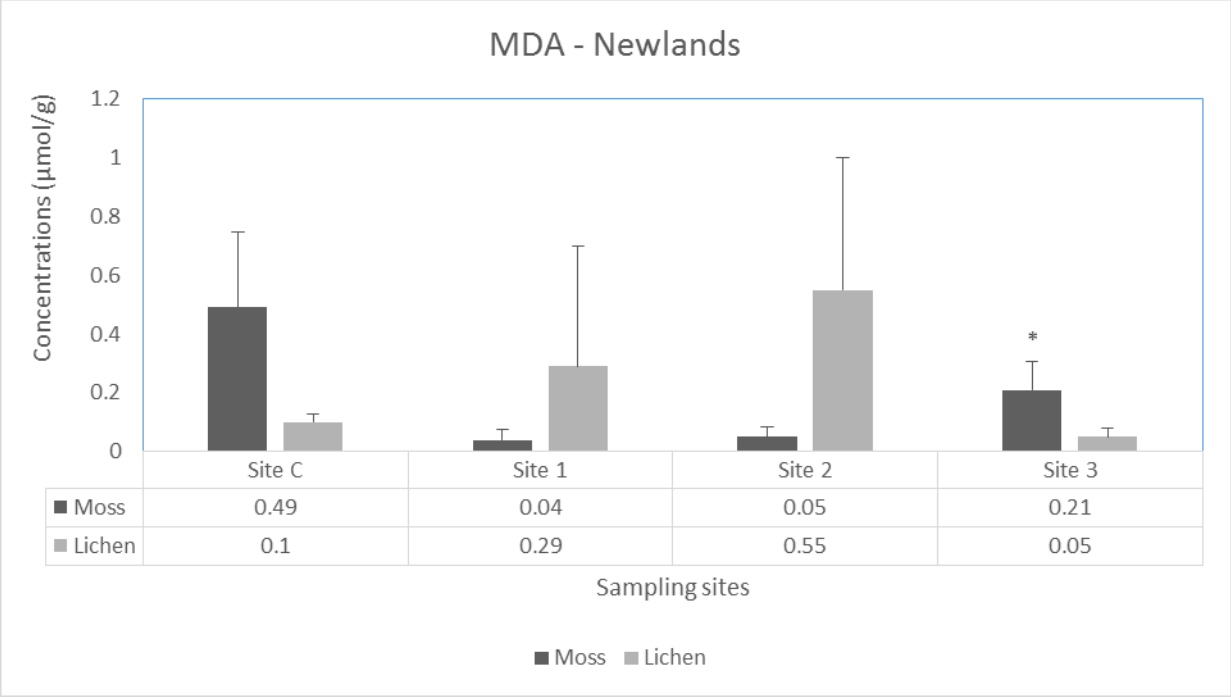


**Figure 4.58:** The mean MDA concentrations (µmol/g) (± SD) in moss and lichen at sites C, 1, 2 and 3 of Newlands forest for the dry sampling occasion in January 2015. MDA (expressed as µmol TBARS per gram material); SD=Standard Deviation; N=5.

Mean tGSH concentrations at sites C (P=0.048) and 1 (P=0.041), showed statistically significant differences between moss and lichen, but no statistically significant differences between moss and lichen were found at sites 2 (P=0.074) and 3 (P=0.101) **(Fig 4.57)**.

There were no statistically significant differences found at sites, C (P=0.185), 1 (P=0.180) and 2 (P=0.064), but significant differences were found at site 3 (P=0.026) at Newlands in terms of mean MDA concentrations in moss vs lichen comparisons **(Fig 4.58)**.

### *4.2.4.2.3) Moisture percentage*

Moisture % of the moss ranged from a relatively moist 45.23 to 55.36 %. Lichen moisture % ranged from a relatively dry 16.22 to 26.4 % and millipede moisture % was a relatively dry 3.66 to 6.74 %.

**Table 4.33:** Moisture % of the moss, lichen and millipedes of Newlands forest for the dry sampling occasion in January 2015.

| NEWLANDS FOREST | % | MOSS | LICHEN | MILLIPEDES |
|---|---|---|---|---|
| Site C | Moisture | 45.23 | 19.95 | 6.74 |
| Site 1 | Moisture | 55.36 | 16.22 | 4.64 |
| Site 2 | Moisture | 49.96 | 20.41 | 4.16 |
| Site 3 | Moisture | 52.85 | 26.4 | 3.66 |

### 4.2.4.3) FORESTS

#### *3.3.4.3.1) Comparisons of tGSH and MDA levels in moss, lichen and millipedes between the forests: Site C, Orange Kloof and Newlands*

The mean tGSH and MDA (measured as TBARS) concentrations in moss, lichen and millipedes for each forest for the dry sampling occasion (January 2015) are presented in **Tables 3.36, 3.37**. The tGSH and MDA concentrations for the sites within each forest were pooled to calculate the mean concentrations for each forest. Concentrations are expressed in µmol/g.

#### *a) tGSH*

**Table 4.34:** The mean tGSH levels (µmol/g) (± SD) in moss, lichen and millipedes for forests for the dry sampling occasion in January 2015.

| FOREST | | MOSS | LICHEN | MILLIPEDES |
|---|---|---|---|---|
| **Site C (N=5)** | **Mean** | **[a]14.36** | **[a]ND** | **[a]542.81** |
| | *SD* | *11.44* | *ND* | *142.90* |
| **Orange Kloof (N=15)** | **Mean** | **[a]51.97** | **[a]ND** | **[a]452.67** |
| | *SD* | *65.53* | *ND* | *123.96* |
| **Newlands (N=15)** | **Mean** | **[b]79.78** | **[a]ND** | **[a]536.74** |
| | *SD* | *54.65* | *ND* | *140.77* |

Statistical signifcant differences (P<0.050) are indicated with different superscripted letters. Comparisons were done separately for moss, lichen and millipedes. ND=Not Detected; SD=Standard Deviation.

When moss were compared between forests, statistically significant differences were found in tGSH concentrations between site C and Newlands and Orange Kloof and Newlands forests (P<0.050). No statistically significant differences were found between site C and Orange Kloof forest (P>0.050). The highest mean tGSH concentrations in moss were found at Newlands forest (79.78 ± 54.65 µmol/g) **(Table 4.34)**.

In terms of tGSH concentrations, there were no statistically differences found between the forests in lichen (P=0.125) and millipedes (P=0.640) **(Table 4.34)**.

***b) MDA***

**Table 4.35:** The mean MDA levels (µmol/g) (± SD) in moss, lichen and millipedes for forests for the dry sampling occasion in January 2015.

| FOREST | | MOSS | LICHEN | MILLIPEDES |
|---|---|---|---|---|
| **Site C (N=5)** | **Mean** | **[a]0.49** | **[a]0.1** | **[a]0.58** |
| | *SD* | *0.26* | *0.03* | *0.28* |
| **Orange Kloof (N=15)** | **Mean** | **[b]0.56** | **[a]0.3** | **[a]1.35** |
| | *SD* | *0.34* | *0.35* | *1.78* |
| **Newlands (N=15)** | **Mean** | **[c]0.1** | **[a]0.3** | **[a]0.67** |
| | *SD* | *0.10* | *0.37* | *0.52* |

Statistical signifcant differences (P<0.050) are indicated with different superscripted letters. Comparisons were done separately for moss, lichen and millipedes. MDA (expressed as µmol TBARS per gram material); SD=Standard Deviation.

Pairwise multiple comparisons showed statistically significant differences in moss between all three forests (P=<0.001) during this sampling occasion. The highest mean MDA concentrations (0.56 ± 0.34 µmol/g) were found at Orange Kloof forest **(Table 4.35)**.

Comparisons in terms of MDA concentrations in lichen (P=0.833) and millipedes (P=0.993) showed no statistically significant differences between any of the forests **(Table 4.35)**.

## 4.2.5) WET SEASON: BIOCHEMISTRY MARKERS OF LIPID PEROXIDATION AND TOTAL GLUTATHIONE

### 4.2.5.1) ORANGE KLOOF FOREST

#### *4.2.5.1.1) Comparisons of tGSH and MDA levels in moss, lichen and millipedes between sites C, 1, 2 and 3*

The mean tGSH and MDA (measured as TBARS) concentrations in moss, lichen and millipedes for sites C, 1, 2 and 3 of Orange Kloof forest for the wet sampling occasion in June 2015 are presented in **Table 4.36**. Concentrations are expressed in µmol/g.

**Table 4.36:** The mean tGSH and MDA levels (µmol/g) (± SD) in moss, lichen and millipedes for sites C, 1, 2 and 3 of Orange Kloof forest for the wet sampling occasion in June 2015.

| REDOX MARKER | | MOSS | | | | LICHEN | | | | MILLIPEDES | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Site C | Site 1 | Site 2 | Site 3 | Site C | Site 1 | Site 2 | Site 3 | Site C | Site 1 | Site 2 | Site 3 |
| **tGSH** | **Mean** | **51.01** | **20.47** | **29.85** | **27.78** | **ND** | **3.00** | **ND** | **ND** | **385.19** | **298.57** | **318.06** | **380.29** |
| | *SD* | *72.25* | *14.87* | *4.86* | *13.68* | *ND* | *14.49* | *ND* | *ND* | *143.67* | *165.92* | *173.69* | *46.86* |
| **MDA** | **Mean** | **0.44** | **0.69** | **0.94** | **0.46** | **2.20** | **1.38** | **1.52** | **0.71** | **1.40** | **1.96** | **1.28** | **3.16** |
| | *SD* | *0.34* | *0.86* | *0.53* | *0.10* | *2.03* | *0.43* | *1.21* | *0.78* | *1.26* | *3.21* | *1.04* | *2.11* |

Statistical signifcant differences (P<0.050) are indicated with different superscripted letters, where differences from: site C=a, site 1=b, site 2=c, site 3=d. Comparisons were done separately for the moss, lichen and millipedes. MDA (expressed as µmol TBARS per gram material). ND=Not Detected; SD=Standard Deviation; N=5.

***a) Moss***

Mean tGSH (P=0.589) and MDA (P=0.740) concentrations in moss between the sites at Orange Kloof forest showed no statistically differences in this sampling occasion **(Table 4.36)**.

***b) Lichen***

Lichen, in terms of mean tGSH (P=0.449) and MDA (P=0.557) concentrations were compared between all the sites, but no significant differences were found **(Table 4.36)**.

***c) Millipedes***

Pairwise multiple comparisons showed no statistically significant differences in mean tGSH (P=0.875) or MDA (P=0.546) concentrations between any of the sites C, 1, 2 and 3 **(Table 4.36)**.

### *4.2.5.1.2) Comparisons of tGSH and MDA levels in moss and lichen at sites C, 1, 2 and 3*

Moss and lichen were compared to determine the more appropriate indicator for this specific environment, due their different accumulation abilities.

Mean tGSH and MDA (measured as TBARS) concentrations in moss and lichen at sites C, 1, 2 and 3 of Orange Kloof forest for the wet sampling occasion in June 2015 are shown in **Figs 4.59 to 4.60**. Statistical signifcant differences (P<0.050) between moss and lichen are indicated with an asterisk above the graph bars.

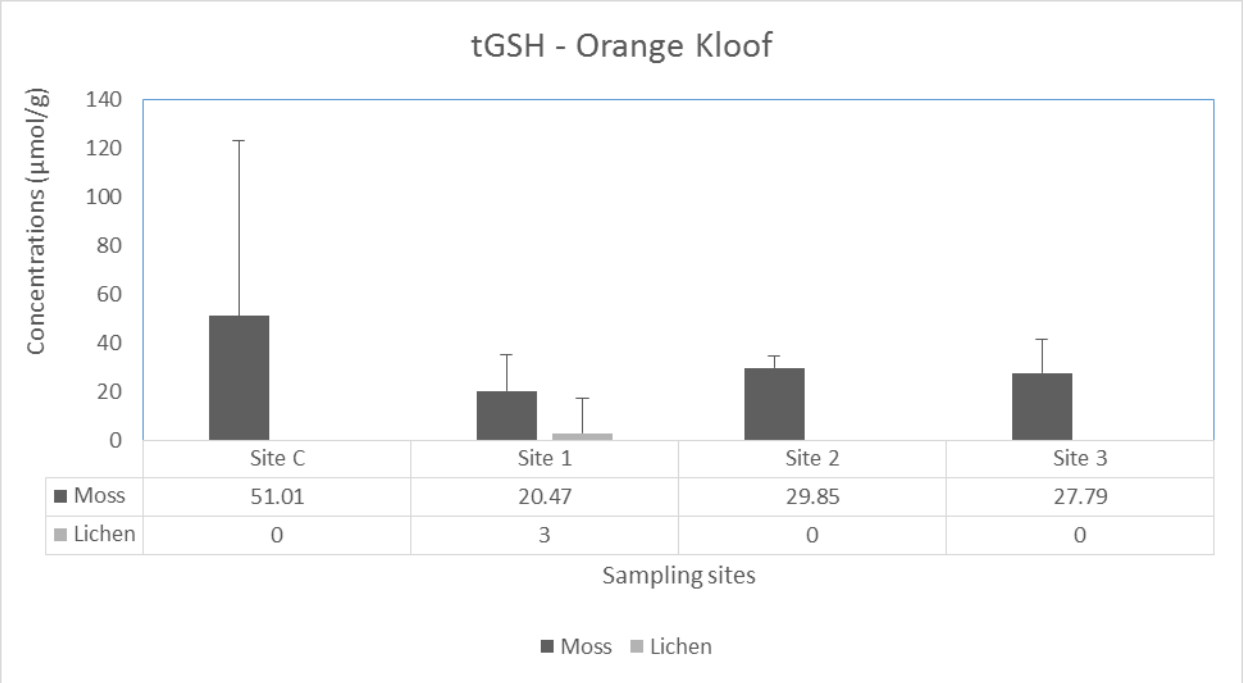


**Figure 4.59:** The mean tGSH concentrations (µmol/g) (± SD) in moss and lichen at sites C, 1, 2 and 3 of Orange Kloof forest for the wet sampling occasion in June 2015. SD=Standard Deviation; 0=Not Detected; N=5.

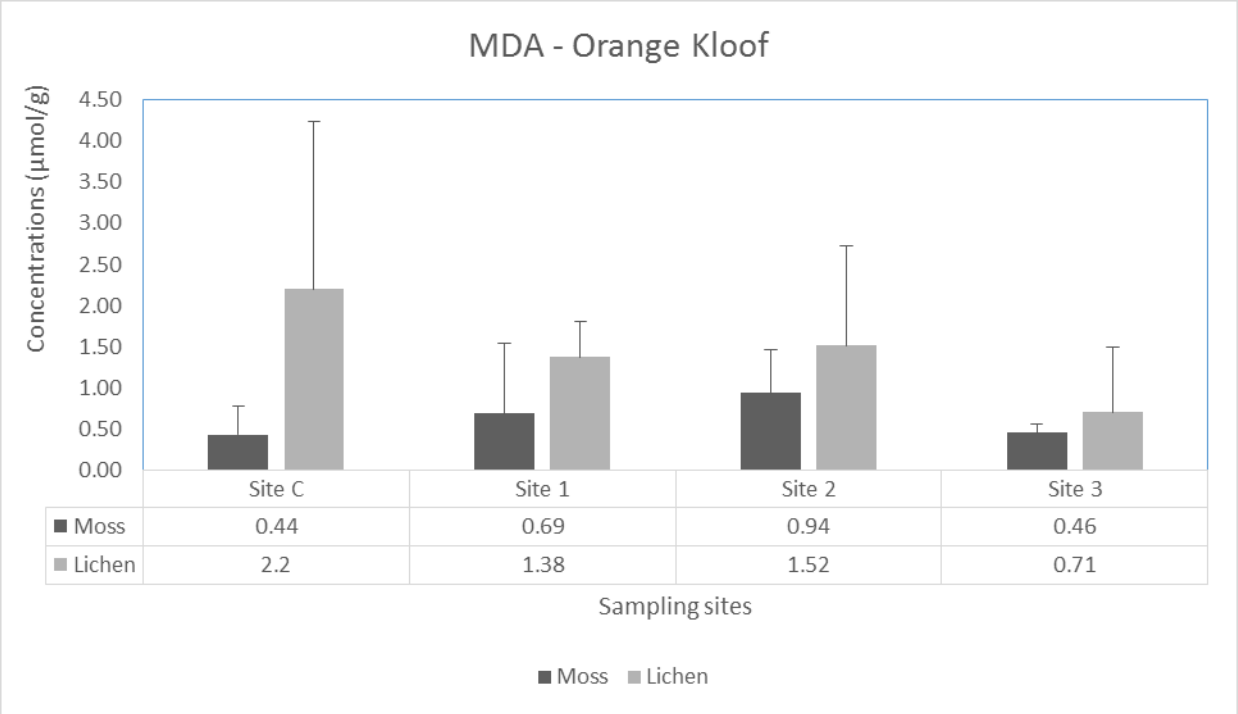


**Figure 4.60:** The mean MDA concentrations (µmol/g) (± SD) in moss and lichen at sites C, 1, 2 and 3 of Orange Kloof forest for the wet sampling occasion in June 2015. MDA (expressed as µmol TBARS per gram material); SD=Standard Deviation; N=5.

No statistically significant differences were found between moss and lichen at all the Orange Kloof sites: C (P=0.144), 1 (P=0.122), 2 (P=0.416) and 3 (P=0.114) in terms of mean tGSH concentrations **(Fig 4.59)**.

Moss and lichen at Orange Kloof showed no statistically significant differences between each other at sites: C (P=0.107), 1 (P=0.142), 2 (P=0.491) and 3 (P=0.620) when mean MDA concentrations were compared **(Fig 4.60)**.

### *4.2.5.1.3) Moisture percentage*

Moisture % of the moss ranged from a relatively moist 45.23 to 53 %. Lichen moisture % ranged from a relatively dry 19.95 to a moist 35.37 % and millipede moisture % was a relatively dry 6.80 to 10.51 %.

**Table 4.37:** Moisture % of moss, lichen and millipedes of Orange Kloof forest for the wet sampling occasion in June 2015.

| ORANGE KLOOF FOREST | % | MOSS | LICHEN | MILLIPEDES |
|---|---|---|---|---|
| Site C | Moisture | 46.89 | 17.54 | 4.85 |
| Site 1 | Moisture | 45.51 | 23.72 | 14.99 |
| Site 2 | Moisture | 48.53 | 34.10 | 8.16 |
| Site 3 | Moisture | 49.56 | 39.08 | 9.02 |

### 4.2.5.2) NEWLANDS FOREST

#### *4.2.5.2.1) Comparisons of tGSH and MDA levels in moss, lichen and millipedes between sites C, 1, 2 and 3*

The mean tGSH and MDA (measured as TBARS) concentrations in moss, lichen and millipedes for sites C, 1, 2 and 3 of Newlands forest for the wet sampling occasion in June 2015 are presented in **Table 4.38**. Concentrations are expressed in µmol/g.

**Table 4.38:** The mean tGSH and MDA levels (µmol/g) (± SD) in moss, lichen and millipedes for sites C, 1, 2 and 3 of Newlands forest for the wet sampling occasion in June 2015.

| REDOX MARKER | | MOSS | | | | LICHEN | | | | MILLIPEDES | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Site C | Site 1 | Site 2 | Site 3 | Site C | Site 1 | Site 2 | Site 3 | Site C | Site 1 | Site 2 | Site 3 |
| **tGSH** | **Mean** | **51.01** | **48.37** | **26.48** | **14.07** | **ND** | **ND** | **ND** | **ND** | **385.19** | **138.41** | **174.89** | **292.26** |
| | *SD* | *72.25* | *47.60* | *34.08* | *5.43* | *ND* | *ND* | *ND* | *ND* | *143.67* | *156.28* | *139.07* | *43.25* |
| **MDA** | **Mean** | **0.44** | **0.53** | **0.21** | **0.16** | **2.20** | **1.00** | **1.85** | **0.46** | **1.40** | **1.52** | **1.26** | **0.60** |
| | *SD* | *0.34* | *0.36* | *0.12* | *0.10* | *2.03* | *0.68* | *1.32* | *0.35* | *1.26* | *0.80* | *1.24* | *0.35* |

Statistical signifcant differences (P<0.050) are indicated with different superscripted letters, where differences from: site C=a, site 1=b, site 2=c, site 3=d. Comparisons were done separately for the moss, lichen and millipedes. MDA (expressed as µmol TBARS per gram material). ND=Not Detected; SD=Standard Deviation; N=5.

***a) Moss***

There were no significant differences found in mean tGSH (P=0.789) or MDA (P=0.248) concentrations in moss between any of the sites during the wet season sampling occasion **(Table 4.38)**.

***b) Lichen***

Pairwise multiple comparisons of lichen showed no statistically significant differences in tGSH (P=0.392) and MDA (P=0.287) concentrations between any of the sites **(Table 4.38)**.

***c) Millipedes***

This forest did not show any significant differences in mean tGSH (P=0.347) and MDA (P=0.589) concentrations in millipede samples between the four sites for this sampling session **(Table 4.38)**.

### *4.2.5.2.2) Comparisons of tGSH and MDA levels in moss and lichen at sites C, 1, 2 and 3*

Moss and lichen were compared to determine the more appropriate indicator for this specific environment, due their different accumulation abilities.

Mean tGSH and MDA (measured as TBARS) concentrations in moss and lichen at sites C, 1, 2 and 3 of Newlands forest for the wet sampling occasion in June 2015 are shown in **Figs 4.61 to 4.62**. Statistical signifcant differences (P<0.050) between moss and lichen are indicated with an asterisk above the graph bars.

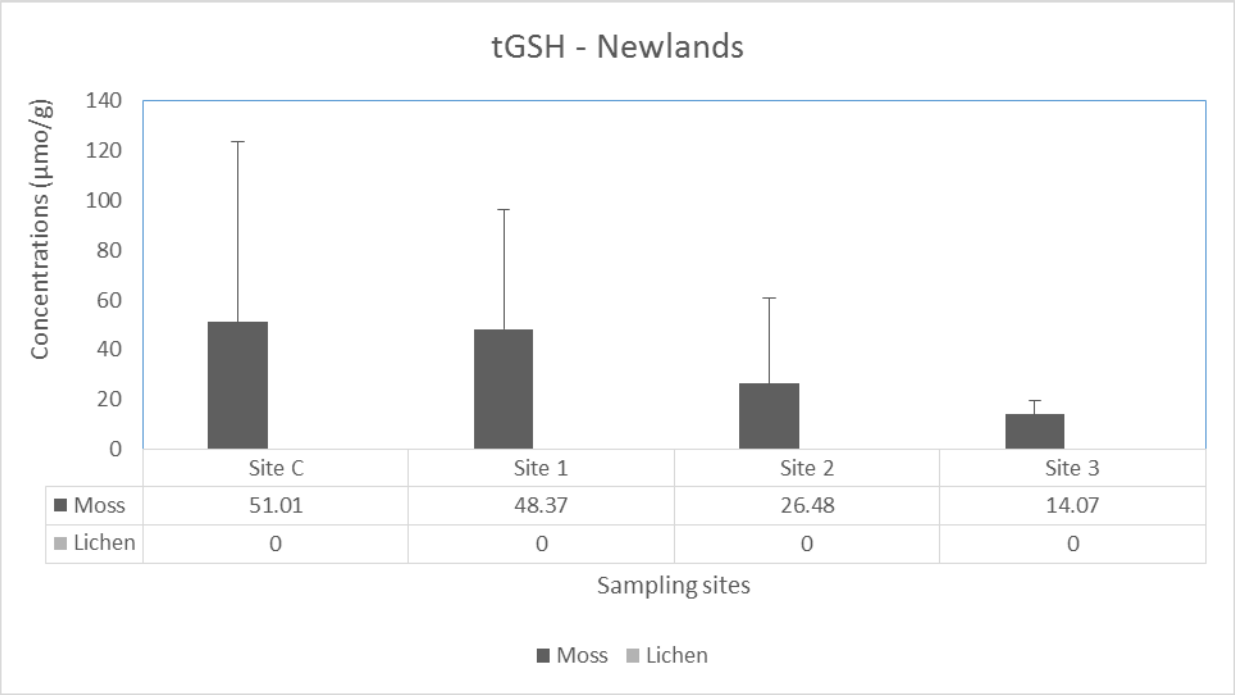


**Figure 4.61:** The mean tGSH concentrations (µmol/g) (± SD) in moss and lichen at sites C, 1, 2 and 3 of Newlands forest for the wet sampling occasion in June 2015. SD=Standard Deviation; 0=Not Detected; N=5.

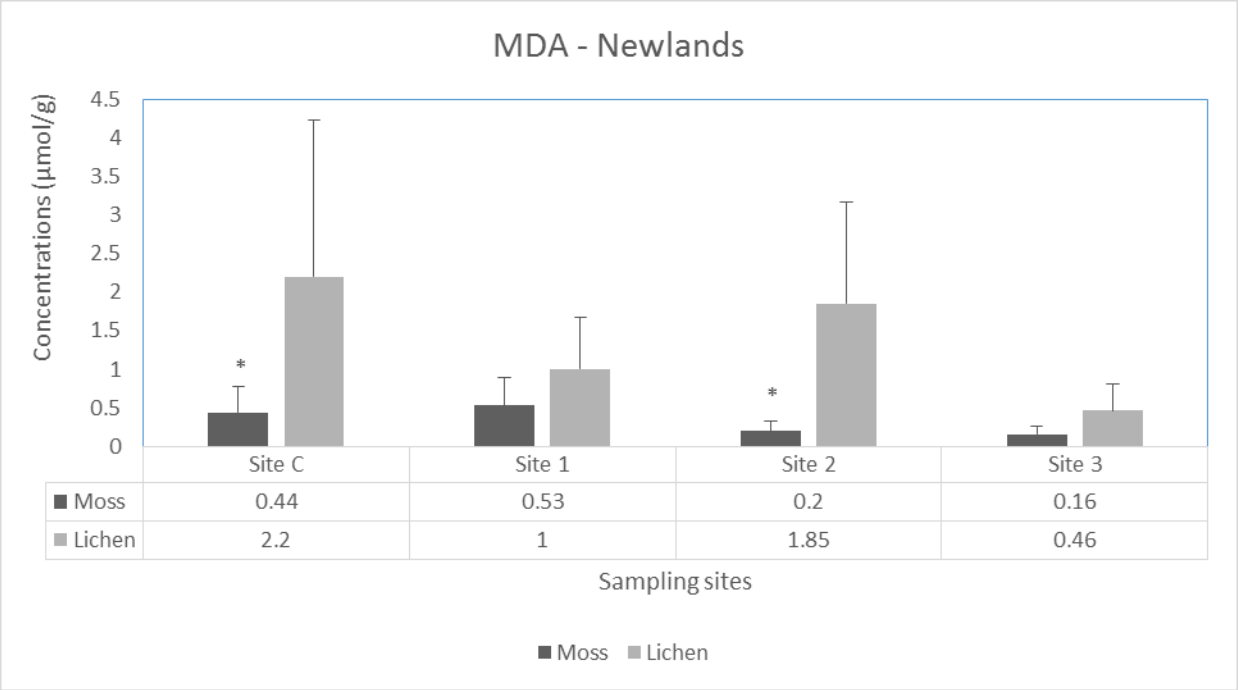


**Figure 4.62:** The mean MDA concentrations (µmol/g) (± SD) in moss and lichen at sites C, 1, 2 and 3 of Newlands forest for the wet sampling occasion in June 2015. MDA (expressed as µmol TBARS per gram material); SD=Standard Deviation; N=5.

At Newlands forest the mean tGSH concentrations at sites C (P=0.144), 1 (P=0.077), 2 (P=0.125) and 3 (P=0.339) showed no statistically significant between moss and lichen **(Fig 4.61)**.

Comparisons showed statistically significant differences at sites, C (P=0.107) and 2 (P=0.050) between moss and lichen in terms of mean MDA concentrations, but not at sites, 1 (P=0.346) and 3 (P=0.216) **(Fig 3.4.62)**.

### *4.2.5.2.3) Moisture percentage*

Moisture % of the moss ranged from a relatively moist 45.23 to 55.36 %. Lichen moisture % ranged from a relatively dry 16.22 to 26.4 % and millipede moisture % was a relatively dry 3.66 to 6.74 %.

**Table 4.39:** Moisture % of the moss, lichen and millipedes of Newlands forest for the wet sampling occasion in June 2015.

| NEWLANDS FOREST | % | MOSS | LICHEN | MILLIPEDES |
|---|---|---|---|---|
| Site C | Moisture | 46.89 | 17.54 | 4.85 |
| Site 1 | Moisture | 49.16 | 41.00 | 4.94 |
| Site 2 | Moisture | 52.50 | 25.60 | 3.37 |
| Site 3 | Moisture | 47.22 | 26.94 | 3.72 |

### 4.2.5.3) FORESTS

#### *4.2.5.3.1) Comparisons of tGSH and MDA levels in moss, lichen and millipedes between the forests: Site C, Orange Kloof and Newlands*

The mean tGSH and MDA (measured as TBARS) concentrations in moss, lichen and millipedes for each forest for the wet sampling occasion (June 2015) are presented in **Tables 4.40, 4.41**. The tGSH and MDA concentrations for the sites within each forest were pooled to calculate the mean concentrations for each forest. Concentrations are expressed in µmol/g.

***a) tGSH***

**Table 4.40:** The mean tGSH levels (µmol/g) (± SD) in moss, lichen and millipedes for forests for the wet sampling occasion in June 2015.

| FOREST | | MOSS | LICHEN | MILLIPEDES |
|---|---|---|---|---|
| **Site C (N=5)** | **Mean** | **$^{a}$51.01** | **$^{a}$ND** | **$^{a}$385.19** |
| | *SD* | *72.25* | *ND* | *143.67* |
| **Orange Kloof (N=15)** | **Mean** | **$^{a}$26.03** | **$^{b}$ND** | **$^{a}$332.3** |
| | *SD* | *11.23* | *ND* | *127.83* |
| **Newlands (N=15)** | **Mean** | **$^{a}$29.64** | **$^{c}$ND** | **$^{b}$201.85** |
| | *SD* | *33.02* | *ND* | *127.50* |

Statistical signifcant differences (P<0.050) are indicated with different superscripted letters. Comparisons were done separately for moss, lichen and millipedes. ND=Not Detected; SD=Standard Deviation.

There were no statistically differences found in mean tGSH concentrations between the forests in moss (P=0.879), as opposed to statistically differences that were found in mean tGSH concentrations between the forests in lichen (P=0.022) **(Table 4.40)**.

Pairwise multiple comparisons of mean tGSH concentrations in millipedes between forests showed statistically significant differences between site C and Newlands and

Orange Kloof and Newlands forests (P<0.050). No statistically significant differences were found between site C and Orange Kloof forest (P>0.050), but the highest mean tGSH concentrations were found at the site C (385.19 ± 143.67 µmol/g) **(Table 4.40)**.

***b) MDA***

**Table 4.41:** The mean MDA levels (µmol/g) (± SD) in moss, lichen and millipedes for forests for the wet sampling occasion in June 2015.

| FOREST | | MOSS | LICHEN | MILLIPEDES |
|---|---|---|---|---|
| **Site C (N=5)** | **Mean** | **[a]0.44** | **[a]2.2** | **[a]1.4** |
| | *SD* | *0.34* | *2.03* | *1.26* |
| **Orange Kloof (N=15)** | **Mean** | **[a]0.7** | **[a]1.2** | **[a]2.13** |
| | *SD* | *0.55* | *0.84* | *2.15* |
| **Newlands (N=15)** | **Mean** | **[a]0.3** | **[a]1.11** | **[a]1.12** |
| | *SD* | *0.26* | *0.97* | *0.86* |

Statistical significant differences are indicated with different superscripted letters. Comparisons were done separately for moss, lichen and millipedes. MDA (expressed as µmol TBARS per gram material); SD=Standard Deviation.

Mean MDA concentrations measured in in moss (P=0.152), lichen (P=0.213) and millipedes (P=0.813) showed no statistically significant differences between any of the forests **(Table 4.41)**.

## 4.2.6) DRY AND WET SEASON: BIOCHEMISTRY MARKERS OF LIPID PEROXIDATION AND TOTAL GLUTATHIONE

### 4.2.6.1) ORANGE KLOOF FOREST

#### *4.2.6.1.1) Comparisons of tGSH and MDA levels of moss between dry and wet seasons at sites C, 1, 2 and 3*

The mean tGSH and MDA (measured as TBARS) concentrations in moss at sites C, 1, 2 and 3 of Orange Kloof forest for the dry and wet sampling occasions in January and June 2015 are shown in **Figs 4.63 to 4.64**. Statistical signifcant differences (P<0.050) between the dry and wet seasons are indicated with an asterisk above the graph bars.

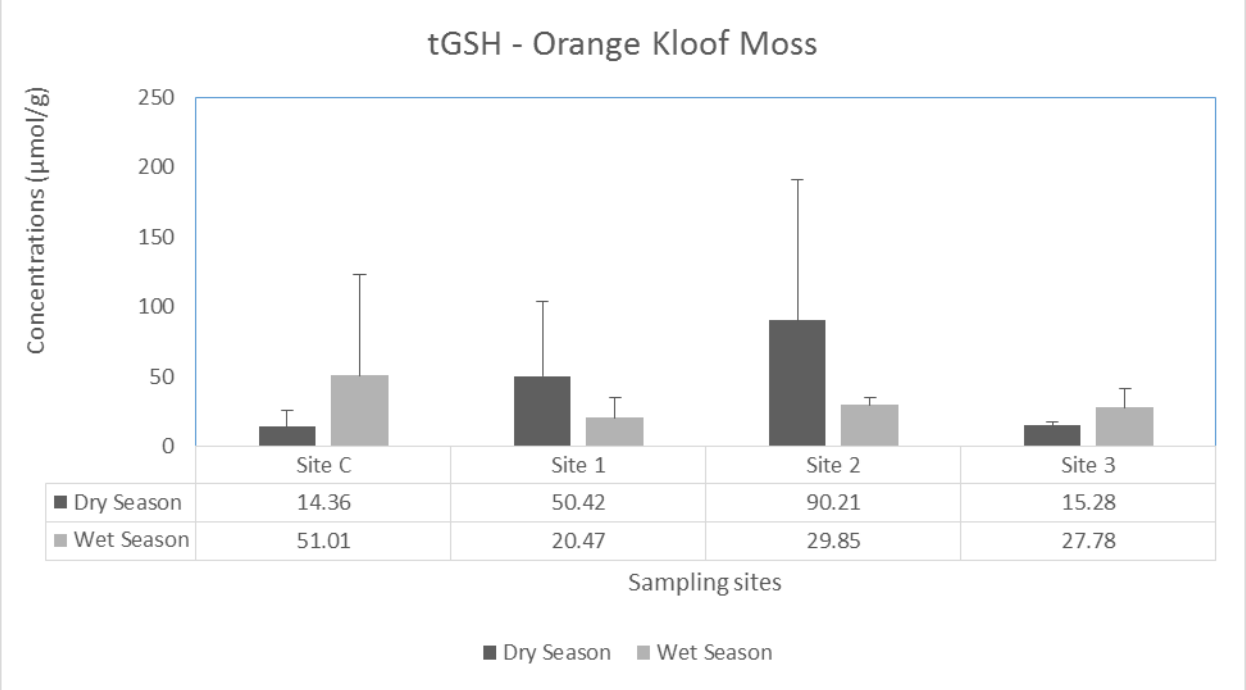


**Figure 4.63:** The mean tGSH concentrations (µmol/g) (± SD) in moss at sites C, 1, 2 and 3 of Orange Kloof forest for the dry and wet sampling occasion in January and June 2015. SD=Standard Deviation; N=5.

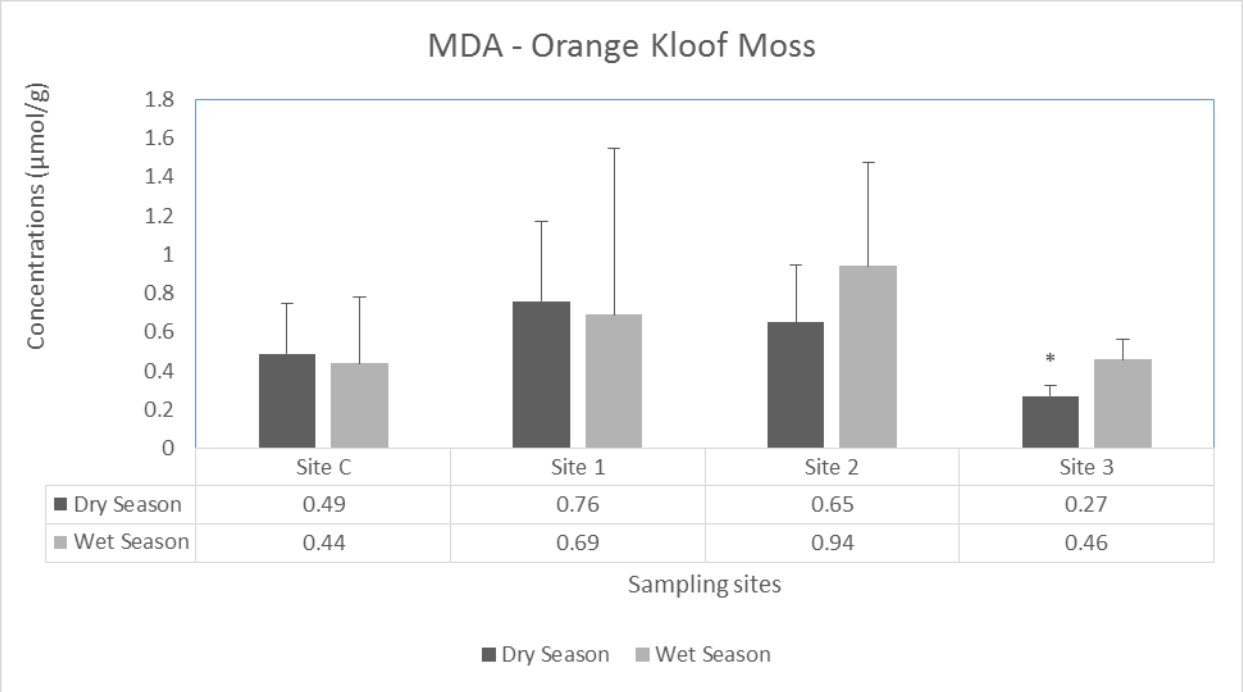


**Figure 4.64:** The mean MDA concentrations (µmol/g) (± SD) in moss at sites C, 1, 2 and 3 of Orange Kloof forest for the dry and wet sampling occasion in January and June 2015. MDA (expressed as µmol TBARS per gram material); SD=Standard Deviation; N=5.

Moss was compared between the dry and wet seasons in terms of mean tGSH concentrations and no statistically significant differences were found between seasons at sites: C (P=0.217), 1 (P=0.201), 2 (P=0.179) and 3 (P=0.947) **(Fig 4.63)**.

In terms of mean MDA content in moss, the dry and wet seasons showed statistically significant differences between each other at Orange Kloof site 3 (P=0.044). The MDA concentrations calculated in moss at site 3 (0.47 ± 0.10 µmol/g) were significantly higher in the wet season as opposed to the dry season (0.27 ± 0.05 µmol/g). No statistically significant differences were, however found at sites C (P=0.373), 1 (P=0.907) and 2 (P=0.228) **(Fig 4.64)**.

### *4.2.6.1.2) Comparisons of tGSH and MDA levels of lichen between dry and wet seasons at sites C, 1, 2 and 3*

Mean MDA (measured as TBARS) concentrations in lichen at sites C, 1, 2 and 3 of Orange Kloof forest for the dry and wet sampling occasions in January and June 2015 are shown in **Fig. 4.65**. Statistical signifcant differences (P<0.050) between the dry and wet seasons are indicated with an asterisk above the graph bars.

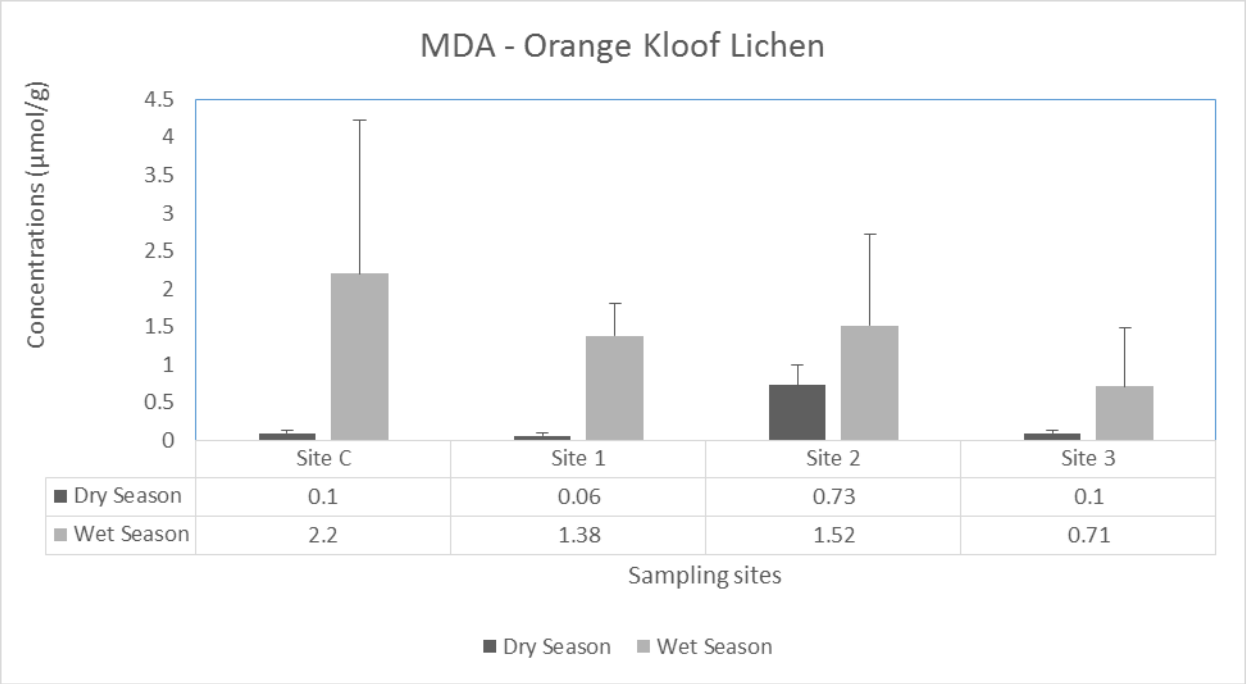


**Figure 4.65:** The mean MDA concentrations (µmol/g) (± SD) in lichen at sites C, 1, 2 and 3 of Orange Kloof forest for the dry and wet sampling occasion in January and June 2015. MDA (expressed as µmol TBARS per gram material); SD=Standard Deviation; N=5.

Mean tGSH comparisons between the dry and wet season of lichen showed a no statistically significant differences between the seasons at sites C (P=1.000), 1 (P=0.072), 2 (P=1.000) and 3 (P=0.427).

Comparisons of mean MDA concentrations between the dry and wet sampling sessions did not differ statistically significantly at sites C (P=0.074), 1 (P=0.100), 2 (P=0.329) and 3 (P=0.254) **(Fig 4.65)**.

### *4.2.6.1.3) Comparisons of tGSH and MDA levels in millipedes between dry and wet seasons at sites C, 1, 2 and 3*

Mean tGSH and MDA (measured as TBARS) concentrations in millipedes at sites C, 1, 2 and 3 of Orange Kloof forest for the dry and wet sampling occasions in January and June 2015 are shown in **Figs 4.66 to 4.67**. Statistical signifcant differences (P<0.050) between the dry and wet seasons are indicated with an asterisk above the graph bars.

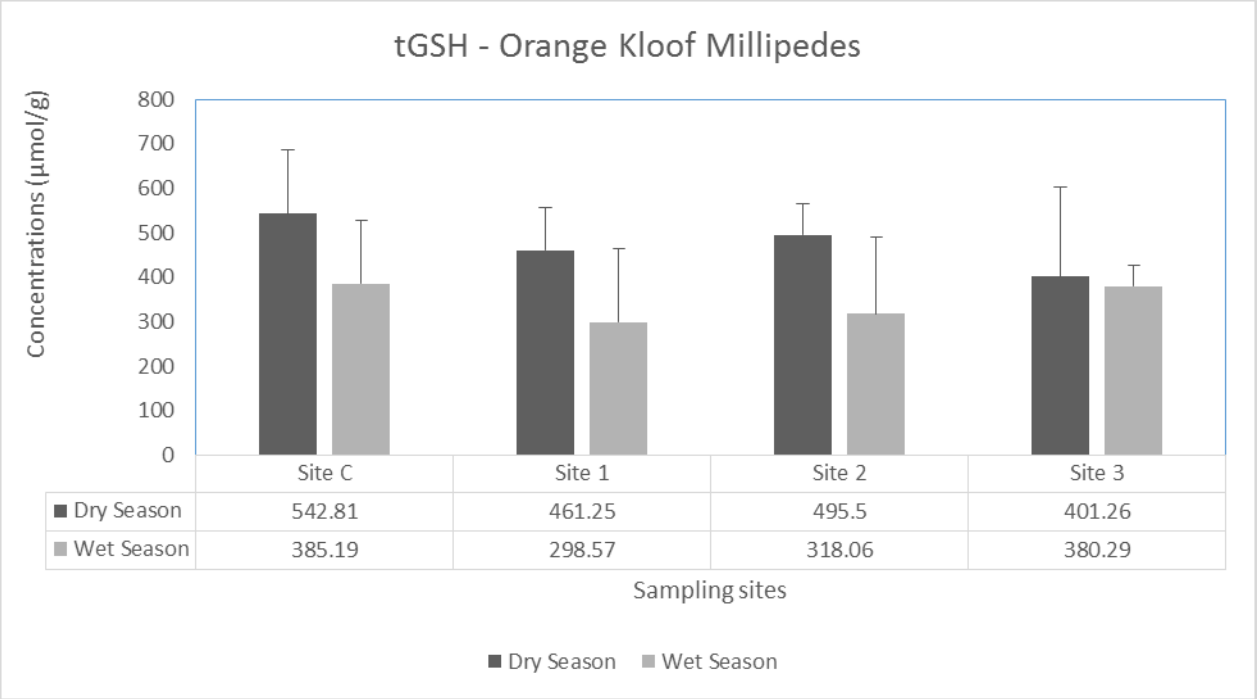


**Figure 4.66:** The mean tGSH concentrations (µmol/g) (± SD) in millipedes at sites C, 1, 2 and 3 of Orange Kloof forest for the dry and wet sampling occasion in January and June 2015. SD=Standard Deviation; N=5.

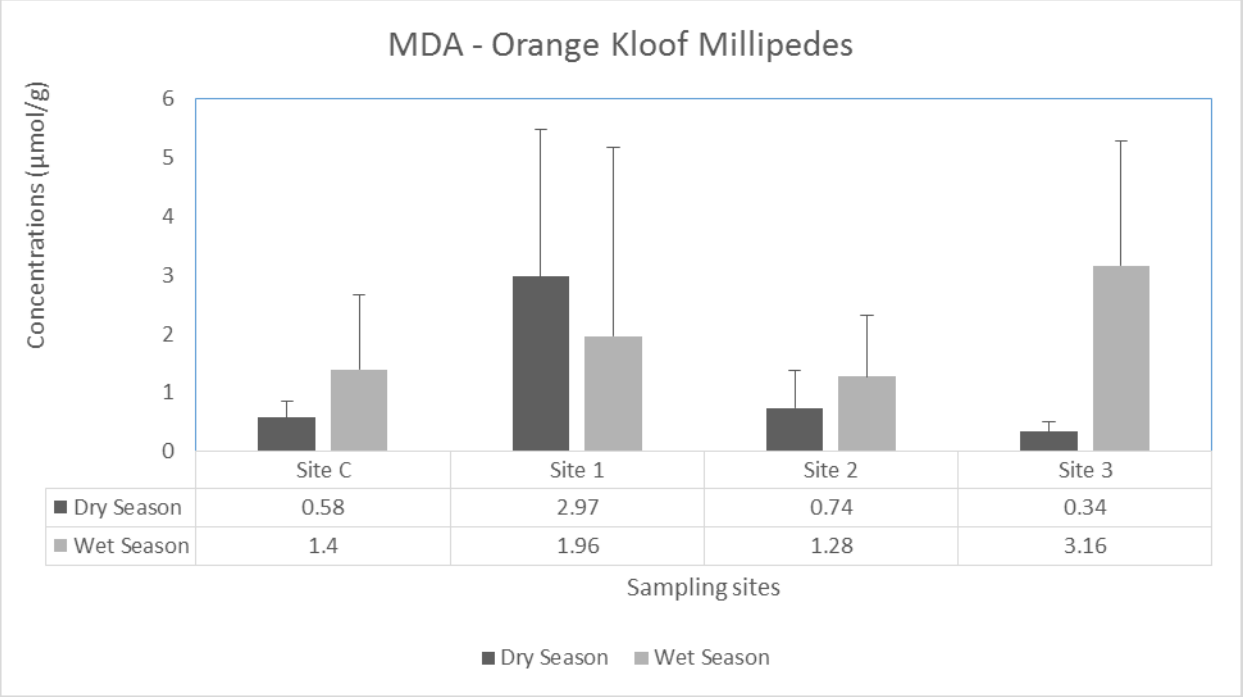


**Figure 4.67:** The mean MDA concentrations (µmol/g) (± SD) in millipedes at sites C, 1, 2 and 3 of Orange Kloof forest for the dry and wet sampling occasion in January and June 2015. MDA (expressed as µmol TBARS per gram material); SD=Standard Deviation; N=5.

Mean tGSH concentrations were compared between the seasons at sites C (P=0.249), 1 (P=0.216), 2 (P=0.175) and 3 (P=0.869) and no statistically significant differences were found between dry and wet seasons **(Fig 4.66)**.

No significant differences were found between the seasons at any of the sites C (P=0.700), 1 (P=0.690), 2 (P=0.482) and 3 (P=0.100) at Orange Kloof forest in terms of mean MDA concentrations **(Fig 4.67)**.

### 4.2.6.2) NEWLANDS FOREST

#### *4.2.6.2.1) Comparisons of tGSH and MDA levels of moss between dry and wet seasons at sites C, 1, 2 and 3*

Mean tGSH and MDA (measured as TBARS) concentrations in moss at sites C, 1, 2 and 3 of Newlands forest for the dry and wet sampling occasions in January and June 2015 are shown in **Figs 4.68 to 4.69**. Statistical signifcant differences (P<0.050) between the dry and wet seasons are indicated with an asterisk above the graph bars.

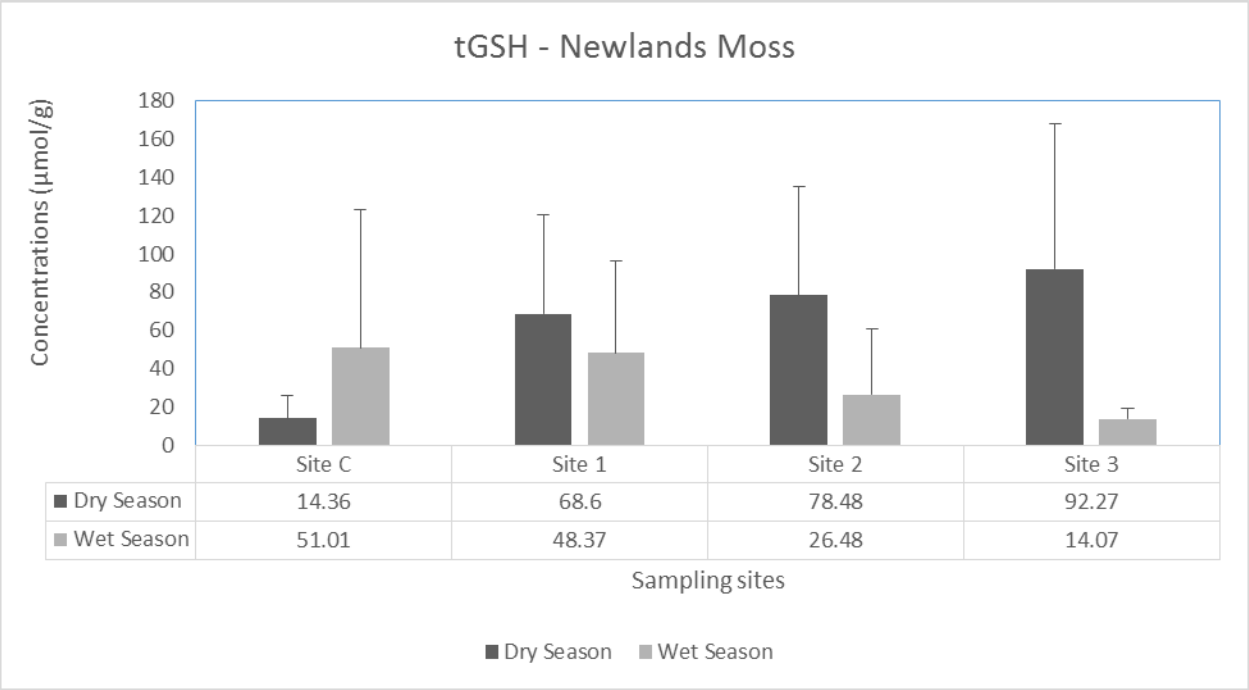


**Figure 4.68:** The mean tGSH concentrations (µmol/g) (± SD) in moss at sites C, 1, 2 and 3 of Newlands forest for the dry and wet sampling occasion in January and June 2015. SD=Standard Deviation; N=5.

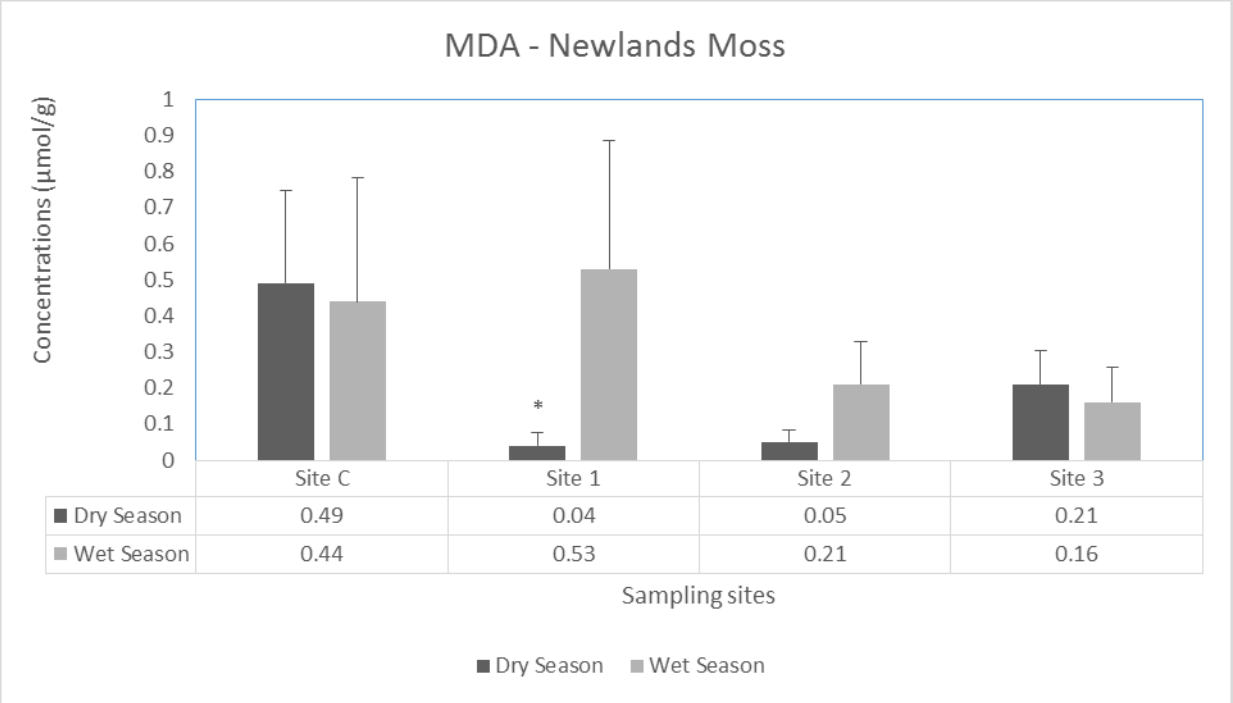


**Figure 4.69:** The mean MDA concentrations (µmol/g) (± SD) in moss at sites C, 1, 2 and 3 of Newlands forest for the dry and wet sampling occasion in January and June 2015. MDA (expressed as µmol TBARS per gram material); SD=Standard Deviation; N=5.

Comparisons of tGSH concentrations between the dry and wet seasons showed no statistically significant differences between the seasons at Newlands forest sites C (P=0.435), 1 (P=0.644), 2 (P=0.244) and 3 (P=0.100) **(Fig 4.68)**.

Moss between the dry and wet seasons were compared in terms of mean MDA concentrations and no significant differences between seasons at the sites: C (P=0.373), 2 (P=0.094) and 3 (P=0.553) were found. Site 1 (P=0.039), however displayed statistical significant differences between seasons **(Fig 4.69)**.

### *4.2.6.2.2) Comparisons of tGSH and MDA levels of lichen between dry and wet seasons at sites C, 1, 2 and 3*

Mean MDA (measured as TBARS) concentrations in lichen at sites C, 1, 2 and 3 of Newlands forest for the dry and wet sampling occasions in January and June 2015 is shown in **Fig. 4.70**. Statistical signifcant differences (P<0.050) between the dry and wet seasons are indicated with an asterisk above the graph bars.

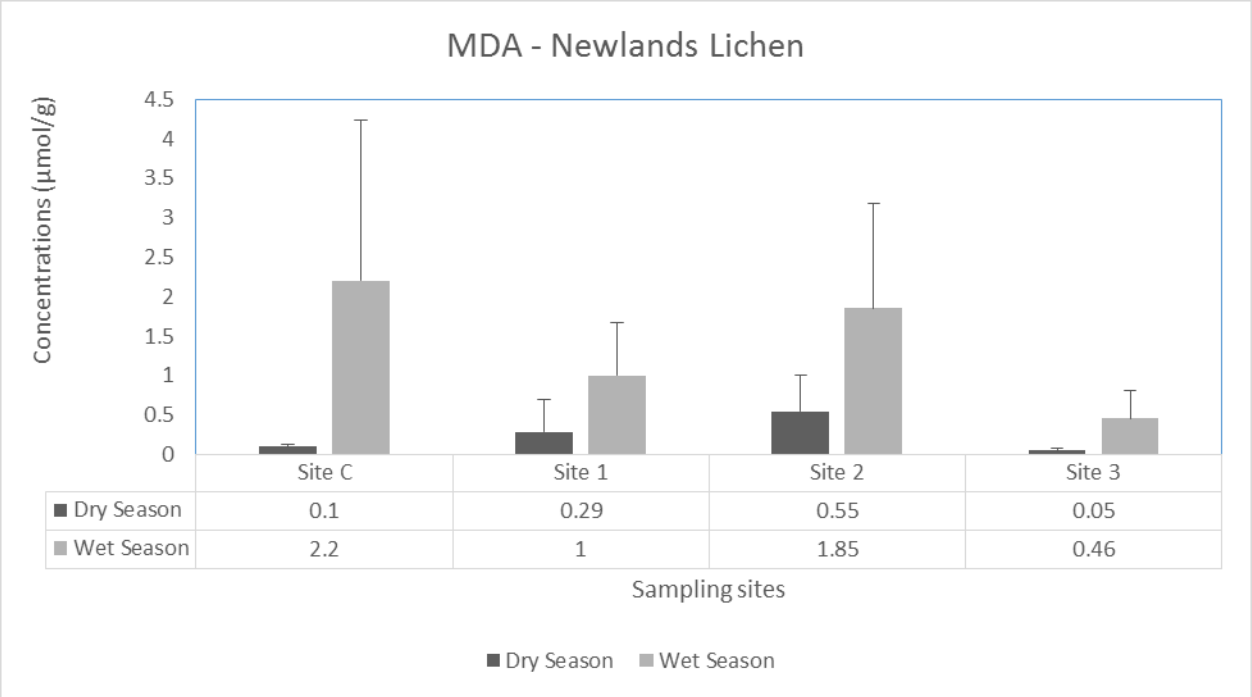


**Figure 4.70:** The mean MDA concentrations (µmol/g) (± SD) in lichen at sites C, 1, 2 and 3 of Newlands forest for the dry and wet sampling occasion in January and June 2015. MDA (expressed as µmol TBARS per gram material); SD=Standard Deviation; N=5.

At Newlands forest, the dry and wet season comparisons showed no statistically significant differences in terms of mean tGSH concentrations at sites C (P=1.000), 1 (P=1.000), 2 (P=0.1000) and 3 (P=0.100).

Comparisons of mean MDA concentrations between the two seasons presented no statistically significant differences at sites C (P=0.147), 1 (P=0.193), 2 (P=0.182) and 3 (P=0.111) **(Fig 4.70)**.

### *4.2.6.2.3) Comparisons of tGSH and MDA levels of millipedes between dry and wet seasons at sites C, 1, 2 and 3*

Mean tGSH and MDA (measured as TBARS) concentrations in millipedes at sites C, 1, 2 and 3 of Newlands forest for the dry and wet sampling occasions in January and June 2015 are shown in **Figs 4.71 to 4.72**. Statistical signifcant differences (P<0.050) between the dry and wet seasons are indicated with an asterisk above the graph bars.

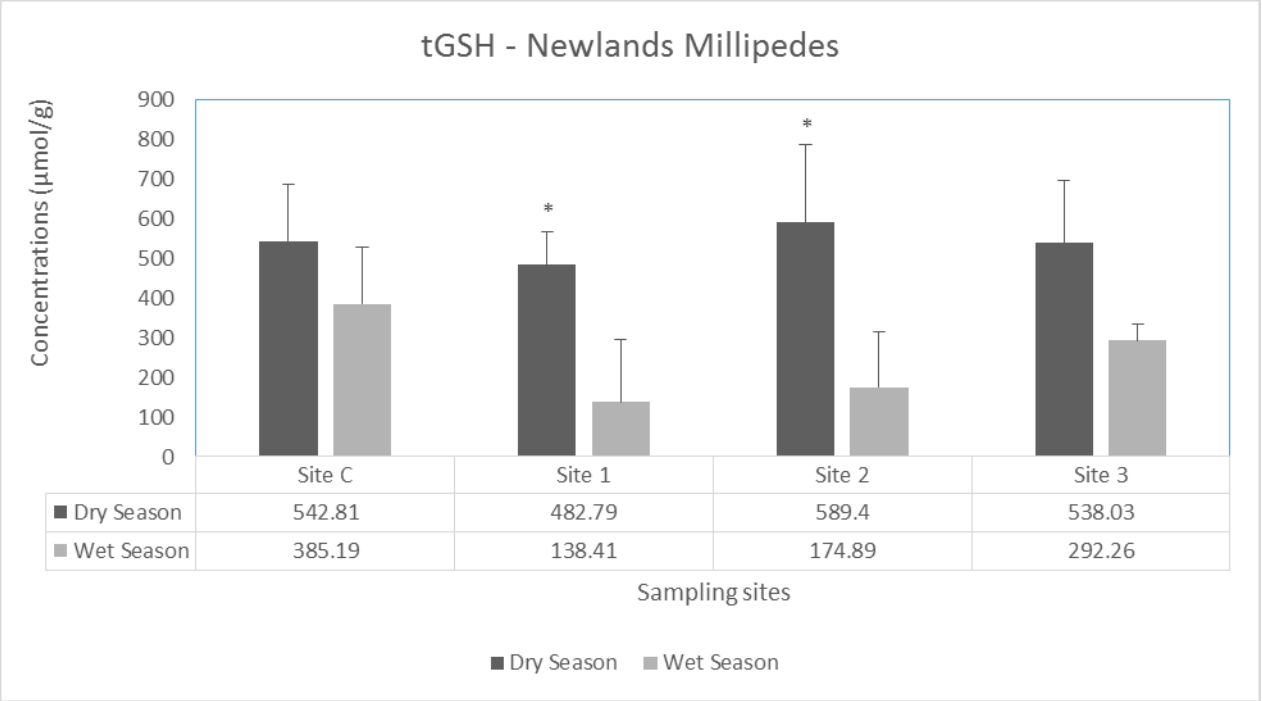


**Figure 4.71:** The mean tGSH concentrations (µmol/g) (± SD) in millipedes at sites C, 1, 2 and 3 of Newlands forest for the dry and wet sampling occasion in January and June 2015. SD=Standard Deviation; N=5.

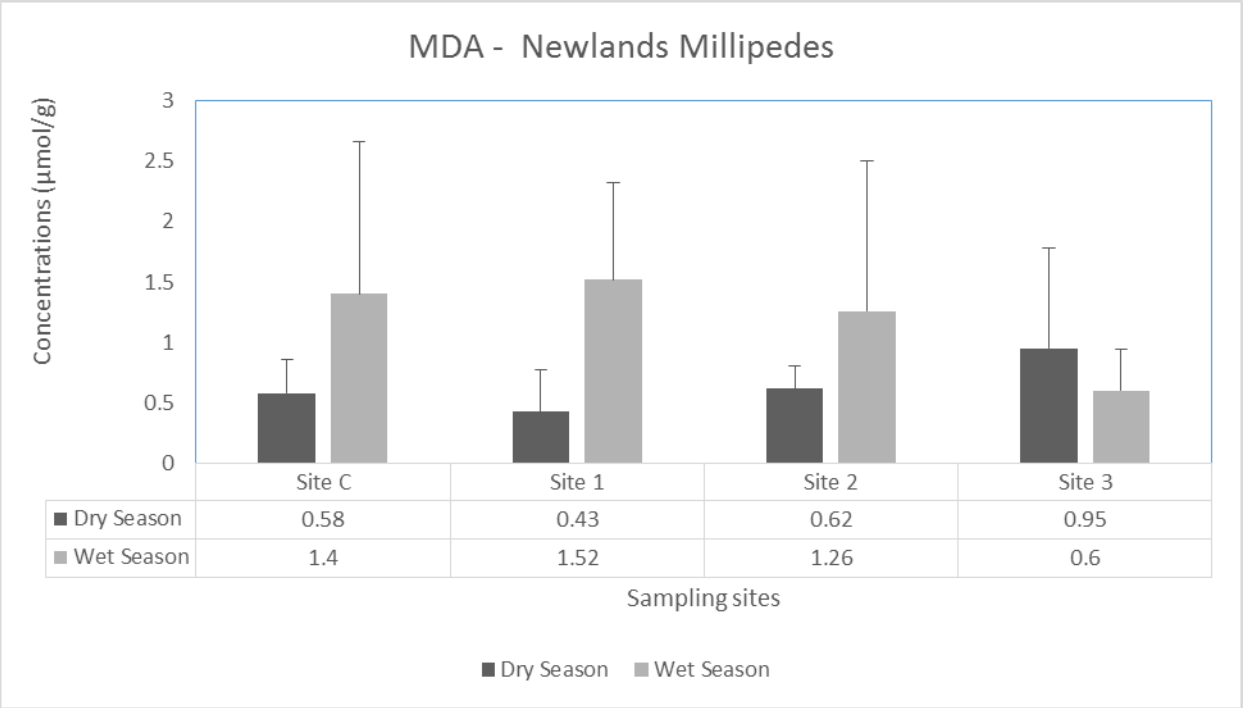


**Figure 4.72:** The mean MDA concentrations (µmol/g) (± SD) in millipedes at sites C, 1, 2 and 3 of Newlands forest for the dry and wet sampling occasion in January and June 2015. MDA (expressed as µmol TBARS per gram material); SD=Standard Deviation; N=5.

Mean tGSH concentrations between the dry and wet seasons were compared and statistically significant differences were found at sites 1 (P=0.028) and 2 (P=0.041). Sites C (P=0.249) and 3 (P=0.059) did not show any statistically significant differences between seasons. Site 2 in the dry season yielded the highest mean tGSH concentration of 589.4 ± 197.85 µmol/g and was significantly higher than the wet season concentration of 174.89 ± 139.0 µmol/g **(Fig 4.71)**.

Mean MDA concentrations compared between the dry and wet seasons at sites C (P=0.700), 1 (P=0.096), 2 (P=0.431) and 3 (P=0.530) presented no statistically significant differences between those seasons **(Fig 4.72)**.

### 4.2.6.3) FORESTS

#### *4.2.6.3.1) Comparisons of tGSH and MDA levels in moss, lichen and millipedes between the forests: Site C, Orange Kloof and Newlands*

The mean tGSH and MDA (measured as TBARS) concentrations in moss, lichen and millipedes for each forest for the dry (January 2015) and wet (June 2015) sampling occasions are presented in **Tables 4.42, 4.43**. The tGSH and MDA concentrations for the sites within each forest were pooled to calculate the mean concentrations for each forest. Concentrations are expressed in µmol/g.

***a) tGSH***

**Table 4.42:** The mean tGSH levels (µmol/g) (± SD) in moss, lichen and millipedes for forests for the dry and wet sampling occasion in January and June 2015.

| FOREST | SEASON | MOSS | LICHEN | MILLIPEDES |
|---|---|---|---|---|
| **Site C (N=5)** | **Dry** | **14.36 ± 11.44** | **ND** | ***542.81 ± 142.90** |
| | *Wet* | *51.01 ± 72.25* | *ND* | *385.19 ± 143.67* |
| **Orange Kloof (N=15)** | **Dry** | **51.97 ± 65.53** | **ND** | **452.67 ± 123.96** |
| | *Wet* | *26.03 ± 11.23* | *ND* | *332.30 ± 127.83* |
| **Newlands (N=15)** | **Dry** | ***79.78 ± 54.65** | **ND** | ***536.74 ± 140.77** |
| | *Wet* | *29.64 ± 33.02* | *ND* | *201.85 ± 127.50* |

Statistical signifcant differences (P<0.050) between dry and wet seasons are indicated with an asterisk. Comparisons were done separately for moss, lichen and millipedes. ND=Not Detected; SD=Standard Deviation.

Moss between the dry and wet seasons were compared in terms of mean tGSH concentrations and significant differences were found between seasons at Newlands forest (P=0.032). There were no statistically significant differences found between the seasons at the site C (P=0.721) and Orange Kloof forest (P=0.724). The highest mean tGSH concentrations were measured in the dry season at Newlands forest (79.78 ± 54.65 µmol/g), which was significantly higher than the concentration found in the wet season of 29.64 ± 33.02 µmol/g **(Table 4.42)**.

Comparisons of mean tGSH concentrations in lichen between the two seasons showed no statistically significant differences at site C (P=1.000), Orange Kloof (P=0.366) and Newlands forests (P=0.0.374) **(Table 4.42)**.

Statistically significant differences in tGSH concentrations in millipedes were detected between the dry and wet seasons at the following forests: site C (P=0.049) and Newlands (P=<0.001). There were no significant differences found in tGSH concentrations between seasons at Orange Kloof forest (P=0.060). The mean tGSH concentration at site C (542.81 ± 142.90 µmol/g) in the dry season was the highest of the three forests and was significantly lower than the opposing wet season concentration of 385.19 ± 143.67 µmol/g. The mean tGSH concentration measured at Newlands forest in the dry season of 536.74 ± 140.77 µmol/g as opposed to the lower wet season concentration (201.85 ± 127.50 µmol/g) was also not much lower than the concentration found at site C **(Table 4.42)**.

***b) MDA***

**Table 4.43:** The mean MDA concentrations (µmol/g) (± SD) in moss, lichen and millipedes for forests for the dry and wet sampling occasion in January and June 2015.

| FOREST | SEASON | MOSS | LICHEN | MILLIPEDES |
|---|---|---|---|---|
| **Site C (N=5)** | **Dry** | ***0.49 ± 0.26** | ***0.10 ± 0.03** | **0.58 ± 0.28** |
| | *Wet* | *0.44 ± 0.34* | *2.2 ± 2.03* | *1.4 ± 1.26* |
| **Orange Kloof (N=15)** | **Dry** | **0.56 ± 0.34** | ***0.03± 0.35** | **1.35 ± 1.78** |
| | *Wet* | *0.70 ± 0.55* | *1.20 ± 0.84* | *2.13 ± 2.15* |
| **Newlands (N=15)** | **Dry** | ***0.1 ± 0.10** | ***0.30 ± 0.37** | **0.67 ± 0.52** |
| | *Wet* | *0.30 ± 0.26* | *1.11 ± 0.97* | *1.12 ± 0.86* |

Statistical signifcant differences (P<0.050) between dry and wet seasons are indicated with an asterisk. Comparisons were done separately for moss, lichen and millipedes. MDA (expressed as µmol TBARS per gram material); SD=Standard Deviation.

The following forests showed statistically significant differences in mean MDA concentrations in moss between the dry and wet seasons: Site C (P=0.049) and Newlands forest (P=0.027). There were no statistically significant differences found between the seasons at Orange Kloof forest (P=0.860). However, the highest mean

MDA concentration of 0.70 ± 0.55 µmol/g was found at Orange Kloof forest in the wet season, as well as in the dry season (0.56 ± 0.34 µmol/g) **(Table 4.43)**.

Comparisons of mean MDA concentrations in lichen presented no statistically significant differences between the seasons at any of the forests: site C (P=<0.001), Orange Kloof (P=0.022) and Newlands (P=0.042) **(Table 4.43)**.

No statistically significant differences in MDA concentrations between seasons in millipedes were found at site C (P=0.245), Orange Kloof (P=0.595) and Newlands forests (P=0.216) **(Table 4.43)**.

## 4.3 DISCUSSION

### 4.3.1 Metal contamination and bioaccumulation

#### 4.3.1.1) Seasonal variations in metal concentrations found at Orange Kloof and Newlands forests

The dry season sampling commenced in January 2015 and was followed by the wet season sampling in June 2015. The data retrieved from the dry and wet season sampling demonstrated that all four sites (C, 1, 2 and 3) at the ancient Orange Kloof forest **(Tables 4.1, 4.14; Figs 4.25 to 4.39)** and all three sites (1, 2 and 3) at Newlands forest **(Tables 4.6, 4.19; Figs 4.40 to 4.54)** received considerable inputs of the metals, Al, Fe and Mn in both seasons, regardless of the fact that these forest ecosystems are hidden away and supposedly untouched (Steinnes and Friedland, 2006; Sen et al., 2017).

Many studies have already established that forest ecosystems are indeed infiltrated by air pollution (McKee et al., 2004; Giam et al., 2010; Sawidis et al., 2011; Pérez-Vega et al., 2012) and that the forest canopies are the first surface to capture atmospheric pollutants (Murray, 1982), through dry and wet deposition (Goossens, 2000; Motelay-Massei et al., 2005). To reiterate this, many adverse impacts of air pollution on vegetation around industrial sources and metropolitan cities have been reported, such as in India (Emberson et al., 2001), France (Motelay-Massei et al., 2005) and Italy (Manes et al., 2016) to name but a few. The afromontane forest pockets on Table Mountain, in the City of Cape Town seems to be no different and it is no surprise when one discovers the extent of the anthropogenic pressure the forests are under throughout the whole year. The pressure is even worse on forests adjacent to major cities, such as Newlands, which is located only approximately nine kilometres away from the centre of Cape Town (Driscoll et al., 1994; Nakazato et al., 2015).

In 1992 a pilot study was conducted in Cape Town by the Energy Research Institute, after which it was concluded that this city has serious air pollution problems compared to other heavily polluted international cities and that the air quality was deemed to deteriorate (Wicking-Baird et al., 1997). The air pollution was identified to

arise from transportation (vehicles, aircraft and shipping), domestic fuel burning of wood and paraffin and industrial activities (petroleum refining, glass manufacturing).

The various sources of industrial pollution in Cape Town includes an Oil Refinery in Milnerton, Glass manufacturer in Bellville, but also smaller industrial sources, landfill sites and agriculture. Other major sources are the townships of Khayelitsha and Mitchell's Plain, the Cape Town International Airport and the Cape Town Central Business District (CBD), which is the dominant area of business, legal and governmental activity within the Cape region and also the main transport center in the city (Dewar, 2004). Airports generate huge amounts of air pollution due to airport operations, vehicle traffic, on-site fuel storage facilities and aircraft maintenance and operation (Dracoulides, 2002) and the aircraft engines are known to produce metals (FAA, 2005). The lack of basic services, within townships and informal settlements, such as electricity and waste removal, require the use of wood, coal and paraffin for cooking and heating purposes (City of Cape Town, 2002) and the resulting particulates contain metals (Maroni et al., 1995). An oil refinery is rated among the most polluting industries, emitting particulate matter that contains metals, amongst others (Nakazato et al., 2015) and the one situated in the Cubatão region of southeast Brazil has reported to inflict major damage to trees in the adjacent Atlantic Rain Forest (CETESB, 2015). The oil refinery in Milnerton Cape Town, that produces gasoline, diesel, jet fuel, liquefied petroleum gas and other products have also been earmarked as a significant contributor to particulate matter and is situated only approximately twenty kilometres from the Cape Town City Centre (Chevron, 2017). High total concentrations of metals have been observed in landfills, containing glass waste or for that matter any areas where waste is not removed efficiently (US EPA, 2016). Studies have indicated that metals may be leached from glass in natural environments (Sterpenich and Libourel, 2001; Silvestri et al., 2005; Colomban et al., 2006; Doménech-Carbó et al., 2006) by way of the original glass structure being altered at the surface. This occurs through various mechanisms, such as hydration, hydrolysis, ion exchange and secondary phase precipitation, resulting in the weathered surface zone to be enriched in those elements which are the basic building blocks of silicate crystalline structures, such as Al and Fe amongst others (Sterpenich and Libourel, 2001). The atmosphere is then contaminated by the metal

enriched surface soils through re-suspension (Cyrys et al., 2003; Tandon et al., 2008).

The metals, Al, Fe and Mn found in the forests may not have arisen from a dominant anthropogenic source, as the majority of aerosols from those metals in the atmosphere arises from natural sources, such as soil and bedrock particulate where they are naturally abundant (Vuai and Tokuyama, 2011). However, based on the dominance of these metals and the concentrations found in the different seasons, it may be proposed that at least three to four sources of the metals can be identified, of which industrial sources seems to dominate. The larger contributions of Fe and Mn arise from industrial sources and Al typically from geogenic source, but the sources may also have been mixed (Pathak et al., 2015).

The opportunity for widespread environmental exposures of Mn in South Africa was provided with the introduction of MMT as an octane booster ("anti-knock" additive) and a replacement for lead in petrol in 2000 (Hanks, 2004). Interestingly, not long after, in 2002, 4.2% of children in Cape Town had blood Mn levels equaled or exceeding 14 mg/L, the upper normal values as specified by the Agency for Toxic Substances Disease Registry (ATSDR, 2000; Röllin et al, 2005). Mn ore is also currently being transported from the multi-purpose terminal in Cape Town at intervals of thirty to ninety days. South Africa holds approximately three quarters of the world's Mn resources, which is used predominantly in steel production to improve hardness, stiffness, and strength. It is further used in carbon steel, stainless steel, and high-temperature steel, along with cast Fe and superalloys. In light of the dangerous neurological effects caused by Mn exposure, it is concerning that ore have been seen loaded on to open bins, lifted by cranes and emptied on to the ship, with clouds of black dust that could be clearly seen while loading (Mtembu, 2016).

Furthermore, a meltshop, in Kuilsriver where steel is melted, consumes approximately 280 000t of scrap steel annually (CISCO, 2015). A Metal Group which is a metal recycling company that processes and recycles all forms of ferrous metals such as Fe and steel and non-ferrous metals, such as Al on site in Epping, Salt River, Kraaifontein, Black Heath and Philippi (SA Metal., 2017). The concentrations of Fe and Mn may thus have originated from sources, such as vehicular traffic and

industrial and smelting industry emissions, battery production, for example in Stikland (Willard Batteries, 2017), Fe and steel foundries, metal welding and landfills (Sterpenich and Libourel, 2001), of which three major landfill sites are situated in Muizenberg, Belville South and Vissershok (City of Cape Town, 2017).

It is also possible that Al and Fe inputs arose from natural soil, road fugitive dust and construction dust (Song et al., 2012; Zhang et al., 2012; Zhou et al., 2014). These crustal elements are known to occur in road dust, which become airborne due to traffic movement (Venkataraman et al., 2005). In Delhi, India, the re-suspension of such contaminated surface soils lead to atmospheric pollution on a regional scale (Cyrys et al., 2003; Gray et al., 2003) and it was reported that nearly 27% of atmospheric aerosols arise from re-suspension of local surface earth materials (Tandon et al., 2008). Some of the major construction projects that took place in 2015 during the sampling period in and around Cape Town was the Cape Town International Convention Centre, an upgrade of the Langa Station South Public space and the construction of a new stage and workshop at Cape Town Film Studios (Future Cape Town, 2017). Particulate matter therefore can be extremely high in Fe concentrations as a result of stationary sources, for example, mechanical plants, construction works and road vehicles that produces high air emissions.

Manganese concentrations in the surface soil on the other hand can be elevated even more (Millaleo et al., 2010; Herndon et al., 2011; Herndon et al., 2015) by vegetation as a result of biological cycling by litterfall, throughfall, and uptake (Watmough et al., 2007; Herndon et al., 2015; Kraepiel et al., 2015). Wild fires, which are common in Cape Town's dry season may also have caused a rise in metal concentrations, as the fires release metals, such as Al, Fe and Mn and deposits them on the soil surface (Parra et al., 1996; Jakubus et al., 2010; Bogacz et al., 2011; Jovanovic et al., 2011; Costa et al., 2014).

There are many factors that impact the concentrations of these metals, for example, meteorological conditions, such as temperature inversions, wind (Goossens, 2000; Motelay-Massei et al., 2005; Mirivel et al., 2011) and precipitation (Bacardit and Camarero, 2010) at the different samplings times. Certain geographic features, such as mountains (Kumar and Mohan, 2002) and even wild fires have a significant effect

on metal accumulation. Other factors include dust particles in the atmosphere that are continuously deposited by dry and/or wet deposition processes (Lawrence and Neff, 2009). The rate of the deposition is also affected by the concentration of the dust in the atmosphere, the energy of dust transporting winds and depositional environment characteristics (Goossens, 2000; Motelay-Massei et al., 2005).

Additional impacting factors include, the type of emission sources and the distance and location of the sampling site (Lawrence and Neff, 2009). It has also been said that extremely high average concentrations of metals are generally found in summer as a result of temperature inversion and the lowest concentrations in winter as a result of washout of the particles (Karar and Gupta, 2006). Al, Fe and Mn have been reported to show lower seasonal dependency, but post-monsoon season have, however been observed to display higher contamination of these metals in soils compared to pre-monsoon (Nriagu and Pacyna, 1988; Rasmussen, 1998; Bhuiyan et al., 2010; Wei and Yang, 2010; Tume et al., 2011), which is an indication that the precipitation and or adsorption processes are more effective than the wash out process by rainwater. It appears that the hydrated insoluble complexes of Al, Fe and Mn attracts other metal ions through adsorption and stays in the soil matrix after rainy periods (Bhuiyan et al., 2010). The distribution of metals in surface and sub-surface soil layers can also be affected by leaching through rainwater on a long term basis (Pathak et al., 2015).

Weather conditions such as low temperature and temperature inversion, or more commonly known as the brown haze have mostly been linked to the higher pollution levels in the colder, wet seasons. This phenomenon has a major effect on the deposition of air pollutants in the wet season. Low temperature and temperature inversion prevents atmospheric convection from happening in the affected area, which may cause the air to become stationary as a result of the collection of dust and pollutants that are unable to be lifted from the surface (Norouzi et al., 2017). The inversion layer contains high levels of ambient atmospheric particulates, which then causes the formation of a visible smog or brown haze (Reddy and Venkataraman, 2000; Zhang et al., 2002; Deng et al., 2011), until heating or winds break up the inversion layer (City of Cape Town - Air Quality, 2007). The brown haze is a common occurrence in Cape Town's winter season (CMA, 2015), which is enhanced by the

calmer conditions during this period and envelops most of the City of Cape Town (Wicking-Baird et al., 1997).

The results in this study, concurred with findings reported by Sen et al. (2017) where part of their study included the Indo-Himalayan mountain Range and other remote and pristine areas in South East Asia's mega cities of the Indo-Gangetic Plains. These areas were reachable through and have been contaminated by long-range transport of continental pollutants (Agnihotri et al., 2011; Ojha et al., 2012; Kumar et al., 2013; Joshi et al., 2016) and the air quality in the two high-altitude mountain sites were deteriorating rapidly during autumn and winter. The causes were attributed to inversion conditions, the advection of copious amounts of pollutants from rural and urban areas, the increase in vehicle usage over the previous couple of decades, agriculture and charcoal for heating purposes (Sarangi et al., 2014; Naja et al., 2014, 2016; Kant et al., 2015).

The aerosols in hazes, contain metals, amongst others (Guilloteau et al., 2009; Alleman et al., 2010; Barrado et al., 2013; Mbengue et al., 2014) that originate from anthropogenic sources, such as agricultural biomass burning and industrial and traffic emissions, as mentioned above (Li and Shao, 2009; Li et al., 2011), but also natural emissions from crustal minerals, soil erosion, forest fires and oceans (Dentener et al., 2006; Ginoux et al., 2012).

Vehicles were found to be the principal source of Cape Town's brown haze, causing over half (65%) of the brown haze experienced. Low-level emitting industries were the next most significant cause (22%) of the brown haze followed by the use of wood (11%) by a significant sector of the population. Natural sources such as wind-blown dust and sea salt were the lowest contributors (2%) towards the brown haze (Wicking-Baird et al., 1997). In various studies done, the sources of the metals Al, Fe and Mn in such hazes have been attributed to its crustal origin (Chinnam et al., 2006), as well as re-suspended surface dust (Allen et al., 2001). Fe and Mn measured in haze have also been traced back to vehicular traffic emissions and the smelting industry (Zhou et al., 2014). According to studies done by See et al. (2006) in South East Asia, most chemical components', concentrations including Al and Fe doubled on hazy days. Notably higher metal concentrations were observed in the

wet season at many of the sites in both forests, but more so at Newlands forest. A viable explanation according to the literature is therefore the anthropogenic activities that escalate in the colder seasons in major cities (Norouzi et al., 2017) that are contaminating the secluded forest areas, even via long-range transport (Agnihotri et al., 2011; Ojha et al., 2012; Kumar et al., 2013; Joshi et al., 2016).

Anthropogenic activities have therefore been earmarked as the major culprits contributing to the excessive dust-borne metal concentrations during the winter seasons (Norouzi et al., 2017). This trend was substantiated by Motelay-Massei et al. (2005) in France when they measured metal concentrations in bulk atmospheric depositions at five stations. They found no seasonal trends, except in a major city, Paris where the higher concentrations of metals were explained by domestic heating in winter (Motelay-Massei et al., 2005). Newlands forest, located around the corner of the City centre, is basically amidst air pollution related sources (Abdul-Wahab and Yaghi, 2004) and showed a tendency towards higher metal concentrations in winter. Authors comparing dry and wet season results in terms of metal pollution (Dan et al., 2004; Motelay-Massei et al., 2005; Feng et al., 2009; Crenn et al., 2017) have found that areas further away from major cities were less likely to show seasonal trends and the results found at the Orange Kloof forest sites reiterated their findings. Orange Kloof forest is situated approximately 25 km away from the centre of Cape Town, which is a major and highly populated city (Abdul-Wahab and Yaghi, 2004), yet the location is far enough away from the city to not show seasonal trends.

By contrast, site C at Orange Kloof forest that supposedly served as a control site, showed extremely high Al and Fe concentrations in relation to the other sites, including the Newlands forest sites. Sites C and 3 are relatively secluded and protected by tree canopies. When the initial pilot study was done in June and November 2014 in order to find an unpolluted site for control purposes, the original site was approximately twenty meters further away. At that stage the pilot data showed significantly lower concentrations of these metals and sufficient millipedes for sampling were found. However, when the dry season sampling sessions commenced in January 2015, extreme difficulties were experienced in obtaining the required number of millipedes necessary for the study. Site C was consequently moved approximately twenty meters closer to site 3 where sufficient amounts of

these millipedes were found. The site could not be moved further away to the opposite original site, due to a river running adjacent to the site. Both the original and new location of site C is densely vegetated and faces the Cape Flats, however the new location was slightly more slanted and exposed to air pollution and wind than the original site.

It is true that wind turbulence with large mixing height aids in proper dilution and dispersion of pollutants during summer. Additionally the sea-breeze in the summer months may also decrease the particulate pollutant concentrations (Gupta et al., 2004). This is also true for the South Easter, known as the “Cape Doctor.” Having said that, this is an extremely strong wind that blows from False Bay over the Peninsula and Cape Flats in summer, funneling through the Hottentots Holland Mountains and the Table Mountain range, leaving very few sheltered places (Van der Velden, 2017). Similar patterns were observed in the Indo-Gangetic Plains during the summer season where mineral dust originating in the arid landscapes of NW-India and SW-Asia were transported across the Indo-Gangetic Plains picking up local pollutants, which accumulated along the southern slopes of the Indo-Himalayan Range (Sen et al., 2017).

Atmospheric dust has a major influence on atmospheric environmental quality (Ren et al., 2004). The dust contains metals (Pope, 2000; Saldiva et al., 2002; Shi et al., 2011), of which the fine particles ($PM_{2.5}$) are significant due to its size, for the reason that it remains airborne for long periods and can be transported over long distances (Ministry of the Environment, 2001). It is thus quite possible that site C at Orange Kloof forest was impacted more by air pollution, which may have been transported from the Cape Flats by the South Easter wind, picking up pollutants from anthropogenic, as well as from natural origin (Sen et al., 2017). This may have caused a micro-climate effect at site C, as the location of the sampling site plays a significant role in the concentrations of the metals. Similarly site 2 metal concentrations at Newlands forest were also constantly higher than at other sites and may be due to its more exposed location on the mountain, close to a contour path and also facing the Cape Flats (Lawrence and Neff, 2009). In India it was found that the metals, Al, Fe and Mn showed higher concentrations around some selected

sampling sites, which suggests that anthropogenic addition of metals in soils is sampling site specific (Pathak et al., 2015).

Another scenario that may have added to the higher Al and Fe concentrations may be attributed to a phenomenon that is locally known as ‘the table cloth,’ which is a huge cloud that hoovers on top of Table Mountain and drips over the mountainside. Vegetation in the suburbs of Constantia and on Table Mountain is visibly green and lush as a result of the ‘table cloth.’ This phenomenon is caused by warm moisture from the False Bay waters, which is picked up by one part of the South Easter wind force and blown around the eastern flank of Table Mountain, pushing up the air against the slopes of Table Mountain, which creates clouds and rain along the eastern slope (Van der Velden, 2017). It is a well-known fact that wet deposition is a critical scavenging process by which metals are returned to terrestrial or aquatic surfaces (Bacardit and Camarero, 2010). Al and Fe concentrations at Cape Point, for example have been reported to reflect a mixed influence of crustal sources and anthropogenic emissions in precipitation (Song and Gao, 2009). Orange Kloof is situated just above Constantia on the eastern slope and precipitation from these clouds that may contain metals may very well have added to the metal loads in these pristine forested areas. Similarly, Newlands forest is situated on the eastern slope of the mountain and on the receiving end of precipitation originating from the “table cloth” containing metals. This phenomenon can therefore be regarded as a positive contributor to the metal loads in the forest pockets, especially as the metal concentrations at the higher sites, 2 and 3 at Newland forest revealed constant higher results.

With regard to soil, understanding the variations of metals in soils pertaining to time or season remain largely unknown (Rovira et al., 2011; Fernández-Caliani, 2012). In view of this, the metal concentrations found in the soil at Orange Kloof forest displayed no patterns of higher concentrations towards a particular season, as were found at Newlands forest that showed a tendency for higher metal concentrations in winter. The explanation thereof lies most likely in the location of Newlands forest, amidst the City Centre (Wicking-Baird et al., 1997), accompanied by the brown haze in winter (Driscoll et al., 1994; Nakazato et al., 2015). Metal concentrations measured at some of the sites in the soil, for example remained fairly similar

between the seasons, displaying no statistically significant differences and may in such cases be ascribed to the climatic characteristics of the sampling area. Similar results were found in a study done in Alcalá de Henares (Spain) where the authors compared urban and industrial soils between seasons, which was explained by the high rainfall rates in combination with the lower evaporation rate that caused decreased metals in the upper layer of the soil, due to leaching of these metals to deep layers. This is a natural process that occurs in highly porous soils (Madrid et al., 2004, 2007) with a sandy loam texture, such as the soils found at both of these forests **(Tables 4.5, 4.18)** (Peña- Fernández, 2011). Another observation was metal concentrations in soil that were substantially higher in relation to the concentrations at the other sites, and may be due to the location of the site, which is functional in the amount of pollution it is exposed to (Lawrence and Neff, 2009). As a result of the site's location, the metals may have accumulated over time in soils as a consequence of pollution due to urbanization and anthropogenic activities that are continuously expanding (Guney et al., 2010; Ali and Malik, 2011).

Wild fires may cause noticeable increases and decreases in metal concentrations in soil. Biomass burning and forest fires are sources of the metals, Al, Fe and Mn, amongst others (Gaudichet et al., 1995; Karthikeyan et al., 2006a, b). Betha et al. (2013) found in their study in Indonesia that Al had the highest and Fe the second highest concentration in the background and peat fire samples measured in $PM_{2.5}$ when they compared it to the other metals. The authors also found that most of the metals, including Al, Fe and Mn increased significantly during the peat fire episodes compared to background concentrations. Khare et al. (2011) attributed the elevated $PM_{10}$ concentrations recorded at certain sites at the Indo-Himalayan Range to the abundance of forest fires. Studies of wildfire ashes also revealed significant differences in concentrations of metals, of which Mn was the highest (Khanna et al., 1994; Someshwar, 1996; Pitman, 2006; Plumlee et al., 2007; Gabet and Bookter, 2011; Viana et al., 2012; Bodi et al., 2014; Costa et al., 2014; Santín et al., 2015). Fires such as those found in Cape Town have in recent years, including the summer of 2015, caused major physical damage to the protected nature reserve on Table Mountain (eNCA, 2015). As previously mentioned, metals from these fires are released and deposited on the soil surface, indirectly via interactions of ashes with the underlying soil or directly by combustion of vegetation and mineralization of soil

organic matter (Parra et al., 1996; Jakubus et al., 2010; Bogacz et al., 2011; Jovanovic et al., 2011; Costa et al., 2014). Both processes can significantly add to the metal load in the soil and thereby, change its chemical properties (Ulery et al., 1993; Antilén et al., 2006; Jakubus et al., 2010; Pereira and Úbeda, 2010). Soil surfaces are the most affected by fire and ash deposition (Mandal and Sengupta, 2006). However, the concentrations of the metals in the soil are influenced by the lengh of time since the fire occurred, usually as a result of new humus formation combined with the leaching or lateral transport of ashes as a result of post-fire rainfall, therefore causing the significant decreases in metal concentrations in soil in winter (Ulery et al., 1993; Certini, 2005; Zavala et al., 2014). Table Mountain endured blazing forest fires, which spread to the southern Peninsula in January and March 2015. Even though the study areas at Orange Kloof and Newlands forest did not burn, it is highly likely that particulate emissions arising from these fires (Siegert et al., 2001; See et al., 2006a, 2007a), containing metals (See et al., 2007) reached the study sites through long range transport and negatively impacted the ecosystems (Nichol, 1998; Fuller et al., 2004; Odihi, 2003; Betha et al., 2013). Thus, soil samples that were taken in the dry season, during or shortly after wild fire episodes in January and March 2015 (Ulery et al., 1993; Certini, 2005; Zavala et al., 2014) at the forests may have been contaminated by the wildfire ashes and account for part of the metal concentrations found in that season. At the same time, it may also explain some of the significant decreases in metal concentrations found, a couple of months later when samples were taken in the wet season.

In terms of leaf litter, both forests showed a pattern of higher Al and Fe concentrations in winter. Mn on the other hand demonstrated a tendency of higher concentrations in summer. The accumulation of metals in decomposing litter, as well as its release from the litter may be dependent on its initial metal concentrations (Lomander and Johansson, 2001; Kaila et al., 2012). Also, most of the metal accumulation is predominantly attributed to atmospheric deposition and throughfall. Other sources of metal input in leaf litter have been ascribed to microbial translocation and immobilization of metals from underlying contaminated soil layers, largely by fungi (Lomander and Johansson, 2001; Lomander, 2002; Tyler, 2005). It is possible for fungi to accumulate huge amounts of metals occurring in their external environment (Gadd and Griffiths, 1978; Berthelsen et al., 1995), even at unpolluted

sites (Lepp, 1992; Tyler, 2005). Fungal mycelia can form a part of a significant pool of organic material, which has a high capacity for metal accumulation and can as a result cause changes in metals in soil systems (Lomander, 2002). However, Scheid et al. (2009) reported that the primary cause for metal enrichment in decomposing litter was due to contact of the underlying polluted soil with the litter and is in accord with most of the results in this study of soil concentrations that correlated with leaf litter concentrations. The authors, Scheid et al. (2009) and Van Nevel et al. (2014) determined in a study done in a fourteen year old forest that “low metal” leaf litter became metal-enriched when the litter decomposed on a contaminated site. Such results suggests that the organic matter of decomposing leaves and needles acted as an efficient metal storage pool. Leaf litter thus acts as a temporary sink for metals from the surrounding soil, as well as the soil below the leaf litter. Metal release from contaminated litter may thus disperse towards the soil or on the other hand metal enrichment of ‘low metal litter’ may be carried away to other areas or serve as a food source for various organisms, which has ecological repercussions.

No statistically significant variations in metal concentrations between seasons were found in mosses and the results found at Orange Kloof forest concurred with results from other studies (Thöni et al., 1996; Berg and Steinnes, 1997; Fernández and Carballeira, 2002). Mn concentrations did, however show a tendency to be higher in the dry season. Conversely, Newlands forest, revealed a seasonal trend of higher metal concentrations in the wet season that is also in line with the whole pattern of higher metal concentrations found in the soil, leaf litter, lichen and millipedes, which may have been brought on by the brown haze effect (Wicking-Baird et al., 1997), which is significantly more prominent in forests, such as Newlands adjacent to major cities (Driscoll et al., 1994; Nakazato et al., 2015). Bioaccumulation of metals in mosses are largely determined by emission sources within study regions (Schroder et al., 2008; Kleppin et al., 2008; Holy et al., 2009). However, the bioaccumulation of elements in moss tissues also depend on factors that affect the amount of pollutants that reach the mosses. Such factors are: elevation, topography of the sampling site and vegetation cover and canopy structure (Fernández et al., 2015). Equally important is the uptake of pollutants by these organisms that include physicochemical characteristics of the pollutants and physiological processes in mosses (Aboal et al., 2010). The total pollutant concentrations in moss tissues are

affected by moss growth, which in turn determines the time length that the portion of the moss shoot sampled for chemical analysis has been exposed to the atmospheric deposition of pollutants (Boquete et al., 2014). Atmospheric and other inputs, such as soil dust and sea spray are thus not definitive in the final concentrations of pollutants in moss tissues (Aboal et al., 2010).

Metal concentrations in lichen did not demonstrate a clear pattern of higher concentrations towards a specific season and the differences between the seasons were also not statistically significantly different at most of the sites. This is in line with literature stating that lichen morphology does not alter with the seasons and accumulation of pollutants can, thus occur throughout the year (Conti and Cecchetti, 2001). There was, nevertheless a tendency for Al and Fe concentrations in lichen to be higher in the wet season and Mn concentrations to be higher in the dry season. With that said, elemental leakage due to precipitation does play a role in wet seasons and cause substantial differences in metal concentrations between the seasons. Particulate entrapped onto the lichen surface can be removed by rainwater, which may result in lower element content during rainy periods. In reverse, higher content of elements are thus found during the dry season in which little or no rain is experienced (Brown and Brown, 1991; Adamo et al., 2008). It was also noticed that Al and Fe, which are indicative of dust emissions, displayed an important relationship with distance from the road in lichen tissue. Notably elevated levels in throughfall have been reported, suggesting that lichens are more influenced by dry/particle deposition than by wet deposition. It was also suggested that adverse impacts may persist at distances greater than 200 m depending on wind direction and traffic density (Angold, 1997; Bernhardt-Römermann et al., 2006; Bignal et al., 2008). It was also observed in this study that lichens are few and far between at Newlands forest and Orange Kloof in the areas before site 1 that are situated approximately 200 m from the main roads.

With reference to the invertebrates, the different seasons, together with the different climatic conditions and different parts of the life cycles of organisms within the given season may affect how soil invertebrates respond to pollution. However, very little information exists on the effects of climatic conditions on soil invertebrates in metal polluted soils (Santorufo et al., 2012). At Orange Kloof there were very little variance

found in millipede metal concentrations between seasons. The metal concentrations at Newlands forest, on the other hand and although mostly not statistically significantly different between seasons, demonstrated a tendency of higher metal concentrations in winter, which is most likely due to its location close to a major city (Driscoll et al., 1994; Nakazato et al., 2015) under more anthropogenic pressure, due to the brown haze (Wicking-Baird et al., 1997).

The dry season was stretched over three months due to the difficulty of finding pill millipedes in the hot, dry season. Similar difficulties were found in India when sampling the pill millipede, *Arthrosphaera,* and was ascribed to sparse canopy and partial dry conditions during the sampling sessions (Attems, 1936; Sakwa, 1974; Achar, 1980; Ashwini, 2003). Stamou et al. (2004) found the optimum temperature for soil arthropods in Mediterranean fields to be around 20 °C, but reported that lower temperatures caused a significant decline of organism activity and arthropod growth. Doblas-Miranda et al. (2007) found arthropods to be more abundant and richer in species during the cold-rainy period, which was more in line with the findings in this study. It was also reported that soil arthropod communities exerted periods of explosive growth at the onslaught of the rainy season and increasing soil moisture content, although in environments different from the Mediterranean (Raghubanshi et al., 1990; Reddy et al., 1994; Ferguson, 2001; Wiwatwitaya and Takeda, 2005; Zhu et al., 2010) or under warm-wet conditions (Harte et al., 1996). Again, the explosive growth of pill millipedes at the onset of the rainy season that was witnessed in this study, was unmistakable in Cape Town's Mediterranean climate. High collembola abundance after a rainy period, for example was possibly due to higher fungal biomass (Hawkes et al., 2011), which is the collembolan main food source (Hopkin, 1997), but also a major part of the millipedes' diet.

The dry season sampling at Orange Kloof and Newlands forests experienced temperatures between 21 and 29 °C **(Tables 4.4, 4.9)**, which according to Stamou et al. (2004), together with the dry conditions are not the most promotive temperature for invertebrate activity (Attems, 1936; Sakwa, 1974; Achar, 1980; Ashwini, 2003). The moisture percentage of the soil at Orange Kloof sites, C, 1 and 2 and 3 ranged from 3.26 to 5.10 % **(Table 4.2)** and the leaf litter moisture percentage at the same sites ranged from 17.56 to 23.23 % **(Table 4.3)**, which is relatively dry. At Newlands

forest the moisture percentage of the soil at sites 1 and 2 ranged from 5.5 to 10.18 % **(Table 4.7)** and the leaf litter moisture percentage at the same sites ranged from 17.56 to 23.41 % **(Table 4.8)**, which is relatively dry. Site 3 at Newlands, which is the highest site and possibly also the site receiving a healthy amount of precipitation from the table cloth effect, due to its location (Van der Velden, 2017), had a soil moisture percentage of 49.10 % **(Table 4.7)** and a leaf litter moisture percentage of 56.10 % **(Table 4.8)**, which is also relatively wet. This high moisture content at site 3 is also evident in the dense, green vegetation and mosses growing at that site. As a result of the moist conditions, pill millipedes at site 3 were easier to find. Pill millipedes found in summer in this study were observed to be more prevalent in close proximity of rocks and in winter they were abundant close to rotting wood, tree stumps and areas with thick layers of leaf litter. Boulders and rocks in foothill forest floors have been reported to support the pill millipedes *Arthrosphaera magna*, possibly due to accumulation of sufficient organic matter around the rocks (David et al., 1993). Further research is, however necessary to clarify the impact of climatic conditions on soil arthropod communities in a Mediterranean context (Hopkin, 1997).

Millipedes ingest leaf litter and produce fecal pellets mainly during two months of the wet season in Southern Africa (Dangerfield and Telford, 1991). Other factors that have an influence on toxic element accumulation in invertebrates depends on food preferences, breeding, development stage, sex, season and physiological conditions (Jelaska et al., 2007; Butovsky, 2011). The excretion ability of the bodies of the invertebrates also play a role in the concentration of these elements (Janssen et al., 1991; Kramarz, 1999; Avgin and Luff, 2000). Studies with snails have demonstrated that feeding preferences have a positive correlation between soil, leaf litter and snail concentration and that metal transfer from leaves to snail are more significant than transfer from soil (Notten et al., 2005). With ground beetles it was found that food preferences cause differences in elemental concentrations and that overwintering species accumulated less toxic elements (Jelaska et al., 2007). Thus, the different food preferences and different breeding patterns may cause differences between the toxic element concentrations in invertebrates (Bednarska et al., 2013).

An observation was made with regard to accumulation differences between ground beetles (Carabids) used in a study done by Simon et al. (2016) and the pill millipedes

used in this study. In their study, the Al, Fe and Mn concentrations, respectively in leaf litter was significantly higher than the metal concentrations found in soil and the metal concentrations in die ground beetles significantly lower than in the concentrations measured in soil. Simon et al. (2016) concluded that ground beetles are poor accumulators of metals. In this study, however Al, Fe and Mn concentrations in soil were found significantly higher than in leaf litter. Al and Fe concentrations found in millipedes were close to and at times even higher than the concentrations found in leaf litter and the concentrations at site C were extremely high in comparison to the concentrations found at the other sites. The concentrations were also higher at Newlands forest close to the city, as mentioned before. Manganese concentrations in millipedes were relative similar to concentrations found in leaf litter. In view of this, it may be suggested that pill millipedes are good accumulators of metals. Information on Al, Fe and Mn accumulation in millipedes or pill millipedes for comparison could not be found in the literature and the effect of organic pollutants and complex mixtures on these invertebrates is relatively unknown (Souza and Fontanetti, 2011)

#### *4.3.1.1.1) Al contamination of soil at Orange Kloof forest*

Al concentrations in soil at sites C and 1 were high in both seasons compared to the other sites and the concentrations measured at site 2 were extremely high in the wet season compared to concentrations found by Peña-Fernández et al. (2015) at industrial sites. The higher concentrations may be due to the fact that the greater part of Al and dry deposition originates from mineral dust, due to its ubiquity in soils (Macdonald and Martin, 1988). Also, the differences and irregularity in Al concentrations, as well as some extreme concentrations that were noticed, could possibly have its clarification in factors such as: the total amount of precipitation, leaching of this element from the canopy (Matzner, 1989) and stemflow. Al fluxes are the lowest in stemflow and is also restricted to a small area around the stem basis, which is not of major significance for the total Al fluxes in the ecosystem, but may considerably impact the local soil chemistry (Koch and Matzner, 1993; Levia and Frost, 2003).

The Al concentrations measured at sites 1 and 3 at Orange Kloof forest remained relatively constant between seasons, with only slight decreases noticed towards the

wet season. This is a natural phenomenon in soils that are highly porous (Madrid et al., 2004, 2007), with a sandy loam texture such as the soil identified in this forest **(Tables 4.5, 4.18)**. The high rainfall rates in combination with the lower evaporation rates in winter causes the metals to leach deeper into such soils, which results in a drop in the metal concentrations in the upper layer of the soil (Peña-Fernández, 2011).

A significant increase in the Al concentration was observed in the wet season at site 2 (22995.01 mg/kg). It was also the highest concentration found in the soil at this forest. The concentration was 17 times higher than the concentration of 1347.92 mg/kg found at the same site in the dry season. Site 2 is situated slightly lower and deeper into the mountain, yet densely covered by tree canopies. The site, however faces the parking area and major road junction, of which pollution from vehicular traffic is generated. Vehicle traffic escalates in winter (Norouzi et al., 2017) and so does the pollution it generates, which account for the higher pollution levels caught up in the thick smog or brown haze in the colder, wet seasons. Brown haze has a major effect on the deposition of air pollutants in the wet season (Reddy and Venkataraman, 2000; Zhang et al., 2002; Deng et al., 2011; CMA, 2015) as were also found in Southeast Asia when Al, amongst other metals doubled during winter brown haze episodes (Li et al., 2011). The wet season Al concentration of 22995.01 mg/kg measured at site 2 was even higher than were found by Peña-Fernández et al. (2015) at an industrial site in Alcalá de Henares, Spain of 11261.40 mg/kg, also in the wet season. Their dry season Al concentration was 10135.90 mg/kg and showed minimal differences between seasons, but was noticeably higher than the dry season concentration in this study of 1347.92 mg/kg. Alcalá de Henares is one of the most densely populated cities in the Comunidad de Madrid where seasonal variations between urban and industrial sites were evaluated (Madrid et al., 2004, 2007; Peña-Fernández, 2011), yet the Al concentrations found at Orange Kloof forest site 2 in the wet season was twice as high as were found at their industrial study site. Even the site C concentrations in both seasons exceeded their industrial site concentrations.

Major differences in Al concentrations were discovered between sites C and 3, located in close proximity of each other in both seasons. Site 3 is situated higher on the mountain than site C and is more densely covered by tree canopies, yet the differences in Al concentrations between these sites in both the seasons were astronomical. The Al concentration of 13450.31 mg/kg at site C in the dry season was thirteen times higher than the concentration of 1030.06 mg/kg found at site 3 in the same season. Similar results were found in the wet season: the Al concentration of 13940.73 mg/kg at site C was nineteen times higher than were found at site 3 with a concentration of 707.24 mg/kg, also in the same season. The high concentrations found at site C in both seasons suggests contamination and accumulation of metals from the atmosphere (Guney et al., 2010; Ali and Malik, 2011) and is probably also location specific, as a result of transported pollutants from the Cape Flats by the South Easter wind (Sen et al., 2017). It also demonstrates the influence that factors such as distance and location of the sampling site (Lawrence and Neff, 2009), dust transporting winds and the depositional environment characteristics (Goossens, 2000; Motelay-Massei et al., 2005) may have on the metal concentrations found at a given site. This may then also clarify the high concentrations found at site 1 in the dry (9990.24 mg/kg) and wet (8305.64 mg/kg) seasons. Site 1 is much closer to the car park and traffic circle, but does not directly face the sources of pollution, as does site C with the higher Al concentration. Al, showing higher concentrations around some selected sampling sites suggests that anthropogenic addition of metals in soils is sampling site specific (Pathak et al., 2015). Deposition of ashes in soil from the forest fires that occurred during the dry season sampling sessions on Table Mountain and the Cape Peninsula (eNCA, 2015) may further have contributed to the higher Al concentrations found in the dry season at sites 1 and 3, as well as cause a general escalation in the Al concentrations in the soil, especially at site C (Parra et al., 1996; Jakubus et al., 2010; Bogacz et al., 2011; Jovanovic et al., 2011; Costa et al., 2014) **(Fig 4.25)**.

#### *4.3.1.1.2) Fe contamination of soil at Orange Kloof forest*

Iron concentrations measured in soil at sites C and 3 in summer and winter showed minimal differences between the seasons, displaying only slight decreases towards the wet season. The texture of the soils at Orange Kloof forest may account for those results as the sandy loam type soil **(Tables 4.5, 4.18)** is highly porous (Madrid et al.,

2004, 2007) and thus conducive to metals leaching deeper in such soils in wet winters (Peña-Fernández, 2011). Decreases in metal concentrations towards winter sometimes also occurs as a result of post-fire rainfall (Ulery et al., 1993; Certini, 2005; Zavala et al., 2014). Soil samples were taken in the wet season of June 2015, shortly after the dry season wild fires in January and March 2015 (eNCA, 2015). The end results regarding soil type and post fire rainfall are similar in the sense that metals are still leached to deeper layers of the soil, as a result of rain in this particular type of soil (Peña-Fernández, 2011). The reverse may also be argued as a slight increase in Fe concentrations in the dry season, which is then generally associated with their common usage in industrial processes and their subsequent presence in the emissions (Pathak et al., 2015).

The site C Fe concentrations in soil in both seasons were significantly higher than were found at sites 1 and 3. Site C is located not far from site 3, yet the wet season concentration (6503.83 mg/kg) was a little more than seven times higher than the Fe concentration measured at site 3 in the same season (874.29 mg/kg). Similarly, the dry season Fe concentration of 8016.55 mg/kg at site C, was almost six times higher than the dry season concentration found at site 3 (1400.82 mg/kg). The denser vegetation and tree canopies at site 3 renders the site more protected than site C facing the Cape Flats. The likeliness of site C being more susceptible to contamination and accumulation of air pollutants (Guney et al., 2010; Ali and Malik, 2011) being transported from the Cape Flats by the South Easter wind (Sen et al., 2017) then becomes a viable explanation for the significantly higher concentrations found at this site compared to the concentrations measured at the other sites in both seasons. The higher Fe concentrations around these sampling sites also demonstrates anthropogenic addition of metals in soils to be sampling site specific (Pathak et al., 2015). Ashes from wild fires that occurred during the dry season sampling sessions on Table Mountain and the Cape Peninsula (eNCA, 2015) may have caused further escalation of Fe concentrations in the dry season at sites C and 3, as well as generally increased the Fe concentrations in the soil at all the sites (Parra et al., 1996; Jakubus et al., 2010; Bogacz et al., 2011; Jovanovic et al., 2011; Costa et al., 2014).

Site 2 Fe concentrations experienced a major rise in winter (13427.4 mg/kg). It was, also the highest Fe concentration found in the soil and twice as high in comparison with urban forest soil concentrations found in a Hungarian study of 6005 mg/kg in the wet season. Additionally, the Fe concentrations at site 2 in the wet season was measured at twelve times higher than the concentration of 1081.88 mg/kg found in the dry season at the same site, which was, however significantly lower than the Hungarian dry season concentration of 4126 mg/kg found in their urban forest study (Simon et al., 2016). Contamination at site 2, facing the direction of the parking area and the traffic circle may have been caused by the resulting vehicular traffic, especially in winter when vehicle traffic increases (Norouzi et al., 2017). Further amplification of the Fe concentrations may have arisen from the thick brown haze, laden with pollutants, arising from the city, which also appears in winter (Reddy and Venkataraman, 2000; Zhang et al., 2002; Deng et al., 2011). Extreme rises in Fe concentrations, such as these were also observed in Southeast Asian studies during these brown haze spells (Li et al., 2011). Site 1 displayed the second highest Fe concentrations, which is understandable, being the site closest to the car park and road junction, but the increase in concentrations towards winter may similar to site 2 mentioned above, be clarified by the higher vehicle traffic from the parking lot and brown haze appearing in the wet season **(Fig 4.26)**.

#### *4.3.1.1.3) Mn contamination of soil at Orange Kloof forest*

No significant differences in Mn concentrations in soil between seasons with minimal decreases towards winter at sites 2 and 3 were detected. The minimal differences in concentrations measured between the seasons, as well as the decreases are generally rationalized by the climatic characteristics of the study sites. The sandy loam texture of the soils found at Orange Kloof **(Tables 4.5, 4.18)** is highly porous (Madrid et al., 2004, 2007), which is conducive to leaching of the metals to the deeper layers of the soils in wet seasons (Peña-Fernández, 2011). Alternatively, the lower concentrations in the wet season may be a result of post-fire rainfall, also causing leaching of the metals in soil in the wet season of June 2015, shortly after the dry season wild fires in January and March 2015 (Ulery et al., 1993; Certini, 2005; Zavala et al., 2014). Similar decreases were noticed at an Alcalá de Henares industrial site. Their wet season concentration of 158.99 mg/kg was significantly lower than the dry season concentration of 192.72 mg/kg. Ferré-Huguet et al. (2007),

however attributed the results to many factors such as the sources of the metals, human activities, weather conditions and mobility of metals at the time of sampling (Peña-Fernández et al., 2015). Their Mn concentrations were significantly lower than the concentrations found in this study at sites 2 and 3 in both seasons. Site 3 in the dry season yielded the highest Mn concentration in soil (465.72 mg/kg). Off course, the decrease in Mn concentrations in winter may also be seen as a minimal increase in concentrations in summer, which is often explained by the general usage of this metal in industrial processes and their successive presence in the emissions (Pathak et al., 2015). Besides atmospheric deposition, the surface soil concentrations can be elevated (Millaleo et al., 2010; Herndon et al., 2011; Herndon et al., 2015) by vegetation due to biological cycling by litterfall, throughfall, and uptake (Watmough et al., 2007; Kraepiel et al., 2015; Herndon et al., 2015), or otherwise enhanced by the occurrence of wild fires during the sampling period (Khanna et al., 1994; Someshwar, 1996; Pitman, 2006; Plumlee et al., 2007; Gabet and Bookter, 2011; Viana et al., 2012; Bodi et al., 2014; Costa et al., 2014; eNCA, 2015; Santín et al., 2015) in January to March 2015. The Mn concentrations measured at these sites between seasons may also be described as uneven and the uneven enrichment of Mn in soils may further be ascribed to the lithologic or vegetation differences, which as such causes accumulation differences (Herndon et al., 2011).

Site C, showed a significant difference in Mn concentrations between seasons. The higher wet season concentration of 186.68 mg/kg, as opposed to the dry season concentration (98.97 mg/kg) may be due to site C being more exposed to air pollution brought on in winter when vehicle traffic escalates and amplified by the brown haze phenomena in the wet season. Similarly site 1, located in close proximity of vehicle traffic, arising from the parking and traffic circle also showed an increased Mn concentration in the wet season (Reddy and Venkataraman, 2000; Zhang et al., 2002; Deng et al., 2011; Norouzi et al., 2017) **(Fig 4.27)**.

#### *4.3.1.1.4) Al contamination of leaf litter at Orange Kloof forest*

Aluminium concentrations in leaf litter were higher in the wet season at sites C, 2 and 3, with significant differences observed between seasons at sites C and 2. The enhanced concentrations in winter may have been caused by the brown haze effect owing to the escalated anthropogenic activities in winter (Reddy and Venkataraman,

2000; Zhang et al., 2002; Deng et al., 2011). In addition, site C is more exposed to pollution from the Cape Flats (Guney et al., 2010; Ali and Malik, 2011) and site 2 is more open to pollution arising from vehicle traffic (Norouzi et al., 2017) generated in the parking lot and major traffic circle in Constantia. Thus, Al accumulation at both these sites may largely have resulted from atmospheric deposition and throughfall (Lomander and Johansson, 2001; Lomander, 2002; Tyler, 2005). The Al concentrations found at site C of 1021.31 mg/kg in the wet season was almost five times higher than the concentration measured in the dry season (220.42 mg/kg). The site 2 Al concentration in the wet season (1050.96 mg/kg), which was also the overall highest concentration measured in leaf litter at this forest, was also more than three times higher than concentrations found in the dry season (303.51 mg/kg). Simon et al. (2016) in Hungary compared Al concentrations in leaf litter between the seasons, autumn and spring at urban, suburban and rural areas and discovered that the concentrations were significantly higher in autumn, which is in accordance with the results found in this study. Al concentrations measured in their study at the rural sites of 38100 mg/kg in autumn and 20800 mg/kg in spring were, however significantly higher than the concentrations found in leaf litter in this study. Metal enhancement in decomposing litter is said to be as a result of the contact of the underlying polluted soil with the litter (Scheid et al., 2009). These findings are in agreement with the results found in this study of Al concentrations, yielding higher results in both the soil and leaf litter in the wet season.

Site 1 showed a slight decrease in Al concentration in the wet season. Studies have shown that the organic matter of decomposing leaves and needles acts as an efficient metal storage pool and leaf litter thus acts as a temporary sink for metals from the surrounding soil, including the soil below the leaf litter. Metal release from contaminated litter may thus disperse towards the soil or carried away to other areas, possibly resulting in a lower metal content in the leaf litter in winter (Scheid et al., 2009; Van Nevel et al., 2014) **(Fig 4.28)**.

#### *4.3.1.1.5) Fe contamination of leaf litter at Orange Kloof forest*

Higher Fe concentrations were measured in leaf litter in the wet season at all the sites, of which sites C, 1, and 2 presented significant differences between the seasons. Sites C and 2, once again yielded the highest Fe concentrations, of which

site 2 displayed the overall highest Fe concentration of 1018.5 mg/kg, which was five times higher than the dry season concentration (218.99 mg/kg) at the same site. The elevated concentrations in winter is most likely due to the escalating vehicle traffic and anthropogenic activities in this season, concurrent with the subsequent brown smog as a result of the concentrated pollutants (Reddy and Venkataraman, 2000; Zhang et al., 2002; Deng et al., 2011). The more exposed locations of site C to the Cape Flats (Guney et al., 2010; Ali and Malik, 2011) and site 2 to the parking lot and traffic circle (Norouzi et al., 2017) more than likely played a major role in the general higher concentrations found at these sites. The fact that the higher Fe concentrations in the leaf litter correlated with the higher Fe concentrations in soil, demonstrates that metals are enriched in the decomposing litter by way of the contaminated soil underneath the litter as reported by Scheid et al. (2009). Studies in Hungary in which Fe concentrations were compared between the seasons, autumn (44000 mg/kg) and spring (17700 mg/kg) at urban, suburban and rural areas in leaf litter revealed higher concentrations in autumn as opposed to spring and is similar to the findings in this study (Simon et al., 2016) **(Fig 4.29)**.

#### *4.3.1.1.6) Mn contamination of leaf litter at Orange Kloof forest*

Manganese concentrations showed higher results in the dry season at sites 1, 2 and 3, with the highest concentration found at site 3 (1695.6 mg/kg) as opposed to the wet season concentration of 968.2 mg/kg at the same site. Leaf litter comparisons between seasons found by Simon et al. (2016) also demonstrated higher concentrations of Mn in the dry (15200 mg/kg) in contrast with the wet season concentrations of 13000 mg/kg. These concentrations were significantly higher than the concentrations found in leaf litter in this study. The enhanced concentrations of Mn in leaves or leave litter in the dry season may be as a result of the vegetation that are cycling existing Mn sources in the forest soil. Mn concentrations may thus be elevated (Millaleo et al., 2010; Herndon et al., 2011; Herndon et al., 2011) by the vegetation due to biological cycling by litterfall, throughfall, and uptake (Watmough et al., 2007; Kraepiel et al., 2015; Herndon et al., 2015). These sites may also have received Mn contributions from the particulate emissions generated by the wild fires that were engulfing the flora on Table Mountain and releasing ashes during the dry sampling period (Siegert et al., 2001; See et al., 2006a, 2007a) of January to March 2015 (eNCA, 2015). With that said, the drop in Mn concentrations in winter

measured at sites 1, 2 and 3 may also be as a result of the winter rains that is said to promote the dilution of metals (Wong et al., 2003; Sharma et al., 2008). Mn is easily leached from leaves (Avila and Rodrigo, 2004), because of its mobility. In some tree species the sun leaves are also reported to contain higher Mn concentrations than shade leaves (McCain and Markley, 1989) and may also be considered as an impacting factor, in both increased and deceased concentrations of Mn in leaf litter.
A significant difference was also measured between the seasons at site C. The higher concentration measured at this site was, however found in the wet season (177.24 mg/kg) and the lower concentration of 64.97 mg/kg in the dry season. This is an indication of higher pollutant levels in winter, associated with vehicle emissions and the brown haze effect (Reddy and Venkataraman, 2000; Zhang et al., 2002; Deng et al., 2011; Norouzi et al., 2017) at a site, which is relatively exposed to the Cape Flats and associated pollution (Sen et al., 2017; Van der Velden, 2017) **(Fig 4.30)**.

#### *4.3.1.1.7) Al contamination of moss at Orange Kloof forest*

Aluminium concentrations in moss did not vary significantly between seasons and similar results were reported by Thöni et al. (1996), Fernández and Carballeira (2002) and Berg and Steinnes (1997). A study done in As Pontes de Garcıa Rodrıgue (Galicia, NW Spain) at four sampling stations located in the surroundings of the largest coal-fired power station also indicated no cyclic variation in the bioconcentration of Al in mosses. The observed fluctuations were irregular and varied at random around the same level. Sampling was done every 28 days and included both a dry and wet season. The Al concentrations found in spring (676 mg/kg) and autumn (712 mg/kg) in Spain in the moss *Hypnum cupressiforme* did not differ significantly between the seasons (Fernández and Carballeira, 2002), but the concentrations were slightly higher in the wet season. Similarly, slightly higher Al concentrations in the wet season were noticed at sites C (3341.43 mg/kg) and 3 (1105.48 mg/kg) in contrast with the lower concentrations in the dry season at site C (1992.11 mg/kg) and site 3 (649.91 mg/kg). The Al concentrations in this study were also significantly higher than were found in the Spanish study. Site C displayed the overall highest Al concentrations in moss in both seasons when compared to the other sites. Interestingly, this is also the site that is exposed to wind and pollution from the Cape Flats (Sen et al., 2017; Van der Velden, 2017). The pollutant laden

brown haze in winter may also have enhanced Al concentrations in moss at sites C and 3 (Reddy and Venkataraman, 2000; Zhang et al., 2002; Deng et al., 2011). Emission sources, thus largely determine bioaccumulation of metals in mosses within study regions (Kleppin et al., 2008; Schroder et al., 2008; Holy et al., 2009).

Conversely the decreased Al concentrations in winter may also be argued as higher concentrations in summer. In this context precipitation from the “table cloth” in summer (Van der Velden, 2017) that contain metals may have contributed to the higher Al concentrations in the dry season in moss at sites 1 and 2, because wet deposition is a critical scavenging process by which metals are returned to terrestrial or aquatic surfaces (Bacardit and Camarero, 2010). With regard to both the dry and wet season concentrations, it should be noted that the physicochemical characteristics of pollutants (Gonzalez and Pokrovsky, 2014), physiological processes in moss (Boquete et al., 2014) and sampling site characteristics are important role players in metal accumulation in moss and should be taken in consideration (Fernández et al., 2015) **(Fig 4.31)**.

#### *4.3.1.1.8) Fe contamination of moss at Orange Kloof forest*

The dry and wet season Fe concentrations in moss were not significantly different between seasons at any of the sites. These findings were in accordance with findings from the authors Thöni et al. (1996), Berg and Steinnes (1997) and Fernández and Carballeira (2002) and also with a study done in Spain at four sampling stations located in the surroundings of the largest coal-fired power station, which revealed no cyclic variation in the bioconcentration of Fe in mosses between dry and wet seasons. Fe concentrations found in spring (555 mg/kg) and autumn (699 mg/kg) in the moss *Hypnum cupressiforme* did not vary significantly between seasons (Fernández and Carballeira, 2002) and the Fe concentrations in their study was exceeded by the Fe concentrations measured in this study at all the sites and in both seasons.

As in the case with Al, higher Fe concentrations were also found in the wet season at sites C (2379.91 mg/kg) and 3 (1204.2 mg/kg) as opposed to the lower concentrations in the dry season at the same sites, C (1694.48 mg/kg) and 3 (723.81 mg/kg). Exposure of the mosses to the brown haze may clarify the higher

concentrations in winter at those sites (Reddy and Venkataraman, 2000; Zhang et al., 2002; Deng et al., 2011). Slightly higher Fe concentrations were found at sites 1 and 2 in the summer when the “table cloth” is a common occurrence (Van der Velden, 2017) and the precipitation thereof is known to contain metals (Bacardit and Camarero, 2010). Both the increases in Fe concentrations in their respective seasons, however may be clarified by the bioaccumulation of metals in mosses within study regions, which are mainly determined by emission sources (Kleppin et al., 2008; Schroder et al., 2008; Holy et al., 2009). Factors such as, physicochemical characteristics of pollutants (Gonzalez and Pokrovsky, 2014), physiological processes in moss (Boquete et al., 2014) and sampling site characteristics influence metal accumulation in moss and should nevertheless be taken into account (Fernández et al., 2015) **(Fig 4.32)**.

#### *4.3.1.1.9) Mn contamination of moss at Orange Kloof forest*

Manganese concentrations measured in moss displayed higher results in the dry season at sites 1, 2 and 3, of which the highest concentration in moss was found at site 3 (673.74 mg/kg). The concentrations at site 2 (527.39 mg/kg), which is the more exposed site to vehicle traffic from the parking lot and traffic circle (Norouzi et al., 2017) were not much lower than the concentrations measured at site 3, which is more secluded. Site 2, also exhibited a significant difference between seasons, of which a lower concentration of 259.86 mg/kg was found in the wet season. It is possible that moss at these sites were impacted by precipitation that contains metals (Bacardit and Camarero, 2010) from the “table cloth” in summer, especially since site 3 is the highest site, lavished with mosses and green lush vegetation (Van der Velden, 2017). Higher Mn concentrations were also found in spring (143 mg/kg) and lower concentrations in autumn (249 mg/kg) in Spain in the moss *Hypnum cupressiforme* (Fernández and Carballeira, 2002), but the concentrations were considerably lower than most of the Mn concentrations found in this study.

Significant differences were found between seasons at sites C. The higher concentrations were measured in the wet season (150.27 mg/kg) in contrast with the lower concentrations found in the dry season (77.51 mg/kg). The higher concentrations in winter may be ascribed to higher vehicle traffic in winter and the brown haze at this more exposed site (Reddy and Venkataraman, 2000; Zhang et

al., 2002; Deng et al., 2011). Both the higher concentrations in their respective seasons is concurrent with evidence of emission sources that largely determine bioaccumulation of metals in mosses within study regions (Kleppin et al., 2008; Schroder et al., 2008; Holy et al., 2009). It is, nevertheless important to consider additional influencing factors that include, the physicochemical characteristics of pollutants (Gonzalez and Pokrovsky, 2014), physiological processes in moss (Boquete et al., 2014) and sampling site characteristics, which is known to have an impact on the metal concentrations in the moss (Fernández et al., 2015) **(Fig 4.33)**.

#### *4.3.1.1.10) Al contamination of lichen at Orange Kloof forest*

With the exception of site 2, Al concentrations in lichen generally did not differ significantly between the seasons, which concurs with other findings of lichen morphology not changing significantly with the different seasons (Conti and Cecchetti, 2001). Malaspina et al. (2014) also reported similar results that displayed very little difference in lichen between seasons in Al concentrations. However, slightly higher concentrations were observed in their study in summer (1030 mg/kg) and lower concentrations in autumn (820 mg/kg), which is similar to the results measured at site C. The exposed location of site C to wind from the Cape Flats (Sen et al., 2017; Van der Velden, 2017) may have contributed to the higher Al concentrations in summer at this site, as the majority of Al and dry deposition is derived from mineral dust, due to its ubiquity in soils (Macdonald and Martin, 1988). Precipitation containing metals (Bacardit and Camarero, 2010) from the “table cloth” in summer (Van der Velden, 2017) may also have contributed to the higher Al concentrations in lichen. Conversely, the concentrations may also be viewed as being lower in winter and in that case elemental leakage caused by precipitation in winter may have been the cause of the lower Al concentrations found at this site in the wet season (Brown and Brown, 1991; Adamo et al., 2008).

The higher Al concentrations in lichen found in winter at sites 1, 2 and 3 may have resulted from anthropogenic activities, such as fossil fuel usage for cooking, heating and road traffic, mounting in the colder seasons in major cities, which results in increased atmospheric pollution (Norouzi et al., 2017) and is visible in the atmosphere as a brown coloured haze (Reddy and Venkataraman, 2000; Zhang et al., 2002; Deng et al., 2011.) The highest concentration was found at site 2 (1792.1

mg/kg) in the wet season and was significantly higher than the dry season concentration of 612.07 mg/kg. Site 2 faces and is also more exposed to vehicle traffic generated at the road junction and car park. The Al concentrations found in this study were also highly comparable with industrial and urban areas from other studies (Adamo et al., 2007; Sorbo et al., 2008) in summer (1030 mg/kg) and autumn (820 mg/kg) (Malaspina et al., 2014) **(Fig 4.34)**.

#### *4.3.1.1.11) Fe contamination of lichen at Orange Kloof forest*

Lichen morphology does not change with the seasons (Conti and Cecchetti, 2001), which means that seasonal differences in metal concentrations are generally minimal. This was witnessed in our findings of insignificant differences recorded in Fe concentrations in lichen between summer and winter at all the sites, except for site 2. In view of this, the Fe concentrations in lichen did display slightly higher Fe concentrations in winter at sites 1, 2 and 3. Site 2, also produced the highest Fe concentration in lichen, which was measured in the wet season (1642.12 mg/kg) as opposed to a much lower concentration found in the dry season (538.47 mg/kg). This is possibly due to the fact that this site incurred more exposure from vehicle emissions arising from the traffic circle and parking lot, it faces. Sites 1 and 3 was nevertheless also impacted by the pollutants (Norouzi et al., 2017), derived from the thick smog, containing metals due to increased vehicle traffic and industrial activities in winter (Reddy and Venkataraman, 2000; Zhang et al., 2002; Deng et al., 2011). The Fe concentrations measured in this study was also notably higher than the concentrations in lichens found in spring (820 mg/kg) and autumn (820 mg/kg by Malaspina et al. (2014).

Site C displayed higher Fe concentrations in summer, which most likely has its clarification in the exposed location to the pollution and wind from the Cape Flats (Sen et al., 2017; Van der Velden, 2017). Metals in precipitation (Bacardit and Camarero, 2010) from the “table cloth” in summer (Van der Velden, 2017) may also have attributed to the higher Fe concentrations in lichen. The opposite scenario of lower Fe concentrations in winter at this site may equally be explained by the element leaking as a result of precipitation during the wet season (Brown and Brown, 1991; Adamo et al., 2008) **(Fig 4.35)**.

#### *4.3.1.1.12) Mn contamination of lichen at Orange Kloof forest*

Manganese concentrations were higher in the dry season at sites 1, 2 and 3, which is in accordance with results outlined by Malaspina et al. (2014) of higher Mn concentrations in spring (191 mg/kg) and lower concentrations in autumn (84 mg/kg). There was a significant difference in Mn concentrations measured between seasons in lichen at site 1. The dry season revealed a higher concentration of 103.25 mg/kg in comparison to the lower wet season concentration of 57.35 mg/kg. Site 2 showed Mn concentrations of up to 4 times higher in the dry season (663.28 mg/kg) than were found in the wet season (144.5 mg/kg) and site 3 Mn concentrations in the dry season (569.81 mg/kg) were twice as high compared to concentrations observed in the wet season (212.36 mg/kg). It is likely that Mn in precipitation (Bacardit and Camarero, 2010) from the “table cloth” in summer (Van der Velden, 2017) may have enhanced Mn concentrations in lichen at these sites. The biological features of lichens render them sensitive to atmospheric contaminants (Conti and Cecchetti, 2001; Wolterbeek, 2002), which causes them to be susceptible to accumulating metals from the atmosphere (Rühling and Tyler 1968; Loppi et al., 1997) and it has alreay been established that all the sites at this forest have been reached by air pollution through natural and anthropogenic sources. However, one may also consider the fact that the element has leached in winter due to the rain, which subsequently caused the lower Mn concentrations (Brown and Brown, 1991; Adamo et al., 2008) at sites 1, 2 and 3 in this season. The higher Mn concentrations measured at site C in the wet season is in all likelihood in reaction to the increased vehicle traffic emissions transported by wind (Sen et al., 2017; Van der Velden, 2017) and magnified by the brown haze phenomena (Norouzi et al., 2017) in winter (Reddy and Venkataraman, 2000; Zhang et al., 2002; Deng et al., 2011) **(Fig 4.36)**.

#### *4.3.1.1.13) Al contamination of millipedes at Orange Kloof forest*

The concentrations of Al in millipedes did not differ significantly between seasons, although slightly higher concentrations were found in the dry season at sites C, 2 and 3, of which the highest concentration was measured at site C (3042.68 mg/kg). This Al concentration was in fact seven times higher than were found at sites 1 and 2 and nine times higher than the concentrations measured at site 3 in the same season. Litter chemistry at small spatial scales (meters to decameters) does have an influence on millipedes (Ashwini and Sridhar, 2008). Hopkin (1989) found with

earthworms that the feeding of soil organic matter causes higher toxic element concentrations as a result of the toxic elements that are typically associated with organic components of soil. In this study, site C constantly showed higher metal concentrations due to its exposed location to the Cape Flats and related pollution (Sen et al., 2017) and thus correlates with Hopkin's findings. Site 1 on the other hand exhibited higher Al concentrations in millipedes in the wet season (554.29 mg/kg) in which the millipedes were noticed to be more active and also in the season where the pollution seemed to be more prominent, as a result of mounting anthropogenic activities in colder seasons (Norouzi et al., 2017). The explanation for these higher Al concentrations in the millipedes, may lie in the polluted litter that they consumed (Da Silva Souza et al., 2014) in the soil, which already displayed a high Al concentration.

Seasonal differences between invertebrates are difficult to determine as a result of many different impacting factors such as sex, feeding preferences and whether or not they are overwintering species. Overwintering species were found to accumulate less toxic amounts of metals, as were found in studies using ground beetles. Aluminium concentrations that were reported in male and female ground beetles ranged between 48.2 and 50.5 mg/kg in highly polluted urban soils, which is significantly lower than the concentrations found in this study. The authors concluded that ground beetles are not good accumulators of metals (Simon et al., 2016). According to the results in this study it seems that pill millipedes are relatively good accumulators of metals **(Fig 4.37)**.

#### *4.3.1.1.14) Fe contamination of millipedes at Orange Kloof forest*

Iron concentrations in millipedes at all the sites did not vary significantly between seasons. It is however, a known fact that the response of soil invertebrates to pollution is affected by the different seasons, together with the different climatic conditions and different parts of the life cycles of organisms within the given season. Information regarding the effects of climatic conditions on soil invertebrates in metal polluted soils are, however limited (Santorufo et al., 2012). With that said, sites 1 and 2 Fe concentrations in millipedes were higher in the wet season when pollutant levels were enhanced due to a rise in anthropogenic activities in winter, combined with the brown smog loaded with metals and other pollutants (Norouzi et al., 2017). Fe concentrations in soil and leaf litter were also higher in winter and thus concurs

with Hopkins' findings of higher toxic element concentrations in, for example earthworms feeding on soil organic matter which are associated with organic components of soil (Hopkin, 1989). Thus, the polluted litter consumed by the millipedes (Da Silva Souza et al., 2014) may have enhanced their Fe concentrations (Hopkin, 1989). This may also have been the reason for the higher Fe concentrations measured in the millipedes at site C in the dry season (1556.15 mg/kg), which was three to seven times higher than the concentrations found at sites 1, 2 and 3, also in the dry season. The Fe concentrations at site C in soil (8016.55 mg/kg) was also high in relation to the other sites, possibly due to the site being constantly exposed to pollution arising from the Cape Flats (Sen et al., 2017).

Even though the higher pollutant levels in a particular season did correspond with the higher concentrations in soil, leaf litter and millipedes, consideration should still be given to the many different factors that have proven to affect metal accumulation in invertebrates such as, sex and food preferences, as well as overwintering habits. Overwintering species accumulate less toxic amounts of metals, leading to lower concentrations, as were found in studies, using ground beetles. Male and female Fe concentrations in ground beetles found in the literature, ranged between 43.7 and 75.7 mg/kg and was significantly lower than were found in millipedes in this study. It was suggested by Simon et al. (2016) that the ground beetles are poor accumulators of metals, which was opposite to the results observed with pill millipedes in this study **(Fig 4.38)**.

#### *4.3.1.1.15) Mn contamination of millipedes at Orange Kloof forest*

Manganese concentrations were higher at sites C, 1 and 3 in the wet season, although the differences between the seasons at all the sites were not found to be significant. The highest concentration in millipedes was measured at site 3 (199.66 mg/kg) in winter. It is noteworthy that site 3 also displayed constant higher Mn concentrations in the soil, leaf litter, moss and lichen, as were found in the millipedes at this site. Manganese concentrations in millipedes at site 2 were, however higher in the dry season, which is also the site facing the vehicle related pollution from the parking lot and traffic circle (Schleicher et al., 2011; Chen et al., 2014). The fact that the millipedes accumulated higher concentrations of this metal in both seasons may be as a result of the polluted litter that they consumed (Da Silva Souza et al., 2014)

when they were observed to be most abundant and active in winter in this study. Organic matter is also associated with organic components of soil as were reported by Hopkin (1989) in earthworm studies.

Concentrations of Mn in ground beetles were found to be dependent on many factors, such as overwintering habits, sex and feeding preferences. During over wintering less toxic amounts of metals are accumulated, which generally leads to lower metal concentrations in these beetles. Mn concentrations were measured separately for males and females in ground beetles in their study and ranged between 46.7 and 18.3 mg/kg, which was significantly lower than were measured in this study. Simon et al. (2016) was of the opinion that ground beetles are not good metal accumulators, but according to the findings in this study, pill millipedes seems to have displayed good accumulation abilities **(Fig 4.39)**.

***4.3.1.1.16) Al contamination of soil at Newlands forest***

With the exception of site 1 that exhibited a significant difference in Al concentrations in soil between seasons, minimal differences in concentrations were otherwise measured between seasons at the rest of the sites. That being said, higher Al concentrations were observed in winter at all the sites, which is more than likely as a result of the brown haze episodes (Reddy and Venkataraman, 2000; Zhang et al., 2002; Deng et al., 2011) that appears without fail in Cape Town's winter seasons (City of Cape Town - Air Quality, 2007). Seasonal trends of higher metal concentrations in winter have been filed by Motelay-Massei et al. (2005) as a result of domestic heating in winter. The escalating anthropogenic activities in major cities, such as fossil fuel usage for road traffic, heating and cooking in the cold seasons, results in increased atmospheric pollution that gives rise to the brown coloured haze (Norouzi et al., 2017). Even secluded forest areas are contaminated by pollutants in the brown haze by means of long-range transport (Agnihotri et al., 2011; Ojha et al., 2012; Kumar et al., 2013; Joshi et al., 2016) and in East Asia Al concentrations, amongst others have been reported to double during such brown haze episodes (See et al., 2006).

The Al concentrations at site 1 (6711.84 mg/kg) found in the wet season exceeded the concentration of 4925.35 mg/kg measured in the dry season by far. This site is

the closest one to the busy De Waal Drive main road, ingoing and outgoing to the city centre, but was interestingly not the site with the highest Al concentrations measured in soil at this forest. Site 2, not only yielded the highest Al concentrations (10395.26 mg/kg) in the wet season, but also in the dry season (8656.02 mg/kg). This site have most likely been impacted by the South Easter wind in the dry season, when one considers the higher and more exposed location (Lawrence and Neff, 2009) on the mountain in closer proximity to the contour path, City Centre and Cape Flats (Van der Velden, 2017). Similar patterns were reported by Sen et al. (2017) in the Indo-Gangetic Plains during the summer season. Mineral dust originating in the arid landscapes of NW-India and SW-Asia were transported across the Indo-Gangetic Plains picking up local pollutants, which accumulated along the southern slopes of the Indo-Himalayan Range. It is therefore highly probable that Al had its inception from mineral dust, because of its ubiquity in soils (Macdonald and Martin, 1988), but also from anthropogenic sources, due to the location of this specific site (Pathak et al., 2015) and dust transporting winds (Goossens, 2000; Motelay-Massei et al., 2005). In addition the multiple forest fires that occurred on Table Mountain and spread to the Cape Peninsula during the dry season sampling occasion (eNCA, 2015) may have added to the Al load at site 2, but also have had a general impact on the Al concentrations in the dry season at all the sites (Parra et al., 1996; Jakubus et al., 2010; Bogacz et al., 2011; Jovanovic et al., 2011; Costa et al., 2014). Factors such as precipitation, leaching of this element from the canopy (Matzner, 1989) and stemflow, nevertheless also influences the total amount of Al concentrations in soil (Koch and Matzner, 1993; Levia and Frost, 2003).

A wet season Al concentration of 11261.40 mg/kg found by Peña-Fernández et al. (2015) at an industrial site in Alcalá de Henares, Spain was only slightly higher than the highest wet season concentration of 10395.26 mg/kg measured in this study at Newlands forest site 2. It is interesting that these high concentrations reported at industrial sites in a major city are actually comparable with the supposedly pristine, secluded forest sites in this study. Their dry season Al concentration (10135.90 mg/kg) was also not significantly higher than the dry season concentration of 8656 mg/kg found at site 2 in this study (Madrid et al., 2004, 2007; Peña-Fernández, 2011) **(Fig 4.40)**.

#### *4.3.1.1.17) Fe contamination of soil at Newlands forest*

Iron concentrations in soil at Newlands forest were predominantly higher in the wet season. The brown haze in winter may account for the higher concentrations found in this season when anthropogenic activities, which are major causes of excessive dust-borne metal inputs during the winter, escalates in the colder seasons (Norouzi et al., 2017) in major cities (Motelay-Massei et al., 2005). Secluded and pristine areas in high-altitude mountain sites have been severely contaminated by air pollution during autumn and winter, by cause of inversion conditions (brown haze), the advection of copious amounts of pollutants from rural and urban areas, the increase in vehicle usage, agriculture and charcoal for heating purposes (Sarangi et al., 2014; Naja et al., 2014, 2016; Kant et al., 2015). Site 3, which is the highest and most densely vegetated site on the mountain, showed the overall highest Fe concentrations in the wet (16243.42 mg/kg) and dry (10225.38 mg/kg) seasons.

The wet season Fe concentration at site 3 (16243.42 mg/kg) was significantly higher in comparison with the concentrations found at the other sites in both seasons, even though this is the most densely vegetated and secluded site, reiterating that very few areas in the forests on Table Mountain are spared from pollution. Similar higher concentrations of Fe was found in India around some selected sampling sites, suggesting that anthropogenic addition of metals in soils is sampling site specific (Pathak et al., 2015). The Fe concentrations found at site 3 was almost three times higher than the concentrations found in a Hungarian urban forest study of 6005 mg/kg, also in the wet season. Their dry season concentration of 4126 mg/kg was also two and a half times lower than the dry season concentration found in this study of 10826.98 mg/kg at the same site and season (Simon et al., 2016). Newlands forest may also have received additional Fe inputs in the upper layers of the soils from the ashes derived from the multiple wild fires (Parra et al., 1996; Jakubus et al., 2010; Bogacz et al., 2011; Jovanovic et al., 2011; Costa et al., 2014) that occurred on Table Mountain and in the Cape Peninsula (eNCA, 2015) during the dry season sampling occasion **(Fig 4.41)**.

#### *4.3.1.1.18) Mn contamination of soil at Newlands forest*

The Mn concentrations measured in soil presented minimal differences and uneven concentrations between seasons, which is generally as a result of the lithologic or

vegetation differences, causing the accumulation differences (Herndon et al., 2011). Site 2 Mn concentrations were only slightly lower in the wet season (486.45 mg/kg) as opposed to the dry season (489.97 mg/kg), possibly by cause of the soil texture, which is sandy loam and highly porous (Madrid et al., 2004, 2007) **(Tables 3.7, 3.20)**. These soils are more prone to metals leaching to the deeper layers of the soils during high rainfall and lower evaporation conditions in winter (Peña- Fernández, 2011). The lower concentrations in the wet season at this site may also have resulted from post-fire rainfall, which also could have caused leaching of the metals in the soil in the winter of June 2015, soon after the dry season wild fires in January and March 2015 (Ulery et al., 1993; Certini, 2005; Zavala et al., 2014).

Sites 1 and 3 showed higher Mn concentrations in the wet season. The highest concentration of 496 mg/kg was found at site 3, although this concentration was not significantly higher than the dry season concentration (340.13 mg/kg). The Mn contribution at both of the sites 1 and 3 may have had their origin from the escalating vehicle traffic during winter (Norouzi et al., 2017) and the concurrent brown haze episodes commonly experienced in Cape Town's winter season (CMA, 2015). Brown haze contains metals that have previously been traced back to vehicular traffic emissions (Zhou et al., 2014). Site 1 is located close to the busy De Waal Drive main road in and outgoing to the City of Cape Town, but site 3 with the higher Mn concentration is relatively secluded and densely vegetated, which leads to a suggestion that the concentrations at site 3 may have been further enhanced by vegetation due to biological cycling by litterfall, throughfall, and uptake (Watmough et al., 2007; Herndon et al., 2015; Kraepiel et al., 2015). The common denominator, however is the fact that Newlands forest is situated close to the city centre, which is known to have a huge impact on adjacent forests, regardless of the additional brown haze input in winter. This have been substantiated by Sen et al. (2017) that remote and pristine areas, as were found in in South Asia's mega cities of the Indo-Gangetic Plains are reachable through and have been contaminated by long-range transport of continental pollutants (Agnihotri et al., 2011; Ojha et al., 2012; Kumar et al., 2013; Joshi et al., 2016).

A study done in Alcalá de Henares at an industrial site, revealed a wet season concentration of 158.99 mg/kg, which was significantly lower than their dry season

concentration of 192.72 mg/kg. Such results were, however attributed to various factors such as the sources of the metals, human activities, weather conditions and mobility of metals at the time of sampling (Ferré-Huguet et al., 2007; Peña-Fernández, et al., 2015). Both of their Mn concentrations were significantly lower than the Mn concentrations found at Newlands forest sites 1, 2 and 3 in both seasons **(Fig 4.42)**.

#### *4.3.1.1.19) Al contamination of leaf litter at Newlands forest*

The wet season displayed overall higher Al concentrations in leaf litter at all the sites and significant differences were noticed between seasons at sites 1 and 3. Site 3 with a concentration of 1067.13 mg/kg was 3 times higher in the wet season than were found in the dry season (323.15 mg/kg) and was also the highest concentration found in leaf litter at Newlands forest. Aluminium concentrations at sites 1 and 2 in the dry season, also almost doubled towards the wet season. The primary cause for metal enrichment in decomposing litter have been ascribed to the litter having contact with the polluted soil underneath the litter (Scheid et al., 2009), which agrees with the findings in this study of higher Al concentrations in soil and leaf litter in the wet season. The enhanced Al concentrations in soil in the wet season were ascribed to vehicular emissions (Zhou et al., 2014) and dust-borne metal inputs derived from escalating anthropogenic activities in winter (Norouzi et al., 2017) in major cities (Motelay-Massei et al., 2005). In leaf litter the rise in Al concentrations from the dry to the wet season may same be attributed to the brown haze in winter. Atmospheric deposition and throughfall is known to be a major cause of metal accumulation in leaf litter, but fungi amongst the leaf litter is also said to cause microbial translocation and immobilization of metals from underlying contaminated soil layers (Lomander and Johansson, 2001; Lomander, 2002; Tyler, 2005). A study done by Simon et al. (2016) in Hungary, which included comparisons of Al concentrations in leaf litter in urban, suburban and rural areas, reported higher Al concentrations in autumn than in spring. The Al concentrations of 38100 mg/kg in autumn and 20800 mg/kg in spring were significantly higher than the concentrations found in leaf litter in this study **(Fig 4.43)**.

#### *4.3.1.1.20) Fe contamination of leaf litter at Newlands forest*

Iron concentrations in leaf litter were found to be higher in the wet season at all the sites. Significant differences were observed in concentrations between seasons at sites 1 and 3, but site 3 showed the highest Fe concentration in the wet season (2344.68 mg/kg), which was 3 times higher than the concentrations measured in the dry season (703.43 mg/kg) at the same site. The higher Fe concentrations found in soil and leaf litter in the wet season is in accordance with reports from Scheid et al. (2009), stipulating that metal enhancement in decomposing litter have their origin from the contact the leaf litter has with the polluted soil underneath the litter. The explanation for the higher Fe concentrations in the wet season may also lie in the location of Newlands forest in close proximity of the major City of Cape Town (Motelay-Massei et al., 2005), which is engulfed by the brown haze in winter, brought on by increased anthropogenic activities in colder seasons (Norouzi et al., 2017). Fe concentrations reported in Hungary between the seasons, autumn and spring at urban, suburban and rural sites in leaf litter were higher in autumn. Both the autumn (44000 mg/kg) and spring (17700 mg/kg) Fe concentrations exceeded the concentrations found in leaf litter in this study (Simon et al., 2016) **(Fig 4.44)**.

#### *4.3.1.1.21) Mn contamination of leaf litter at Newlands forest*

The Mn concentrations in leaf litter were relatively constant between the seasons at sites 1, 2 and 3, showing minimal differences. There were some decreases noticed in concentrations towards the wet season at sites 1 and 3 that may have evolved from the rain in the winter season that is known to promote the dilution of metals (Wong et al., 2003; Sharma et al., 2008). It should also be noted that Mn is easily leached from leaves (Avila and Rodrigo, 2004), because of the mobility of this element, which makes comparisons between leaf litter samples difficult. In some tree species studies also show that the sun leaves can contain higher Mn concentrations than shade leaves (McCain and Markley, 1989).

Site 2, which is more exposed to pollutants from the city centre, yielded the highest Mn concentrations in both seasons, but the wet season concentration of 987.63 mg/kg was higher than the dry season concentration (733.34 mg/kg). The higher Mn concentrations in the wet season most probably had its origin in the brown haze, containing metals, such as Mn (Zhou et al., 2014) caused by the increased vehicle

traffic emissions and anthropogenic activities in winter (Norouzi et al., 2017). However, Mn cycling may also have enhanced the concentrations (Millaleo et al., 2010; Herndon et al., 2011; Herndon et al., 2011) by litterfall, throughfall, and uptake (Watmough et al., 2007; Herndon et al., 2015; Kraepiel et al., 2015). Leaf litter was compared between seasons in terms of Mn concentrations by Simon et al. (2016). The authors reported higher Mn concentrations in spring, (15200 mg/kg) and lower concentrations in autumn (13000 mg/kg), which was significantly higher than the concentrations found in this study **(Fig 4.45)**.

#### *4.3.1.1.22) Al contamination of moss at Newlands forest*

Moss displayed the highest Al concentrations in the wet season at sites 1, 2 and 3 with site 3 (6364.45 mg/kg) showing the highest concentrations. The concentrations were three times higher than the Al concentrations found at the same site in the dry season (1822.76 mg/kg). Brown haze containing metals derived from vehicle traffic emissions (Zhou et al., 2014) and dust-borne metal inputs from anthropogenic activities in winter (Norouzi et al., 2017) may have contributed to the higher metal concentrations in moss in this season. This forest is situated in close proximity of a major city (Motelay-Massei et al., 2005). Resulting emission sources is thus said to be a determinant factor in the bioaccumulation of metals in mosses within study regions (Schroder et al., 2008; Kleppin et al., 2008; Holy et al., 2009). Influencing factors such as the uptake of pollutants by mosses, which include physicochemical characteristics of the pollutants and physiological processes in mosses, also play a deciding role in final concentrations (Aboal et al., 2010). Significantly lower Al concentrations in moss were found by Fernández and Carballeira (2002) in Spain than were measured in this study. The authors also reported similar lower concentrations in spring (676 mg/kg) as opposed to higher concentrations in autumn (712 mg/kg) in in the moss *Hypnum cupressiforme* as were found in this study **(Fig 4.46)**.

#### *4.3.1.1.23) Fe contamination of moss at Newlands forest*

Iron concentrations yielded higher results in moss in the wet season and site 3 Fe concentrations (7683.84 mg/kg) were twice as high in the wet season as opposed to the dry season (3547.25 mg/kg). The brown haze, containing metals originating from vehicle traffic emissions (Zhou et al., 2014) and dust-borne metal inputs from

anthropogenic activities arising from Cape Town and surrounding area (Norouzi et al., 2017) may have enhanced the Fe concentrations. Such emission sources is said to be a determinant factor in the bioaccumulation of metals in mosses within study regions (Schroder et al., 2008; Kleppin et al., 2008; Holy et al., 2009). With that said, metal accumulation in mosses are influenced by a number of factors that should be taken into account, such as physicochemical features of pollutants (Gonzalez and Pokrovsky, 2014), physiological activities in moss (Boquete et al., 2014) and sampling site traits (Fernández et al., 2015). Iron concentrations in moss found in the highly populated city of Spain in spring (555 mg/kg) and autumn (699 mg/kg) in the moss *Hypnum cupressiforme* (Fernández and Carballeira, 2002) were significantly lower than the Fe concentrations measured in in moss in this study **(Fig 4.47)**.

#### *4.3.1.1.24) Mn contamination of moss at Newlands forest*

Manganese concentrations in moss were higher in the wet season at sites 1, 2 and 3, although there were minimal differences measured between seasons. Site 2 Mn concentrations in the wet season (515.1 mg/kg) showed the highest overall results and was also higher than were found in the dry season (437.67 mg/kg). The elevated Mn concentrations in moss in winter most likely have their origin from vehicle traffic emissions that escalates in winter. Mn measured in haze have been traced back to vehicle traffic emissions and the smelting industry (Zhou et al., 2014) and dust-borne metal inputs from anthropogenic activities (Norouzi et al., 2017). Emission sources does to a large extend dominate the bioaccumulation of metals in mosses within a given area (Schroder et al., 2008; Kleppin et al., 2008; Holy et al., 2009), but physicochemical traits of pollutants (Gonzalez and Pokrovsky, 2014), physiological processes in moss (Boquete et al., 2014) and sampling site features should not be excluded (Fernández et al., 2015). Once again, the Mn concentrations found in spring (143 mg/kg) and autumn (249 mg/kg) in Spain in the moss *Hypnum cupressiforme* was exceeded by the concentrations found in this study (Fernández and Carballeira, 2002) **(Fig 4.48)**.

#### *4.3.1.1.25) Al contamination of lichen at Newlands forest*

Aluminium concentrations in lichen were higher in the wet season at sites 1 and 2 and the highest concentrations were measured at site 1 (2377.85 mg/kg). These concentrations were almost twice as high as the Al concentrations found in the dry

season (1342.57 mg/kg) at the same site. The increased anthropogenic activities in populated cities, such as Cape Town during the colder seasons, naturally causes increased atmospheric pollution and the subsequent brown haze (Norouzi et al., 2017). In view of this, See et al. (2006) announced that most chemical components', concentrations including Al in South East Asia doubled on days when the brown smog makes its appearance. Metal concentrations in lichen have been reported to be higher in winter seasons (Motelay-Massei et al., 2005, Norouzi et al., 2017), but lichen morphology as such is not usually affected by the different seasons and accumulation of pollutants can occur throughout the year (Conti and Cecchetti, 2001). One may therefore suggest that the brown haze in the winter season of Cape Town may have caused the higher Al concentrations in lichen at those sites.

Site 3, on the other hand showed a marginal decrease in Al concentrations in the wet season, of which the clarification can be two-fold. Metals in precipitation (Bacardit and Camarero, 2010) from the "table cloth" in summer (Van der Velden, 2017) may have been a positive contributor to the higher Al concentrations measured in lichen in the dry season. In reverse, the elements could have leaked by cause of precipitation in winter and resulted in the lower Al concentrations found at site 3 in the winter (Brown and Brown, 1991; Adamo et al., 2008). The concentrations found in this study were highly comparable with industrial and urban concentrations reported by Adamo et al. (2007) and Sorbo et al. (2008) of Al concentrations in summer (1030 mg/kg) and autumn (820 mg/kg) (Malaspina et al., 2014), suggesting that Newlands forest is under substantial anthropogenic pressure **(Fig 4.49)**.

#### *4.3.1.1.26) Fe contamination of lichen at Newlands forest*

Sites 1 and 2 revealed higher Fe concentrations in lichen in the wet season, of which site 1 yielded the overall highest concentration (3105.6 mg/kg). This wet season Fe concentration was almost double the amount measured in lichen in the dry season concentration (1701.6 mg/kg). The Fe contribution in the wet season most probably have their origin from mounting anthropogenic activities in the colder season (Norouzi et al., 2017) and the subsequent concentrated pollutants in the brown haze. This commensurate with a study done in South East Asia, where Fe concentrations have been reported to double on days when the brown haze made its appearance (See et al., 2006). Malaspina et al. (2014) reported Fe concentrations in lichen in

their study of 820 mg/kg in summer and 820 mg/kg in autumn, which is significantly lower than were measured in this study.

Opposite to the results found at sites 1 and 2, site 3 displayed lower Fe concentrations in the wet season. The different seasons are, however not an influencing factor with regard to the accumulation of pollutants in lichen (Conti and Cecchetti, 2001), but elements are known to leak during periods of rainy weather, resulting in lowered metal concentrations in lichen in that season (Brown and Brown, 1991; Adamo et al., 2008). When one reverses the scenario, the higher concentrations in the dry season may have resulted from metals in precipitation (Bacardit and Camarero, 2010) from the 'table cloth" in summer (Van der Velden, 2017) **(Fig 4.50)**.

#### *4.3.1.1.27) Mn contamination of lichen at Newlands forest*

Manganese concentrations in lichen were slightly higher in the dry season at sites 2 and 3, with site 3 in the dry season (159.27 mg/kg) showing a notably higher concentration as opposed to the lower wet season concentration of 77.84 mg/kg. The overall highest Mn concentration that was found in the dry season at site 2 (234.52 mg/kg), could quite possibly have arisen from metals in precipitation (Bacardit and Camarero, 2010) from the 'table cloth" in summer (Van der Velden, 2017). Other studies also revealed higher Mn concentrations in lichen in summer (191 mg/kg) as opposed to lower concentrations in the colder season (84 mg/kg) (Malaspina et al., 2014). These concentrations are comparable to the concentrations found in this study. On the other hand, the lower concentrations in the wet season at sites 2 and 3 may be ascribed to element leakage during the wet season, as a result of precipitation. Rainwater may remove particulate that are entrapped onto the lichen surface, which may have caused the lower Mn content during the wet season (Brown and Brown, 1991; Adamo et al., 2008). The higher Mn concentrations found at site 1 in the wet season may be due to anthropogenic activities, in particular the vehicle related emissions, arising from De Waal Drive mainroad in and outgoing to the City of Cape Town during the colder seasons and enhanced by the concentrated pollutants in the brown haze (Norouzi et al., 2017) **(Fig 4.51).**

#### *4.3.1.1.28) Al contamination of millipedes at Newlands forest*

Millipedes displayed higher Al concentrations in winter at sites 1, 2 and 3 and a considerable difference was noted between seasons at site 1, which was also where the overall highest Al concentrations were measured (3007.07 mg/kg). This concentration was almost seven times higher than the concentration found in millipedes in the dry season (1072.3 mg/kg) at this site. Millipedes are influenced by litter chemistry at small spatial scales (meters to decameters) (Ashwini and Sridhar, 2008) and with earthworms, (Hopkin, 1989) it was found that the feeding of soil organic matter causes enhanced toxic element concentrations due to the toxic elements that are generally associated with organic components of soil. The Al concentrations in soil and leaf litter in this study were all significantly higher in the wet season, which correlates with Hopkin's findings. Site 2 Al concentrations (1717 mg/kg) were six times higher than were found in the dry season and site 3 Al concentrations (1581.9 mg/kg) almost twice as high as were found in summer. The enhanced Al concentrations in millipedes in this season, may have their explanation in the consumption of polluted litter (Da Silva Souza et al., 2014) in a polluted soil during a season that receives additional exposure from the concentrated pollutants in the brown haze (Norouzi et al., 2017).

Simon et al. (2016) did not make comparisons between seasons in ground beetles, as it was found that Al concentrations in these invertebrates were dependant on a variety of factors of which food preferences, sex and overwintering species play a significant role. Overwintering species have been found to accumulate less toxic amounts of metals. Male and female Al concentrations measured in their study ranged between 48.2 and 50.5 mg/kg. According to Simon et al. (2016) the ground beetles are poor accumulators of metals, but the results in this study suggests pill millipedes to be relatively good accumulators of metals **(Fig 4.52)**.

#### *4.3.1.1.29) Fe contamination of millipedes at Newlands forest*

Iron concentrations in millipedes were the highest in the wet season at sites 1, 2 and 3 and a significant difference between seasons were found at site 1 with a wet season concentration of 2735.58 mg/kg and a significantly lower concentration of 555.33 mg/kg in the dry season. Site 3 in the wet season had a concentration of 3384.41 mg/kg, which was the overall highest concentration found between the sites

and seasons. The fact that litter chemistry influences millipedes in their environment (Ashwini and Sridhar, 2008) and that polluted litter consumed by millipedes (Da Silva Souza et al., 2014) may enhance their metal concentrations, can be observed in the pattern observed in this study of high pollutant levels and brown haze in winter (Norouzi et al., 2017), corresponding with the high Fe concentrations in the soil, leaf litter and millipedes in this season. Similar patterns have been noticed in earthworms feeding on soil organic matter, which exerted higher toxic element concentrations associated with organic components of soil (Hopkin, 1989).

Regardless of the obvious pattern found in this study, Simon et al. (2016) reports of various influencing factors, on Fe concentrations in invertebrates, in for example ground beetles, which makes comparisons between seasons difficult. Factors such as sex, food preferences and overwintering species play a significant role in metal accumulation. Overwintering species also accumulate less toxic amounts of metals, consequently leading to lower metal concentrations. The Fe concentrations found separately in males and female ground beetles ranged between 43.7 and 75.7 mg/kg of which was concluded that ground beetles are not good accumulators of metals (Simon et al., 2016). The pattern of correlating Fe concentrations in soil, leaf litter and millipedes found in this study, however suggests that pill millipedes are good accumulators of metals **(Fig 4.53)**.

#### *4.3.1.1.30) Mn contamination of millipedes at Newlands forest*

Higher Mn concentrations in millipedes at sites 1, 2 and 3 were observed in the wet season that is associated with higher pollutant levels and the brown haze (Norouzi et al., 2017). Millipedes at site 2, which is more exposed to pollution from the city centre showed a significant difference between seasons in concentrations. The wet season concentration (173.13 mg/kg), which is also the highest Mn concentration found between sites, were significantly higher than the dry season concentrations of 72.72 mg/kg. Leaf litter showed higher Mn concentrations at site 2 in the wet season and these concentrations correlate with the higher concentrations in millipedes at site 2 in the wet season. Da Silva Souza et al., (2014) is in agreement that millipedes that consume polluted litter accumulate higher concentrations of this metal. The millipedes in this study were observed to be highly active in winter when litter fall is

higher and may thus be the reason for the higher Mn concentrations in them. Hopkin (1989) also confirms organic matter's association to the organic components of soil.

Many factors contribute to the metal concentrations found in invertebrates of which feeding preferences, sex and overwintering habits play a major role. Over wintering species have been found to accumulate less toxic amounts of metals, consequently leading to lower metal concentrations. Mn concentrations for male and female ground beetles were thus measured separately in their study and ranged between 46.7 and 18.3 mg/kg, which was significantly lower than were measured in this study (Simon et al., 2016). The pattern of correlating Mn concentrations in soil, leaf litter and millipedes found in this study leads to the assumption that pill millipedes exhibit good accumulation abilities, which is not reported to be the case with ground beetles (Simon et al., 2016) **(Fig 4.54)**.

#### 4.3.1.2) Comparisons of site C, Orange Kloof and Newlands forests in terms of metal concentrations in soil, leaf litter and sentinel organisms

The whole of Table Mountain that includes the three study areas and all of the other flora and fauna not mentioned here for that matter, are exposed to continuous air pollution generated in the Cape Metropolitan area during the entire year (Scorgie, 2003; Guney et al., 2010; Ali and Malik, 2011). Such findings were apparent in Nepal's pristine, high altitude areas, showing the impacts of metal pollution by both long-range and short-range transport (Xu et al., 2009; Cong et al., 2010). In addition, Sakata et al. (2006), Chen et al. (2008) and Huang et al. (2013a), (2013b) reiterated the probability of anthropogenic emissions contributing to elevated metal deposition through short range transport within a small geographic area. Similarly, Orange Kloof forest and site C was impacted through long range transport of metals, as this site and forest is located further away from the City Centre. The short range transport of metals and subsequent impact is more applicable to Newlands forest, which is significantly closer to Cape Town. Be that as it may, both of these forests, as well as the control site, site C were to a higher or lesser degree impacted by the metals, Al, Fe and Mn in both seasons. This leads to a concern for the eventual health and survival of these indigenous forest pockets and its organisms, as it seems that very few areas, regardless of remoteness or seasons are spared from the impact of pollution, be it from natural or anthropogenic origin.

The metal concentrations found in the soil, leaf litter and sentinel organisms at site C, Orange Kloof and Newlands forests in the dry and wet seasons are indicative of air pollutants being captured by forest canopies (Steinnes and Friedland, 2006; Sen et al., 2017) and the origin of these metals may be two-fold. Al, Fe and Mn may either have stemmed from its crustal origin (Tripathee et al., 2014), as natural soil, road fugitive dust and/or construction dust (Song et al., 2012; Zhang et al., 2012; Zhou et al., 2014) is known to occur in road dust, which becomes airborne due to traffic movement (Venkataraman et al., 2005). Also, these mineral elements are associated with the crustal fraction of $PM_{10}$ and their occurrence in $PM_{10}$ is predominantly as a result of local and regional dust re-suspension, which have been generated by wind, convection, and other natural processes (Negi et al., 1987; Song et al., 2006; Kar et al., 2010; Shridhar et al., 2010; Pant and Harrison, 2012). The anthropogenic sources of these metals may typically have sprung from the various combustion and non-combustion sources generated from the City of Cape Town. The combustion-related sources include industrial activities, transportation, fuel-burning appliances, wild fires, tyre burning and domestic fuel burning. Non-combustion emission sources include evaporative losses and landfill operations, waste water treatment and fugitive emissions from wind erosion and agriculture (Scorgie, 2003), all of which are prevalent sources in and around Cape Town as discussed in the section of 3.4.1. The main sources of particulate matter, however is said to originate from vehicle exhaust, road dust, and industrial production (Dzierzanowski et al., 2011; Gunawardena et al., 2012). The Cape Metropolitan Area with a population count of 3.74 million (StatsSA, 2011) and 1.93 million vehicles that are registered (Wheels24, 2017) is the most congested city in South Africa (BusinessDay, 2017). Further research is, however necessary to evaluate the prospective impacts of airborne particulate matter on surface ecosystems, water resources, via leaching and human health (Cao et al., 2011). Even so, understanding the capture and accumulation process of both course ($PM_{10}$) and fine ($PM_{2.5}$) particulate matter, its origin and its behavior in different seasons does shed some light when one analyzes the results in this study.

Forests have proven their effectiveness for absorbing and filtering particulate matter (Slinn, 1982; Draaijers et al., 1994; Yang et al., 2005; Nowak et al., 2006; Escobedo and Nowak, 2009; Terzaghi, 2013; Ji and Zhao, 2014; Liu et al., 2016a). This is

significant as particulate matter consist of inhalable particles of which the fine particles ($PM_{2.5}$) is specifically harmful to health as a result of the highly toxic metals and other toxic materials it contains (Jouraeva et al., 2002). Dry particulate matter can be increased by the forest canopy by airflow (Giorgi, 1986), but studies in Mexico also revealed forests to potentially remove an annual amount of 100 tonnes of $PM_{10}$ (course particles) from the air, which amounts to 2% of the annual emissions in that country (Baumgardner et al., 2012). Deposition of particulate matter to the forest and the attenuation processes of particles are influenced by characteristics of the forest, such as canopy density and plant species. Sparse forests were shown to be more efficient in the deposition of particulate matter (Shan, 2007; Tiwary et al., 2008; Sæbø, 2012; Popek, 2013; Terzaghi, 2013). Additional factors influencing the deposition of particulate matter in forests are meteorological conditions, particularly wind speed, humidity, and temperature (Croxford et al., 1996; Ould-Dada, 2002; Erisman and Draaijers, 2003). Also, the entrance pathways of particulate matter to forest ecosystems occurs in two ways: onto the forest canopy and within the canopy. Deposition within the canopy entails collection via the vegetation (Petroff et al., 2008). In other words, plants collect more particulates, but the collection rates are influenced by the plant structures (Liu et al., 2016b). It so happens that deposition of particulate matter occurs more within the forest canopy. Also, the coarse particle ($PM_{10}$) deposition in a forest canopy is more than the fine particle ($PM_{2.5}$). It was found by the authors that fine particle deposition is elevated in winter, as opposed the coarse particles that are higher in summer (Liu et al., 2016b). Metals of anthropogenic origin have a tendency to accumulate in fine mode (<1.0 μm), having a relatively long lifespan in the atmosphere, claryfying the visibility effects of atmospheric aerosols. Metals that originate from crustal origin, on the other hand, mostly dominate in coarse mode (>2.5 μm). Due to their large size, the particles have a shorter lifespan in the atmosphere and thus impact on surface rather fast (Allen et al., 2001; Handler et al., 2008). This concurs, in short with the results found in this study of a tendency toward higher metal concentrations found in winter with escalating anthropogenic activities and the subsequent brown haze (Norouzi et al., 2017). In summer, some of the higher metal concentrations may have been caused by dust transporting winds containing the crustal elements Al, Fe and Mn, (Allen et al., 2001; Handler et al., 2008). It therefore also makes sense that the coarse particle ($PM_{10}$) deposition in these forest pockets most likely dominated the

concentrations found in the forested areas. Many important factors, however influenced, but also clarified the individual results found in the soil, leaf litter and different organisms at their respective study areas.

With regard to the dry season, site C displayed a tendency towards higher Al, Fe and Mn concentrations in this season. The elevated concentrations of these metals in $PM_{10}$, which are associated with crustal origin, showed seasonal variability as reported by Li (2013). The concentrations were higher in summer and lower in monsoon and the pattern was ascribed to seasonal variation of crustal source emissions and regional weather conditions. The higher concentrations during summer was also a reflection of the dominance of crustal components over anthropogenic elements (Cheng et al., 2005; Xia and Gao, 2011). It was, nevertheless also suggested that construction, vehicular, and farming activities in the vicinity of their study area may further have enhanced crustal/soil re-suspension in summer (Li, 2013). Increases in Al and Fe concentrations in the dry season, are as a rule associated with their common usage in industrial processes and their subsequent presence in the emissions, as were further substantiated by Pathak et al. (2015). These findings are in accordance with studies done at the Indo-Gangetic Plains in the summer season where mineral dust originating in the arid landscapes of NW-India and SW-Asia were transported across the Indo-Gangetic Plains, while picking up local pollutants that accumulated along the southern slopes of the Indo-Himalayan Range (Sen et al., 2017). Similar patterns were noticed with long-range transport during summer in India and a possible mixing of anthropogenic pollutants during transport of dust was affirmed by Chinnam et al. (2006). Soil dust in the Indo-Gangetic Plains have also been earmarked as a prime contributor in aerosol concentrations during summer and monsoon seasons (Dey, 2004; Chinnam et al., 2006; Singh and Beegum, 2013). The combination of mineral dust and anthropogenic pollutants mixing with the local winds may, thus have contributed largely to the elevated metal concentrations in the dry season (Kulshrestha et al., 2009), at this site. It should be noted that proper dilution and dispersion of pollutants during summer are assisted by wind turbulence with large mixing height, as well as the sea-breeze, which may decrease the particulate pollutant concentrations (Gupta et al., 2004) and therefore summer dust appears to be less harmful and polluted due to the lower concentrations of toxic metals (Norouzi et al., 2017). Other factors that

may have contributed to the overall higher metal concentrations in the dry season that have been of specific reference to this study in Cape Town are the wild fires that occurred on Table Mountain and the Cape Peninsula during the dry sampling period that are known sources of the metals, Al, Fe and Mn (Gaudichet et al., 1995; Karthikeyan et al., 2006a, b). The 'table cloth,' which is a huge cloud that hoovers on top of Table Mountain and drips over the mountainside, creates clouds and rain along the eastern slope (Van der Velden, 2017) and the precipitation thereof contains metals, which are returned to terrestrial or aquatic surfaces and may further have enhanced metal concentrations (Bacardit and Camarero, 2010) in this season.

In terms of the wet season, Orange Kloof and Newlands forests presented a tendency towards a pattern of higher metal concentrations in this season, which went hand in hand with the higher metal concentrations found in soil, leaf litter and sentinel organisms at these forests, in winter. Cape Town is situated at the south-western tip of Africa and edged by the Table Mountain range along the west coast, which concurrently with its position, between False Bay and Table Bay, impact air flow within the region. Cape Town's high pollution levels during winter with the brown haze episodes from April to September is as a result of strong temperature inversions and calm conditions during this period. Most of the City of Cape Town is covered by this haze and shifts according to the prevailing wind direction (Wicking-Baird et al., 1997). The brown haze made its first appearance on 17 April 1992 for approximately twelve days, which had the public of Cape Town concerned, confused and at the doorstep of the Minister of Health and Population Development. Since then there has been clear trends of the visible smog in winter, which increases every year (Popkiss, 1992), so much so that the haze now appears from April to September each year (Wicking-Baird et al., 1997). Haze pollution in China is regarded as the most serious environmental air issue in China at present, due to its detrimental effect on public health (Tie et al., 2009; Chen et al., 2013; Yao et al., 2014; Huo et al., 2015) not to mention the adverse impacts it has on remote ecosystems (Chameides et al., 1999; Zhang et al., 2013) and the climate at large (Solomon et al., 2007; Tainio et al., 2013). The smog is an indication of high concentrations of atmospheric particulate matter (Cheng et al., 2013) that arise from human activities such as vehicle traffic, construction sites, and resuspension processes related to urban surfaces, various industries, the burning of fossil fuels for

heating and cooking. Additionally the geogenic particles contribute to the overall mass concentration of airborne particles and they include minerals transported from regions, which are arid, semi-arid or bare soils within or surrounding cities (Schleicher et al., 2011; Chen et al., 2014), similar to the Cape Flats. The higher metal concentrations measured at these forests, surrounded by the City of Cape Town in winter, is therefore an indication of severe haze pollution brought on predominantly by escalating anthropogenic activities, as the fine particle deposition ($PM_{2.5}$) have been found to be elevated in winter (Liu et al., 2016b). This is a serious health warning due to the inhalable particles of which the fine particles are particularly harmful to health as a result of the highly toxic metals and other toxic materials it contains (Jouraeva et al., 2002). Norouzi et al. (2017) confirmed that atmospheric dust in winter is significantly to very high contaminated with metals. The authors also suggested that the harmful effects of atmospheric deposition in an area be minimized by implementing appropriate measures to reduce the concentration of metals in dust. This could be done more effectively in winter when stable and cold air to a large extend prevents the rapid movement of polluted atmosphere to the neighboring areas.

“Site specific” factors were of special interest in this study at Newlands forest and site C. Pathak et al. (2015) in India also found that the metals, Al, Fe and Mn showed higher concentrations at some of the sampling sites, and in view of that, suggested anthropogenic addition of metals in soils to be sampling site specific. One such case was found with Newlands forest, which displayed overall higher metal concentrations in the wet season compared to Orange Kloof and site C. The clarification lies in its location closer to the city center. It has been found that the level of contaminants are generally higher in samples from forest ecosystems adjacent to industrialized and heavily populated areas (Driscoll et al., 1994; Nakazato et al., 2015), which is subjected to various sources of metals due to major anthropogenic activities (São Paulo, 2013). Similar findings were reported in Paris and was due to domestic heating, which escalates in winter in a populated city (Motelay-Massei et al., 2005). Major cities, such as the Indo-Gangetic Plains in India also reported remarkably high loading of ambient particulates that includes both fine and coarse fractions in winter, which quite often breached the set NAAQS levels (Pant et al., 2006; Massey et al., 2012; Pandey et al., 2012; Murari et al., 2015; Das et al., 2016; Panda et al., 2016;

Sharma et al., 2016a, b). Site C was initially designated as the control site, but turned out to be the most metal contaminated site and it was later determined that it was by reason of its slightly more exposed location on the mountain. This site seemed to be impacted by natural and anthropogenic pollutants in both seasons transported from the Cape Flats by the South Easter wind (Sen et al., 2017), creating a micro-climate effect at this site (Lawrence and Neff, 2009).

An important season-specific factor that may have altered metal concentrations between seasons and observed at all three study areas is leaching of metals in the wet season. In soils, decreased metals in the upper layer is often due to high rainfall rates in combination with the lower evaporation rate, which causes leaching of these metals to deep layers. This is especially true in highly porous soils (Madrid et al., 2004, 2007) with a sandy loam texture, such as the soils characterized at Orange Kloof and Newlands forests (Peña- Fernández, 2011) **(Tables 4.5, 4.18)**. Leaching in soil could also have occurred as a result of post-fire rainfall, where significant decreases in metal concentrations in soil in winter have been reported after the occurrence of wild fires in the summer (Ulery et al., 1993; Certini, 2005; Zavala et al., 2014), such as were experienced during the dry sampling occasion in January to March 2015 (eNCA, 2015). There were instances when lower concentrations were measured in lichen in winter, which could have been caused by leakage of elements during the wet periods as a result of precipitation. Rainwater can remove particulate that are entrapped onto the lichen surface, which may thus cause lower element content during rainy periods (Brown and Brown, 1991; Adamo et al., 2008). Mn is also easily leached from leaves, which could have resulted in lower concentrations of this metal in leave litter (Avila and Rodrigo, 2004).

Non-season specific factors that may have influenced the metal concentrations are, Mn concentrations in the surface soil that may have been elevated even more (Millaleo et al., 2010; Herndon et al., 2011; Herndon et al., 2011) by vegetation as a result of biological cycling by litterfall, throughfall, and uptake (Watmough et al., 2007; Herndon et al., 2015; Kraepiel et al., 2015). In some tree species the sun leaves are also reported to contain higher Mn concentrations than shade leaves (McCain and Markley, 1989) and may also be considered as an impacting factor, in both increased and deceased concentrations of Mn in leaf litter in both seasons. The

initial metal concentrations in leaf litter plays an important role in the accumulation and flow of metals in the decomposing leaf litter (Lomander and Johansson, 2001; Kaila et al., 2012). Metal accumulation thus occurs mainly from atmospheric depositions and throughfall, but also from microbial translocation and immobilization of metals from contaminated soil layers underneath the litter, primarily by fungi (Lomander and Johansson, 2001; Lomander, 2002; Tyler, 2005). Metal enrichment in decomposing litter is, however mainly ascribed to contact of the underlying polluted soil with the leaf litter (Scheid et al., 2009), which is in accordance with most of the results in this study, as the soil concentrations correlated with the leaf litter concentrations between seasons. In moss, emission sources largely determines the bioaccumulation of metals in mosses within study regions (Kleppin et al., 2008; Schroder et al., 2008; Holy et al., 2009). Several other factors does have an influence on metal accumulation in mosses, such as the physicochemical characteristics of the pollutants and the moss binding surfaces (Gonzalez and Pokrovsky, 2014), physiological processes, such as moss growth (Boquete et al., 2014) sampling site characteristics, such environmental conditions, elevation, gradient and level of exposure and canopy structure (Fernández et al., 2015).

General influencing factors in this study, with special reference to millipedes are, development stage, food preferences, breeding, season, sex and physiological conditions, which all influences toxic element accumulation in invertebrates (Jelaska et al., 2007; Butovsky, 2011). Additionally, the excretion ability of the bodies of the invertebrates also influences the concentration of these elements (Janssen et al., 1991; Kramarz, 1999 Avgin and Luff, 2000). This study was, however not designed to allow for research into these differences, but differences between sexes, does seem to play a significant role in accumulation of metals (Stone et al., 2002). There are many factors that influence invertebrate response to pollution. Such impacting factors are: the different seasons, in combination with the different climatic conditions and different stages of the life cycles of organisms within the given season. Information on the effects of climatic conditions on soil invertebrates in metal polluted soils are, however scarce (Santorufo et al., 2012). The effect of organic pollutants and complex mixtures on invertebrates is relatively unknown and available literature is generally on the use of diplopods as bioindicators of soil and usually metal related (Souza and Fontanetti, 2011). Be that as it may, the metal concentrations in the pill

millipedes were observed to be higher at site C and Newlands forests, where the highest concentrations of the metals, Al, Fe and Mn in soil and leaf litter were measured, suggesting that these specific pill millipedes are good accumulators of metals. Unfortunately, literature relevant to this study, using pill millipedes is to the best of my knowledge scarce to non-existent.

#### *4.3.1.2.1) Al contamination of soil between site C, Orange Kloof and Newlands forests*

Higher Al concentrations in soil were found in the wet season at all three of the study areas, with a significant difference in concentrations found at Newlands forest between the dry (5846.29 mg/kg) and the wet (8049.87 mg/kg) season. The overall highest Al concentrations were, however found at site C in the wet (13940.73 mg/kg), as well as in the dry (13450.31 mg/kg) season. The dry season concentration at site C exceeded concentrations reported in Alcalá de Henares, one of the most densely populated cities in Spain, at an industrial site of 10135.90 mg/kg also in summer (Peña-Fernández et al., 2015). Similarly the wet season Al concentrations at this site exceeded the wet season concentrations found in the Spanish city of Alcalá de Henare's industrial study site of 11261.40 mg/kg (Peña-Fernández et al., 2015). The elevated Al concentrations in winter is in all likelihood owed to brown haze spells during the colder winter season in the City of Cape Town (CMA, 2015). Similar reports were announced by authors in East Asia, who found Al concentrations to double during such brown haze episodes (See et al., 2006). Fine particle deposition is also said to be elevated in winter, as opposed the coarse particles that are generally higher in summer (Liu et al., 2016b), which suggests that the higher Al concentrations in winter at these afromontane forests are predominantly from anthropogenic origin (Allen et al., 2001; Handler et al., 2008). These findings concur with research in the literature stating that anthropogenic activities, such as vehicle traffic, construction work, resuspension processes related to urban surfaces, various industries and the burning of fossil fuels for cooking and heating escalates during winter, causing high pollution levels in the atmosphere (Schleicher et al., 2011; Chen et al., 2014).

The overall enhanced Al concentrations measured in both seasons at site C, may also have originated from mineral dust, owing to its ubiquity in soils (Macdonald and

Martin, 1988) and transported by wind (Goossens, 2000; Motelay-Massei et al., 2005), such as the South Easter in the dry season. The location of site C (Lawrence and Neff, 2009), being more exposed to pollutants, arising from the Cape Flats (Van der Velden, 2017) may therefore clarify the higher Al concentrations at this site. Higher Al concentrations around some selected sampling sites does suggest that anthropogenic addition of metals in soils is sampling site specific (Pathak et al., 2015). Similar patterns were reported by Sen et al. (2017) in the Indo-Gangetic Plains during the summer season. In addition, ashes from the wild fires that occurred on Table Mountain and the Cape Peninsula (eNCA, 2015), containing Al and occurring simultaneously with the dry sampling period may have added to the Al budget (Gaudichet et al., 1995; Karthikeyan et al., 2006a, b) in the study areas, but even more so at the site C **(Table 4.27)**.

#### *4.3.1.2.2) Fe contamination of soil between site C, Orange Kloof and Newlands forests*

The wet season displayed higher Fe concentrations in soil at Orange Kloof and Newlands forests. The brown haze contribution of higher pollution levels in the wet seasons in Cape Town (Wicking-Baird et al., 1997) may be of relevance to these results, as See et al. (2006) in South East Asia also reported most chemical components', concentrations including Fe to have increased by double on days that experienced brown haze episodes. Fine particle deposition, representing anthropogenic inputs have been reported to be enhanced in winter (Liu et al., 2016b), suggesting that the enhanced Fe concentrations in winter at these forests were mainly derived from human activities (Allen et al., 2001; Handler et al., 2008). Vehicle traffic, construction work, resuspension of road dust, industries and the burning of fossil fuels for heating and cooking is known to escalate during colder seasons, inevitably causing high pollution levels in the atmosphere (Schleicher et al., 2011; Chen et al., 2014). The highest Fe concentrations in soil were found at Newlands forest in the wet (11856.77 mg/kg) and dry season (9029.98 mg/kg), which may be due to its closer location to the city centre (Driscoll et al., 1994; Nakazato et al., 2015). The Newlands Fe concentration was almost twice as high as were found in the Hungarian urban forest study of 6005 mg/kg in the wet season. Their dry season concentration of 4126 mg/kg was also significantly lower than the dry season concentration found in this study (Simon et al., 2016). The Fe

concentrations in this forest may also in part be from crustal origin (Tripathee et al., 2014). Natural soil, road fugitive dust and construction dust (Song et al., 2012; Zhang et al., 2012; Zhou et al., 2014) occurs in road dust, which becomes airborne due to traffic movement (Venkataraman et al., 2005). These mineral elements are also associated with the crustal fraction of $PM_{10}$ and their occurrence in $PM_{10}$ is mainly as a result of local and regional dust re-suspension, which are generated by wind, convection, and other natural processes (Negi et al., 1987; Song et al., 2006; Kar et al., 2010; Shridhar et al., 2010; Pant and Harrison, 2012).

The significant decline in Fe concentrations at the site C in the wet season, may be due to leaching or lateral transport of ashes as a result of post-fire rainfall. Such declines are visible in metal concentrations of soil samples taken in the wet season and in this case, shortly after wild fire episodes in the dry season (Ulery et al., 1993; Certini, 2005; Zavala et al., 2014). Alternatively the decrease may have been as a result of leaching of these metals in the highly porous soils (Madrid et al., 2004, 2007) **(Table 4.28)**.

#### *4.3.1.2.3) Mn contamination of soil between site C, Orange Kloof and Newlands forests*

Manganese concentrations in soil were higher in the wet season at site C and Newlands forest. The enhanced wet season concentrations were more than likely by cause of the concentrated pollutants in the brown haze (Reddy and Venkataraman, 2000; Zhang et al., 2002; Deng et al., 2011) that appears at regular intervals in winter (City of Cape Town - Air Quality, 2007) in Cape Town (CMA, 2015). The brown smog contains metals, such as Mn (Zhou et al., 2014), which have been reported to be of vehicle related origin (Norouzi et al., 2017). Site C showed a significant difference in Mn concentrations between the wet (186.67 mg/kg) and dry (98.97 mg/kg) seasons. According to Landre et al. (2010), these findings are in line with their results obtained from a study done at a rural site in central Ontario. The authors found that Mn deposition was twice to four times higher even at the furthest site from the road than the throughfall deposition that was measured. They therefore ascribed the higher Mn concentrations to possible general urban influence. Their suggestion makes sense in this study when bearing in mind the location of site C facing the Cape Flats, as well as the Newlands forest's location at the foot of the De

Waal Drive main road. Newlands forest in the wet (449.49 mg/kg) and dry (354.15 mg/kg) seasons displayed the overall highest Mn concentrations. Besides the possible urban (Landre et al., 2010) and brown haze influence (Reddy and Venkataraman, 2000; Zhang et al., 2002; Deng et al., 2011), the atmospheric deposition in the surface soil concentrations could also have been elevated (Millaleo et al., 2010; Herndon et al., 2011; Herndon et al., 2011) due to biological cycling by litterfall, throughfall, and uptake (Watmough et al., 2007; Kraepiel et al., 2015; Herndon et al., 2015). The levels of contaminants are as a rule also found to be higher in samples from forest ecosystems adjacent to industrialized and heavily populated areas (Driscoll et al., 1994; Nakazato et al., 2015), such as Newlands forest.

A decline in Mn concentrations in the wet season at Orange Kloof forest was noticed and could be ascribed to high rainfall rates in combination with the lower evaporation rates in winter (Madrid et al., 2004, 2007), thus causing leaching of the metals to deeper layers (Peña- Fernández, 2011). Leaching of the metals in soil due to post-fire rainfall, may same be of relevance in this study (Ulery et al., 1993; Certini, 2005; Zavala et al., 2014), because of the occurrence of wild fires during the dry sampling occasion in January and 2015 (eNCA, 2015). Similar results were reported in Alcalá de Henares (Spain) at an industrial site. Mn concentrations of 158.99 mg/kg in winter was lower than the dry season concentration of 192.72 mg/kg. The Mn concentrations in this study exceeded the concentrations found at the aforementioned industrial site in Spain **(Table 4.29)**.

#### *4.3.1.2.4) Al contamination of leaf litter between site C, Orange Kloof and Newlands forests*

Aluminium concentrations in leaf litter measured at site C, Orange Kloof and Newlands forests were higher in winter, which is evident of the brown haze influence during the cold wet weather in Cape Town's winter season (CMA, 2015). From the literature is also appears that the higher concentrations in winter are most likely from anthropogenic origin (Allen et al., 2001; Handler et al., 2008) as a result of the escalating human activities in winter (Schleicher et al., 2011; Chen et al., 2014) associated with major cities (Motelay-Massei et al., 2005). Significant differences were found between seasons at site C and Newlands forest, but Newlands forest

showed the overall highest concentrations in both the dry (447.11 mg/kg) and the wet (980.00 mg/kg) seasons, which is synonymous with other findings of contaminant levels being higher in samples from forest ecosystems adjacent to industrialized and heavily populated areas (Driscoll et al., 1994; Nakazato et al., 2015). Metal concentrations in leaf litter are quite often an indication of metal concentrations in the soil underneath the litter, as polluted soil underneath leaf litter is known to enrich the leaf litter above the soil, which may add to the metal load in the litter (Scheid et al., 2009). These findings are in accordance with the findings in this study of leaf litter Al concentrations correlating with the soil Al concentrations. Al concentrations in leaf litter was compared between the seasons, autumn and spring in Hungary at urban, suburban and rural study sites. Simon et al. (2016) found higher Al concentrations of 38100 mg/kg in autumn and lower concentrations of 20800 mg/kg in spring, which is significantly higher than the concentrations found in leaf litter in this study, but concurs with the higher concentrations found in winter in this study **(Table 4.27)**.

#### *4.3.1.2.5) Fe contamination of leaf litter between site C, Orange Kloof and Newlands forests*

Higher Fe concentrations in the wet season in leaf litter, as well as significant differences in concentrations between the seasons were observed at all three of the study areas. Newlands forest, however showed the highest concentrations in the wet (1466.95 mg/kg) and the dry season (552.64 mg/kg), which is synonymous with reports of higher contamination levels found in samples from forest ecosystems in major populated cities (Driscoll et al., 1994; Motelay-Massei et al., 2005; Nakazato et al., 2015). Simon et al. (2016) reported similar findings of higher Fe concentrations in leaf litter in the wet season, although the concentrations were extremely high in comparison to the concentrations found in this study. Their rural study areas revealed Fe concentrations of 44000 mg/kg in autumn and 17700 mg/kg in spring. The elevated wet season concentrations are ascribed to the occurrence of brown haze episodes in winter in the City of Cape Town (CMA, 2015), due to inversion conditions. Major amounts of pollutants from rural and urban areas, the increase in vehicle usage, agriculture and charcoal for heating purposes are known to enhance metal pollution in winter (Sarangi et al., 2014; Kant et al., 2015; Naja et al., 2014, 2016), so much so that Fe concentrations have been found to double during brown

haze episodes (See et al., 2006). Polluted soil underneath the leaf litter is known to enhance the metal concentrations in the leaf litter above the soil, adding to the existing metal concentrations in the litter (Scheid et al., 2009). Metal accumulation originates predominantly from atmospheric deposition and throughfall, but also from microbial translocation and immobilization of metals from underlying contaminated soil layers by fungi (Lomander and Johansson, 2001; Lomander, 2002; Tyler, 2005) **(Table 4.28)**.

#### *4.3.1.2.6) Mn contamination of leaf litter between site C, Orange Kloof and Newlands forests*

Manganese concentrations in leaf litter showed higher results in the wet season at site C and Newlands forest. A significant difference was found between seasons at site C. The wet season concentration (177.24 mg/kg) was significantly higher than the dry season concentration (64.97 mg/kg). Brown haze episodes containing metals from escalating vehicle traffic in winter may have contributed to the higher wet season levels detected (Wicking-Baird et al., 1997; Norouzi et al., 2017) at both site C and Newlands forest, as well as account for the significant differences found.

The highest Mn concentration was found at Orange Kloof forest in the dry season (792.98 mg/kg) as opposed to the wet season concentration of 461.57 mg/kg. Mn inputs in summer could have come from ashes derived from burnt vegetation (Parra et al., 1996), as Mn is known to accumulate in tree leaves and especially needles of resinous species (Kabata-Pendias, 2011). Furthermore the atmospheric deposition in the surface soil concentrations can be elevated (Millaleo et al., 2010; Herndon et al., 2011; Herndon et al., 2011) by vegetation as a result of biological cycling by litterfall, throughfall, and uptake (Watmough et al., 2007; Kraepiel et al., 2015; Herndon et al., 2015). As a further consequence, the metal concentrations in the leaf litter can be enhanced by the polluted soil underneath the leaf litter (Scheid et al., 2009). Mn concentrations in leaf litter were compared between seasons by (Simon et al., 2016) and higher concentrations of this metal were reported in spring. Mn concentrations in autumn (13000 mg/kg) and spring (15200 mg/kg) were significantly higher than the concentrations found in leaf litter in this study **(Table 4.29)**.

#### *4.3.1.2.7) Al contamination of moss between site C, Orange Kloof and Newlands forests*

Insignificant variations in Al concentrations were noticed between the seasons at site C, Orange Kloof and Newlands forest, but the minimal differences measured are in line with literature from other authors using mosses as biomonitors of metal deposition (Thöni et al., 1996; Berg and Steinnes, 1997; Fernandez and Carballeira, 2002). With mosses it is also expected to consider other factors that may influence metal accumulation, such as physicochemical features of pollutants (Gonzalez and Pokrovsky, 2014), sampling site traits (Fernández et al., 2015), as well as physiological processes in moss (Boquete et al., 2014). Orange Kloof and Newlands forests displayed, nonetheless higher Al concentrations in moss in the dry season and may be clarified by the fact that the bioaccumulation of metals in mosses within study regions are predominantly determined by emission sources (Kleppin et al., 2008; Schroder et al., 2008; Holy et al., 2009). Emissions arising from mineral dust (Macdonald and Martin, 1988) and human activities (Driscoll et al., 1994; Nakazato et al., 2015), containing Al may have contributed to the elevated dry season concentrations (Gaudichet et al., 1995; Karthikeyan et al., 2006a, b). The 'table cloth,' in summer, which creates clouds and rain along the eastern slope of Table Mountain, where these forests are situated (Van der Velden, 2017) could also quite possibly have contributed to the higher Al concentrations in the dry season, due to the fact that wet deposition is a critical scavenging process by which metals are returned to terrestrial or aquatic surfaces (Bacardit and Camarero, 2010).

Site C showed the highest Al concentrations in the wet season (3341.43 mg/kg), which is also where the overall highest concentrations were found. The highest dry season concentration of 1992.10 mg/kg was also found at site C. The brown haze containing metals from escalating vehicle traffic in winter may have contributed to the higher wet season levels (Wicking-Baird et al., 1997; Norouzi et al., 2017). Further enhancement may have resulted from pollutants arising from anthropogenic, as well as from natural origin, transported by wind over the Cape Flats (Sen et al., 2017) at this slightly more exposed site. Al concentrations found in spring (676 mg/kg) and autumn (712 mg/kg) in Spain in the moss *Hypnum cupressiforme* were exceeded by the results found in this study (Fernández and Carballeira, 2002) **(Table 4.27)**.

#### *4.3.1.2.8) Fe contamination of moss between site C, Orange Kloof and Newlands forests*

Moss at site C and Orange Kloof forest displayed higher Fe concentrations in the wet season. Human activities increases drastically in winter due to the cold weather, resulting in increased pollution and subsequent brown smog laden with metals and other toxic pollutants (Wicking-Baird et al., 1997; Norouzi et al., 2017). It must be noted that mosses accumulate metals directly from the atmosphere, therefore the bioaccumulation of metals in mosses is said to be regulated by emission sources within study regions (Kleppin et al., 2008; Schroder et al., 2008; Holy et al., 2009). Other factors are also considered in moss metal accumulation, for example physicochemical characteristics of pollutants (Gonzalez and Pokrovsky, 2014), physiological processes in moss (Boquete et al., 2014) and sampling site features (Fernández et al., 2015). Newlands forest, nevertheless displayed higher concentrations in moss in the dry season, which was also where the Fe concentrations were the highest (2254.81 mg/kg). Higher contamination levels are found in samples from forest ecosystems in major populated cities (Motelay-Massei et al., 2005; Driscoll et al., 1994; Nakazato et al., 2015). Metals in precipitation from the ‘table cloth (Van der Velden, 2017) may also have been instrumental in the enhancement of Al concentrations in the dry season, because of the wet deposition being a critical scavenging process by which metals are returned to terrestrial or aquatic surfaces (Bacardit and Camarero, 2010).

In general, only small variations in metal concentrations between seasons at site C and the two forests were found, which is not uncommon according the other authors using mosses as biomonitors of metal deposition (Thöni et al., 1996; Berg and Steinnes, 1997; Fern!andez and Carballeira (2002). Fe concentrations found in spring (555 mg/kg) and autumn (699 mg/kg) in the moss *Hypnum cupressiforme* (Fernández and Carballeira, 2002) were significantly lower than were observed in this study **(Table 4.28)**.

#### *4.3.1.2.9) Mn contamination of moss between site C, Orange Kloof and Newlands forests*

Moss at site C and Newlands forest showed higher Mn concentrations in the wet season and significant differences were found at site C between the dry (77.51

mg/kg) and wet (150.27 mg/kg) season. Brown haze episodes, as a result of mounting anthropogenic activities and vehicle traffic in winter (Wicking-Baird et al., 1997; Norouzi et al., 2017) may have attributed to the higher Mn concentrations observed in the wet season. Orange Kloof, on the other hand yielded higher Mn concentrations in the dry season (445.76 mg/kg), which is also where the overall highest Mn concentrations were found. The vehicle traffic (Schleicher et al., 2011; Chen et al., 2014) from the parking lot and major road junction and a general urban influence have been suggested to enhance Mn concentrations at even the furthest site from a road and even more so than throughfall in a forest (Landre et al., 2010). The bioaccumulation of metals in mosses are also mainly determined by emission sources within study regions (Kleppin et al., 2008; Schroder et al., 2008; Holy et al., 2009). Precipitation from the 'table cloth (Van der Velden, 2017) may also have added to the Mn load in the dry season, because of the wet deposition being a critical scavenging process by which metals are returned to terrestrial or aquatic surfaces (Bacardit and Camarero, 2010). It is, however recommended that the sampling site characteristics (Fernández et al., 2015), physicochemical features of pollutants (Gonzalez and Pokrovsky, 2014) and physiological processes be considered with regard to metal accumulation in moss (Boquete et al., 2014). Concentrations found in spring (143 mg/kg) and autumn (249 mg/kg) in Spain in the moss *Hypnum cupressiforme* was exceeded by the Mn concentrations found in this study (Fernández and Carballeira, 2002) **(Table 4.29)**.

#### *4.3.8.1.2.10) Al contamination of lichen between site C, Orange Kloof and Newlands forests*

Newlands and Orange Kloof forests displayed higher Al concentrations in the wet season and a notable difference between seasons was found at Orange Kloof forest. The wet season concentration (1269.4 mg/kg) was significantly higher than the dry season concentration (671.66 mg/kg). Newlands forest, however displayed the overall highest concentration of 1283.52 mg/kg in the wet season as opposed to the dry season concentration (839.49 mg/kg), which is in accordance with other studies of contaminants showing higher results in samples from forest ecosystems adjacent to industrialized and heavily populated areas (Driscoll et al., 1994; Nakazato et al., 2015) as in the case with Newlands forest around the corner of the City of Cape Town. Lichen morphology is not impacted by the different seasons and accumulation

of pollutants occurs throughout the year (Conti and Cecchetti, 2001). However, the lichens, containing higher metal concentrations in winter is most likely as a consequence of higher pollutant levels in this season, caused by the surge in anthropogenic activities in the colder seasons in major cities (Norouzi et al., 2017). A slight decrease in Al concentration was observed at site C in the wet season, which is quite normal, as elements in lichen are known to leak during rainy cycles (Brown and Brown, 1991; Adamo et al., 2008). The higher concentration at site C in the dry season may to the contrary be due to metals contained in precipitation (Bacardit and Camarero, 2010) from the “table cloth” in summer adding to the existing Al load (Van der Velden, 2017). The concentrations found in this study were comparable with industrial and urban areas (Adamo et al., 2007; Sorbo et al., 2008) as were reported in summer of 1030 mg/kg and autumn of 820 mg/kg by Malaspina et al. (2014) **(Table 4.27)**.

#### *4.3.1.2.11) Fe contamination of lichen between site C, Orange Kloof and Newlands forests*

Orange Kloof and Newlands forests showed higher Fe concentrations in lichen in the wet season, of which a considerable difference was found between seasons at Orange Kloof forest. The Fe concentration of 1119.43 mg/kg in the wet season was almost double the dry season concentration (566.54 mg/kg), but such increases in concentrations are not abnormal during winter brown haze episodes when human activities (Norouzi et al., 2017) increases in colder weather (See et al., 2006). The overall highest Fe concentration was, however found at Newlands forest in the wet (1660.31 mg/kg) season and is also not uncommon as contaminant levels have been found to be higher in samples from forest ecosystems adjoining industrialized and heavily populated areas (Driscoll et al., 1994; Nakazato et al., 2015) such as Cape Town. Significantly lower Fe concentrations of 820 mg/kg in summer and 820 mg/kg in autumn were reported by Malaspina et al. (2014) than were found in this study. The lower Fe concentrations measured at site C in the wet season, may have resulted from the elements leaking in rainy periods (Brown and Brown, 1991; Adamo et al., 2008), as the different seasons is not known to have an impact on the accumulation of metals in lichen (Conti and Cecchetti, 2001). On the other hand, the increase in Fe concentrations at site C in the dry season may also have been

caused by metals in precipitation from the “table cloth” in summer (Van der Velden, 2017) **(Table 4.28)**.

#### *4.3.1.2.12) Mn contamination of lichen between site C, Orange Kloof and Newlands forests*

Site C, displayed higher Mn concentrations in lichen in winter, possibly owing to the brown haze phenomena caused by the escalating anthropogenic activities and vehicular traffic in the colder seasons associated with major cities (Norouzi et al., 2017). Orange Kloof and Newlands forests on the other hand, displayed higher concentrations in the dry season and the highest Mn concentrations were measured at Orange Kloof in the dry (445.44 mg/kg) and significantly lower concentrations in the wet (138.07 mg/kg) season. The lower Mn concentrations in winter could have resulted from excessive rain that causes the elements to leach from the lichen (Brown and Brown, 1991; Adamo et al., 2008), but the results may be considered in reverse, which means the the concentrations were higher in summer. In this way, clarification could be found in metals in precipitation from the “table cloth” in summer that caused the higher Mn concentrations in lichen at this site (Van der Velden, 2017). Mn concentrations of 191 mg/kg in summer and 84 mg/kg autumn were reported by Malaspina et al. (2014). These concentrations are significantly lower than were found in this study **(Table 4.29)**.

#### *4.3.1.2.13) Al contamination of millipedes between site C, Orange Kloof and Newlands forests*

Aluminium concentrations in millipedes were higher in winter at Orange Kloof and Newlands forests. Significant differences were noticed between seasons at Newlands forest, of which the wet season concentration (2101.99 mg/kg) in millipedes was almost four times higher than the dry season concentration (528.41 mg/kg). Higher pollutant levels are found in the winter season, consequently causing the pollutant laden brown haze, as a result of escalating human activities and vehicle traffic (Norouzi et al., 2017). The results obtained from the millipede Al concentrations correlates with the soil and leaf litter concentrations, which is in agreement with reports from Da Silva Souza et al. (2014) that higher metal concentrations in millipedes are caused by consumption of polluted litter in a polluted soil. Similarly, Hopkin (1989) found that earthworms that feed on soil organic matter,

causes enhanced toxic element concentrations in them as a result of the toxic elements that are associated with organic components of soil. Site C presented higher Al concentrations in the dry season (3042.68 mg/kg), which was significantly different to the lower wet season concentration of 1072.29 mg/kg and was also the overall highest concentration found in millipedes. Site C is more prone to contamination and accumulation of metals from the atmosphere (Guney et al., 2010; Ali and Malik, 2011) and transported pollutants from the Cape Flats by the South Easter wind (Sen et al., 2017), which then explains the higher Al concentrations found in soil, leaf litter and millipedes in this season.

Even though the Al concentrations in soil, leaf litter and millipedes correlated with each other, there are many influencing factors that determine the metal concentrations in invertebrates, such as physiological conditions, feeding habits, breeding, sex, development stage, season (Jelaska et al., 2007; Butovsky, 2011) and excretion ability of the bodies (Janssen et al., 1991; Kramarz, 1999; Avgin and Luff, 2000). The authors, Simon et al. (2016) found that overwintering species of ground beetles accumulate less toxic amounts of metals. The male and female Al concentrations in ground beetles measured, ranged between 48.2 and 50.5 mg/kg and was thus considered poor metal accumulators. The reverse was found with pill millipedes who seemed to exhibit good accumulation abilities **(Table 4.27)**.

#### *4.3.1.2.14) Fe contamination of millipedes between site C, Orange Kloof and Newlands forests*

Iron concentrations in millipedes were higher in the Orange Kloof and Newlands forests in the wet season, which could be by cause of the excessive pollution that occurs in winter in Cape (CMA, 2015), resulting from increased anthropogenic activities in colder weather (Allen et al., 2001; Handler et al., 2008; (Schleicher et al., 2011; Chen et al., 2014). The highest concentrations in millipedes were found at Newlands forest, as well as a significant difference observed between the seasons, of which the wet season concentration (2890.29 mg/kg) was three times higher than the dry season concentration (922.63 mg/kg). Newlands forest is situated adjacent to the City of Cape Town, which makes this forest more susceptible to higher contaminant levels, as were reported from results obtained in samples from forest ecosystems adjoining industrialized and heavily populated areas (Driscoll et al.,

1994; Nakazato et al., 2015). On the other hand, the Fe concentrations in millipedes at site C was significantly higher in the dry season (1556.14 mg/kg) as opposed to the wet season (623.14 mg/kg), which again follows the same pattern as were found with soil and leaf litter, being higher at this site, which is also more exposed to wind and pollutants from the Cape Flats (Sen et al., 2017; Van der Velden, 2017). Soil and leaf litter patterns in this study corroborate with the patterns found in the millipede concentrations at the same study areas, which is evident of the relationship between litter chemistry and the millipedes in their immediate environment (Ashwini and Sridhar, 2008). This is also true for earthworms feeding on soil organic matter, thus exerting higher toxic element concentrations as it is associated with the organic components of soil (Hopkin, 1989). Thus, the polluted litter consumed by the millipedes (Da Silva Souza et al., 2014) may have enhanced their Fe concentrations as a result of the already high Fe concentrations in the soil and leaf litter in the respective seasons.

Be that as it may, other factors does play a role in the metal accumulation, such as the sex and feeding habits of the respective invertebrates. Over wintering species of ground beetles also plays a major role in metal accumulation, as they naturally accumulate less toxic amounts of metals, thus leading to lower metal concentrations. The authors reported Fe concentrations found separately in males and female ground beetles in urban areas, which ranged between 43.7 and 75.7 mg/kg Simon et al. (2016). These concentrations were exceeded by the Fe concentrations found in millipedes in this study. They also reported these beetles to be poor accumulators of metals. In this study, however, pill millipedes were found to be good accumulators of metals **(Table 4.28)**.

#### *4.3.1.2.15) Mn contamination of millipedes between site C, Orange Kloof and Newlands forests*

Higher Mn concentrations in millipedes were found in the wet season at site C, Orange Kloof and Newlands forests and a significant difference in millipedes between seasons were found at Newlands forest. The wet season concentration (129.02 mg/kg) was twice as high as the dry season concentration of 58.31 mg/kg. Site C also showed a significant difference between seasons in millipedes with a wet season concentration of 47.1 mg/kg verses a dry season concentration of 33.28

mg/kg. Except for Orange Kloof, the leaf litter and soil Mn concentrations correlate with the concentrations found in millipedes in both seasons, which may be due the polluted litter that the millipedes feed on causing higher metal accumulation (Da Silva Souza et al., 2014). It is also confirmed by Hopkin (1989) that organic matter is associated to the organic components of soil. Factors that may have influenced the Mn concentrations are overwintering habits, sex and food preferences. Less toxic amounts of metals, are accumulated by overwintering species, which leads to lower metal concentrations. Pill millipedes were thus considered to be good accumulators of metals. Mn concentrations for males and female ground beetles in their study ranged between 46.7 and 18.3 mg/kg, which was significantly lower than were measured in this study (Simon et al., 2016) **(Table 4.29)**.

### 4.3.2 Biomarkers of oxidative stress

#### 4.3.2.1) Seasonal variations in antioxidant levels and biomarkers of oxidative damage found at Orange Kloof forest

##### *4.3.2.1.1) tGSH levels in moss at Orange Kloof forest*

Oxidative stress in forest ecosystems are largely driven by climate related factors such as, extreme temperatures, drought and high irradiance, as well as well-defined wet and dry periods similar to Cape Town's summer and winter seasons. However, the additional exposure to complex mixtures of anthropogenic air pollutants are also known to induce oxidative stress to these ecosystems (Tausz et al., 1998, 2007a, b; Bussotti, 2008; Encyclopedia Britannica, 2017). With reference to the climatic conditions, it should be noted that moss are more tolerant to shady conditions, as it prefers a moister environment (Düll, 1991). The moisture percentage calculated from the moss samples, however did not disclose any abnormally moist or dry conditions in these organisms in both seasons **(Tables 4.31, 4.37)**. On the other hand, a possible correlation was observed with the naked eye between the concentrations of the metals Al, Fe and Mn and the glutathione (tGSH) levels in moss in their respective seasons **(Figs 4.31, 4.32, 4.33)**, suggesting a possible anthropogenic input (Tausz et al., 1998, 2007a, b; Bussotti, 2008).

The tGSH levels measured in moss at this forest presented significant differences between seasons at sites C, 1 and 2, of which sites 1 (50.42 µmol/g) and 2 (90.21 µmol/g) showed higher concentrations in moss in the dry season, as opposed to the lower wet season concentrations at the same sites: 1 (20.47 µmol/g) and 2 (29.85 µmol/g). The tGSH concentrations measured in moss at site 2 in the wet season were also the overall highest found in moss at this forest. Conversely, site C displayed higher tGSH concentrations in the wet season (51.01 µmol/g) as opposed to the lower dry season concentration (14.36 µmol/g) and a similar higher tGSH concentration in the wet season was found at site 3 (27.78 µmol/g). The increased tGSH activities observed in moss demonstrate an effort of the antioxidant defense system to prevent peroxidation effects in moss cells in their respective seasons (Wilce and Parker, 1994; Van Der Oost et al., 2003; Ezemonye and Ikpesu, 2011; Paulino et al., 2012) and the decreases in tGSH levels noticed at those sites in the moss, more than likely points to tissue damage in moss that have already occurred, also in their respective seasons. Damages such as these generally result from moss being subjected to chronic toxicant exposure and both the increases and decreases in the tGSH levels in both seasons, thus suggests that an overproduction of reactive oxygen species (ROS) in moss cells have occurred (Loguercio et al., 1996; Barbaro et al., 1999).

Looking more closely at the results of higher tGSH concentrations found in moss in winter at sites C and 3 in conjunction with the higher Al and Fe concentrations measured in moss at these sites, also in winter **(Figs 4.31, 4.32)** it appears that this antioxidant defense system was activated to prevent peroxidation damage in moss cells (Wilce and Parker, 1994; Van Der Oost et al., 2003; Ezemonye and Ikpesu, 2011; Paulino et al., 2012) possibly in defense of the metals, Al and Fe (Ercal et al., 2001; Koivula and Eeva, 2010; Sanchez-Virosta et al., 2015). The fact that lower tGSH concentrations, concurrent with lower metal concentrations were measured in the dry season at the same sites, suggests that damage in the moss tissues have occurred (Loguercio et al., 1996; Barbaro et al., 1999), however at lower metal concentrations. In view of this a deduction can be made that the cellular antioxidant defense system was more efficient in its protection of the moss cells in the wet season in the moss's preferred environment. The higher Al and Fe concentrations

found in moss at sites 1 and 2 **(Figs 4.31, 4.32)** concurrent with the higher tGSH concentrations in that season, however suggests that the antioxidant defense system in this case was also efficient in protecting the moss cells, even in the dry season. This may be due to the fact that the increasing levels of ROS in tissues of the organisms can be controlled by activating the cellular antioxidant defense system (Gomes et al., 2014) and tGSH performs an essential role in neutralizing and/or detoxifying the oxidative damage caused by ROS (Valko et al., 2006; Regoli et al., 2011).

tGSH levels in moss were also found to be higher than the MDA content in both seasons, also suggesting that this non-enzymatic antioxidant is successfully protecting cells against oxidative stress by means of scavenging ROS (Winston and Di Giulio, 1991; Choudhury and Panda, 2004, 2005; Singh et al., 2006) and is also sufficient in the process of metal detoxification (Sanita di Toppi et al., 2008). Organisms have the ability to eliminate ROS and also to protect cells and tissues from injury and dysfunction by way of antioxidant defense systems (Sugiyama, 1994). Similar results of higher tGSH concentrations as opposed to the lower MDA content in moss have been reported in other studies after moss have been exposed to the metals Pb and Cr, of which the tGSH levels ranged between 25 µmol/g to 38 µmol/g (Choudhury and Panda, 2005). Even though a comparison cannot be made with regard to the particular metals, as different metals were measured in this study, it should be noted that the tGSH levels in moss found at Orange Kloof forest, which ranged from 14.36 µmol/g to 90.21 µmol/g in a field situation is still higher than were found in the above mentioned study, which may propose that in this study tGSH was displaying an effort to prevent peroxidation effects (Wilce and Parker, 1994; Van Der Oost et al., 2003; Ezemonye and Ikpesu, 2011; Paulino et al., 2012) and in their study it would seem that damage to moss tissues have already occurred (Loguercio et al., 1996; Barbaro et al., 1999). It should also be noted that metal retention capacities vary with different moss species and therefore also respond differently to metal stress (Bleuel et al., 2005; Sun et al., 2007) **(Fig 4.63)**.

#### *4.3.2.1.2) MDA content in moss at Orange Kloof forest*

MDA content measured in moss were higher at sites C and 1 in the dry season. Significant differences were, however found between seasons at site 3, of which the

highest concentrations were measured in the wet season and the overall highest MDA concentrations was found at site 2 (0.94 µmol/g). The lower MDA content measured in moss in their respective seasons is still an indication that lipid peroxidation (LPO) have occurred. This is due to the fact that MDA, being a decomposition product of polyunsaturated fatty acids, was produced during peroxidation of membrane lipids (Mittler, 2002). Malondialdehyde (MDA) is formed as a result of oxidation processes (Caňas et al., 1997; Gonsález and Pignata, 1997; Majumder et al., 2013) and the amount formed is based on the estimation of the MDA content, derived from the reaction between MDA and thiobarbituric acid (Buege and Aust 1978). Therefore, the amount of MDA formed due to oxidation processes is, as a rule used as a parameter, which signifies the damage induced by oxidant atmospheric pollutants on organisms (Caňas et al., 1997; Gonsález and Pignata, 1997; Majumder et al., 2013). Sun et al. (2011), in their study found significantly higher MDA contents of 10 µmol/g to 30 µmol/g after exposing moss to Pb and Ni, than were found in this study of 0.27 µmol/g to 0.95 µmol/g. The higher MDA concentration demonstrates exposure of the organisms to high concentrations of toxic elements (Bačkor et al., 2010; Pisani et al., 2011), such as metals, which may cause lipid peroxidation by means of the generation of free radicals (Choudhury and Panda, 2005). If MDA accumulation in mosses are induced or increased it demonstrates considerable lipid peroxidation in moss cells, which is an indication of oxidative stress (Ohkawa et al., 1979; Dazy et al., 2009). The lower MDA content found in this study compared to Sun's study, therefore is an indication that lipid peroxidation have indeed occurred (Mittler, 2002) and is known to occur even at the lowest levels of pollution (Ahmed et al., 2013; Martins and Costa, 2015). LPO is the most common effect of ROS (Ahmad and Ahmad, 2015; Javed et al., 2015) and the damage incurred seems to be minimal in comparison with Sun's study (Winston and Di Giulio, 1991; Choudhury and Panda, 2004, 2005; Singh et al., 2006).

Hermenean et al. (2015) found that moss in an urban environment experienced stress when the MDA content remained higher in the urban environmental sites, compared to the control site, with no significant differences detected between the sites. This finding was ascribed to moss being able to cope with the environmental stress. In this study, the MDA content at site C was similar to slightly lower than the concentrations found at the other sites, even though site C displayed the highest Al

and Fe concentrations **(Figs 4.31, 4.32),** which may similarly imply that moss at these sites were coping with the stress incurred by the metals **(Fig 4.64)**.

#### *4.3.2.1.3) tGSH levels in lichen at Orange Kloof forest*

tGSH levels in lichen in both seasons were too low for detection and gives the impression of depletion, with the exception of lichen at site 1 (3 µmol/g) in the wet season. tGSH is an extremely important cell constant, as it directly quenches the reactive hydroxyl radicals (Tabrez and Ahmad, 2009). Depleted tGSH levels, therefore could increase sensitivity of cells to metal toxicity (Valko et al., 2005; Cohen et al., 2006) and the depleted antioxidant activity then causes cells to be susceptible to ROS-mediated stress (Jerome et al., 2017). Metals have proven to generate ROS, as well as deplete the major antioxidants of cells such as tGSH, (Ercal et al., 2001; Koivula and Eeva, 2010; Sanchez-Virosta et al., 2015). In all likelihood, the undetected tGSH levels in the lichen tissue could be the result of damage, brought on by chronic toxicant exposure (Loguercio et al., 1996; Barbaro et al., 1999). This assumption may be made in light of the fact that the Al, Fe and Mn concentrations **(Figs 4.34, 4.35, 4.36)** measured in lichen in this study can be considered as relatively high, due to the fact the concentrations were similar to concentrations found at urban and industrial sites in various studies (Adamo et al., 2007; Sorbo et al., 2008; Malaspina et al., 2014). It may further be suggested that the concentration level of these metals are able to cause excessive ROS production and induce oxidative stress in association with lipid peroxidation in lichen (Cakmak and Horst, 1991; Foyer et al., 1994; Jing et al., 2009; Sen et al., 2014). The slight increase in tGSH levels at site 1 may signify the activation of the cellular antioxidant defense system in an effort to control the increasing levels of ROS in the tissues of the organisms (Gomes et al., 2014). tGSH is essential in neutralizing and detoxifying oxidative damage inflicted by ROS (Valko et al., 2006; Regoli et al., 2011). This occurs in order to protect cells and tissues from injury and dysfunction (Sugiyama, 1994).

Both the increase, decrease and depletion in the tGSH levels, nevertheless points to the overproduction of ROS, which could have resulted from either the differences in temperatures, experienced in the dry and wet seasons (Tausz et al., 1998, 2007a, b; Bussotti, 2008) or metal contamination (Ercal et al., 2001; Koivula and Eeva, 2010;

Sanchez-Virosta et al., 2015). Lichen is tolerant to warm and dry habitats (Wirth, 1991), being able to withstand drought (Düll, 1991), but the temperatures recorded in either seasons were not abnormally low or high **(Tables 4.4, 4.17)**. In contrast to moss, the tGSH levels measured in lichen in this study was lower than the MDA content in lichen and concurs with findings from Cuny et al. (2003) who exposed lichen to the metals Cd, Pb and Zn, which presented lower tGSH levels (0.13 µmol/g to 0.49 µmol/g) as opposed to the higher MDA content. The significantly lower tGSH levels measured in lichen in this current study (0 µmol/g to 3 µmol/g) in comparison with Cuny's study signifies more damage in the lichen tissue in this current study (Loguercio et al., 1996; Barbaro et al., 1999)**.**

***4.3.2.1.4) MDA content in lichen at Orange Kloof forest***

MDA content presented higher concentrations in lichen in winter at all the sites, with the overall highest concentration found at site C (2.2 µmol/g). The dry season MDA concentration at site C (0.1 µmol/g) was lower than the wet season concentration. When the MDA content increases, it indicates exposure of the organisms to high concentrations of toxic elements (Bačkor et al., 2010; Pisani et al., 2011), of which metals generally causes lipid peroxidation through the production of free radicals (Choudhury and Panda, 2005). The depletion of antioxidants, as were found with tGSH levels in lichen, leads to oxidative stress and LPO thus occurs due to the generation of hydroxyl radicals, which is evident in the higher levels of LPO. Therefore, the increase in LPO levels proposes that excess in production of ROS occurred, due to a less effective antioxidant defense mechanism (Velma and Tchounwou, 2010). LPO is the most common effect of ROS (Ahmad and Ahmad, 2015; Javed et al., 2015). Organisms are therefore subjected to oxidative stress by cause of the low levels of antioxidants and high LPO levels, which can be regarded as one of the biomarkers for cell death (Dreiem et al., 2005; Sayes et al., 2005). The effect of toxicants on MDA content in lichens are as a matter of fact, well known (Bačkor et al., 2009; 2010; Unal et al., 2010).

In this study higher MDA content was measured at sites 1, 2 and 3 in winter where the higher Al and Fe concentrations were found in winter. There was a similarly found in the higher Al and Fe concentrations at site C in the dry season and the higher MDA content, also in the dry season **(Figs 4.34, 4.35)**. It may be proposed

that metal contamination was instrumental in causing lipid peroxidation through the generation of free radicals (Choudhury and Panda, 2005). Aluminium have been reported to induce MDA accumulation, as well as increase the MDA content in lichen (Unal et al., 2010). In addition LPO in lichen have been reported at sites facing landfills, which were incidentally also lower in lichen diversity, which already points to a polluted environment (Garty et al., 2000). Similar reports were filed of induced oxidative stress and increased MDA concentrations in lichens in urban areas (Caňas et al., 1997; Majumder et al., 2013). Furthermore, MDA concentrations were also found to be the highest in major transport and industry sites (Gonsález et al., 1996). MDA concentrations in lichen in this study were higher than the tGSH levels, which is in accordance with findings from Cuny et al. (2003) who exposed lichen to the metals Cd, Pb and Zn, which presented lower tGSH levels and higher MDA content (103.04 µmol/g to 262.21 µmol/g). MDA concentrations in this study ranged from 0.1 µmol/g to 2.2 µmol/g and is minimal in comparison with the above mentioned study, suggesting minor peroxidation of membrane lipids **(Fig 4.65)**.

#### *4.3.2.1.5) tGSH levels in millipedes at Orange Kloof forest*

Increased tGSH levels in millipedes were noticed in summer at all the sites. Site C showed the highest tGSH concentration of 542.81 µmol/g in this season and is also the site where the highest Al and Fe **(Figs 4.37, 4.38)** concentrations were measured, suggesting that these metals could have played a role in the induction of ROS in these organisms (Ercal et al., 2001; Koivula and Eeva, 2010; Sanchez-Virosta et al., 2015). Similar findings were reported in studies done with ribbed mussels where the highest tGSH content in both the digestive gland and gills were found in the mussels from the polluted sites. More studies confirmed these findings of increased tGSH levels in bivalve mollusks that were exposed to metals (Yan et al., 1997; Cheung et al., 2002; Irato et al., 2003).

The higher tGSH levels of this antioxidant shows that it is successfully scavenging ROS in an attempt to protect cells against oxidative stress (Winston and Di Giulio, 1991; Choudhury and Panda, 2004, 2005; Singh et al., 2006) and at the same time detoxifying metals (Sanita di Toppi et al., 2008). Similar increased tGSH levels were also reported in tGSH activities in crab tissue in aid of preventing peroxidation effects (Wilce and Parker, 1994; Van Der Oost et al., 2003; Ezemonye and Ikpesu, 2011;

Paulino et al., 2012), as well as with mussels. It was, however found with mussels that the defense systems, although activated, are not effective in actually preventing oxidative damage from occurring (Di Salvatore et al., 2013). The lower tGSH levels, as were found in millipedes in the wet season then may signify that tissue damage have already occurred in the millipedes, most likely as a result of exposure to toxicants over a period of time (Loguercio et al., 1996; Barbaro et al., 1999). Both the rise and fall of tGSH levels may point to an overproduction of ROS that may even have occurred from extreme temperatures (Tausz et al., 1998, 2007a, b; Bussotti, 2008). This may be applicable to the pill millipedes, especially in the dry season, as these invertebrates prefer a moist environment (SANBI, 2016). Increased tGSH levels in the millipedes were in fact measured in the dry season, even though the differences in concentrations between the seasons were not significant. It has, however been found in studies with mussels that the different seasons caused no significant differences int tGSH levels (Di Salvatore et al., 2013).

It was not possible to make direct comparisons with pill millipedes, due to the lack of literature found on these millipedes. Even literature on soil invertebrates with regard to this type of study were scarce. A recent study done with earthworms, nonetheless determined that the antioxidant system was indeed activated, when the earthworms were exposed to silver nanoparticles, though not enough to prevent oxidative damage (Gomes et al., 2015). Also, exposure studies of pesticides on earthworms have been reported to increase the levels of ROS in earthworms after exposure to toxic chemicals (Xu et al., 2013; Liu et al., 2014; Wang et al., 2015). tGSH levels measured in the digestive glands of land snails contaminated by Fe, Cu, Zn, Cd, Pb and Ca ranged between 25.05 µmol/g and 53.81 µmol/g at non-polluted and polluted sites, which is much lower than the concentrations found in the millipedes at Orange Kloof forest and in both seasons, which ranged from 298.57 µmol/g to 542.81 µmol/g. These findings may indicate that the antioxidant system is successfully scavenging ROS in an effort to protect cells against oxidative stress (Winston and Di Giulio, 1991; Choudhury and Panda, 2004, 2005; Singh et al., 2006) and more so in this study than the above mentioned studies **(Fig 4.66)**.

#### *4.3.2.1.6) MDA content in millipedes at Orange Kloof forest*

MDA content in millipedes showed higher results in the wet season, but no significant differences were noticed between the seasons. Similar findings were reported by Di Salvatore et al. (2013) with mussels, not being affected by the different seasons, but rather by highly polluted sites such as harbor areas. The MDA concentration at site 3 (3.16 µmol/g) in the wet season was the highest concentration measured at these sites. Increases in MDA content, such as were observed in the wet season, indicates that the organisms were exposed to elevated concentrations of toxic elements (Bačkor et al., 2010; Pisani et al., 2011), causing lipid peroxidation by means of the generation of free radicals (Choudhury and Panda, 2005), which is an indication of oxidative stress (Ohkawa et al., 1979; Dazy et al., 2009). The Al, Fe and Mn concentrations **(Figs 4.37, 4.38, 4.39)** measured at the sites may have been instrumental in causing the ROS production and induced oxidative stress, associated with peroxidative damage of membrane lipids in the organisms (Cakmak and Horst, 1991; Foyer et al., 1994; Jing et al., 2009; Sen et al., 2014). Examples of metal exposure that induces oxidative stress in, especially aquatic invertebrates are numerous. These findings are based on an increase in lipid peroxidation products (MDA) in, for example clams (Geret et al., 2002), vent mussels (Company et al., 2004) and ribbed mussels. Evidence of increased oxidative damage to lipids, due to metals have further been confirmed in laboratory experiments (De Almeida et al., 2004) and field studies (Belcheva et al., 2011). By contrast, MDA content in snails were measured at 45.47 µmol/g to 143.26 µmol/g, which were much higher than were found in MDA content measured in millipedes in this study of 0.34 µmol/g to 3.17 µmol/g in both seasons, suggesting minimal peroxidative damage in millipedes in this study in comparison with the above mentioned study. tGSH levels are also generally found to be lower in snails, as opposed to the MDA levels, but seems to be a natural response for snails (El-Shenawy et al., 2012). The reverse was, however found in this study and various other soil and aquatic invertebrates **(Fig 4.67)**.

### 4.3.2.2) Seasonal variations in antioxidant levels and biomarkers of oxidative damage found at Newlands forest

#### *4.3.2.2.1) tGSH levels in moss at Newlands forest*

Higher tGSH levels in moss were observed in the dry season at sites 1, 2 and 3, although the differences between summer and winter were insignificant. Site 3 presented the highest tGSH concentration (92.27 µmol/g) and site 2 the second highest concentration (78.48 µmol/g) in this season. The lowest tGSH concentration was found at site 3 (14.07 µmol/g) in the wet season. The higher tGSH activities in the moss cells at all the sites in summer indicates an attempt of the cellular antioxidant defense system to prevent peroxidation effects (Wilce and Parker, 1994; Van Der Oost et al., 2003; Ezemonye and Ikpesu, 2011; Paulino et al., 2012). The lower tGSH levels on the other hand, is evident of damage in moss tissues and may speak of persistent exposure to toxic pollutants. Be that as it may, both the increases, as well as the decreases in tGSH levels observed in moss at the Newlands forest sites proposes that an overproduction of ROS in moss cells have occurred (Loguercio et al., 1996; Barbaro et al., 1999).

In an attempt to pinpoint the particular reason for the overproduction of ROS in forest ecosystems, one defaults immediately to climate related factors. The wet winters and dry summer seasons, such as in Cape Town (Encyclopedia Britannica, 2017), as well as extreme temperatures, drought and high irradiance are known to be the main factors involved in the occurrence of oxidative stress in forest ecosystems, but the extra exposure to complex mixtures of anthropogenic air pollutants have also been reported to induce oxidative stress to these ecosystems (Tausz et al., 1998, 2007a, b; Bussotti, 2008). Specifically with regard to moss, the high summer temperatures, cannot totally be excluded as a possible influencing factor in the results obtained in this study, (Tausz et al., 1998, 2007a, b; Bussotti, 2008), as moss is shade tolerant, preferring a moister environment (Düll, 1991). However, no extreme or untypical moisture percentages were observed in moss samples in either winter or summer **(Tables 4.33, 4.39)**. Further investigation into the anthropogenic pollutant side of the matter, in particular the metal concentrations found at sites 1, 2, 3 reveals that the highest Al, Fe and Mn concentrations **(Figs 4.46, 4.47, 4.48)** were found in the wet season, as opposed to the lowest tGSH levels found in the same season, suggesting

that damage in moss cells have occurred, most likely by cause of those metals (Loguercio et al., 1996; Barbaro et al., 1999). To further substantiate these findings, Al, Fe and Mn are known to cause excessive ROS production and induce oxidative stress associated with lipid peroxidation (Cakmak and Horst, 1991; Foyer et al., 1994; Jing et al., 2009; Sen et al., 2014).

tGSH levels in moss were also found to be higher than the MDA content in both seasons at all the sites and similar results were found in other studies (Choudhury and Panda, 2005). This in actual fact means that the antioxidant, tGSH is working hard to protect cells against oxidative stress by scavenging ROS (Winston and Di Giulio, 1991; Choudhury and Panda, 2004, 2005; Singh et al., 2006). It is also an indication of tGSH successfully detoxifying metals (Sanita di Toppi et al., 2008). Organisms, by means of antioxidant defense systems are well equipped to eliminate ROS and protect cells and tissues from injury and dysfunction (Sugiyama, 1994). Increased levels of ROS in the tissues of the organisms can therefore be controlled by activating the cellular antioxidant defense system (Gomes et al., 2014). A paper in the literature was found of tGSH levels in moss that, after exposure to Pb and Cr ranged between 25 µmol/g to 38 µmol/g (Choudhury and Panda, 2005). These concentrations, were lower than were found in the Newlands forest moss that ranged from 14.08 µmol/g to 92.27 µmol/g. This may indicate that tGSH was exhibiting an effort to prevent peroxidation effects in moss cells in this study (Wilce and Parker, 1994; Van Der Oost et al., 2003; Ezemonye and Ikpesu, 2011; Paulino et al., 2012), but propose that damage to moss tissues have already occurred in their study (Loguercio et al., 1996; Barbaro et al., 1999). Metal retention capacities do differ with various moss species and it should be kept in mind that they therefore also respond differently to metal stress (Bleuel et al., 2005; Sun et al., 2007) **(Fig 4.68)**.

#### *4.3.2.2.2) MDA content in moss at Newlands forest*

MDA content was measured higher in moss at sites C and 3 in the dry season and higher at sites 1 and 2 in the wet season. The overall highest MDA concentration was found at site 1 (0.53 µmol/g), of which this site also displayed a significant difference between seasons. The lowest MDA concentration was also found at this site in the dry season (0.04 µmol/g). In addition, sites 1 and 2 displayed the highest Al, Fe and Mn concentrations in moss in the wet season **(Figs 4.46, 4.47, 4.48)** and

the assumption can thus be made that these metals were functional in causing a rise in the MDA content and consequent lipid peroxidation in the wet season (Choudhury and Panda, 2005; Ohkawa et al., 1979; Dazy et al., 2009). MDA content measured in mosses, albeit low still shows lipid peroxidation, because it is a decomposition product of polyunsaturated fatty acids, which is produced during peroxidation of membrane lipids (Mittler, 2002). The amount of MDA formed as a result of oxidation processes (Caňas et al., 1997; Gonsález and Pignata, 1997; Majumder et al., 2013) is determined by estimating the content of MDA that is derived from the reaction between MDA and thiobarbituric acid (Buege and Aust 1978). The amount of MDA formed as a result of oxidation processes is, therefore generally used as a parameter, which demonstrates the damage induced by oxidant atmospheric pollutants on organisms (Caňas et al., 1997; Gonsález and Pignata, 1997; Majumder et al., 2013). The higher MDA content found in the wet season at sites 1 and 2 and dry season at sites 1 and 3, thus imply that moss at those sites and seasons incurred more damage than at the sites and seasons where the lower concentrations were measured.

In the literature it was found that Pb and Ni exposed moss presented MDA contents of 10 µmol/g to 30 µmol/g (Sun et al., 2011), which is significantly higher than the MDA concentrations found in moss in this study of 0.04 µmol/g to 0.54 µmol/g. The increases found in MDA content in Sun's study shows considerable lipid peroxidation in moss cells, which is an indication of oxidative stress (Ohkawa et al., 1979; Dazy et al., 2009). In contrast, the lower MDA concentrations measured in this study, points to the occurrence of lipid peroxidation, but in to a lesser degree (Mittler, 2002). The fact is that ROS occurs even when pollution levels are low (Ahmed et al., 2013; Martins and Costa, 2015) and LPO is the most common consequence of ROS (Ahmad and Ahmad, 2015; Javed et al., 2015) **(Fig 4.69)**.

#### *4.3.2.2.3) tGSH levels in lichen at Newlands forest*

tGSH levels in lichen in both the dry and wet seasons were totally depleted. It is not uncommon for metals to generate ROS and cause depletion of the major antioxidants of cells such as tGSH, (Ercal et al., 2001; Koivula and Eeva, 2010; Sanchez-Virosta et al., 2015). Depleted tGSH levels in tissue is the result of damaged tissue, generally brought on by persistent exposure to toxicants (Loguercio

et al., 1996; Barbaro et al., 1999). Extreme temperatures in the dry and wet seasons (Tausz et al., 1998, 2007a, b; Bussotti, 2008) may be an unlikely explanation as lichen is tolerant to warm and dry habitats (Wirth, 1991) and no untypical high or low temperatures were experienced in either seasons **(Table 4.9, 4.22)**. The concentrations of the metals, Al, Fe and Mn **(Figs 4.49, 4.50, 4.51)** in lichen were in fact comparable to metal concentrations in lichen found at urban and industrial sites (Adamo et al., 2007; Sorbo et al., 2008; Malaspina et al., 2014). In addition these metals have proven to cause excessive ROS production and induce oxidative stress associated with lipid peroxidation in membrane lipids in organisms (Cakmak and Horst, 1991; Foyer et al., 1994; Jing et al., 2009; Sen et al., 2014). It may therefore be proposed that metal contamination (Ercal et al., 2001; Koivula and Eeva, 2010; Sanchez-Virosta et al., 2015) were instrumental in the induction of ROS and eventual depletion of the tGSH levels in lichen at this forest.

#### *4.3.2.2.4) MDA content in lichen at Newlands forest*

Lichen displayed increased MDA content in the wet season at all the sites, with site 2 (1.86 µmol/g) displaying the overall highest MDA concentration. The increased MDA content suggests that the organisms have been exposed to high toxic element concentrations (Bačkor et al., 2010; Pisani et al., 2011), that includes metals, which is known to cause lipid peroxidation through the generation of free radicals (Choudhury and Panda, 2005). A visual observation was that, except for site 3, the higher MDA content measured in winter seemed to show a relationship with the high Al and Fe concentrations, also measured in winter. The differences in Al and Fe concentrations between the seasons were, however minimal **(Figs 4.49, 4.50)**. Al is known to induce MDA accumulation and increase MDA content in lichen (Unal et al., 2010), and it can therefore be proposed metal contamination may have been functional in causing lipid peroxidation (Choudhury and Panda, 2005).

tGSH levels in lichen were depleted and such depletion of antioxidants leads to oxidative stress and LPO. This is known to occur when hydroxyl radicals are produced, which subsequently leads to the higher LPO levels. The increase in LPO levels thus indicates an excess in production of ROS, due to a less effective antioxidant defense mechanism (Velma and Tchounwou, 2010). Organisms are thus subjected to oxidative stress due to low levels of antioxidants. High LPO levels can

as such be regarded as one of the biomarkers for cell death (Dreiem et al., 2005; Sayes et al., 2005). To further substantiate this, the lichen samples showed higher MDA content and lower tGSH levels, which is yet another indication of damage to cellular membranes and lipid peroxidation (Mittler, 2002) that occurred in lichen in both seasons. These findings are also concurrent with findings from Cuny et al. (2003) who exposed lichen to the metals Cd, Pb and Zn, which presented lower tGSH levels and higher MDA content (103.04 µmol/g to 262.21 µmol/g). Much lower MDA concentrations were measured in this study (0.1 µmol/g to 2.2 µmol/g), suggesting minor lipid peroxidation in lichen compared to the lichen in the study done by Cuny et al. (2003). Garty et al. (2000) reported LPO in lichen correlating with sites facing landfills that were lower in lichen diversity, which in itself is a sign of a polluted environment. Studies also confirm that polluted environments induced oxidative stress in lichens and increased MDA concentrations in lichens have been measured in urban areas (Caňas et al., 1997; Majumder et al., 2013). In addition, MDA concentrations were also found to be the highest in large transport and industry sites (Gonsález et al., 1996) **(Fig 4.70)**.

#### *4.3.2.2.5) tGSH levels in millipedes at Newlands forest*

tGSH content in millipedes showed higher levels in summer at all the sites. Significant differences were measured in tGSH concentrations between seasons at site 1 (482.79 µmol/g), as opposed to the lower wet season concentration (138.42 µmol/g), as well as site 2 (589.4 µmol/g) in contrast with the lower wet season concentration (174.89 µmol/g). Site 2 in summer displayed the overall highest tGSH concentrations. In view of the above, Al, Fe and Mn concentrations were all higher in the wet season **(Figs 4.52 to 4.54)** and the tGSH levels were found lower in the wet season. The lower tGSH levels, thus implies that damage to tissues in millipedes have occurred at Newlands forest in that season, possibly due to the chronic exposure to those metals (Loguercio et al., 1996; Barbaro et al., 1999), subsequently causing the induction of ROS in these organisms (Ercal et al., 2001; Koivula and Eeva, 2010; Sanchez-Virosta et al., 2015). Exposure to organisms inaugurates the increase in tGSH content, as were found in studies done with ribbed mussels collected from polluted sites and other bivalve mollusks that were exposed to metals (Yan et al., 1997; Cheung et al., 2002; Irato et al., 2003). Significantly lower tGSH levels were measured in the digestive glands of land snails contaminated by Fe, Cu,

Zn, Cd, Pb and Ca than were found in the pill millipedes at Newlands forest. The tGSH concentrations in snails ranged between 25.05 µmol/g and 53.81 µmol/g at non polluted and polluted sites, while tGSH levels in the millipedes in this study ranged from 138.42 µmol/g to 589.4 µmol/g. Against this background, the non-enzymatic antioxidant in this study seems to successfully scavenge ROS and protect cells against oxidative stress (Winston and Di Giulio, 1991; Choudhury and Panda, 2004, 2005; Singh et al., 2006), as well as detoxifying metals, which is clear from the higher tGSH levels measured in the millipedes (Sanita di Toppi et al., 2008). Similar increases in tGSH activities in crab tissue have been found, suggesting an effort to prevent the effects of peroxidation (Wilce and Parker, 1994; Van Der Oost et al., 2003; Ezemonye and Ikpesu, 2011; Paulino et al., 2012).

Oxidative damage cannot always be prevented and this is evident in studies done with mussels, where the defense systems were not all that effective to stop the damage from occurring (Di Salvatore et al., 2013). When tissue damage then have actually taken place, possibly due to being exposed to toxicants for a period of time, it is normally evident in the lower tGSH levels measured in the organisms (Loguercio et al., 1996; Barbaro et al., 1999) as were found in millipedes in the wet season. Climate and extreme temperatures may also have been partly responsible for both the lower and higher tGSH levels and the overproduction of ROS (Tausz et al., 1998, 2007a, b; Bussotti, 2008). With pill millipedes one would assume the dry season to play a role, as pill millipede habitats are characterized by moist soil and thick layers of leaf litter, where they were found in abundance during the sampling periods (SANBI, 2016). It has, however been said that seasons did not present significant differences in tGSH levels, as were found with mussels (Di Salvatore et al., 2013). Direct comparisons with pill millipedes could not be made, due to the absence of literature of this nature. It was, however reported that the antioxidant system in earthworms, exposed to silver nanoparticles were activated, but was unsuccessful in preventing oxidative damage from occurring, as were found with millipedes in this study (Gomes et al., 2015). Other authors, doing studies with earthworms exposed to pesticides have also confirmed that ROS levels in earthworms have been increased after exposure to toxic chemicals (Xu et al., 2013; Liu et al., 2014; Wang et al., 2015) **(Fig 4.71)**.

#### *4.3.2.2.6) MDA content in millipedes at Newlands forest*

MDA content at sites C, 1 and 2 were all slightly higher in winter, with site 1 showing the highest MDA concentration (1.52 µmol/g). No seasonal differences in MDA content in millipedes were noticed and concurs with studies done with mussels by Di Salvatore et al. (2013). Highly polluted areas, such as harbors, however affected the MDA content. A high MDA content suggests exposure of the organisms to high concentrations of toxic elements (Bačkor et al., 2010; Pisani et al., 2011), thereby inducing lipid peroxidation by means of the production of free radicals (Choudhury and Panda, 2005) and is, thus indicative of oxidative stress (Ohkawa et al., 1979; Dazy et al., 2009). Of the highest Al and Fe concentrations in millipedes **(Figs 4.52, 3.69)** were measured at site 1 in the wet season where the highest MDA content was measured and may have been functional in causing ROS production and inducing oxidative stress, associated with peroxidative damage of membrane lipids (Cakmak and Horst, 1991; Foyer et al., 1994; Jing et al., 2009; Sen et al., 2014). Metal exposure induces oxidative stress in invertebrates and is based on an increase in lipid peroxidation products (MDA) in the invertebrates. Aquatic invertebrates, such as clams (Geret et al., 2002), vent mussels (Company et al., 2004) and ribbed mussels provide ample evidence of increased oxidative damage to lipids due to metals, which have also been reported from laboratory experiments (De Almeida et al., 2004) and field studies (Belcheva et al., 2011). MDA content in the land snails ranged from 45.47 µmol/g to 143.26 µmol/g, which was significantly higher than the MDA content measured in millipedes in this study that ranged between 0.6 µmol/g to 1.52 µmol/g, suggesting much less peroxidative damage in millipedes in this study. The authors also reported that tGSH levels in snails were generally lower than MDA levels, which seems to be a natural response for snails (El-Shenawy et al., 2012). The reverse was, however true for this study **(Fig 4.72)**.

### 4.3.2.3) Seasonal variations in antioxidant levels and biomarkers of oxidative damage found between Site C and the Orange Kloof and Newlands forests

#### *4.3.2.3.1) tGSH levels in moss at site C and the Orange Kloof and Newlands forests*

Exploring the various reasons for the overproduction of ROS in forest ecosystems, one should consider the predominant factors involved, such as weather conditions,

radical temperatures and excessive dry and wet spells. Even so, the composite mixtures of atmospheric pollutants, arising from the numerous anthropogenic activities are also instrumental in the overproduction of ROS in these ecosystems (Tausz et al., 1998, 2007a, b; Bussotti, 2008). Organisms in forests are nevertheless subjected to oxidative damage as a result of increased production of ROS in the plant cells (Gill and Tuteja, 2010; Sharma et al., 2012; Contin et al., 2014). The Mediterranean climate in Cape Town goes hand in hand with hot dry summers and cold wet winters (Encyclopedia Britannica, 2017). In this study these seasons were, as per normal accompanied by high temperatures in the dry- **(Tables 4.4, 4.9)** and low temperatures in the wet **(Table 4.17, 4.22)** season at the time of sampling and may have been partly responsible for the occurrence of oxidative stress observed in the moss sampled at site C, Orange Kloof and Newlands forests (Tausz et al., 1998, 2007a, b; Bussotti, 2008). Moss do prefer moister conditions (Düll, 1991), but the temperatures recorded at the time of sampling during both seasons cannot as such be regarded as “radical” and the moisture percentage obtained from these samples were also not unusually moist or dry **(Tables 4.31, 4.33, 4.37, 4.39)**.

Both Orange Kloof and Newlands forests displayed higher tGSH levels in moss in the dry season, but only Newlands forest showed a significant difference in tGSH levels between seasons. The tGSH concentration measured in moss at Newlands in the dry season (79.78 µmol/g) was the highest found at all three of the study areas and a significantly lower tGSH concentration was measured at this forest in the wet season (29.64 µmol/g). Site C presented higher tGSH concentrations in the wet season (51.01 µmol/g) and the overall lowest tGSH concentration (14.36 µmol/g) was measured at this site in the dry season. The higher tGSH activities in moss cells displayed at site C, Orange Kloof and Newlands forests is an indication of the cellular antioxidant defense system attempting to prevent the effects of peroxidation (Wilce and Parker, 1994; Van Der Oost et al., 2003; Ezemonye and Ikpesu, 2011; Paulino et al., 2012) in both seasons and the lower tGSH levels, on the other hand signifies damage to moss tissues, possibly resulting from long term exposure to pollution (Loguercio et al., 1996; Barbaro et al., 1999) also in their different seasons. Both the rise and fall in tGSH levels, nonetheless points to an overproduction of ROS in moss cells (Tausz et al., 1998, 2007a, b; Bussotti, 2008).

A closer view of the results found in moss at site C of higher tGSH concentrations in the wet season in conjunction with the higher Al, Fe and Mn concentrations measured in this season (**Tables 4.27, 4.28, 4.29)**, may suggest that the antioxidant defense system was activated in order to prevent the effects of peroxidation (Wilce and Parker, 1994; Van Der Oost et al., 2003; Ezemonye and Ikpesu, 2011; Paulino et al., 2012), more than likely as a result of the metals that are known causes of the over production of ROS (Ercal et al., 2001; Koivula and Eeva, 2010; Sanchez-Virosta et al., 2015). In addition, the dry season metal concentrations were not much lower than the wet season concentrations at site C **(Tables 4.27, 4.28, 4.29)**, yet the tGSH concentration was lower in this season. The lower tGSH concentration in moss, thus indicates damage to the moss tissues, probably from exposure to these metals (Loguercio et al., 1996; Barbaro et al., 1999). In light of these results it seems that the antioxidant system was more efficient in its function as protector in the wet season where moss was in its preferred environment, as the lower metal concentrations seemed to have caused more damage in moss tissues in the dry season. With that said, the higher Al, Fe and tGSH concentrations measured in moss at Newlands in the dry season, suggests that the antioxidant defense system in this case successfully protected the moss cells, even in the dry season. This may be clarified by means of the increasing levels of ROS in tissues of the organisms that can be controlled by activating the cellular antioxidant defense system (Gomes et al., 2014). The all important role of tGSH to neutralize and detoxify the oxidative damage caused by ROS can therefore not be under estimated (Valko et al., 2006; Regoli et al., 2011). This explanation may same be of relevance to Orange Kloof forest, showing a similar pattern.

tGSH levels in moss showed higher results in both seasons at all three study areas, as opposed to the lower MDA content measured, also in both seasons. These results are in accordance with other studies where tGSH successfully, protects the sentinel organisms against oxidative stress by scavenging ROS (Winston and Di Giulio, 1991; Choudhury and Panda, 2004, 2005; Singh et al., 2006), as well as sufficiently detoxifying metals (Sanita di Toppi et al., 2008). Organisms are well equipped with antioxidant defense systems to eliminate ROS and protect cells and tissues from impairment and dysfunction (Sugiyama, 1994). By activating the cellular

antioxidant defense system it is thus possible for the organisms to control the increased levels of ROS in tissues (Gomes et al., 2014) **(Table 4.42)**.

#### *4.3.2.3.2) MDA content in moss at site C and the Orange Kloof and Newlands forests*

Site C displayed higher MDA concentrations in moss in the dry season and Orange Kloof and Newlands forests showed higher MDA content in moss in the wet season. Significant differences between seasons were found in the MDA content in moss at site C and Newlands forest, but the overall highest MDA content was measured at Orange Kloof forest in the wet season (0.70 µmol/g). The higher MDA concentrations in any given season signifies exposure of the organisms to toxic element concentrations that are extremely high (Bačkor et al., 2010; Pisani et al., 2011). Metals, as such are recognized toxicants to cause lipid peroxidation by producing free radicals (Choudhury and Panda, 2005). Substantial lipid peroxidation in moss cells occurs when MDA accumulation is induced or increased and is a sure sign that oxidative stress has occurred (Ohkawa et al., 1979; Dazy et al., 2009). It would therefore seem that moss at Orange Kloof in the wet season incurred higher levels of oxidative stress than at the other study areas, but it was interestingly not the forest that showed the highest metal concentrations in moss. Just from comparing results with the naked eye one could not observe obvious links between the MDA contents and the Al, Fe and Mn concentrations **(Tables 4.27, 4.28, 4.29)** at the three study areas. The metal concentrations were, however relatively similar between seasons. In view of this one may presume that the cellular antioxidant defense system was activated efficiently in order for the organisms to control the increased levels of ROS in tissues (Gomes et al., 2014) **(Table 4.43)**.

#### *4.3.2.3.3) tGSH content in lichen at site C and the Orange Kloof and Newlands forests*

All three study areas displayed depleted tGSH levels in lichen in summer and winter. It is noteworthy that the Al, Fe and Mn concentrations measured in lichen in this study were comparable with urban and industrial concentrations found in other studies, in which one may assume that the concentrations are deemed relatively high (Adamo et al., 2007; Sorbo et al., 2008; Malaspina et al., 2014). One may further suggest that the metals at such concentrations were able to cause excessive

ROS production and subsequently induced oxidative stress, causing lipid peroxidation (Cakmak and Horst, 1991; Foyer et al., 1994; Jing et al., 2009; Sen et al., 2014). Metals are known to generate ROS, which may have led to the depletion of antioxidants in cells such as tGSH (Ercal et al., 2001; Koivula and Eeva, 2010; Sanchez-Virosta et al., 2015). The depleted tGSH levels in the lichen tissue is, therefore an indication of damaged tissue that normally occurs when the organisms are exposed to toxicants over a long period of time (Loguercio et al., 1996; Barbaro et al., 1999).

Extreme temperatures and climate can cause an overproduction of ROS in forests and lead to the depletion in the tGSH levels in plant cells (Tausz et al., 1998, 2007a, b; Bussotti, 2008), but lichen is relatively tolerant to warm, dry habitats (Wirth, 1991) and drought (Düll, 1991). It is therefore unlikely that the normal temperatures experienced in both winter and summer, had a major effect on ROS induction **(Tables 4.4, 4.9, 4.17, 4.22)**. In this study, one would rather lean toward metal contamination (Ercal et al., 2001; Koivula and Eeva, 2010; Sanchez-Virosta et al., 2015) as a possible cause of the induction of ROS and eventual depletion of the tGSH levels in lichen **(Tables 4.27, 4.28, 4.29, 4.42)**.

#### *4.3.2.3.4) MDA content in lichen at site C and the Orange Kloof and Newlands forests*

Lichen at site C, Orange Kloof and Newlands forests displayed higher MDA content in winter, of which site C (2.2 µmol/g) and Newlands forest (1.11 µmol/g) displayed significant differences between seasons. The overall highest MDA concentration was measured at site C (2.2 µmol/g) in winter. In contrast, significantly lower MDA concentrations were measured in the dry season at sites C (0.10 µmol/g) and Newlands (0.30 µmol/g). It is generally evidenced in the increased MDA content that lichen endured exposure to high concentrations of toxic elements (Bačkor et al., 2010; Pisani et al., 2011), of which these metals have been reported to cause lipid peroxidation (Choudhury and Panda, 2005). With the exception of site C, higher Al and Fe concentrations, as well as higher MDA content were measured in lichen in winter. The dry season metal concentrations were not much different to the wet season concentrations **(Tables 4.27, 4.28)**. In addition, Al is said to induce MDA accumulation and increase MDA content in lichen (Unal et al., 2010). In this study,

metal contamination may therefore have contributed largely to the peroxidation of lipids through the induction of ROS (Choudhury and Panda, 2005).

tGSH levels in lichen were totally depleted, which in all likelihood induced oxidative stress and LPO. This occurs as result of the generation of hydroxyl radicals and is therefore evident in the higher levels of LPO. Increased LPO levels, thus signifies an excess in production of ROS due to an antioxidant defense mechanism that has become less effective (Velma and Tchounwou, 2010). Due to low levels of antioxidants and high LPO levels, organisms are thus subjected to oxidative stress, which in turn can be considered a significant biomarker for cell death (Dreiem et al., 2005; Sayes et al., 2005). The literature mentions of many incidences of LPO occurrence correlating with sites facing landfills lower in lichen diversity (Garty et al., 2000). Many authors also report of polluted environments inducing oxidative stress in lichens and increased MDA concentrations in lichens in urban areas (Caňas et al., 1997; Majumder et al., 2013). Many reviews of MDA concentrations that were found to be the highest in heavy transport and industry sites (Gonsález et al., 1996) have been cited in the literature **(Table 4.43)**.

#### *4.3.2.3.5) tGSH levels in millipedes at site C and the Orange Kloof and Newlands forests*

The levels of tGSH in millipedes were higher in summer at all three forests. Site C and Newlands forest displayed significant differences between the dry and wet seasons, with site C displaying the overall highest concentration in the dry (542.81 µmol/g) and wet season (385.19 µmol/g). It is evident that the higher tGSH levels found in millipedes in summer that this antioxidant scavenges ROS with success and protects cells against oxidative stress (Winston and Di Giulio, 1991; Choudhury and Panda, 2004, 2005; Singh et al., 2006), as well as simultaneously detoxifying metals (Sanita di Toppi et al., 2008). Likewise, crab tissue displayed high tGSH activities in order to avert peroxidation effects (Wilce and Parker, 1994; Van Der Oost et al., 2003; Ezemonye and Ikpesu, 2011; Paulino et al., 2012). Ribbed mussels found at polluted sites, also showed higher tGSH content than mussels that were found in unpolluted areas. This is also true for bivalve mollusks that were exposed to metals (Yan et al., 1997; Cheung et al., 2002; Irato et al., 2003). Conversely, the lower tGSH levels in millipedes in the wet season signifies damage to tissues and may be

by cause of chronic exposure to contaminants (Loguercio et al., 1996; Barbaro et al., 1999), such as metals, which are known to cause the induction of ROS in organisms (Ercal et al., 2001; Koivula and Eeva, 2010; Sanchez-Virosta et al., 2015). Even though the antioxidant systems are activated, oxidative damage may still occur, as were found in mussels and it is because the defense systems are not always that effective in stopping the damage from happening (Di Salvatore et al., 2013).

Increased or decreased tGSH levels causes an overproduction of ROS, (Tausz et al., 1998, 2007a, b; Bussotti, 2008) and although metals are proven culprits, weather conditions should also be considered as possible influencing factors. This is especially relevant to pill millipedes' moist habitat requirements (SANBI, 2016), but in, for example mussels it was reported that the seasons provided no differences in tGSH levels (Di Salvatore et al., 2013 **(Table 4.42)**.

#### *4.3.2.3.6) MDA content in millipedes at site C and the Orange Kloof and Newlands forests*

All three study areas showed higher MDA levels in winter with Orange Kloof displaying the highest concentration (2.12 µmol/g). No significant differences were, however detected between the seasons. Seasonal differences with regard to MDA content in mussels were also found to be insignificant. To the contrary, highly polluted areas, such as harbors rather affected the MDA content (Di Salvatore et al., 2013). A high MDA content, such as were found in winter at the study areas, suggests exposure of the organisms to high concentrations of toxic elements (Bačkor et al., 2010; Pisani et al., 2011), thereby inducing lipid peroxidation (Choudhury and Panda, 2005) and is indicative of oxidative stress (Ohkawa et al., 1979; Dazy et al., 2009). Except for Al at site C, the Al, Fe and Mn concentrations were slightly higher in the wet season **(Tables 4.27, 4.28, 4.29)** and points to a possible explanation for the excessive ROS production and induction of oxidative stress, associated with LPO in these organisms (Cakmak and Horst, 1991; Foyer et al., 1994; Jing et al., 2009; Sen et al., 2014). Exposure to metals causes induction of oxidative stress in invertebrates. This is based on an increase in lipid peroxidation products (MDA) in the invertebrates. Aquatic invertebrates seems to be well studied as reports of clams (Geret et al., 2002), vent mussels (Company et al., 2004) and ribbed mussels of increased oxidative damage to lipids due to metals, are well

documented, as are laboratory experiments (De Almeida et al., 2004) and field studies (Belcheva et al., 2011) **(Table 4.43)**.

#### 4.3.2.4) Recommendation

Multiple oxidation events are initiated in cells, which can trigger a series of damaging changes (Cheng et al., 2007; Feng and Kobayashi, 2009; Singh et al., 2010; Wujeska et al., 2013). In a forest ecosystem set-up, plant growth may as a result change and in extreme cases, tree death occurs. Additionally, changes in the competition of species, in population and community structures and in sequential processes may eventually be noticed (Karnosky et al., 2003; Shriner and Karnosky, 2003). The intensity of injury level in plants, for example is dependent on the efficient mobilization of antioxidant defenses scavenging the ROS, which would minimize oxidative effects on such plants (Iriti and Faoro, 2008; Foyer and Shigeoka, 2011; Gill et al., 2013) and the same could apply for invertebrates or any other forest organism for that matter (Regoli and Principato, 1995; Verlecar et al., 2008).

It is thus advisable, as were done in this study, with moss, lichen and millipedes, to test organisms in their natural habitat. This is because measuring antioxidant responses in plants for example, that grows in their natural environment, such as forests, is an effective indicator of their redox potential and induced-stress resistance against numerous oxidative stresses. In this way, the ability of plants and trees to sustain themselves in disturbed ecosystems are visible in these measurements (Tausz et al., 2001, 2003; Bussotti, 2008; Wujeska et al., 2013). This is also true for animals in field conditions where, similar to plants and trees in forests, oxidative stress biomarkers are used to assess the impact of pollutants or seasonal variation (Regoli and Principato, 1995; Verlecar et al., 2008). Thus, analyzing the indicators of the organism's tolerance to oxidative stress at cell level, may provide essential information on the susceptibility of plants to natural and anthropogenic oxidative stress (Pignata et al., 2002; Gratão et al., 2012).

## 4.4 CONCLUSION

It can be concluded that even the most secluded sites chosen in the afromontane forest pockets on Table Mountain were infiltrated by air pollution and contaminated by the metals Al, Fe and Mn in both the dry and the wet seasons.

Aluminium and Fe inputs most probably had its origin from natural soil, road fugitive dust and construction dust. Manganese may have originated from vehicular traffic and was also possibly elevated in soil by vegetation as a result of biological cycling by litterfall, throughfall and uptake. Wildfires may also have contributed to the metal loads in the forests.

Aluminium concentrations revealed the highest concentrations in this study, possibly as a result of its ubiquity in soils. Manganese concentrations did have a tendency to be lower in the wet seasons and may be as a result of the mobility of this element. Manganese is also easily leached from leaves and in some tree species the sun leaves contain higher Mn concentrations than shade leaves. The percentage contribution of natural as opposed to anthropogenic inputs from these crustal elements were not determined in this study, but authors in the literature confirmed that the natural inputs tend to dominate in summer and the anthropogenic inputs in winter, which concurred with the results found in this study.

Clear trends of higher metal concentrations were found in all three of the study areas in winter, which is indicative of fine particle deposition (PM 2.5) and severe haze pollution brought on mostly by a general increase in anthropogenic activities in the colder season. This pattern of higher metal concentrations in soil, leaf litter, moss, lichen and millipedes were significantly higher in winter and more so at Newlands forest, which is the closest forest to the City of Cape Town and therefore also the forest more impacted by air pollution genetated in the city. The metal concentrations could, therefore only have been enhanced by the increased traffic and anthropogenic activities that normally escalates in colder seasons in major cities, which leads to the conclusion that winter in Cape Town is a relatively unhealthy place, due to maximum toxic metal content that seemed to have occurred in this season.

Many factors have been observed to influence the metal concentrations. The South Easter wind and sea-breeze in summer helps with dilution and dispersion of pollutants and decreases particulate pollutant concentrations, which is evident in the lower metal concentrations that were found in summer. At the same time the South Easter wind may also have been responsible for transporting pollutants over the cape flats towards the mountain where, especially Al and Fe concentrations at the control site were found to be at their highest. Additionally, precipitation from the 'Table cloth' and metals from wildfires, may further have added to the metal loads in these forests. Unusual decreases in metal concentrations observed in soil, leaf litter and lichen in the wet season from one site to another and even from one season to another may have been caused by leaching leaching. Unusual high metal concentrations may be ascribed to the location of the site. When one considers the the heterogeinity of the study areas in these forests it is surprising that the metal concentrations between sites and seasons did not show more dissimilarities or irragularities.

The pill millipedes showed seasonal trends of higher metal concentrations in winter, which was an important finding in this study, especially as there are many impacting factors that may affect how they respond to pollution, as well as the limited information available on the effects of climatic conditions on soil invertebrates and more so pill millipedes in metal polluted soils.

From this study it appears that the forest organisms, moss, lichen and millipedes have indeed incurred oxidative stress in both seasons, which was observed in the MDA content measured in the organisms. Even though the concentrations were relatively low, it is still an indication that lipid peroxidation have occurred.

Both increased and decreased tGSH levels in the organisms were noticed. The increased levels signified to the activation of the cellular antioxidant defence system in an effort to control the increasing levels of ROS in the tissues of the organisms and the decreased levels suggested that oxidative damage have occurred. Nevertheless, both increases and decreases in the tGSH levels is evidence of the overproduction of ROS in these forest organisms at all three the study areas.

The tGSH levels were higher than the MDA content in moss and millipedes in both seasons, suggesting that tGSH have been activated in moss and millipedes, but more so in millipedes, judging from the higher tGSH concentrations in an effort to protect the cells from damage. tGSH was, however not successful in the total prevention of oxidative damage, which can be concluded from the lower MDA concentrations measured in these organisms.

In lichen, on the other hand, the tGSH levels in both seasons were too low for detection, which indicate that excessive tissue damage in these organisms have occurred. The higher MDA content measured in lichen thus shows evidence of damage to cellular membranes and the occurrence of lipid peroxidation. The MDA concentrations in lichen were also higher than were measured in moss and millipedes, suggesting that lichen incurred more oxidative damage.

Climatic related factors are said to be a major factor in the overproduction of ROS, but the temperatures experienced in either summer or winter, or even the moisture percentages measured in the organisms were not found to be extreme or abnormal. It may thus be concluded that climate and seasons were most likely not pivotal in causing the overproduction of ROS in organisms in this study, but also cannot be totally excluded as partly contributing, especially due to the sensitivity of the organisms, such as moss and pill millipedes to hot and dry circumstances.

The high concentrations of the metals, Al, Fe and Mn measured in the organisms, points to a more viable explanation for the generation of ROS leading to oxidative stress and consequent activation or depletion of tGSH and occurrence of lipid peroxidation. Although it was not specifically measured, a link was visually observed between the tGSH and MDA concentrations with the more contaminated sites and seasons and also, because of the already demonstrated effects of metal contamination, specifically Al, Fe and Mn, with regard to oxidative stress, even at low pollutant levels.

It may also be suggested that studies of this sort be done in the test organism’s natural habitat, as were done in this current study in order to monitor the ability of organisms to sustain themselves in disturbed ecosystems and in so doing may

determine the overall health of, in this case, the forest ecosystem. Other biomarkers for oxidative stress exists and future studies should include those as they may differ for each organism in the environment. For example: a range of antioxidant responses of ascorbate-glutathione cycle in forest trees exposed to air pollutants and different seasons have been used as indicators of their tolerance to oxidative stress and biochemical leaf traits variations (oxidativedamage indicators: hydrogen peroxide and lipid peroxidation indicator; antioxidant defenses: ascorbate peroxidase, catalase, superoxide dismutase, glu-tathione reductase, ascorbate and glutathione; pigments: chlorophyll a, b and carotenoid) have been determined successfully. Clear signs of stress in lichens, such as decreased photosynthetic efficiency, altered chlorophyll integrity and production of secondary metabolites, discoloration, necrosis, lower ergosterol content and higher dehydrogenase activity have been seen. Superoxi dedismutase (SOD), catalase (CAT) and peroxidase (POX) revealed reliable results in mosses. For invertebrates, glutathione peroxidase (GPx) and glutathione reductase (GR) have been recommended. Extracellular soil enzymatic activities with regard to corresponding metal concentrations at sites contaminated over a long period of time, measuring soil enzymatic potential (alkaline phosphatase, cellobiohydrolase, and L-leucine-aminopeptidase) have been reliably.

Significant findings in this study were the seasonal differences in metal concentrations and in particular the higher metal concentrations found in winter, which is also the season plagued by severe brown haze episodes. In addition was the finding of Newlands forest on the city's doorstep, of higher metal concentrations in general, but more so in winter.

Of great value was that pill millipedes showed good potential as bioindicators and biomonitors of metal pollution. Their response to oxidative stress (oxidative lipid damage and altered levels of the endogenous antioxidant tGSH) can additionally be seen as good biomarkers of exposure to the metals, Al, Fe and Mn. This information is especially valuable in light of the fact that pill millipede studies in terms of metal contamination and oxidative stress are virtually non-existent.

Of further value was the pronounced differences between seasons in tGSH levels in moss and millipedes, of which the dry season mostly revealed the higher

concentrations. It would seem that the environmental conditions, both natural and anthropogenic in this season, activated their defence mechanism. MDA levels in moss and millipedes were mostly higher in the wet season when the metal concentrations were higher, as a result of the prominent brown haze episodes in winter. It therefore appears that the moss and millipedes incurred more damage during this season, as a result of the higher concentrations of metals. In addition, moss seemed to be more tolerant to environmental stressors than lichen.

# CHAPTER FIVE

# EXPOSURE EXPERIMENT

# METAL CONTAMINATION, INDUCED OXIDATIVE DAMAGE AND BIOACCUMULATION OF MILLIPEDES, EXPOSED TO A COCKTAIL OF AL, FE AND MN

## 5.1 INTRODUCTION

Metals in remnant patches, such as forest pockets adjacent to cities arise mostly from emission sources that are associated with urban land uses. There is, therefore a well-documented relationship between metal concentrations in remnant patches, such as forest pockets, with urbanized cities (Pouyat et al., 2008). This is a legitimate cause for concern, as there is no more doubt or debating that metals in environmental pollution is one of the most critical problems in urban regions (Pinto et al., 2003).

The Global Health Observatory data (GHO, 2016) reported that in Europe, more than 50% of the population in 2014 lived in urban areas and thát percentage was expected to increase. The cities continue to expand and grow rapidly (EEA, 2006), exposing those inhabitants to high levels of environmental pollution, which increases health risks. The adverse effects on public health, both short- and long-term are grave and range from altered lung function to increased mortality (Martuzzi et al., 2006; WHO, 2013; EEA, 2014). The situation in South Africa is no different and the population, traffic and pollution is increasing at an alarming rate (Popkiss, 1992; Wicking-Baird et al., 1997; StatsSA, 2011; BusinessDay, 2017; Wheels24, 2017).

Forest soils are particularly affected by atmospheric pollution, amongst others, especially near industrial and urban areas, as were reported in many countries for example: South Korea (Kim, 2002), the Amazon rainforest (Goudie and Middleton, 2001; Garrison et al., 2003), forests in southern Chile (Kennedy et al., 2002) and also forests in Europe (EC, 2016). Forests in South Africa are of the most species-rich temperate forests worldwide (Lawes et al., 2004), but the Peninsula forests, that include the Western Cape afromontane forests have been neglected in terms of research and conservation planning (Von Maltitz et al., 2003).

Nature is instrumental in coping with these environmental problems and is mostly cost effective, while simultaneously providing environmental, social and economic benefits, as well as to help build resilience (EC, 2016). Urban and periurban forests are key players in the enhancement of environmental quality, due to the all-important ecosystem services they provide (Costanza et al., 1998; de Groot et al., 2002; MA, 2005; Gómez-Baggethun and Barton, 2013). These benefits are economically measurable for urban dwellers (Escobedo et al., 2011; Millward and Sabir, 2011; Manes et al., 2012, 2014; Costanza et al., 2014; Silli et al., 2015), of which air purification, mitigation of near-road air pollution impact, urban temperature regulation, run-off mitigation, noise reduction and recreation are the most significant (Bolund and Hunhammar, 1999; Dobbs et al., 2011; Baró et al., 2014; Tong et al., 2016). It is therefore without a doubt of the highest importance to the well-being of humans to ensure the maintenance and health of these green areas that are so rich in biodiversity (Fuller and Gaston, 2009).

Soils are fundamental to the delivery of virtually every terrestrial ecosystem service on earth. Critical ecosystem functions and services such as litter decomposition, nutrient cycling and diverse aboveground vegetation processes are sustained by soil biodiversity (Wardle et al., 2004; Gardi et al., 2009; Wall et al., 2012; Bardgett and Van der Putten, 2014). Soil community composition is eminent in processes such as carbon cycling (Nielsen et al., 2011) and at the same time drives soil processes and functions (Wurst et al., 2012). It is also evident that soil-dwelling groups form a major and significant proportion of total biodiversity (Decaëns et al., 2006).

Authors have reported tirelessly on species richness and diversity of detritivores that decreased along a gradient of metal pollution (Pižl and Josens, 1995; Nahmani and Lavelle, 2002; Lukkari et al., 2004), which may cause an ecosystem to struggle with changes in the environment. A decrease in detrivore biodiversity may cause adverse effects on ecosystem functions if species or species combinations that play an important role in the maintenance of an ecosystem function are lost (Brussaard et al., 1997; Heemsbergen et al., 2004).

Soil ecosystems determine the productivity of forests, which are globally under pressure of diverse environmental stresses that include natural, as well as anthropogenic disturbances. The influence these stresses have on living organisms in forest soils are remarkable. It is therefore imperative to engage in long-term monitoring studies to evaluate the environmental stresses on soil fauna (Moldenke and Lattin, 1990; Battigelli et al., 2004; Langor and Spence, 2006; Lee et al., 2009), which would certainly work towards ensuring the health of the rich biodiversity contained in forests (Fuller and Gaston, 2009).

Apart from some work on termites, dung beetles and antlions (Van Jaarsveld et al., 1998), soil dwelling groups have not been used in making major decisions, with regard to conservation or land-use planning in South Africa. This is largely because of the lack of monitoring resources available on which to base indicators of biodiversity (McGeoch et al., 2011). Millipedes have only been studied sporadically since the description of the first three South African millipede species by Brandt (1841) and only one publication by Van den Spiegel et al. (2002) reviewed some of the Sphaerotheriida *(Sphaerotherium)* (pill millipede) species. Natural resource management decisions in South Africa are therefore directed with little information on soil biota. Uninformed decisions may thus lead to diminished functionality, a reduction in ecosystem services and in extreme cases, permanent damage to ecosystems (MEA, 2005; Cardinale et al., 2012), which may jeopardize endeavors to reach the Sustainable Development Goals for human prosperity (Griggs et al., 2013).

Soil invertebrates are well known indicators of pollutant levels (Dallinger, 1994; Nahmani and Lavelle, 2002). The results are, however influenced by numerous factors (Beyer and Cromartie, 1987; Nahmani et al., 2007), such as their response to metal exposure, as a result of their physiology, mobility, feeding habits and microhabitat preferences (Beyer and Cromartie, 1987; Pižl and Josens, 1995; Scharenberg and Ebeling, 1996; Kamitani and Kaneko, 2007). Some diplopod species will also develop strategies to minimize the effects of metals on their survival, which may include a decrease in food intake, a decrease in nutrient assimilation, or both (Hopkin et al., 1985).

There are, however also additional effects of accumulated metals in soil fauna and that is the potential for the bioconcentration of metals up the food chain (Hopkin and Martin, 1985; Raczuk and Pokora, 2008). This is because soil organisms are a primary food source for many invertebrate and vertebrate predators. Heavily contaminated areas therefore pose an increased risk of secondary poisoning (Spurgeon and Hopkin, 1996; Reinecke et al., 2000; Maerz et al., 2005).

Accumulation of metals in forest ecosystems are more pronounced in the litter layer (Martin et al., 1982), which subjects soil organisms inhabiting the humus horizon to higher metal levels (Hopkin, 1989). A substantial proportion of metal particles in dust deposits on leaves are also conveyed to the soil by precipitation and shedding of the leaves (Glavač et al., 1987; Bloemen et al., 1995). Diplopods perform an essential role in the decomposition of vegetal organic matter, as well as in soil aeration and enrichment and stimulation of microbial activity, which is essential for the cycling of nutrients (Hopkin and Read, 1992). It is thus, because of their habit, that they have been relatively widely used to assess the quality of soils (Hopkin and Read, 1992; Godoy and Fontanetti, 2010; Nogarol and Fontanetti, 2010).

Metal cytotoxicity in organisms have been, to a large extend linked to oxidative damage (Valko et al., 2005) and a number of metals have already been recognized to induce oxidative stress (Halliwell, 1992). Organisms have developed complex antioxidant defense mechanisms of both enzymatic and non-enzymatic nature in an attempt to minimize ROS-induced damage. The non-enzymatic antioxidant defenses contain molecules of low molecular weight that act as free radical scavengers as ascorbic acid, tocopherols and glutathione. Conversely, lipid peroxidation is a common mechanism of cellular injury in invertebrates, and acts as an indicator of oxidative damage in cells and tissues (Pampanin et al., 2005).

The synergy between metals and the constituents of the antioxidant defense systems is essential in the ecotoxicological response of an organism to its environment (Regoli et al., 2006). Once again, studies on these interactions are imperative for the identification of biomarkers that can serve as early warning systems for environmental monitoring (Radwan et al., 2010).

The effect that organic pollutants and complex mixtures in atmospheric pollution have on soil arthropods have not been investigated adequately (Godoy and Fontanetti, 2010; Nogarol and Fontanetti, 2010). Likewise, the pill millipedes in the afromontane forest pockets in Cape Town are exposed to a combination of metals, arising from atmospheric pollution and the effects thereof are in question.

In the current study the forest situation was mimicked in a laboratory experiment through exposure of the pill millipedes to a combination of the metals Al, Fe and Mn at high, low and control concentrations as it would naturally occur in the environment. These particular metals were chosen, because of the higher concentrations found in the millipedes, soil and the leaf litter in the field investigation of this study (Chapters 1 and 3).

The aim of the exposure experiment was to determine a) the metal concentrations accumulated in the millipedes after a period of 6 weeks and b) to assess whether responses linked to oxidative stress measured in the pill millipede, *Spaerotherium compressum* **(Fig 4.1)** may be utilized as potential biomarkers of exposure to the metals Al, Fe and Mn by using two markers associated with oxidative stress: i.e. MDA (measured as TBARS) as marker for oxidative lipid damage, which is a product formed between MDA and thiobarbituric acid (TBA) (Pisani et al., 2011) and glutathione (tGSH) levels, a non-enzymatic endogenous antioxidant, which performs an essential role in neutralizing and or detoxifying the oxidative damage caused by ROS (Valko et al., 2006; Regoli et al., 2011).

The purpose of this experiment was also a monitoring effort to determine the effects of metal concentrations on pill millipedes, which play a significant role in the soil processes of these forests. In turn the information may shed light as to the health of the afromontane forest ecosystems or at the very least, provide warning signs of possible detrimental effects to follow in the future.

## 5.2 RESULTS

### 5.2.1 BIOACCUMULATION OF METALS IN MILLIPEDES

#### *5.2.1.1) Comparisons of metal concentrations in soil, leaf litter and millipedes between different exposure groups*

The mean metal concentrations in soil, leaf litter and millipedes of the control group, as well as the low and high metal exposure groups are presented in **Tables 5.1, 5.2**. Metal concentrations are expressed in mg/kg.

**Table 5.1**: The mean metal concentrations (mg/kg) (± SD) in soil, leaf litter and millipedes at the start (week 0) and the end (week 6) of the experimental exposure period for all three exposure groups.

| WEEK 0 | | SOIL | | | LEAF LITTER | | | MILLIPEDES | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Control | Low | High | Control | Low | High | Control | Low | High |
| Al | Mean | [a]4008.40 | [a]4414.21 | [a]4414.21 | [a]825.44 | [a]946.06 | [a]946.06 | [a]585.62 | [a]366.65 | [a]307.55 |
| | *SD* | *0.01* | *0.00* | *0.00* | *0.00* | *0.01* | *0.01* | *315.77* | *139.56* | *57.78* |
| Fe | Mean | [a]3166.60 | [a]3492.28 | [a]3492.28 | [a]640.96 | [a]740.37 | [a]740.37 | [a]301.86 | [a]256.10 | [a]216.27 |
| | *SD* | *0.00* | *0.00* | *0.00* | *0.00* | *0.00* | *0.00* | *158.05* | *57.86* | *44.61* |
| Mn | Mean | [a]24.45 | [a]50.99 | [a]50.99 | [a]97.64 | [a]134.63 | [a]134.63 | [a]0.00 | [a]16.77 | [a]19.07 |
| | *SD* | *0.00* | *0.00* | *0.00* | *0.00* | *0.00* | *0.00* | *9.43* | *7.47* | *8.40* |

Statistical signifcant differences (P<0.050) between groups are indicated with different superscripted letters. Comparisons were done separately for the soil, leaf litter and millipedes. N=5.

**Table 5.2:** The mean metal concentrations (mg/kg) (± SD) in soil, leaf litter and millipedes at the end (week 6) of the experimental exposure period for all three exposure groups.

| WEEK 6 | | SOIL | | LEAF LITTER | | MILLIPEDES | | |
|---|---|---|---|---|---|---|---|---|
| | | Low | High | Low | High | Control | Low | High |
| Al | Mean | [a]5014.40 | [a]6228.80 | [a]1305.42 | [a]1448.71 | [a]391.84 | [a]471.69 | [a]368.72 |
| | *SD* | *0.00* | *0.00* | *0.00* | *0.00* | *310.54* | *30.41* | *178.71* |
| Fe | Mean | [a]4286.38 | [a]5431.27 | [a]882.29 | [a]909.62 | [a]228.88 | [a]310.11 | [a]254.53 |
| | *SD* | *0.00* | *0.00* | *0.00* | *0.00* | *164.12* | *33.07* | *84.38* |
| Mn | Mean | [a]77.59 | [a]80.83 | [a]120.45 | [a]338.10 | [a]0.00 | [a]9.15 | [a]12.68 |
| | *SD* | *0.00* | *0.00* | *0.00* | *0.00* | *16.16* | *3.57* | *4.47* |

Statistical signifcant differences (P<0.050) between groups are indicated with different superscripted letters. Comparisons were done separately for the soil, leaf litter and millipedes. N=5.

***Week 0***

***a) Soil***

There were no statistically significant differences found in the soil between any of the three exposure groups in terms of Al (P=0.067), Fe (P=0.067) and Mn (P=0.067) concentrations at the start of the exposure period **(Table 5.1)**.

***b) Leaf litter***

No statistically significant differences were found in leaf litter between the three exposure groups with reference to Al (P=0.067), Fe (P=0.067) and Mn concentrations (P=0.067) on week 0 of the exposure period **(Table 5.1)**.

***c) Millipedes***

All three exposure groups displayed no statistically significant differences between them with regard to Al (P=0.361), Fe (P=0.829) and Mn (P=0.050) concentrations when compared **(Table 5.1)**.

***Week 6***

***a) Soil***

The soil in all three exposure groups presented no statistically significant differences from each other with reference to Al (P=0.333), Fe (P=0.067) and Mn (P=0.333) concentrations at the end of the six week exposure period. A steady increase in metal concentrations were, however observed towards the high exposure group **(Table 5.2)**.

***b) Leaf litter***

Leaf litter in all three exposure groups were compared, but no significant differences were found in Al (P=0.333), Fe (P=0.333) and Mn (P=0.067) concentrations after the exposure period. The metal concentrations, nevertheless increased towards the high exposure group **(Table 5.2)**.

***b) Millipedes***

No statistically significant differences in Al (P=0.829), Fe (P=0.629) and Mn (P=0.071) concentrations between the three exposure groups were found after six weeks, but millipedes showed the highest concentrations in the low exposure group **(Table 5.2)**.

### *5.2.1.2) Comparisons of metal concentrations in soil between week 0 and week 6 of exposure groups*

Mean metal concentrations in soil between week 0 and week 6 of the control group, as well as the low and high metal exposure groups are shown in **Figs 5.1 to 5.3**. Statistical signifcant differences (P<0.050) between week 0 and week 6 are indicated with an asterisk above the graph bars.

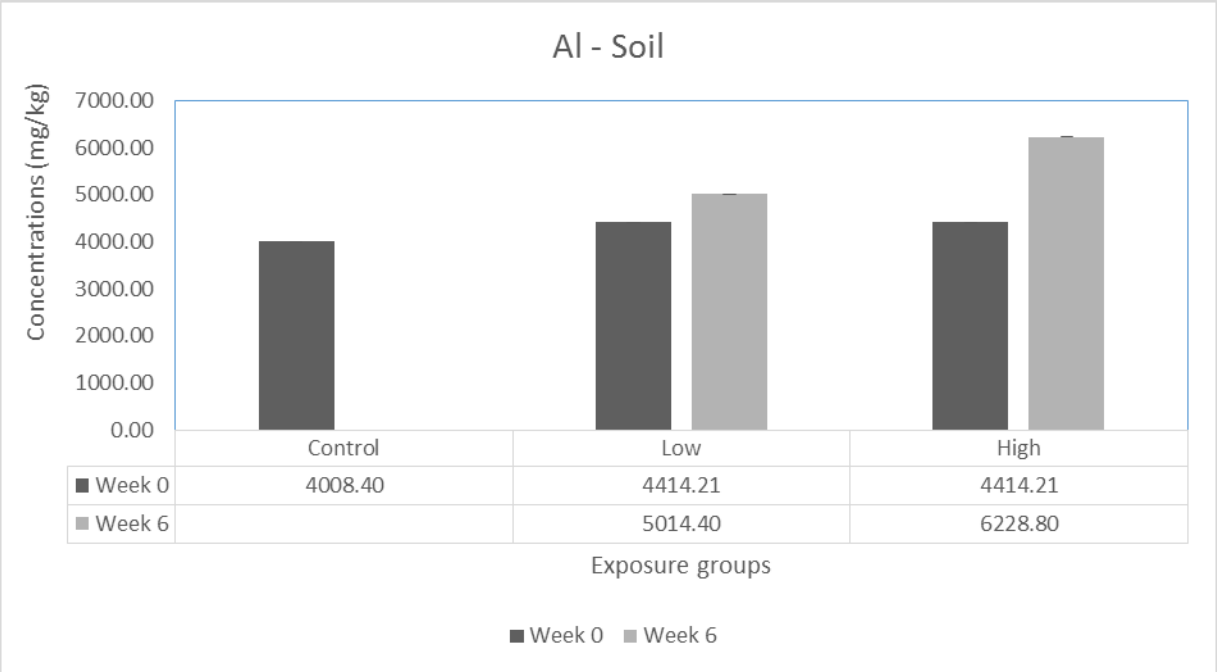


**Figure 5.1:** The mean Al concentrations (mg/kg) (± SD) in soil of all three exposure groups between week 0 and week 6. N=5.

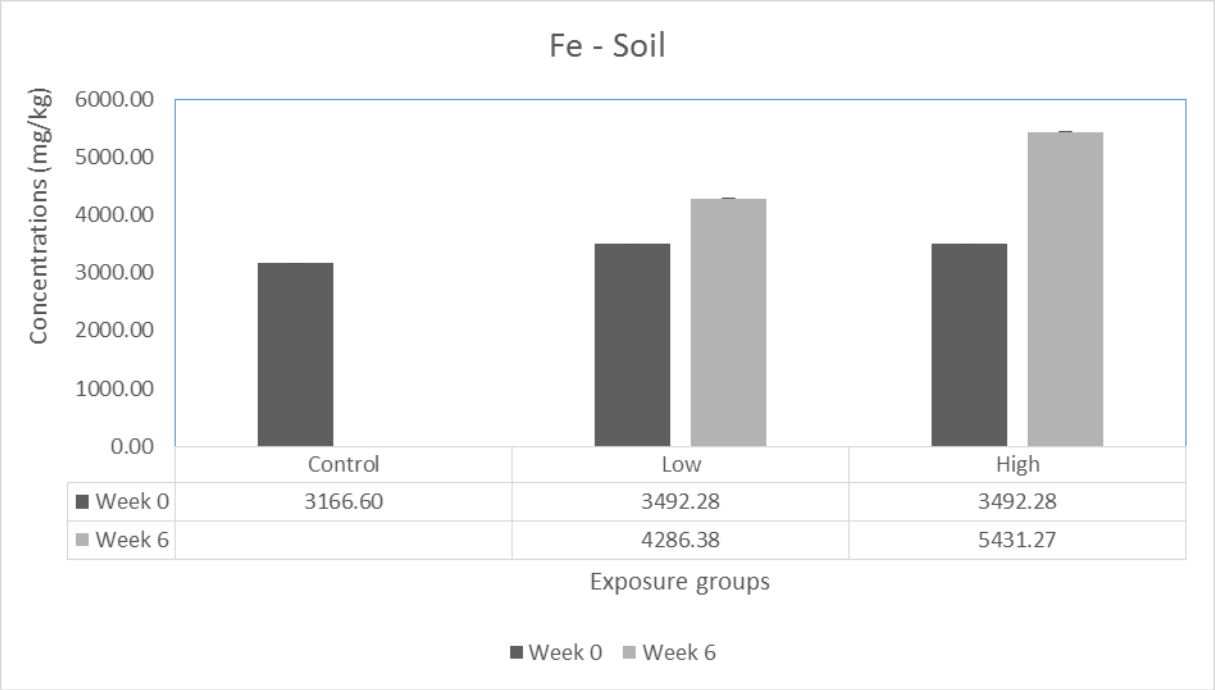


**Figure 5.2:** The mean Fe concentrations (mg/kg) (± SD) in soil of all three exposure groups between week 0 and week 6. N=5.

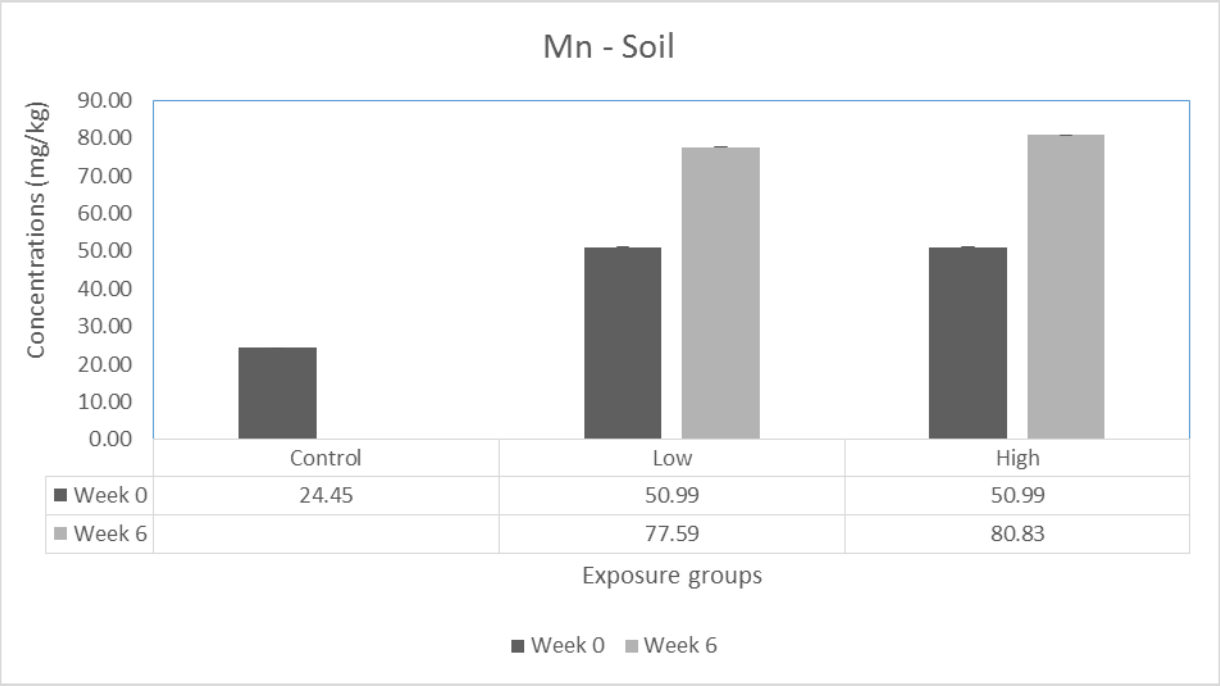


**Figure 5.3:** The mean Mn concentrations (mg/kg) (± SD) in soil of all three exposure groups between week 0 and week 6. N=5.

Aluminium concentrations found in soil between week 0 and week 6 did not differ significantly from each other in the exposure groups low (P=0.333) and high (P=0.333), but higher Al concentrations were noticed in soil after six weeks in the low and high exposure groups. An Al concentration of 6228.8 ± 0.0028 mg/kg in the high exposure group yielded the highest results **(Fig 5.1)**.

Higher Fe concentrations in soil were measured in the low and high groups after the exposure period, with the highest Fe concentration (5431.27 ± 0.001 mg/kg) found in the high exposure group. The Fe concentrations found in soil, however showed no statistically significant differences between week 0 and week 6 in the exposure groups low (P=0.333) and high (P=0.333) **(Fig 5.2)**.

When Mn concentrations in soil were compared between week 0 and week 6, there were no statistically significant differences found between each other in the exposure groups: control: low: (P=0.333) and high: (P=0.333). Elevated Mn concentrations in soil were, nevertheless observed after 6 weeks of exposure with the highest concentration measured in the high exposure group (80.83 ± 0.0003 mg/kg) **(Fig 5.3)**.

### *5.2.1.3) Comparisons of metal concentrations in leaf litter between week 0 and week six of exposure groups*

Mean metal concentrations in leaf litter on week 0 and week six of the control group, as well as the low and high metal exposure groups are shown in **Figs 5.4 to 5.6**. Statistical signifcant differences (P<0.050) between week 0 and week 6 are indicated with an asterisk above the graph bars.

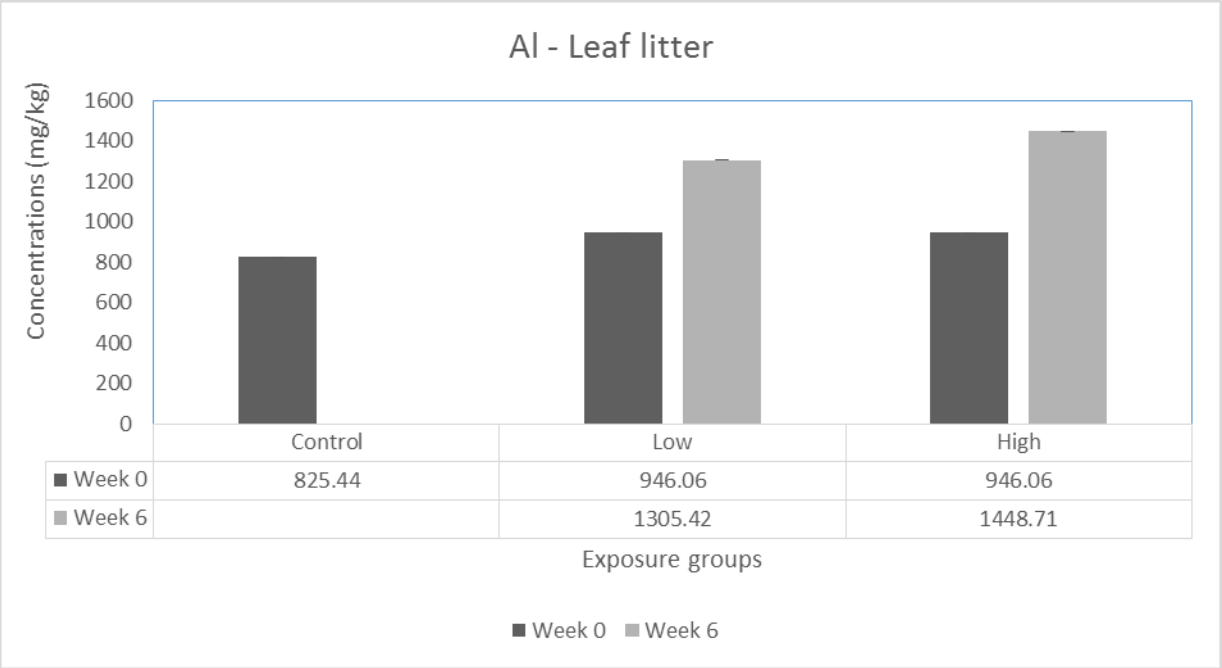


**Figure 5.4:** The mean Al concentrations (mg/kg) (± SD) in leaf litter of all three exposure groups between week 0 and week 6. N=5.

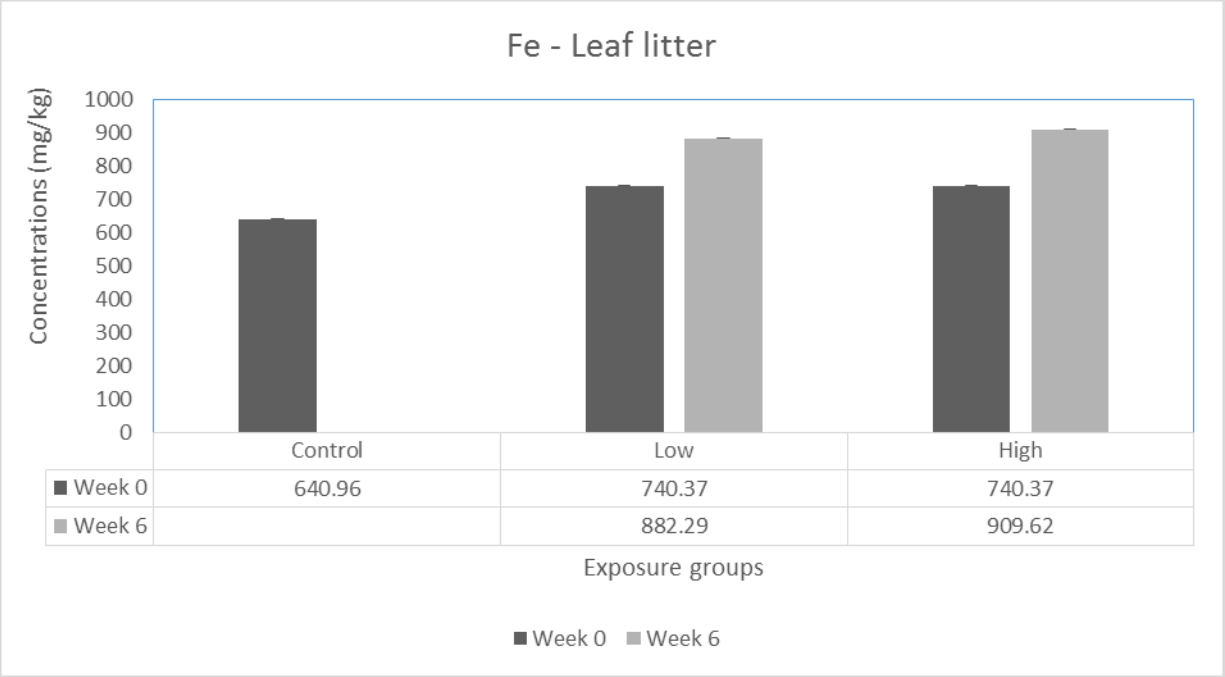


**Figure 5.5:** The mean Fe concentrations (mg/kg) (± SD) in leaf litter of all three exposure groups between week 0 and week 6. N=5.

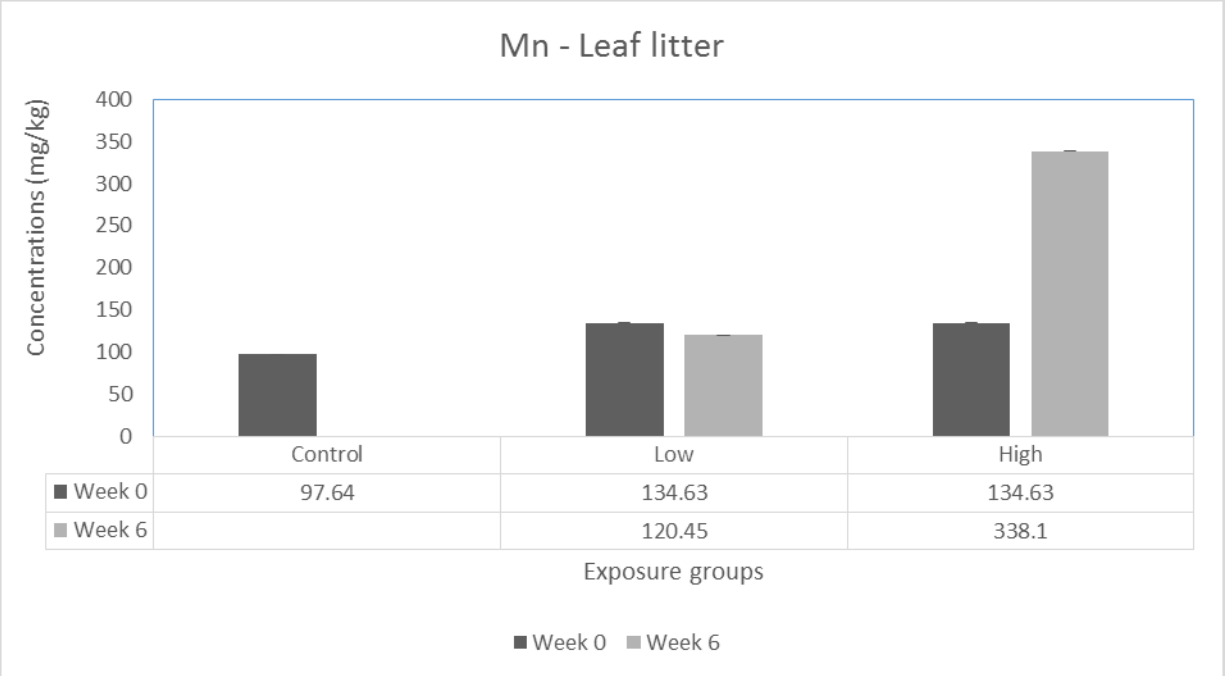


**Figure 5.6:** The mean Mn concentrations (mg/kg) (± SD) in leaf litter of all three exposure groups between week 0 and week 6. N=5.

When Al concentrations in leaf litter between week 0 and week 6 were compared, there were no statistically significant differences found between each other in the groups: low: (P=0.333) and high: (P=0.333). Aluminium concentrations in leaf litter were, however higher after the exposure period in those groups, of which the latter group displayed the highest concentrations (1448.71 ± 0.0027 mg/kg) **(Fig 5.4)**.

No statistically significant differences in leaf litter with reference to Fe concentrations between week 0 and week 6 were measured in the exposure groups low (P=0.333) and high (P=0.333). Leaf litter, nevertheless displayed higher concentrations in those groups after six weeks, with the high exposure group showing the highest Fe concentrations (909.62 ± 0.0027 mg/kg) **(Fig 5.5)**.

Manganese concentrations showed no statistically significant in leaf litter between week 0 and week 6 in the low (P=0.333) and high (P=0.333) exposure groups **(Fig 5.6)**.

### *5.2.1.4) Comparisons of metal concentrations in millipedes between week 0 and week six of exposure groups*

Mean metal concentrations in millipedes on week 0 and week six of the control group, as well as the low and high metal exposure groups are shown in **Figs 5.7 to 5.9**. Statistical signifcant differences (P<0.050) between week 0 and week 6 are indicated with an asterisk above the graph bars.

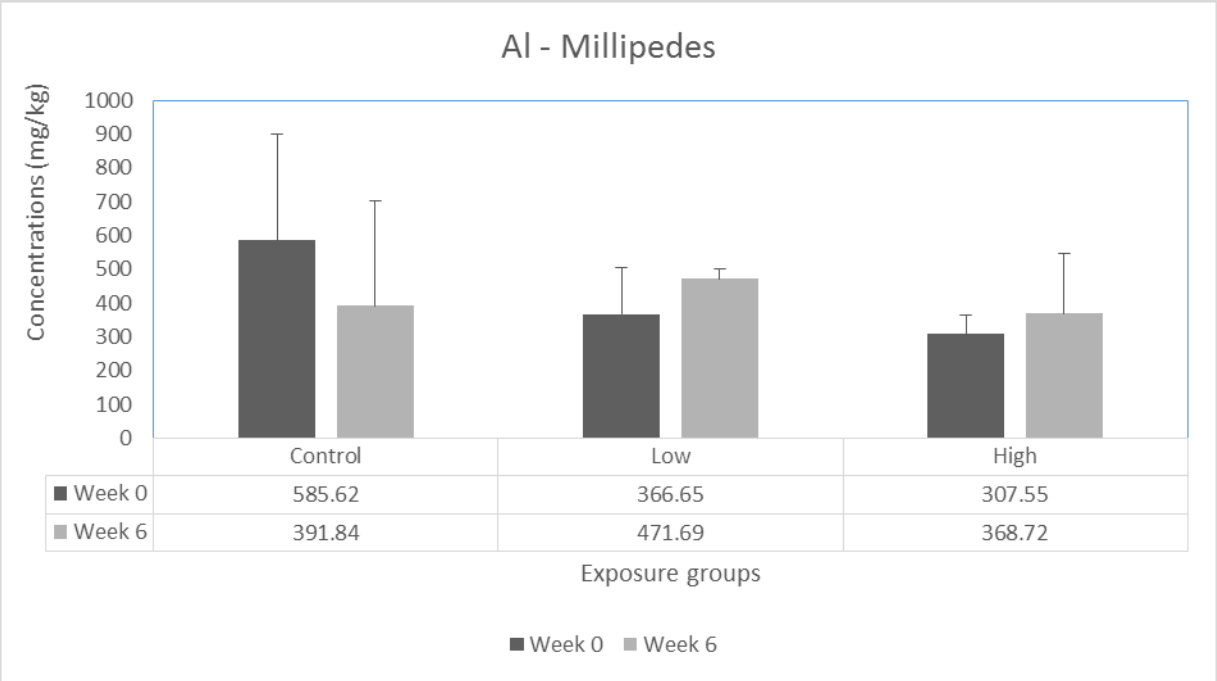


**Figure 5.7:** The mean Al concentrations (mg/kg) (± SD) in millipedes of all three exposure groups between week 0 and week 6. N=5.

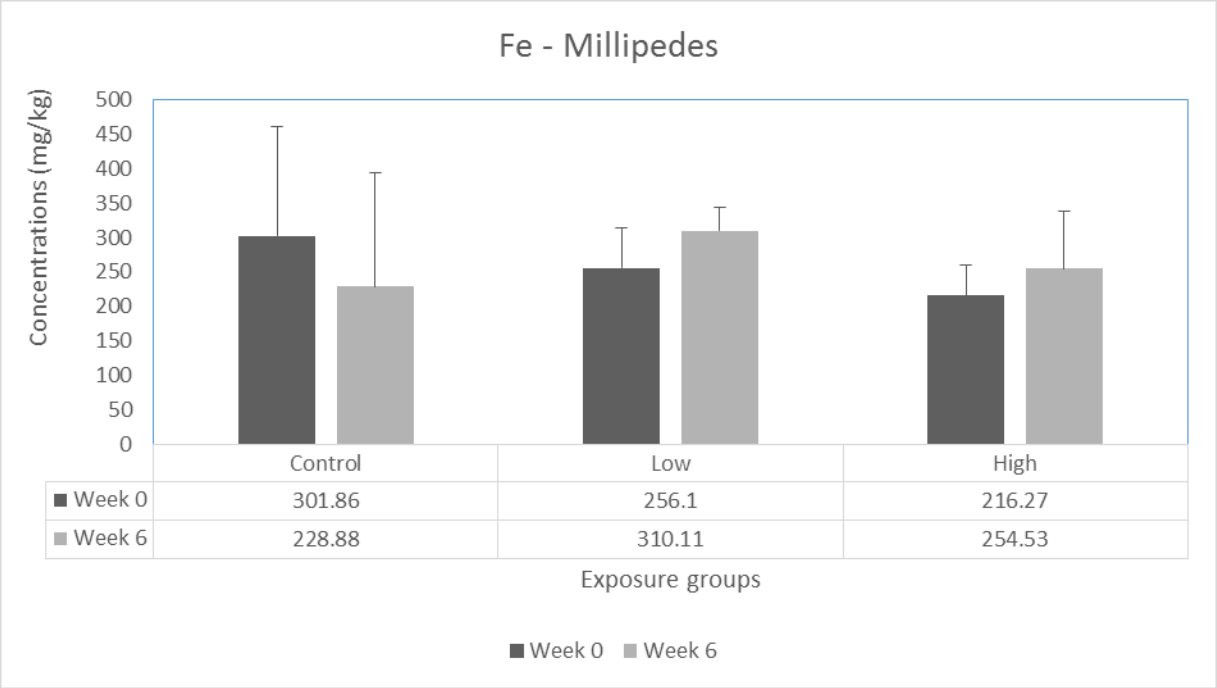


**Figure 5.8:** The mean Fe concentrations (mg/kg) (± SD) in millipedes of all three exposure groups between week 0 and week 6. N=5.


**Figure 5.9:** The mean Mn concentrations (mg/kg) (± SD) in millipedes of all three exposure groups between week 0 and week 6. N=5.

No statistically significant differences in millipede Al concentrations between week 0 and week 6 were found in the control (P=0.491), low (P=0.272) and high (P=0.603) exposure groups. The low exposure group at week 6 showed the highest Al concentrations in millipedes (471.69 ± 30.41 mg/kg) **(Fig 5.7)**.

Iron concentrations between week 0 and week 6 in millipedes were compared, but no statistically significant differences in the exposure groups control (P=0.609), low (P=0.233) and high (P=0.526) were presented. Millipedes did, however display higher concentrations in the low and high groups after six weeks, with the low exposure group showing the highest Fe concentrations (310.11 ± 0.0027 mg/kg) **(Fig 5.8)**.

Manganese concentrations showed no statistically significant differences in millipedes between week 0 and week 6 in the exposure groups: control (P=374), low (P=0.186) and high (P=0.309). Enhanced Mn concentrations were noted on week 0 **(Fig 5.9)**.

### *5.2.1.5) Mass of the millipedes before and after the six week exposure period and percentage mass change after exposure*

**Table 5.3:** Mass (mg/kg) and % mass change in millipedes of the exposure groups at week 0 and week 6.

| EXPOSURE PERIOD (6 WEEKS) | | | | |
|---|---|---|---|---|
| **GROUP** | | **WEEK 0** | **WEEK 6** | **% MASS CHANGE** |
| **Control** | **Mean** | **0.81** | **0.84** | **3.72** |
| | *SD* | *0.16* | *0.26* | *66.64* |
| **Low** | **Mean** | **0.91** | **0.95** | **4.69** |
| | *SD* | *0.11* | *0.14* | *28.20* |
| **High** | **Mean** | **1.09** | **0.89** | **-18.69** |
| | *SD* | *0.36* | *0.11* | *70.41* |

### *5.2.1.6) Comparisons of metal concentrations in millipedes between week 0 and week 6 of exposure groups and percentage mass change after exposure*

Mean metal concentrations in millipedes on week 0 and week six of the control group, as well as the low and high metal exposure groups and percentage mass change are shown in **Figs 5.10 to 5.12**. Statistical signifcant differences (P<0.050) between week 0 and week 6 are indicated with an asterisk above the graph bars. The % mass change is indicated on the secondary axis with a marked line.

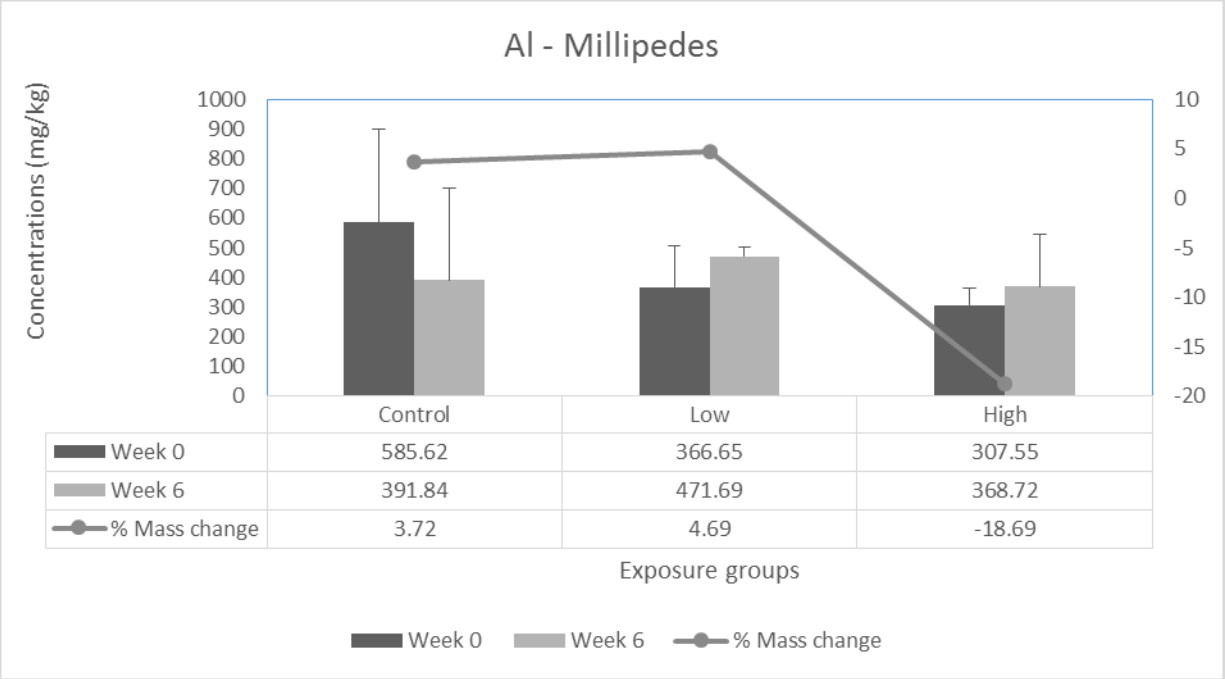


**Figure 5.10:** The mean Al concentrations (mg/kg) (± SD) in millipedes of all three exposure groups and the % mass change between week 0 and week 6. N=5

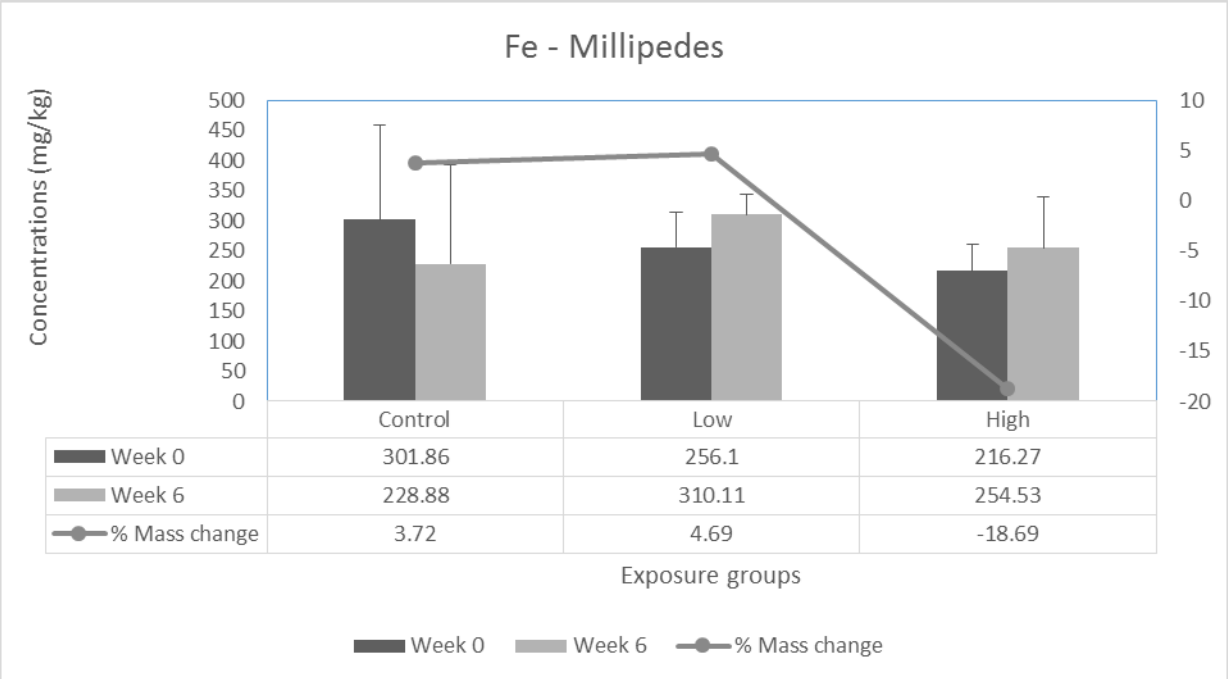


**Figure 5.11:** The mean Fe concentrations (mg/kg) (± SD) in millipedes of all three exposure groups and the % mass change between week 0 and week 6. N=5

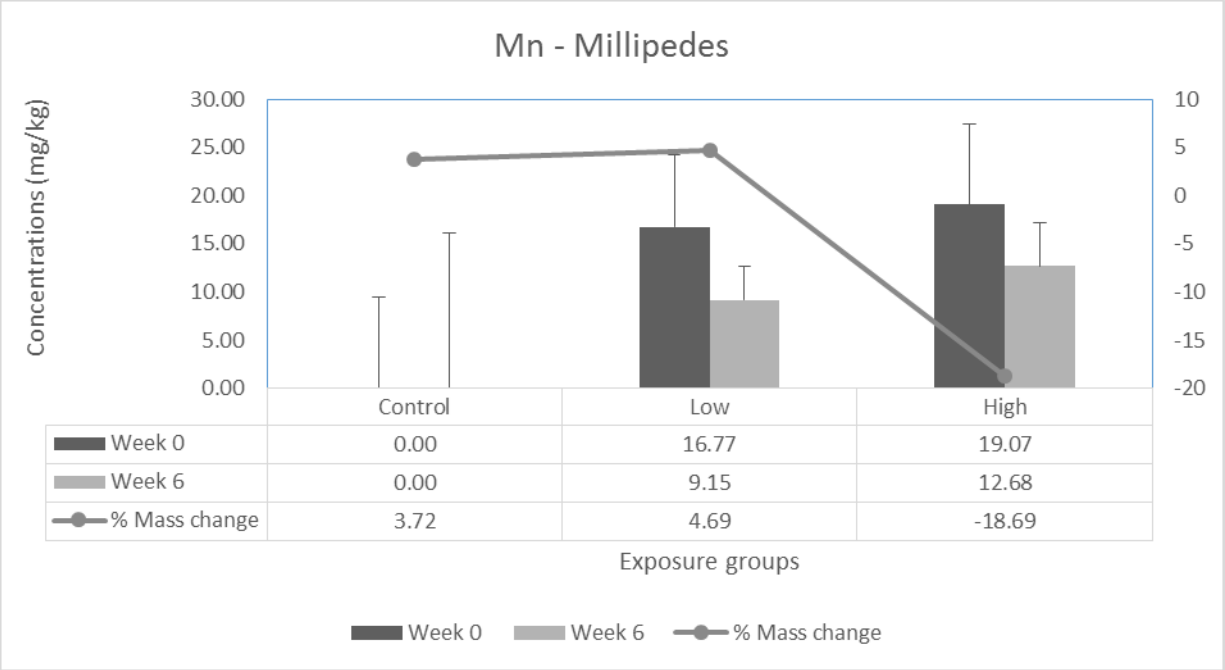


**Figure 5.12:** The mean Mn concentrations (mg/kg) (± SD) in millipedes of all three exposure groups and the % mass change between week 0 and week 6. N=5

### *5.2.1.7) pH and Moisture*

pH of the soil of exposure groups ranged from an alkaline (7.21) to (7.28) and the moisture % of the soil ranged from a relatively moist 20.39 % to 21.89 % at week 0

At week 6 the pH of the soil of exposure groups ranged from slightly acidic (6.76) to alkaline (7.29) and the moisture % of the soil ranged from a moist 22.35 % to 23.11 %.

**Table 5.4:** pH and moisture % of soil of exposure groups at week 0 and week 6**.**

| EXPOSURE GROUPS | | SOIL | |
|---|---|---|---|
| | | **WEEK 0** | **WEEK 6** |
| Control | **pH** | 7.28 | 7.29 |
| | *Moisture* | 21.72 | 23.11 |
| Low | **pH** | 7.28 | 7.05 |
| | *Moisture* | *20.39* | *22.84* |
| High | **pH** | 7.21 | 6.76 |
| | *Moisture* | *21.89* | *22.35* |

Moisture % of leaf litter at week 0 ranged from a moist 64.39 % to 65.38 %. At week 6 the moisture % of leaf litter ranged from a moist 54.35 % to a moist 55.93 %.

**Table 5.5:** Moisture % of the leaf litter of the exposure groups at week 0 and week 6.

| EXPOSURE GROUPS | % | LEAF LITTER | |
|---|---|---|---|
| | | WEEK 0 | WEEK 6 |
| Control | Moisture | 64.39 | 55..76 |
| Low | Moisture | 64.98 | 55.93 |
| High | Moisture | 65.38 | 54.35 |

### *5.2.1.8) Soil characterization of control group*

**Table 5.6:** Characterization % of soil used for different exposure groups collected from the control site

| EXPOSURE | (%) | | | | | | | WATER RETENTION | | |
|---|---|---|---|---|---|---|---|---|---|---|
| SOIL | Clay | Silt | Fine sand | Medium sand | Coarse sand | Rock (v/v) | Classification | 10kPa | 100kPa | mm/m |
| | | | | | | | | | | |
| Control | 9 | 12 | 31.6 | 26 | 21.4 | 21 | LmSa | 18.28 | 10.77 | 75.1 |

### 5.2.2 OXIDATIVE STRESS INDICATORS

#### *5.2.2.1) Comparisons of tGSH and MDA levels in millipedes between different exposure groups*

The mean tGSH and MDA (measured as TBARS) concentrations in millipedes of the control group, as well as the low and high exposure groups are presented in **Table 5.7**. Concentrations are expressed in µmol/g.

**Table 5.7:** The mean tGSH and MDA concentrations (µmol/g) (± SD) in millipedes at the start (week 0) and the end (week 6) of the experimental exposure period for all three exposure groups.

| WEEK 0 | | MILLIPEDES | | |
|---|---|---|---|---|
| | | Control | Low | High |
| tGSH | Mean | [a]425.43 | [b]201.20 | [b]274.70 |
| | *SD* | *35.25* | *50.37* | *39.15* |
| MDA | Mean | [a]0.60 | [a]0.31 | [a]0.84 |
| | *SD* | *0.21* | *0.37* | *1.26* |
| **WEEK 6** | | **MILLIPEDES** | | |
| | | Control | Low | High |
| tGSH | Mean | [a]198.17 | [a]286.53 | [a]291.28 |
| | *SD* | *99.55* | *8.06* | *26.42* |
| MDA | Mean | [a]0.10 | [a]0.12 | [a]0.28 |
| | *SD* | *0.04* | *0.02* | *0.38* |

Statistical signifcant differences (P<0.050) between groups are indicated with different superscripted letters.
MDA (expressed as µmol TBARS per gram material); SD=Standard Deviation; N=5.

***Week 0***

***Millipedes***

Statistically significant differences were found in mean tGSH ($P<0.05$) concentrations between the exposure groups control vs low and control vs high, but no significant differences were found between exposure groups, low and high ($P>0.05$) at the start of the exposure period. The highest tGSH concentration of 425.43 ± 35.25 µmol/g in millipedes were measured in the control group, which was significantly higher than were found in the low and high exposure groups **(Table 5.7)**.

Pairwise multiple comparisons showed no statistically significant differences in mean MDA ($P=0.829$) concentrations in millipedes between any of the exposure groups **(Table 5.7)**.

**Week 6**

***Millipedes***

Millipedes showed no statistically significant differences in terms of mean tGSH ($P=0.071$) and MDA ($P=0.879$) concentrations between all three exposure groups, at the end of the 6 week exposure period **(Table 5.7)**.

### *5.2.2.2) Comparisons of tGSH and MDA levels in millipedes between week 0 and week 6 of exposure groups*

Mean tGSH and MDA (measured as TBARS***)*** concentrations in millipedes between week 0 and week 6 of the control group, as well as the low and high exposure groups are shown in **Figs 5.13 to 5.14**. Statistical significant differences (P<0.05) between week 0 and week 6 are indicated with an asterisk above the graph bars.

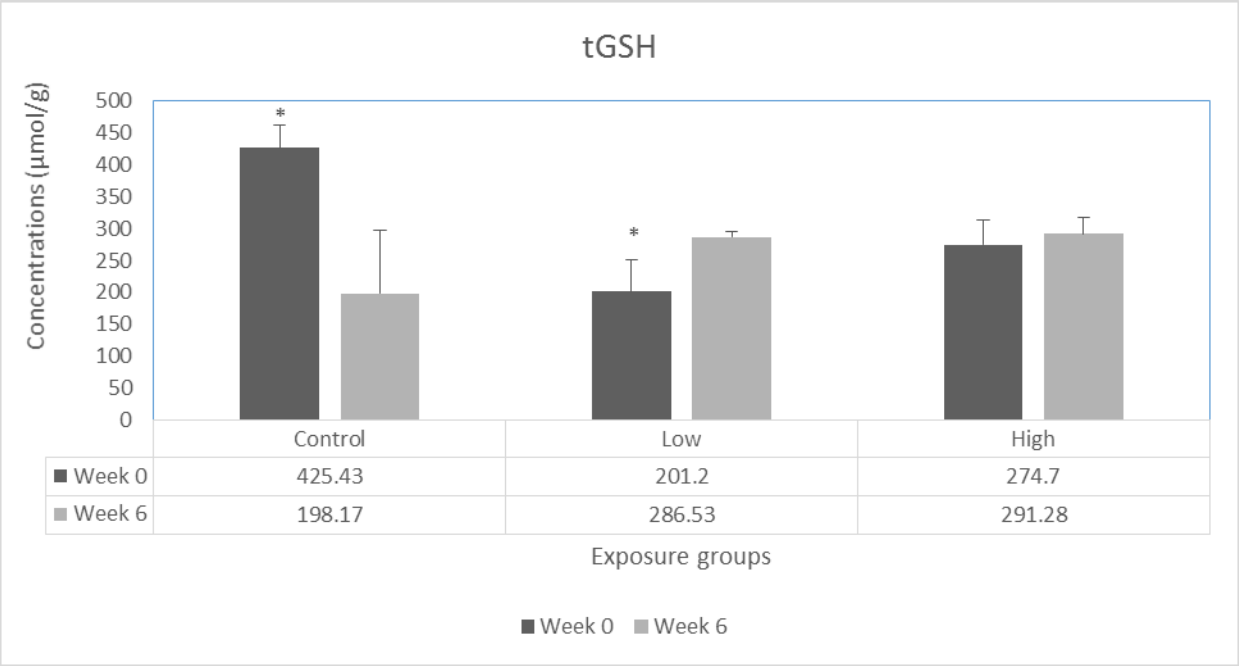


**Figure 5.13:** The mean tGSH concentrations (µmol/g) (± SD) in millipedes of all three exposure groups between week 0 and week 6. SD=Standard Deviation; N=5.

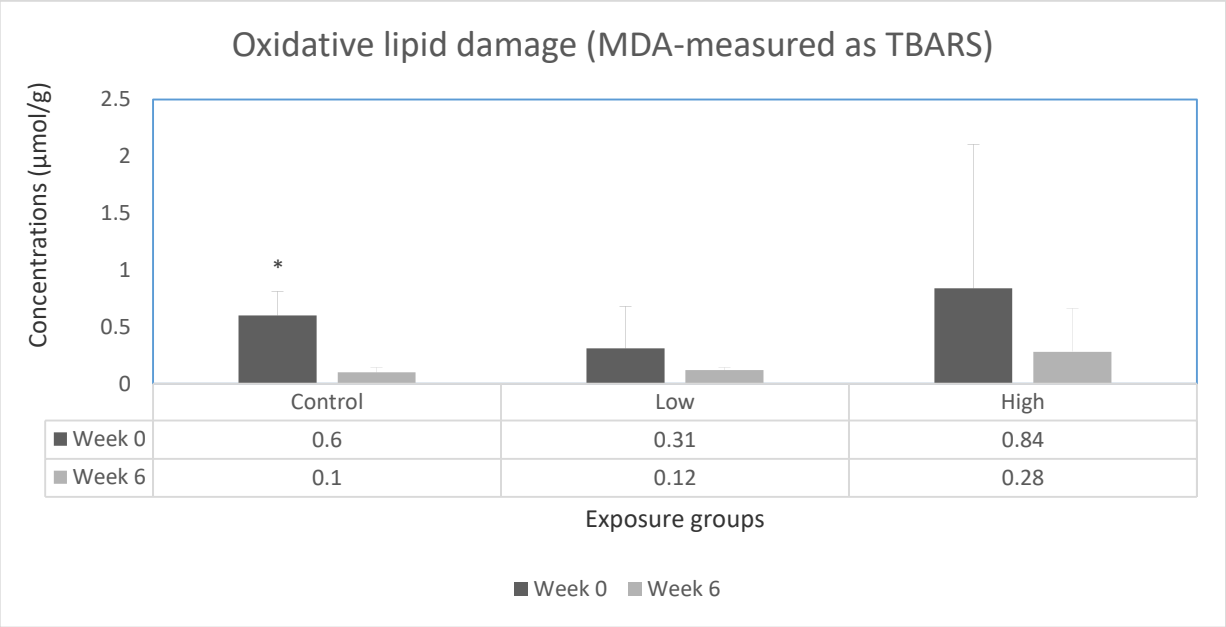


**Figure 5.14:** The mean MDA concentrations (µmol/g) (± SD) in millipedes of all three exposure groups between week 0 and week 6. MDA (expressed as µmol TBARS per gram material); SD=Standard Deviation; N=5.

Statistically significant differences in tGSH concentrations in millipedes between week 0 and week 6 were found in the exposure groups control (P=0.020) and low (P=0.044). There were, however no statistically significant differences found in tGSH concentrations between week 0 and week 6 in millipedes in the high exposure group (P=0.576). Millipedes showed the highest concentrations of tGSH in the control group (425.43 ± 35.25) on week 0, as opposed to the significantly (P=0.020) lower concentration of 198.17 ± 99.55 µmol/g at the end of week six **(Fig 5.13)**.

Millipedes showed statistically significant differences between week 0 and week 6 in the exposure group: control (P=0.018) in terms of mean MDA concentrations. No statistically significant differences were found in MDA concentrations between week 0 and week 6 in millipedes in the low (P=0.434) and high (P=0.254) exposure groups. The highest MDA concentration was found in millipedes in the high exposure group on week 0 (0.84 ± 1.26), in contrast with the significantly lower concentration (0.28 ± 0.38) at the end of week six **(Fig 5.14)**.

### *5.2.2.3) Comparisons of tGSH and MDA levels, as well as metal concentrations in millipedes at week 0 and week 6 of exposure groups*

Mean tGSH and MDA (measured as TBARS), as well as metal concentrations in millipedes at week 0 and week 6 of the control group, as well as the low and high exposure groups are shown in **Figs 5.15 to 5.20**.

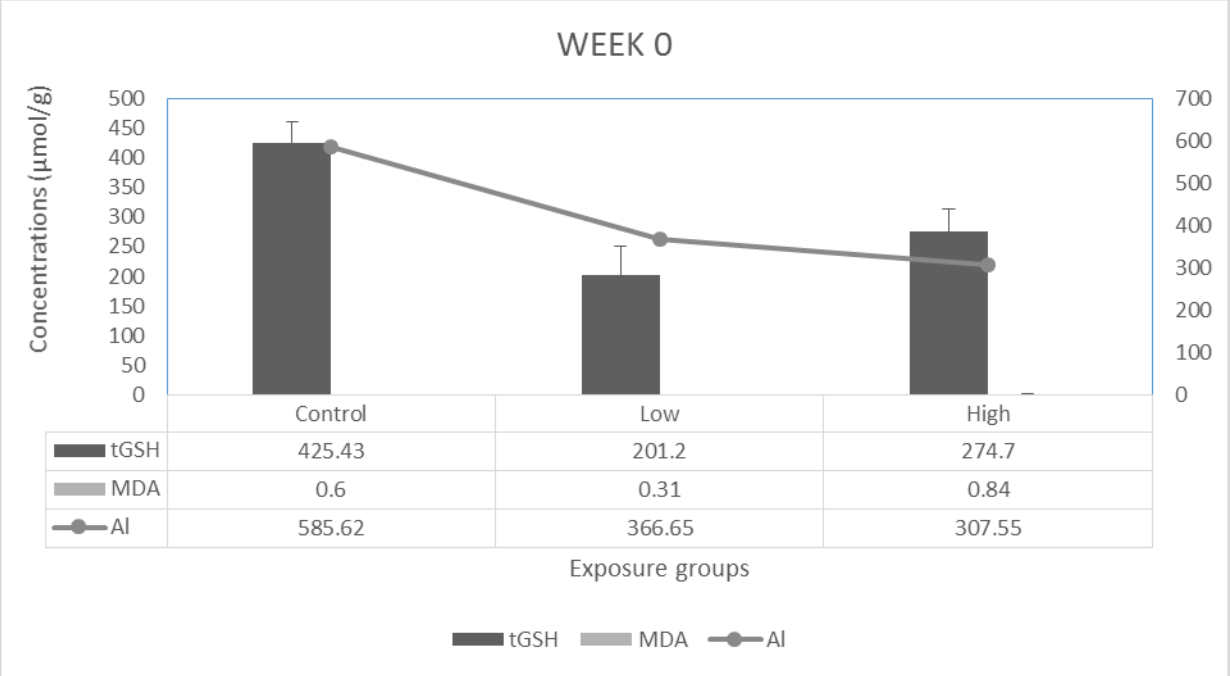


**Figure 5.15:** The mean tGSH, MDA (µmol/g) (± SD) and Al (mg/kg) (± SD) concentrations in millipedes of all three exposure groups at week 0. MDA (expressed as µmol TBARS per gram material); SD=Standard Deviation; N=5.

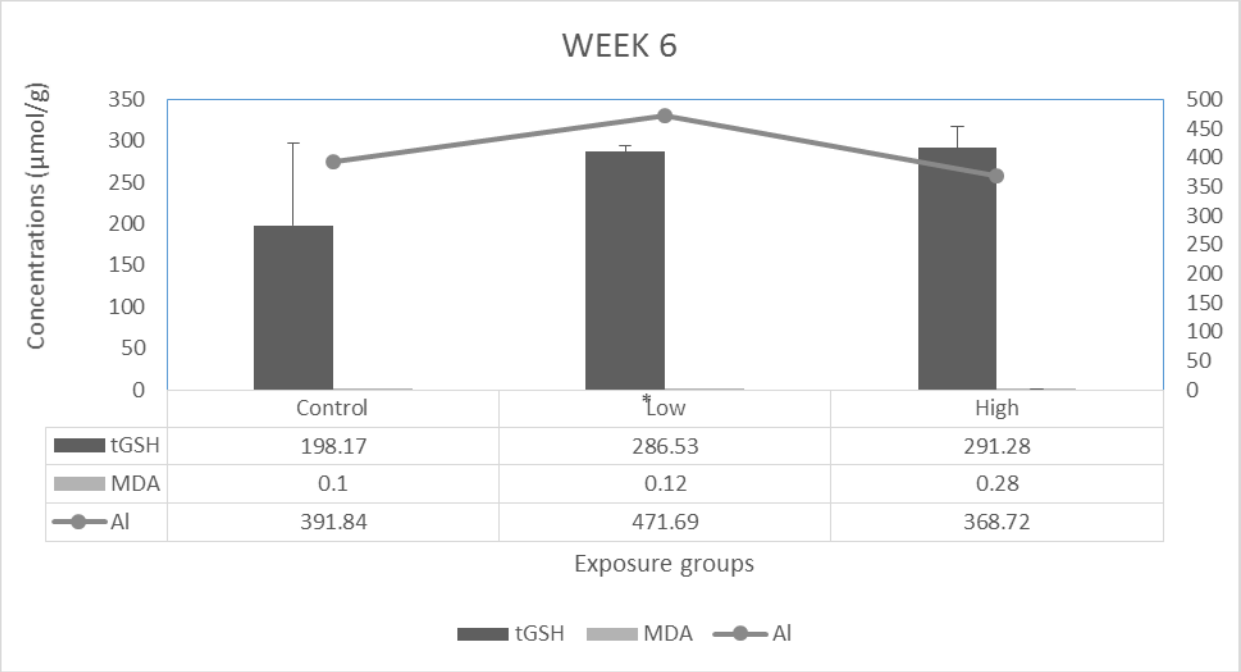


**Figure 5.16:** The mean tGSH, MDA (µmol/g) (± SD) and Al (mg/kg) (± SD) concentrations in millipedes of all three exposure groups at week 6. MDA (expressed as µmol TBARS per gram material); SD=Standard Deviation; N=5.

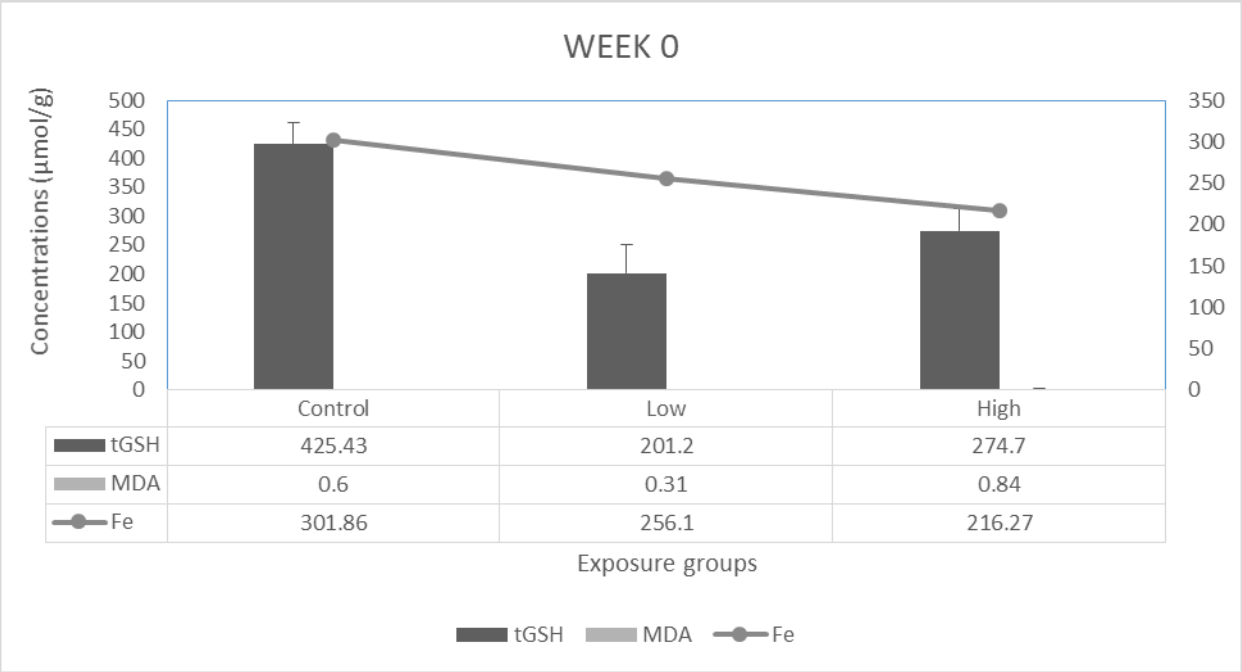


**Figure 5.17:** The mean tGSH, MDA (µmol/g) (± SD and Fe (mg/kg) (± SD) concentrations in millipedes of all three exposure groups at week 0. MDA (expressed as µmol TBARS per gram material); SD=Standard Deviation; N=5.

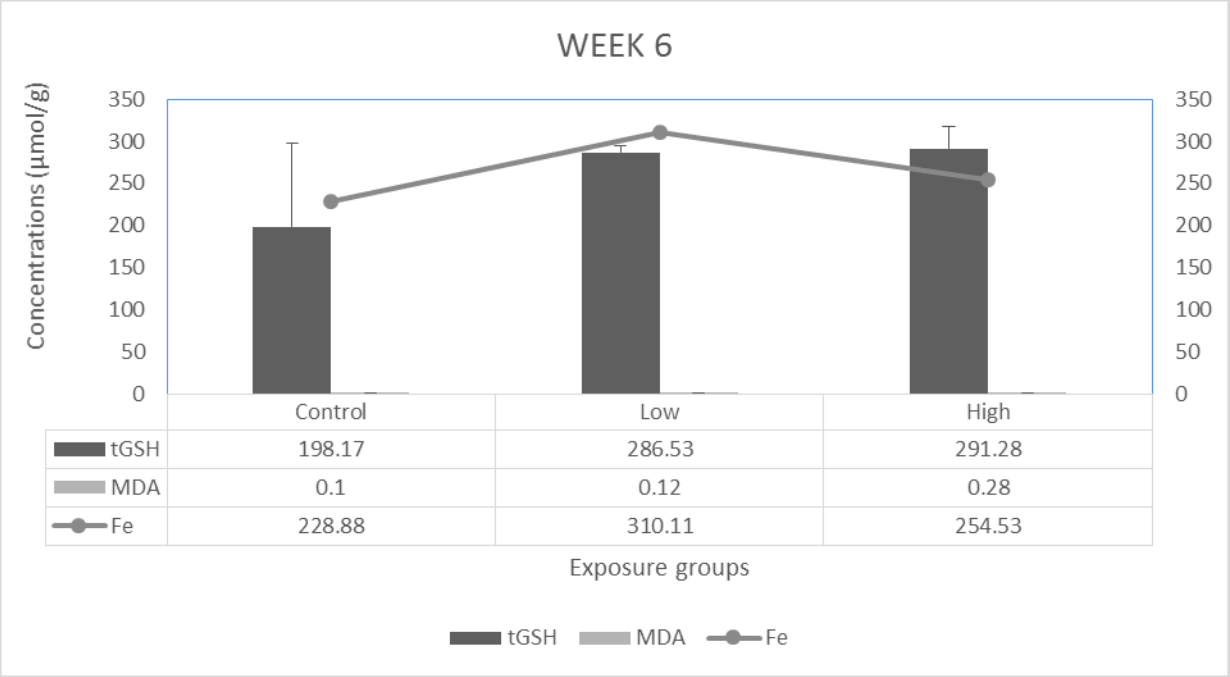


**Figure 5.18:** The mean tGSH, MDA (µmol/g) (± SD) and Fe concentrations (µmol/g) (± SD) in millipedes of all three exposure groups at week 6. MDA (expressed as µmol TBARS per gram material); SD=Standard Deviation; N=5.

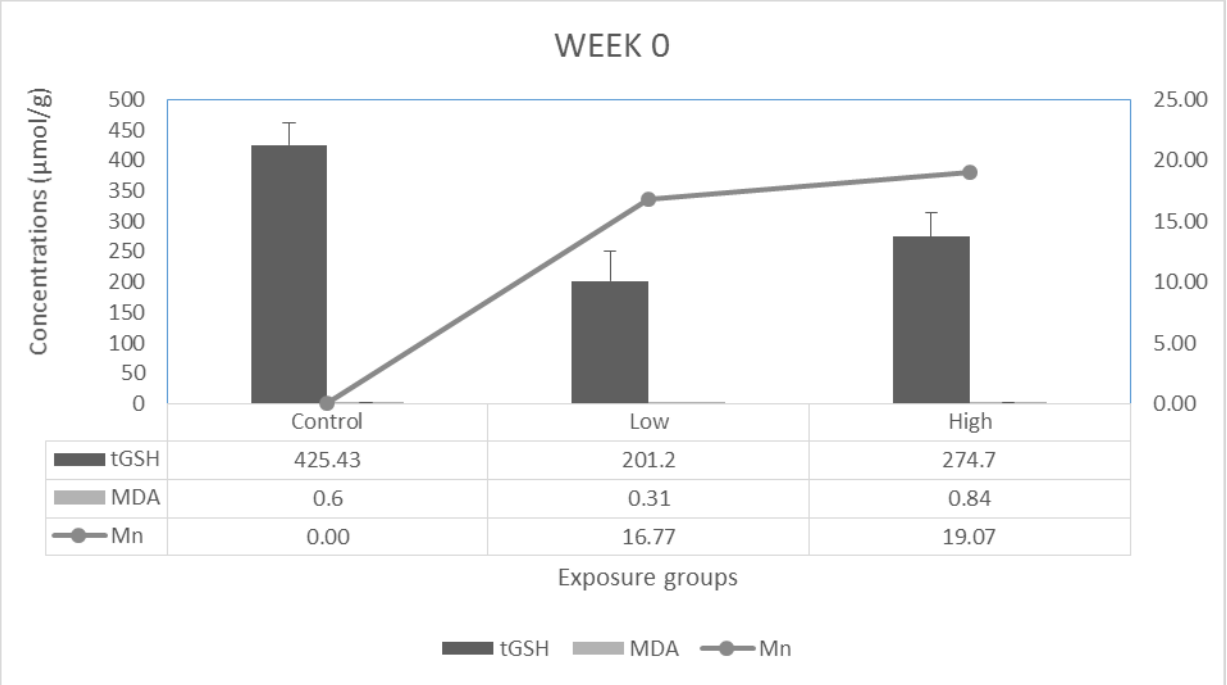


**Figure 5.19:** The mean tGSH, MDA (µmol/g) (± SD) and Mn (mg/kg) (± SD) concentrations in millipedes of all three exposure groups at week 0. MDA (expressed as µmol TBARS per gram material); SD=Standard Deviation; N=5.

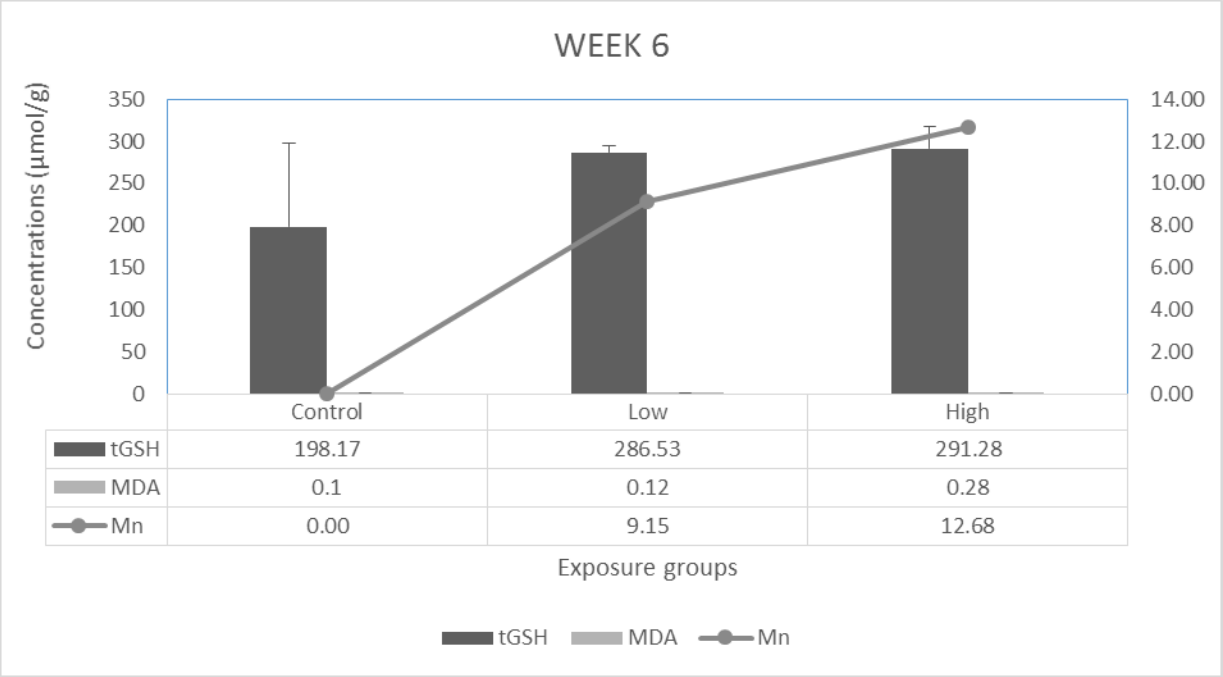


**Figure 5.20:** The mean tGSH, MDA and Mn concentrations (µmol/g) (± SD) in millipedes of all three exposure groups at week 0 week 6. MDA (expressed as µmol TBARS per gram material); SD=Standard Deviation; N=5.

### *5.2.2.4) Moisture % of millipedes at week 0 and week 6*

***Moisture % ranged from a moist 5.76 % to 15.13 % at week 0. At week 6 the moisture % of millipedes ranged from 3.94 % to 10.61 %.***

**Table 5.8:** Moisture % of the millipedes of exposure groups at week 0 and week 6.

| EXPOSURE GROUPS | % | MILLIPEDES | |
|---|---|---|---|
| | | WEEK 0 | WEEK 6 |
| Control | Moisture | 8.01 | 3.94 |
| Low | Moisture | 5.76 | 10.61 |
| High | Moisture | 15.13 | 5.67 |

## 5.3 DISCUSSION

### 5.3.1 Bioaccumulation of metals in experimentally exposed millipedes

The impact of toxicants on invertebrates largely depend on the bioavailability of the individual substances for the organism. A number of other factors, however are at play with regard to the bioavailability of a toxicant, such as the specific toxicant, characteristics of the environment, the organism itself (Crommentuijn et al., 1994) and the particular composition of the intestinal microflora of the invertebrate (Hopkin and Martin, 1984). In addition, the concentration present in the diet, is predominantly dependent on the rate of resorption of these substances (Dallinger, 1993). Research with regard to metals in their biology is, however few and far between, which is surprising as millipedes are wide spread terrestrial invertebrates in soil/leaf litter ecosystems (Hopkin, 1989).

It was clear from the results retrieved from the six week exposure experiment that bioaccumulation of the metals, Al, Fe and Mn in millipedes in their individual exposure groups had indeed occurred. The metal concentrations measured in the millipedes after the exposure period also corresponded with the metal concentrations found in the soil and leaf litter of each respective exposure groups, which is an indication of the millipedes consuming contaminated litter in a contaminated soil (Da Silva Souza et al., 2014) **(Tables 5.1, 5.2)**. Leaf litter is known to accumulate an exceeding amount of metals (Bengtsson, 1986). This is evident in the concentrations of Al, Fe and Mn measured in the leaf litter of the exposure groups, including the control group, before and after the soil had been spiked with the Al, Fe and Mn cocktail **(Figs 5.4 to 5.6).** The large quantities of metals, thus found in decaying leaves alone exposes millipedes to much higher metal concentrations than one would anticipate (Hopkin, 1989), considering that they digest just about half of the bacteria and fungal hyphae in the litter (Anderson and Bignell, 1982). The concentrations of metals in the bodies of diplopods do, however vary with different species (Siegel et al., 1975; Beyer et al., 1985; Poboszny, 1985) and depending on the metal content in the food, their assimilability also differs (Hopkin et al., 1985). Their metal concentration levels can also be elevated remarkably when they have been exposed to heavily metal-contaminated soils over a long period of time (Köhler and Alberti, 1990). This is reiterated in the metal concentrations measured in the millipedes collected from the control site, that were not all that different from the

concentrations measured in them after a period of six weeks **(Figs 5.7 to 5.9)**. Terrestrial invertebrates do not have much control over the uptake of metals from the food pulp (Hopkin, 1993), but millipedes have shown to discriminate their diet and regulate the rate of consumption in an effort to avoid the uptake of metal contaminated food (Hopkin et al., 1985). With regard to metal poisoning of the animals and of great significance, is the prospect of detoxification of undesirable elements and, hence the capacity to inactivate, store, and/or to excrete them (Hunziker and Kägi, 1985; Kägi and Kojima, 1987; Dallinger et al., 1989; Janssen and Dallinger, 1991).

The natural behavioral activities of the pill millipedes being buried in the soil during the day, with high activity above the leaf litter at night was observed in the control group. After being placed in the control tank at the start of the exposure period, the millipedes disappeared into the soil underneath the litter. At the same time, most of the millipedes that were placed in the tanks with the soil exposed to the lower and higher concentrations of the Al, Fe and Mn cocktail, came up to the surface, remaining there and climbing onto the decayed wood and branches, whilst exerting high activity and fast movements, similar to findings from Da Silva Souza and Fontanetti (2011). The pill millipede genus, *Sphaerotherium* are known tree-climbers, especially at night when they are most active, (Lawrence, 1966), however this specific behavior during the day is not the norm for nocturnal animals, but rather an attempt to escape, which concurs with findings reported by Da Silva Souza and Fontanetti (2011). This behavior also makes sense when pill millipedes in southern India were found to be exceedingly abundant in organically managed mixed plantations (Ashwini and Sridhar, 2002; Ashwini, 2003), as well as in forests. Chemically managed plantations were, on the other hand devoid of pill millipedes (Dangerfield, 1990; Dangerfield and Telford, 1992), suggesting that millipedes are sensitive to litter chemistry at narrow spatial scales (meters to decameters) (Warren and Zou, 2002). This also clarifies their attempt to escape the tanks, spiked with the low and high concentrations of the Al, Fe and Mn cocktail. Contrary to the findings of Da Silva Souza and Fontanetti (2011), of this behavior being magnified after the 15th day of exposure in their study, the abnormal behavior of the millipedes in the current study subsided after a couple of days. It seemed as if the millipedes reacted to the initial, sudden shock of exposure, but adapted to their environment soon thereafter, which can occur, as certain species have developed the ability to overcome stress

factors by inducing cellular processes of metal detoxification (Hopkin, 1989). At the same time this rapid adaptation to its environment may also be on account of the lower metal concentrations in the substrate of this study of Al (5014.4 mg/kg), Fe (4286.38 mg/kg) and Mn (77.59 mg/kg) in the low exposure group and Al (6228.8 mg/kg), Fe (5431.27 mg/kg) and Mn (80.83 mg/kg) in the high exposure group **(Table 5.2)** in contrast with the higher concentrations of, amongst others, Fe (39545 mg/kg) and Mn (228 mg/kg) found in Da Silva Souza and Fontanetti's 100% landfarming soil. The authors also reported some deaths occurring already during the 7th and 21$^{st}$ day of exposure in the 100%, 50% and 30% landfarming soil. Their exposure period lasted ninety days, as opposed to the six week exposure period in the current study, of which none of the millipedes died in any of the exposure groups, which may demonstrate extreme tolerance to the metal-containing food. They may further not have required any need to reduce food uptake (Hopkin, 1989), clarifying the increase in the mass of the millipedes in the control and low exposure groups after six weeks. A decrease in mass was, however observed in the millipedes of the high exposure group after the exposure period, suggesting that the millipedes were regulating the rate of consumption in an effort to avoid the uptake of the food contaminated with higher concentrations of metals (Hopkin et al., 1985; Read and Martin, 1990) **(Table 5.3 & Figs 5.10 to 5.12**).

The millipede species used in Da Silva Souza and Fontanetti's study, *Rhinocricus padbergi* have been well studied and is easily maintained in the laboratory (Camargo-Mathias et al., 1998; Camargo-Mathias and Fontanetti, 2000; Arab et al., 2003; Fontanetti and Camargo-Mathias, 2004; Fontanetti et al., 2004, 2006). In contrast, the pill millipede species used in the current study have, to the best of my knowledge not been used in studies of this kind. On the grounds of that fact, the pill millipedes were put through a trial period in unexposed control forest soil, leaf litter, and decaying wood in four different tanks to determine their stamina in a laboratory situation. This trail period was significant, as the natural habitat of pill millipedes are in moist forest soil and leaf litter. Their sensitivity to dry conditions (Attems, 1936; Sakwa, 1974; Achar, 1980; Ashwini, 2003) were witnessed as the millipedes in two of the tanks, succumbed at the first sign of dry conditions, whilst the millipedes in the remaining two tanks that were kept at steady moist conditions survived. This observation was a deciding factor in the determination of the length of the exposure period, as the required moist conditions in small tanks with a limited amount of

substrate were difficult to maintain at longer periods than six weeks. In their natural forest habitat of high moisture and dense leaf litter in a loam soil they can easily escape dry spells by burrowing deeper into the soil (SANBI, 2016), which is difficult to attain in tanks with such a limited amount of soil. The observation was in fact also essential, to rule out oxidative stress mostly due to dry conditions as opposed to metal induced stress in the millipedes (Tausz et al., 1998, 2007a, b; Bussotti, 2008).

The soil spiked with the low dosage of 1.4 g of Al, 1.4 g of Fe and 0.06 g of Mn and the high dosage of 20 g of Al, 20 g of Fe and 1.2 g of Mn yielded steady increases in the metal concentrations in both the soil and leaf litter when measured after the six week exposure period, which was to be expected. The Mn concentrations were higher in leaf litter in the low exposure group on week 0, as opposed to the expected higher concentrations after six weeks, which could in part be explained by physico-chemical processes in the tree canopy (Avila and Rodrigo, 2004) from the control site. The soil and leaf litter metal concentrations were higher in each respective group and corresponded with the dosages applied on week 0. However, there were no statistically significant differences found in the metal concentrations in soil and leaf litter, respectively after week 6 **(Tables 5.1, 5.2 & Figs 5.1 to 5.6)**, which may suggest possible leaching of the elements to the surface of the tank, as a result of the daily mist spray to accommodate the moisture requirement of the millipedes. This may have resulted in the lower metal concentrations in the topsoil and is a natural occurrence in highly porous soils (Madrid et al., 2004, 2007), used in this experiment **(Table 5.6)** (Peña-Fernández, 2011). Metals are similarly released from contaminated litter (Van Nevel et al., 2014) and may in all probability also serve as clarification for the same results. Although not significantly, the pH of soil declined slightly after six weeks in the low and high exposure groups, but the moisture percentage increased slightly **(Table 5.4)**. The soil becoming more acidic may have been caused by the daily spray of water, which is known to happen to soil subjected to constant precipitation. It is explained by increased leaching of basic cations, as a result of increased rainfall that transfers alkalinity from the soil exchange complex into surface waters and groundwater, causing acid soils (Rengel, 2011). The moisture in leaf litter decreased in percentage after the exposure period, which was anticipated, due to the loss of moisture in the leaves, it being fed on by the millipedes, as well as a result of the artificial circumstances. The addition of fresh leaf

litter weekly and the daily mist spray, therefore compensated for most of the moisture loss **(Table 5.5)**.

### *5.3.1.1) Al contamination of millipedes in all three exposure groups*

Aluminiumn concentrations were the highest in millipedes in the control group (585.62 mg/kg) at the start of the exposure period, but lower in this same group (391.84 mg/kg) after six weeks. Although a mass increase was observed in the millipedes for this group, after the exposure period, their metal concentrations were lower. This may suggest that they did not reduce their food intake, but detoxification of unwanted elements and, consequent inactivation, storage, and/or excretion may have played a role (Hunziker and Kägi, 1985; Kägi, 1987; Dallinger et al., 1989; Janssen and Dallinger, 1991). Aluminium concentrations in millipedes were higher in both the low and high exposure groups after six weeks of exposure. The higher concentrations were most likely caused by a higher rate of diffusion, as a result of the addition of metals to the diet of the millipedes, which as a consequence increases the concentration of the respective metals in the bodies of the millipedes (Cavallero and Ravera 1966; Ryder and Bowen, 1977; Ireland, 1982). The millipedes in the low exposure group (471.69 mg/kg) showed the highest Al concentrations after six weeks, even though the high exposure group had the higher application of the metal. The difference between the two groups might be explained by a reduction in food uptake in the millipedes of the higher exposure group (Cavallero and Ravera, 1966; Ryder and Bowen, 1977; Ireland, 1982) to avoid further uptake of the toxins and is a normal response to metal contaminated food (Hopkin, 1989). This was further verified by the mass decrease witnessed in the millipedes of the high exposure group **(Tables 5.1, 5.2, 5.3 & Figs 5.7, 5.10)**.

### *5.3.1.2) Fe contamination of millipedes in all three exposure groups*

The control group (301.86 mg/kg) of millipedes showed the highest Fe concentrations at the onset of the exposure experiment, but the millipedes decreased in Fe concentrations in this same group (228.88 mg/kg) after the experiment. A mass increase was noticed in the millipedes for this group after six weeks, but their metal concentrations were lower, suggesting that a reduction in food intake did not occur. However, the detoxification of the undesirable elements, inactivation, storage, and/or excretion may have been instrumental in the lower concentrations (Hunziker and Kägi, 1985; Kägi, 1987; Dallinger et al., 1989; Janssen and Dallinger, 1991). Both the

low and high exposure groups showed higher Fe concentrations in millipedes after six weeks of exposure, most likely by cause of a higher diffusion rate, due to the application of metals to the millipedes' diet. Consequent increases in the concentration of the respective metals in the bodies of the millipedes occurs as a result thereof (Cavallero and Ravera, 1966; Ryder and Bowen 1977; Ireland, 1982). The highest Fe concentrations were observed in the low exposure group (310.11 mg/kg) after six weeks, although the high exposure group received the higher addition of metals. This difference between the two exposure groups might be clarified by the millipedes in the high exposure group reducing their food intake (Cavallero and Ravera, 1966; Ryder and Bowen, 1977; Ireland, 1982) in an attempt to avoid further uptake of the toxins, which is an excepted response to metal contaminated food (Hopkin, 1989). The mass decrease in the millipedes of the high exposure group reiterated the results **(Tables 5.1, 5.2, 5.3 & Figs 5.8, 5.11)**.

#### *5.3.1.3) Mn contamination of millipedes in all three exposure groups*

The control group of millipedes displayed zero Mn concentrations in millipedes at the start and end of the period. In the low and high exposure groups, on the other hand, Mn showed higher concentrations in millipedes at the onset of the experiment, in comparison with the lower Mn concentrations in millipedes after the exposure period. The highest Mn concentrations were found in the high exposure group on week 0 (19.07 mg/kg), but also after the period in the same group (12.68 mg/kg). When the metal concentrations decrease with the addition of metals it be as a consequence of an accelerated saturation of the uptake mechanisms in animals subjected to extremely high metal burdens. They may also have reduced their food intake (Cavallero and Ravera, 1966; Ryder and Bowen, 1977; Ireland, 1982), as were noticed in the mass decrease of the millipedes in the high exposure group **(Tables 5.1, 5.2, 5.3 & Figs 5.9, 5.12)**.

### 5.3.2) Antioxidant levels as biomarkers of oxidative stress in experimentally exposed millipedes

#### *5.3.2.1) tGSH levels in millipedes of all three exposure groups*

The ability to overcome stress factors either by induction of cellular processes or metal detoxification have been noticed in some species of invertebrates (Hopkin, 1989). Induced detoxification mechanisms may therefore allow the animals to survive

certain acute shocks and avoid being poisoned by metals (Köhler, et al., 1992), as were found with the pill millipedes in this study. Both increases and decreases in tGSH levels in millipedes in their respective exposure groups, as well as between week 0 and week 6 were found, indicating that an overproduction of ROS in these organisms had occurred. However, the tGSH concentrations found in the millipedes at the start of the exposure period may have arisen from the control soil collected from the control site, at the forest, caused by accompanying stress factors in their environment (Tausz et al., 1998, 2007a, b; Bussotti, 2008). The highest tGSH concentration measured in the millipedes of the control group (425.43 µmol/g) at the start of the exposure period, was therefore possibly by chance, due to contamination over a long period, as the soil and millipedes came from the same control site. Of interest though is the fact that significantly lower tGSH concentrations in this group of millipedes were measured after the six week period (198.17 µmol/g). The lower tGSH levels implies that damage to tissues in millipedes had occurred and could have occurred due to the chronic exposure to the pollutants, such as metals (Loguercio et al., 1996; Barbaro et al., 1999) from the site that it was collected from. This is further reiterated in **Figs 5.15, 5.17** where the highest tGSH concentrations at week 0 in the control group millipedes, although not statistically measured, seemed to have shown a link with the highest Al and Fe concentrations, also measured in the millipedes from the control group.

Nevertheless, enhanced tGSH concentrations in millipedes were noticed after 6 weeks in the low (286.53 µmol/g) and high (291.18 µmol/g) exposure groups that have been spiked with the Al, Fe and Mn cocktail, at the start of the exposure period. These results on the other hand suggest that these metals were instrumental in the further induction of ROS in these organisms (Ercal et al., 2001; Koivula and Eeva, 2010; Sanchez-Virosta et al., 2015). This was evident in **Figs 5.16, 5.18, 5.20** where the highest tGSH concentrations were found in millipedes with the highest Al, Fe and Mn concentrations from the low and high exposure groups. The Al and Fe concentrations in the low exposure group millipedes were slightly higher, even though the tGSH concentrations in the millipedes showed the highest concentrations in the high exposure group, demonstrating that this endogenous antioxidant is successfully scavenging ROS in an attempt to protect the cells against the induced oxidative stress (Winston and Di Giulio, 1991; Choudhury and Panda, 2004, 2005; Singh et al., 2006) and simultaneously detoxifying metals (Sanita di Toppi et al.,

2008) at the initial high application of the metals. It thus seems, judging from the initial shock evidenced in the erratic millipede behaviour when they were first placed in the tanks of spiked soil, an overproduction of ROS was caused, which activated the antioxidant defence mechanism in order to protect cells from the induced oxidative stress **(Table 5.7 & Fig 5.13)**.

***5.3.2.2) MDA levels in millipedes of all three exposure groups***

MDA content (measured as TBARS) in millipedes showed higher levels at the start of the exposure period in all three groups, without the additional metal application. Significant differences in concentrations in millipedes were noticed between week 0 and week 6 of the exposure group, control (0.6 µmol/g). The high (0.84 µmol/g) exposure group displayed the overall highest MDA concentration. The increased MDA content in the millipedes at the start of the exposure period, indicates that they have already been exposed to elevated concentrations of toxic elements (Bačkor et al., 2010; Pisani et al., 2011) over an extended period of time. Metal contamination of the millipedes may already have occurred at their collection site, as the metal concentrations measured from the collection site was high when collected. Lipid peroxidation by means of the generation of free radicals (Choudhury and Panda, 2005) is an indication of oxidative stress (Ohkawa et al., 1979; Dazy et al., 2009). The lowest MDA concentration was found in the control group after week 6 (0.1 µmol/g). Even a low MDA count is an indication that lipid peroxidation (LPO) had occurred. This is due to the fact that MDA, being a decomposition product of polyunsaturated fatty acids, was produced during peroxidation of membrane lipids (Mittler, 2002).

The lowest MDA concentrations were measured in millipedes from the control group and the highest MDA content in the millipedes from the high exposure group, which is, therefore an indication that the millipedes in the group, exposed to the highest concentrations of the metals, Al, Fe and Mn experienced induced oxidative stress, associated with peroxidative damage of membrane lipids in the organisms (Cakmak and Horst, 1991; Foyer et al., 1994; Jing et al., 2009; Sen et al., 2014). Metal exposure does induce oxidative stress in invertebrates, which have been reported from laboratory experiments (De Almeida et al., 2004) and the stress behaviour observed when they were initially exposed to the high dosage reiterates it **(Table 5.7 & Figs 5.14; 5.15 to 5.20)**.

## 5.4. CONCLUSION

To conclude, it was clear that bioaccumulation of the metals, Al, Fe and Mn in millipedes in their individual exposure groups have taken place, through the contaminated litter, in the spiked soil that they have consumed during a six week period. From their erratic behaviour when initially placed in the tanks exposed to the low and high dosages, one can assume that the millipedes have experienced stress due to the toxicants they were subjected to. Their adaptation soon after, mass loss in millipedes in the high exposure group and zero mortalities, were possibly due to detoxification of the metals and a reduction in food intake, to avoid being poisoned.

According to the tGSH concentrations measured in the millipedes, it can be suggested that an overproduction of ROS in these organisms have occurred during the six week exposure period. The higher metal and tGSH concentrations in the respective exposure groups that were observed, indicated that the elevated metal concentrations in the millipedes activated the endogenous antioxidant system to scavenge ROS in an effort to protect cells against the induced oxidative stress and promote detoxification of the metals. The enhanced MDA content in the millipedes at the start of the exposure period, is an indication that these organisms have already been exposed to high concentrations of toxicants, (including metals) over a long period, which may already have occurred at the control site, from which the soil, leaf litter and the millipedes were collected. The higher MDA levels measured in millipedes in the group, exposed to the highest metal dosage also suggests lipid peroxidation possibly by means of the increased generation of free radicals, therefore shows that the pill millipedes have experienced induced oxidative stress, which was also confirmed by their erratic, escape attempts when initially exposed.

In view of this, the pill millipedes demonstrated extreme tolerance to the metal-containing food. It should, however be noted that these pill millipedes have been found to be more sensitive to the laboratory conditions and great care should be taken to comply with their living requirements before exposing them to extreme dosages and extended periods. In view of the observations and information gathered in the exposure experiment, as well as the fact that they have demonstrated to be good accumulators of metals, it may be suggested that they rather be used in a field situation, unless one could provide much larger and more natural, moist conditions

when using a laboratory set up. Even though pill millipedes poses a challenge in the laboratory, it may be suggested that they be used in ecotoxicological research, especially with regard to their important function in a forest ecosystem, their abundance and their sensitivity to metal contamination. Including pill millipedes in an ongoing program to monitor the health of forest ecosystems may very well provide invaluable information in terms of forest health in urbanized cities.

Further long term research, using pill millipedes in laboratory studies, especially over longer periods and even higher dosages may provide valuable information with regard to their response to the increasing amounts of pollutants these forests are exposed to. However, the dosages must fit the situation and using extremely high dosages that is out of the norm for these forests in order to determine the lethal dosage in this experiment would have defeated the purpose of this study, which is to determine the health of the forest ecosystems in the Western Cape at present. It would be of great significance though to monitor the metal contamination yearly and adapt the exposure experiment to the increased dosages found in the field, along with the increased urbanization, vehicle traffic and brown haze in winter. As an early warning system, it would then be advisable to apply slightly higher dosages as to determine when the sub-lethal effects are leaning towards lethal effects. It is also advisable to investigate other biomarkers for induced oxidative stress as it is always suggested to use a battery of markers to accurately assess the induced-oxidative stress or changes in redox status of these indicator organisms, such as millipedes. One has to understand that these forests are not situated next to industrial plants where they are subjected to extreme or abnormal situations or sudden spillages, which would yield extremely high metal concentrations. They are rather subjected to steady and increasing rates of pollutants over the years, which yields results, of which the metal concentrations seems insignificant. Against this background one may even suggest that Cape Town’s afromontane forests, situated on Table Mountain, one of the seven wonders of the world is still in good health and the pollution is not yet as severe, which is more than one could say for the forests in the rest of the world.

As a final thought, even though there were no statistical differences in millipede metal concentrations between the start and the end of the exposure period, the metal concentrations still increased and the millipedes reacted accordingly. This occurred

at dosages that have already been determined in the field and from the field studies it has been reiterated through the several pollutant patterns found that there is indeed cause for concern for the future of these forest ecosystems, as pollution is on the increase.

# CHAPTER SIX

# CAPE TOWN'S BROWN HAZE AND THE LINK BETWEEN FOREST AND HUMAN HEALTH

## 6.1 GENERAL DISCUSSION

Through urbanization, humans have within the last century showed unparalleled dominance over their ecosystems (Vitousek et al., 1997; Curran et al., 2002; Wei et al., 2015), exerting enormous pressure on the remaining natural resources (Atmis et al., 2007). It caused significant changes in the relationships between humans and the natural world (Miller, 1997; Miller, 2005; Maller et al., 2005; Nisbit and Zelenski, 2011; The Royal Society, 2012). Forests, once wide-reaching and remote, boasting of pristine biodiversity, have now been reduced to a small part of the urban infrastructure. Being fragmented and degraded, they share the same boundary with the residential and commercial areas (Farahwaheeda et al., 2009; Foo and Kidokoro, 2011). Yet, forests within cities are able to mitigate the majority of environmental impacts caused by urban development. They moderate the climate, reduce building energy use and atmospheric carbon dioxide ($CO_2$). They improve air quality, reduce rainfall runoff and flooding, and minimize noise levels (Nowak and Dwyer, 2007). However, the focus of their value has been reduced to counter-balancing the low quality urban environment, such as high traffic volumes and air pollution instead of being treasured for their biodiversity importance (Farahwaheeda et al., 2009; Foo and Kidokoro, 2011).

In this study it was found that the indigenous afromontane forest pockets on Table Mountain, receive considerable metal inputs through atmospheric deposition (Mason et al., 2000; Schwesig and Matzner, 2000), throughout the year (Wicking-Baird et al., 1997; CMA, 2015). One can almost not fathom air pollution reaching and accumulating in soil, leaf litter, moss, lichen and millipedes in such secluded and pristine areas, lush with plant growth and blanketed by dense tree canopies. These forests are surrounded by the major City of Cape Town, inundated with pollutant related sources (De Villiers et al., 2005), of which the most significant finding in this study was the enhanced concentrations of the metals, Al, Fe and Mn in soil, leaf litter and sentinel organisms in winter at Newlands forest, closest to the City of Cape Town where the brown haze appears most prominent. Haze is an indication that there are high concentrations of particulate matter in the atmosphere (Cheng et al., 2013) and

in Cape Town this smog is a common occurrence every winter, appearing as low as 30 m above the surface (CMA, 2015). The haze episodes in Cape Town escalate every year (Popkiss, 1992) and goes hand in hand with atmospheric deposition, which is instrumental in the biogeochemical cycling of metals, amongst others (Bari et al., 2014; Liang et al., 2016; Smets et al., 2016). Moreover, is the fact that much of the metal concentrations found at both Orange Kloof and Newlands forests surpassed concentrations found at industrial sites in major cities around the world (Peña-Fernández, 2011; Malaspina et al., 2014). It is therefore no wonder the ecosystems on Table Mountain are regarded as one of the world's most threatened (Underwood et al., 2009).

At the very least the health of these forest ecosystems are at risk, due the toxic metal levels it has been and still are subjected to. In such a vulnerable state, soil, biota and plants may be affected in terms of abundance, diversity, distribution (Hopkin, 1989), respiratory processes, photosynthesis and protein synthesis (Marschner, 1995). Respiration rates in forests may decrease (Bewley and Stotzky, 1983; Laskowski et al., 1994; Fritze et al., 1996), tree growth itself could be reduced (Aznar et al., 2007). Natural populations may be impacted (Van Hook and Yates, 1975; Hunter et al., 1987b; Posthuma and Van Straalen, 1993; Lock et al., 2003) and in turn show effects at the community level and alter critical ecosystem functions (Coughtrey et al., 1979; Hunter et al., 1987a; Tyler et al., 1989; Read et al., 1998; Nahmani and Lavelle, 2002; Creamer et al., 2008; Clements and Rohr, 2009). These ecosystems may further encounter induced oxidative stress (Tausz et al., 1998, 2007a, b; Bussotti, 2008), which was indicated in this study through the increased levels of the endogenous antioxidant, tGSH in moss, lichen and millipedes, by which these organisms demonstrated an attempt to prevent and/or modulate the induced peroxidation effects (Wilce and Parker, 1994; Van Der Oost et al., 2003; Ezemonye and Ikpesu, 2011; Paulino et al., 2012). Decreased tGSH levels were also found in these organisms, reflecting tissue damage, already occurred as a result of being subjected to chronic toxicant exposure. Both the increases and decreases in the tGSH levels shown in the organisms of these forests suggested that an overproduction of reactive oxygen species (ROS) in their cells had occurred (Loguercio et al., 1996; Barbaro et al., 1999). The organisms also showed different levels in MDA content, of which the higher concentrations demonstrated exposure of the organisms to high concentrations of toxic elements (Bačkor et al., 2010; Pisani et

al., 2011), such as metals, which caused lipid peroxidation by means of the generation of free radicals (Choudhury and Panda, 2005). The lower MDA content displayed in the organisms suggested that lipid peroxidation had occurred (Mittler, 2002). Both the higher and lower levels, nonetheless suggests that the organisms in these forests incurred induced oxidative stress, in all likelihood by cause of the metal contamination (Ohkawa et al., 1979; Dazy et al., 2009).

The metal concentrations found in this study, together with the contributing sources of the metals towards particulate matter in the haze concurs with many other studies done world-wide. Vehicle emissions are the main culprits, followed by industries, wood-, wild- and forest fires (Dentener et al., 2006; Ginoux et al., 2012) and natural sources such as wind-blown dust and sea salt (Wicking-Baird et al., 1997). The brown haze or smog, being a worldwide phenomenon is therefore encouraging researchers to investigate this, because of the negative impacts it may have on ecosystems (Chameides et al., 1999; Zhang et al., 2013), the global climate, on visibility, cloud formation and on public health (Okada et al., 2001; Menon et al., 2002; Yadav et al., 2003; Andre, 2005). Metals are regarded as one of the most critical and universal forms of environmental contamination (Pruski and Dixon, 2002) and the well-studied detrimental impacts on human and ecosystem health (Herngren et al., 2006; Wijesiri et al., 2016) cannot be ignored, or in fact underestimated. A brown haze study conducted in Auckland, New Zealand in 2001 revealed similar results to those found in a study done in the winter of 1992 in Cape Town (Pineda and Villiers, 1995), of which the main anthropogenic sources of the brown haze were as mentioned above (Senaratne and Shooter, 2004). Haze has engulfed Southeast Asian regions, especially in Malaysia, Singapore, Indonesia and Thailand, as a result of recurrent slash-and-burn agricultural activities (Betha et al., 2013). In Sri Lanka predominant sources of the metals, Al, Fe and Mn to the atmosphere and in hazes in the urban environment (Ziyath et al., 2016) arises from natural sources such as soil and anthropogenic activities such as traffic, industrial processes, and incineration of sewage sludge and solid waste (Azimi et al., 2003; Tian et al., 2015). The additional traffic influence of tourism in holidays and weekends in Sri Lanka adds to the anthropogenic pressure and may be of relevance to Cape Town, also being a popular tourist destination (Meetiyagoda, 2016). Haze resulting from rapid urbanization and industrialization along the elevated site of the Himalaya (Lau et al., 2006, 2008; Gautam et al., 2009; Guleria et al., 2011a, 2011b; Guleria and Kuniyal,

2016) have grave impacts on Himalayan glaciers and consequently the climate system (Ramanathan at el., 2001, 2007; Lau et al., 2006, 2008). The glaciers feed the rivers, which are the lifelines of millions of people that live in the downstream (Singh and Bengtsson, 2004; Qiu, 2008; Xu et al., 2009). Also in the United States the patterns and trends of haze were demonstrated by Schichtel et al. (2001). In China haze pollution, with similar contributing sources are regarded as the most serious environmental air issue, because of the detrimental effects on public health (Tie et al., 2009; Chen et al., 2013; Yao et al., 2014; Huo et al., 2015), ecosystems (Chameides et al., 1999; Zhang et al., 2013) and the climate as a whole (Solomon et al., 2007; Tainio et al., 2013). Serious illnesses as a result of the haze has been reported, of which respiratory illnesses, heart disease, premature death, and cancer are but a few (Duan and Tan, 2013; Li et al., 2016a, 2016b). Admission rates to hospitals are high and unexpected infant mortality during the weather conditions that contribute to haze have increased greatly (Thach et al., 2010; Ge et al., 2011; Guttikunda and Goel, 2013; Liu et al., 2014; Othman et al., 2014; Gao et al., 2015). Numerous studies have also indicated that the oxidative damage of plasmid DNA in humans is the result of free radicals generated by particle components such as metals (Villalobos et al., 1995; Li et al., 1996; Prahalad et al., 2001; Lü et al., 2006; Wei et al., 2009). Even the reduced number of hours of sunshine caused by haze has an effect on the growth of crops (Moran et al., 2014). The impact of haze pollution further results in serious economic losses (Gultepe et al., 2007), of which the total economic cost of health impacts caused by air pollution in Shanghai was estimated at approximately 625.40 million dollars in 2001 (Kan and Chen, 2004), and increasing in value by four folds in 2004 (Zhang et al., 2008). The fine particles have, furthermore a significant ecotoxicity during high pollution episodes (Liu and Zhang, 2015; Lu et al., 2008; Turóczi et al., 2012). In an attempt to address the adverse impacts caused by the brown haze, China have appointed a growing body of researches that focusses on size distributions and chemical components of the haze particles (Sun et al., 2006; Han et al., 2015; Zhao et al., 2015), of which Hu et al. (2015) reported that metals, such as Fe in particles during the haze episodes in Beijing originated mostly from local industrial emissions. It was also said that effective air pollution control measures are crucial in order to improve atmospheric visibility, protect human health, and reduce ecological damage and that investigation into the characteristics of particulate matter should be done during brown haze episodes (Huang et al., 2011). Mitigation strategies, such as easing congestion, improving fuel

and vehicle quality would have to form a significant part of the strategies (Weerasundara, et al., 2017).

There is an extremely strong link between the environment and human health and well-being, of which scientific research supports the positive effect of interacting with forests with respect to mental and physical health promotion (Bowler, et al., 2010; Nilsson et al., 2011; Bratman, et al., 2015). Awareness campaigns regarding the adverse impacts of human technology to natural systems are also causing a change in people's perspectives towards synchronizing their lifestyles with nature (Farahwaheeda et al., 2009; Foo and Kidokoro, 2011). A shift have therefore been made in finding a balance between both sustainable forest management and ecosystem-based management paradigms to maintain ecosystem integrity, while preserving opportunities for humans to procure benefits from forests (Higman et al., 1999; Price et al., 2009). This important link between human health and well-being and the environment have caused growing political interest in promoting natural environments, such as forests for public health in aid of creating sustainable cities (European Commission, 2014; World Health Organization, 2006).

## 6.2 CONCLUSION AND RECOMMENDATION

The research question, principle aim and objectives in this study have each been answered and met. The soil, leaf litter and sentinel organisms used in the current study were excellent bioindicators and biomonitors of metal contamination in the indigenous afromontane forest pockets on Table Mountain, within the City of Cape Town. They were even more effective in revealing typical pollutant related patterns, especially with regard to the brown haze experienced during Cape Town's winter seasons, suggesting that Cape Town in South Africa is no different to the rest of the world in terms of pollution and the brown haze. The degree of pollution differs in every town and country, but the sources and impacts on the climate, ecosystems and human health remain the same. The health of these forest ecosystems, within this major city are definately at risk in the future, which was demonstrated in the metal contamination and bioaccumulation patterns signified in the soil, leaf litter and sentinel organisms, as well as in the antioxidant response from the sentinel organisms to metal contamination, but also in the expansion of industries, population, vehicle traffic usage and urbanization.

Continuing to monitor the effects of brown haze on Cape Town's forest ecosystems, using the same, as well as the many other indicator organisms available on the mountain are, therefore imperative as an early warning system with respect to forest ecosystem health and to the health of Cape Town's inhabitants for that matter. The research is also essential in implementing effective air pollution control measures in terms of metals in order to improve atmospheric visibility, protect human health, and reduce ecological damage in a country that does not measure metals. This study have also provided a basis of metal concentrations in vital components of a forest ecosystem for future reference from which to work with, that was not otherwise available. Pill millipedes that have also, to the best of my knowledge not been used in studies of this kind, provided valuable information for future research in this field. And of utmost importance is the link between the environment, human health and well-being, due to the many health benefits these ecosystems offer, whether it be physical or psychological, social or economical, which may develop a conservation mindset in humans.

Some limilations and practicle problems expienced during this research were a) the lack of data that exists in South Africa from which to compare the metal concentrations found in this study with; b) the rocky en rugged forest terrain made finding suitable sites difficult in terms of reachbility and availability of lichens and mosses; c) difficultly in finding pill millipedes in the dry summer season was not anticipated and caused practical and time constraint problems, especially at Platbos where they were totally unavailable during the pilot study. This was also the reason for moving the control site about 30 m from the original site at short notice where pill millipedes were found in abundance during the first scouting excersize; d) the control site turned out to be the most contaminated site, because of its slightly more exposed location to wind and this was also not anticipated. It did, however make sense after the higher metal concentrations lead to the realization that the site received emmense exposure from the wind blowing from the cape flats against the side of the mountain; e) the pill millipedes were quick to show stress in the laboratory experiment, especially with regard to their moisture requirements; f) it was difficult to pinpoint exact sources of the metal contamination in the forests, due to the fact that the forests are not situated next to industries or even encountered toxic spillages; g) percentage metal contributions from either natural or anthropogenic origin did not form part of this study, but such information would be valuable in determining the exact sources; h) the heterogeneity of the forest terrain at times caused significant differences in metal concentrations from one site to another and even from one organism to another.

In view of the above mentioned limitations, one has to realize that this is the nature of forest research. The terrain is heterogenous and there are no huge overnight contamination scares. There is however a silent and slowly increasing pollution problem, that if not monitored consistently over the years, using a wide aray of bioindicators and monitors in combination with a battery of biomarkers, could cause surprises in the form of irreparable damages to these ecosystems in ten or twenty years time or even less. Pollution related patterns in these forests and a database of metal concentrations in soil, leaf litter and some important sentinel organisms have been established. It is therefore recommended to continue this research and create a comprehensive database of metal concentrations to track changes in metal contamination and more so in winter during the brown haze episodes.

Metals of particular interest that need addressing from this current study would be Al, which causes soil acidity as Al toxicity in soils with very low acidity may cause serious forest damage. High Mn bioavailability in soils may cause Mn toxicity in plants and also eventual dieback trees. It is thus recommended to monitor these metals in particular: Al in combination with soil pH, as well as Mn in the gasoline additive methylcyclopentadienyl Mn tricarbonyl (MMT), due to the increasing vehicle traffic. It is therefore then advisable to distinguish whether metal contamination are of natural or anthropogenic origin.

Using pill millipedes had its challenges, but with minor practical adaptations in terms of their moisture requirements they are still highly recommended for use in the monitoring program, due to their important function and abundance in a forest ecosystem. They are good indicators and monitors of metal pollution, are easily sampled under the leaf litter layer in the shallow soil and their shiny ball shapes are easily spotted. In a histological experiment done during this study, but not included in this study they were also easily euthanized, dissected and their intestinal tracts removed and midguts analysed.

Responses at lower levels of biological organization, such as molecular and biochemical are preventative techniques and are therefore recommended as an integral part of such an ongoing monitoring program (see Chap 4: Conclusion for recommended biomarkes of oxidative stress).

Monitoring metal accumualion and contamination in forest leaf litter is important because it can retard litter decomposition processes, which plays a major role in the health of forest ecosystems. Finally, soils are not renewable, soil biodiversity sustains critical ecosystem functions above and below ground and soil ecosystems determine the productivity of forests. It is therefore recommended see the evaluation of the environmental stresses on soil fauna as a high priortity when monitoring the health of forest ecosystems.

The specific species of moss, lichen and pill millipedes used in the current study are commonly found in the afromontane forests in the Western Cape and South Africa. Using the same species as bioindicators of air pollution, in the different forests all over South Africa in urbanized cities would provide valuable information for

comparison between forests with regard to the health of each forest ecosystem individually, but on a much larger scale, thereby creating a database and network of information not currently available in South Africa. The effects of air pollution in forests can further be assessed by monitoring millipedes, lichens and mosses for a) changes in community, such as species composition, disappearance and density, b) physiological and biochemical changes in lichen and mosses, such as degradation of chlorophyll and photosynthesis decline and c) morphological biomarkers in the midgut of millipedes, such as histological and histochemical changes. One could expand research further by making use of other species of moss, lichen, millipedes or invertebrates that commonly occur in the same forests.

Monitoring programs in forests should include the responses of multiple indicators to make more accurate assessments of the pollution status in a forest. There are many multifaceted and interlinked environmental factors that impact the organisms in mountain forested areas. Mountain communities are also likely to respond to disturbance in different ways and species richness and diversity will quite often decrease in response to the various types of chemical pollution. In that light long term forest degradation or health could possibly be better evaluated by monitoring changes in the whole invertebrate community, using the right indicators and endpoints with each specific purpose in mind (Pearson 1994; McGeoch 1998).

## CHAPTER SEVEN

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