The impact of mine tailings on snouted harvester termites, *Trinervitermes trinervoides* (Sjostedt) (Isoptera: Termitidae), in the Vaal river region.

By

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Declaration

I declare that this Dissertation is my own, unaided work. It is being submitted for the Degree of Master of Science at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.

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20 January 2012

Abstract

Mining activities are well known for their negative effects on the environment and animals, due to the deposition of large volume of wastes in the form of tailings on the soil. These wastes or tailings contain high levels of heavy metals, sulphides and cyanide. Heavy metals are one of the most persistent pollutants in the environment and have been shown to bioaccumulate in animals. The purpose of this investigation was to evaluate the impact of mine tailings on the snouted harvester termite, *Trinervitermes trinervoides*, inhabiting the Vaal River region. These termites play a significant role in the food chain as they provide a protein- and energy-rich food source to numerous predators therefore any bioaccumulation of heavy metals may adversely impact the food chain. Three aspects of the termite biology was studied, namely, the density and distribution of the termite mounds, the temperature profile of the mounds and the heavy metal content of the termites, mounds and surrounding soil. Three sites were chosen in accordance to their position relative to a tailings dam with the furthest site being the Control site. The most contaminated site and site closest to the tailings dam (AEL site) had the highest termite mound density, followed by the least contaminated site (Control site) and then the intermediately contaminated site (West Complex). The AEL site had many incipient mounds but few large mounds indicating that although there was a high turnover of new mounds, the longevity of these mounds was low. Higher densities at the AEL site may be explained by the water table being closer to the surface as a result of the tailings dam, allowing the termites easier access to water and hence a more favourable environment within the mound. The centre temperatures of the mounds at all three sites were kept constant on a monthly basis but fluctuated on a seasonal basis. The West Complex site had the highest and most variable centre mound temperatures. The average heavy metal content of the surface layer did not differ significantly from the average heavy metal content of the mounds at the AEL and West Complex site, indicating that the termites are not making heavy metals more bioavailable to the environment. The termites at the AEL site had the highest levels of Cu and Zn out of all the sites and accumulated these metals to levels toxic to mammals. Alates (a major food source for many animals) however, did not accumulate any heavy metals therefore it is unlikely the food chain is being negatively impacted by the termites. From this study there is no indication that the snouted harvester termite density or behaviour are being impacted by the tailings dams.

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CHAPTER 1 – GENERAL INTRODUCTION

1.1. Introduction

South Africa is considered a treasure trove because the country boasts an abundance of mineral resources, producing and owning a significant proportion of the world's minerals (Winde and van der Walt, 2004). Among minerals such as platinum, manganese and chrome; gold is one of South Africa's most economically important minerals and has played a significant role in the economic development of the country over the past 120 years (Richardson and van Helten, 1984). Almost 50 % of the world's gold reserves are found in South Africa, rendering it the largest gold producer of the world (Rosner and van Schalkwyk, 2000). The most prominent and largest gold mining company in South Africa is the Anglogold Ashanti Mining Company. It is the third largest gold mining company in the world boasting 21 operations that produce some 6 million ounces of gold combined each year (AngloGold Ashanti, 2006a). Headquartered in Johannesburg, the South African operations are comprised of seven underground mines along the Witwatersrand Basin, four of which are located in the Vaal River and three in the West Wits region (AngloGold Ashanti, 2006a).

AngloGold Ashanti South Africa owes its prolific and efficient gold production to the gold cyanidation process. This process involves the crushing of the ore into a fine powder and subsequently a dilute cyanide solution is percolated through it, leaching out the gold from the slurry. The gold is completely extracted using the "carbon-in-pulp" process (Naicker *et al.*, 2003). Gold is not the only element extracted from the slurry and sold commercially; three of the four Vaal river mines extract large amounts of uranium as a by-product of gold mining operations owing to the much higher ratio of uranium to gold in the ore. Uranium is leached with sulphuric acid, instead of cyanide (AngloGold Ashanti, 2005).

Each mine in the AngloGold Ashanti Vaal river region is equipped with a tailing storage facility (TSF) to store all the waste generated by the mining and processing operations. All the wastes (tailings) are transported via tailing pipes that stretch over several kilometres to designated TSF's situated in specific locations (Figures 1.1 & 1.2). Once the tailings have been stored in the TSF, it is common practise to add water, creating a tailings dam that allows for sedimentation of the solid particles from the refuse material (Figure 1.3, Van Niekerk and Viljoen, 2005). One of the largest tailings dams of the region is 32 metres in height, currently holding 22 million tonnes of tailings and is expected to increase to 60 metres over its remaining lifespan of 12 years (AngloGold Ashanti, 2007a). All the tailings dams are unlined, uncovered and almost all are unvegetated as most plants cannot withstand the tailing substrate. Tailings consist mainly of waste rock of the mined ore that contains large quantities of heavy metals such as cadmium, arsenic, copper, manganese, lead and zinc and unprocessed uranium. Other substances present include sulphides and cyanide, from the gold cyanidation process (Wonga *et al.*, 1999).

Figure 1.1. The Tailing Storage Facilities (yellow blocks) and associated tailing pipes (red lines) found in the AngloGold Ashanti Vaal river region (AngloGold Ashanti, 2007a).

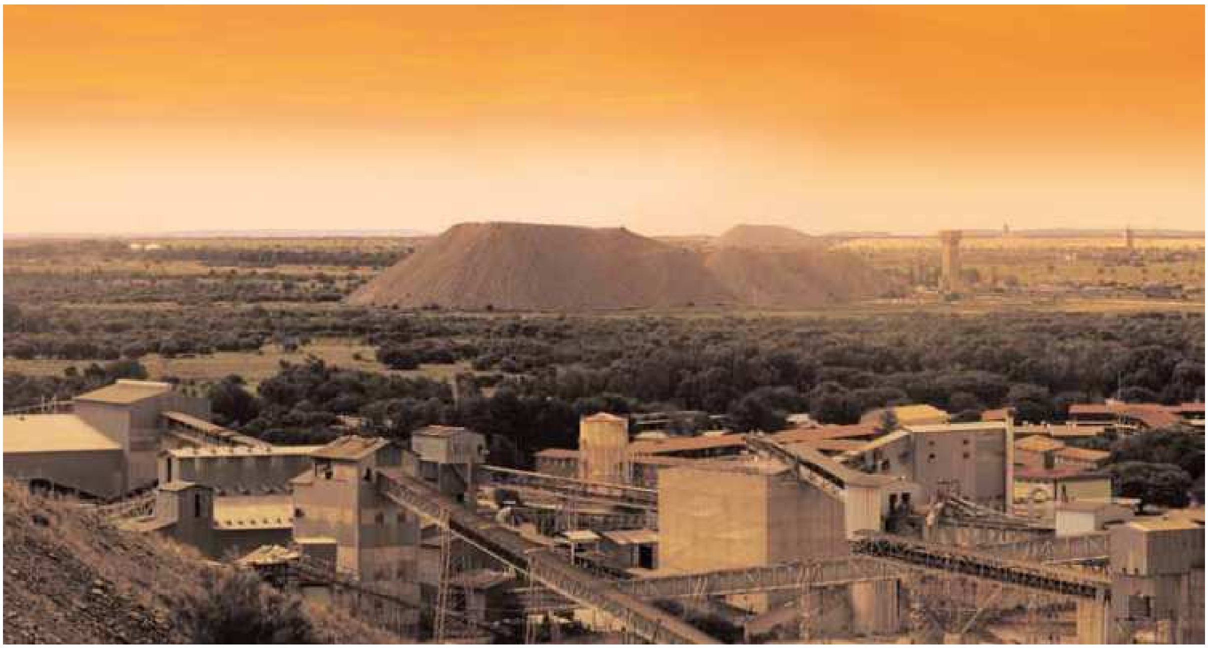


Figure 1.2. Photograph of a typical tailings storage facility (or tailings dam) (AngloGold Ashanti, 2007a).

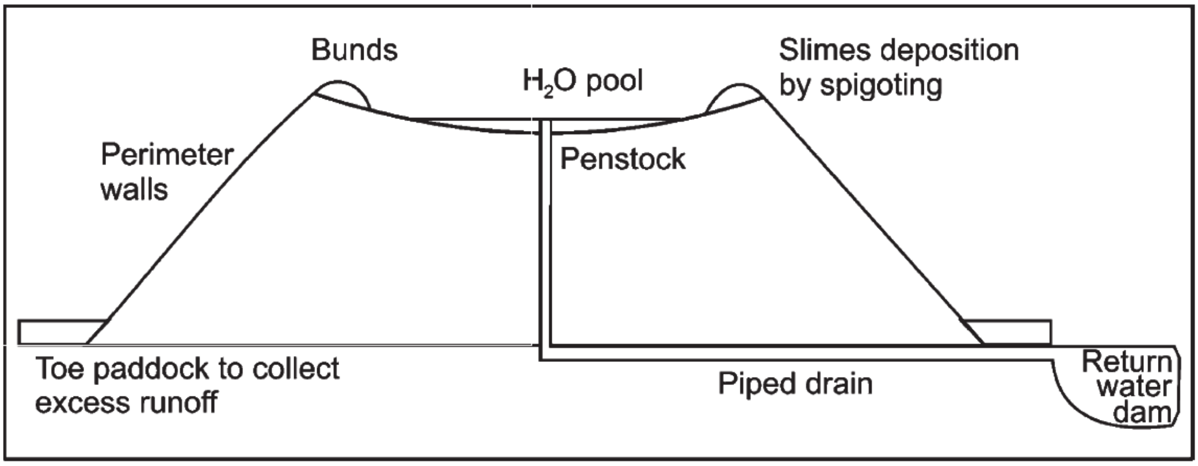


Figure 1.3. A diagrammatic representation of a typical gold-tailings dam (not to scale) taken from Van Niekerk and Viljoen (2005).

Most of the substances present in tailings are extremely toxic and pose a major threat to the environment. Sulphides found in tailings weather and dissolve to form an acid discharge also known as acid mine drainage, which is a major environmental issue in South Africa (Naicker *et al.*, 2003). This acid discharge reacts with the tailings to solubilise heavy metals and ultimately produce more contaminants. There are numerous reported cases in which acid mine drainage has caused serious environmental problems in South Africa, particularly when ground and surface waters are involved. For instance, in 2003, acid mine drainage from an active gold mine in the Witwatersrand region, heavily contaminated and acidified the surrounding ground and surface waters of the mining district which in turn contaminated water 10 km beyond the source of pollution (Naicker *et al.*, 2003).

The production of millions of tonnes of tailings as a result of gold mining is unavoidable. For every tonne of gold produced 200 000 tonnes of waste is generated and deposited into a tailing storage facility (Rosner and van Schalkwyk, 2000). This is a colossal amount when one considers that the Vaal river operations produce approximately 35 tonnes of gold per annum (AngloGold Ashanti, 2007a). In 2007, 14 tailing spillages occurred in the Vaal river region, causing consequent environmental damage, soil degradation and water pollution that is still, in some cases, evident some two years later (AngloGold Ashanti 2007b). It is therefore of outmost importance that these tailings are monitored, contained and managed properly to ensure that these damaging tailing spillages occur as infrequently as possible.

Cyanide is hypertoxic to humans and many other living creatures. It is quickly absorbed and distributed throughout the body of vertebrates where it acts rapidly as an asphyxiant, causing hypoxia of cells (Bhattacharya and Flora, 2009). Although cyanide reacts readily in the environment and degrades or forms complexes and salts of varying stabilities, it is still toxic to many living organisms at low concentrations, making it a hazardous contaminant (Way *et al.*, 1988; Wonga, 1999; Macklin *et al.*, 2003; Ritcey, 2005; Donato *et al.*, 2007). Numerous studies have illustrated the devastating effects cyanide on the environment. For example, in 1995, a goldmine tailings dam collapsed in the Yining County of XinJiang Region of China and the surrounding farmlands and rivers were seriously polluted by cyanide (Shehong *et al.*, 2005). Shehong *et al.* (2005) showed that some four years after the collapse, the polluted farmland soils were still highly enriched with cyanide and in some cases, showed a concentration higher than that of the fresh tailing products.

When dealing with the environmental impact of uranium, two important properties of the element need to be taken into consideration; it is both a radioactive and a chemical toxin. Uranium as a toxic metal, along with other metals such as cadmium and zinc, negatively impacts the quality of the environment, affecting mainly soils and surface and ground waters while simultaneously polluting great areas of lands and endangering the catchments of available drinking water (Keith *et al.*, 2000). The radioactive properties of uranium dramatically increase its potential to negatively impact the environment as it produces decay products that, in themselves, are highly toxic (Gavrilescu *et al.*, 2009).

A central concern when dealing with the environmental impact of tailing dams is the introduction of heavy metals, sulphides, cyanide and uranium into the food chain. According to Solomon *et al.* (2005), the degrees of impact of these contaminants are the result of three characteristics; their persistence, bioaccumulation and biomagnification. Contaminants that are classified as persistent are extremely stable and may take many years to be broken down into simpler forms by natural processes. Bioaccumulation and biomagnification are sometimes used interchangeably however, an important distinction is drawn between the two. Bioaccumulation refers to the accumulation of or increase in concentration of a persistent substance (i.e. contaminants) in an organism within a trophic level; whereas biomagnification pertains to the accumulation of the substance as it passes through successive levels of the food chain (Solomon *et al.*, 2005). Therefore, organisms at higher trophic levels in the food chain tend to store greater concentrations of bioaccumulated contaminants in their bodies than do those at lower levels. If the contaminants released from the Vaal River tailing dams are persistent and tend to bioaccumulate (hence biomagnify), the resultant scenario could, on a smaller scale, resemble the DDT calamity that began in the 1940's (Turusov *et al.*, 2002).

It is well known that mine contaminants, particularly heavy metals, can accumulate in both invertebrates such as snails and insects and a variety of plants (O'Shea *et al.*, 2001; Yanqun *et al.*, 2004; Liu *et al.*, 2006; Ping *et al.*, 2009; Li *et al.*, 2010). Ping *et al.* (2009) found that plant-eating insects provided important links in transferring pollutants to their predators resulting in biomagnification as pollutant levels were highest in the predators. Due to the potential for hazardous and persistent mine wastes to bioaccumulate and biomagnify in the environment surrounding the tailings, it is considerably important to engage in methods that allow for the monitoring of such wastes. Such methods could involve an in-depth analysis and sampling of prominent organism/s found in the food chain inhabiting the environment surrounding the tailings.

AngloGold Ashanti acknowledges the need to work in an environmentally responsible way by incorporating sound environmental management practises in its everyday operations (AngloGold Ashanti, 2006b). In fact, the company conducted an enormous environmental impact assessment of all its mining operations in South Africa. This involved the determination of soil types and plant and animal biodiversity at applicable regions as well as a rating system that accorded to activities that may detrimentally affect biodiversity and the environmental profile at "demarcated biodiversity management units" (AngloGold Ashanti, 2006b). In order to implement these tasks, the company has to examine how the contaminants, released during their operations, impact and interact with the natural environment.

Of all the animals inhabiting the Vaal river region, the most obvious are the termites. One cannot travel more than 5 km without seeing a landscape speckled with dome-shaped termite mounds. These mounds are part of the nests of the snouted harvester termite, *Trinervitermes trinervoides*, a ubiquitous grass harvesting termite found throughout southern Africa (Uys, 2002). These termites play a significant role in the food chain as they provide a protein- and energy-rich food source to numerous predators. These include small rodents, mongooses, aardwolves, aardvarks, reptiles, birds and various invertebrates (Dean and Siegfried, 1991; Richardson and Levitan, 1994; Haddad and Dippenaar-Schoeman, 2002). The predators that are adapted to feed exclusively on termites (i.e. aardvarks and aardwolves) are at a particular risk due to the large volumes of termites they consume each day. An aardwolf has been known to eat more than 300 000 termites in a day (Richardson and Levitan, 1994). Snouted harvester termite alates (flying termites) offer an easily obtainable food source to many flying animals as well as ground dwelling animals after the shedding of their wings (Abe *et al.*, 2000).

Although there are no published data regarding the impacts of mine tailings on termites and in turn their impact on the food chain, it has been shown that bioaccumulation of mining contaminants such as uranium and heavy metals (i.e. Pb, Cd, Cu and Zn) does take place in various insect species (Hull Sieg *et al.*, 1987; O'Shea *et al.*, 2001; Gongalsky, 2006; Ping *et al.*, 2009). O'Shea *et al.* (2001) concluded that numerous bat predators died as a direct result of feeding on insects that accumulated toxic elements in streams with mine drainages. One may surmise that if the soil were contaminated with persistent substances, the snouted harvester termite may be absorbing the substances from the soil and may play an indirect role in negatively impacting those animals that consume them. *Trinervitermes trinervoides* is a grass harvester termite therefore the may also bioaccumulate contaminants via the intake contaminated plant material. However this is unlikely as according to Weiersbye (personal communication), grasses do not bioaccumulate contaminants. Provided the type and level of contaminants are sufficient, consuming toxic termites may even lead to the animal's death. There are no published data on the accumulation of harmful chemicals in termites. However, Gongalsky (2006) has showed that other soil-dwelling invertebrates such as saprophagous tenebrionid beetles accumulated concentrations of uranium that were 12 times higher than those found in the control site.

Snouted harvester termites form a vital part of the ecology of certain arthropod families, as they not only provide a source of nutrition but also shelter in the form of their abandoned mounds. Haddad and Dippenaar-Schoeman (2002) collected a total of 771 spiders represented by 21 families and 82 species from 30 abandoned *T. trinervoides* mounds and concluded that the symbiotic association between spider and termite is essential as part of the spiders ecology. Reptilian species have also been known to inhabit *T. trinervoides* mounds (B. Maritz, personal communication). Thus animals using termitaria as a refuge could be susceptible to contamination as the mound interior may be laden with contaminated faeces.

Trinervitermes trinervoides has an intimate association with their surrounding soil environment as the termites spend most of their lives in mounds built from the surrounding soil. They are considered as an epigeous mound building species, meaning they build mounds that subtend subterranean nests that extend up to half a metre into the soil (Adam, 1993). With such a strong association coupled with high population densities, the mound building termites (unlike most other soil animals), exert a significant influence on soil properties and processes. The influence the termites have on soil physico-chemistry, has in some instances, dramatically altered both plant and animal communities and their interactions. It is therefore apt that they have been named “the engineers of ecosystems” (Dangerfield *et al.*, 1998).

The fact that mound building termite activities alter soil profiles and properties would have important implications regarding the dispersion of potential contaminants found in the soil. Firstly, there is the consideration that they may be bringing up contaminated subsoil (subsoil is contaminated by leaching from rain) to the surface, exposing flora and fauna that would have otherwise been shielded by top layers of uncontaminated soil. This phenomenon has been exploited by various central African communities as termites act as “nature’s little miners”, bringing valuable minerals such as gold and diamonds to the surface (Prasad *et al.*, 1987). Secondly, the repacking and cementing of soil particles during mound and nest building usually results in a higher bulk density and reduced porosity of the soil (Dangerfield *et al.*, 1998). This in turn may affect the solubility of the contaminants; making them potentially more hazardous and bioavailable to the environment.

Mound building species such as *T. trinervoides* need to be studied to establish possible presence of contaminants in their mounds. Assessing the potential contaminant-related impacts on *T. trinervoides* biology, will not only provide insight as to how the termites respond to a contaminated environment and impact the food chain but the responses of the termites will also provide information on their effectiveness as bioindicators or biomonitors of contamination in the environment. A bioindicator is defined as an organism whose function, population or status can be used to monitor the health of an environment (Markert, 2007). Using the termite colonies as potential bioindicators will be especially useful during minesite rehabilitation as they provide a way to test the effectiveness of restoration treatments (Alexandra and de Bruyn, 1997; Andres and Mateos, 2006). All mining operations eventually cease therefore the occupation of the tailing dams in the Vaal river region is temporary. The company is charged with the responsibility of rehabilitating any land disturbed or occupied by its operations in accordance with appropriate post-mining land uses (AngloGold Ashanti, 2008). Although there is limited literature available on the use of termites as bioindicators, other soil macrofauna such as ants and earthworms have been used successfully (Majer, 1983; Alexandra and de Bruyn, 1997; Veiga *et al.*, 1999; Andres and Mateos, 2006).

1.2. Aim and objectives

The aim of the project was to investigate the impact of a mine tailings dam (produced by AngloGold Ashanti Vaal River mining operations) on the snouted harvester termite, *T. trinervoides,* biology. These termites occurred on land that is known to be contaminated by plumes radiating from tailing storage facilities (tailing dams).

This study compared the physical, chemical and physiological aspects of the snouted harvester termites and their environment between two contaminated sites and one less contaminated site. The following aspects were investigated:

- the distribution and density of the mounds
- "mound status" i.e. are the mounds uninhabited or inhabited by termites
- the dimensions of the mounds to assess age
- the temperature profiles of the mounds
- the chemical constituents of the mound; the termites and the soil surrounding the mounds
- using *T. trinervoides* as a potential bioindicator of contamination

The distribution, density, dimension, "mound status" and temperature of the mounds allowed me to determine the impacts (if any) of nearby tailing storage facilities on the termite colonies. Dimension of the mounds gave a rough estimate of their age, a technique adopted by Korb and Linsenmair (1999) during their study of *Macrotermes michaelseni*. Determining age of the mounds at each site indicated population turnover, i.e. whether the older mounds were successful in producing alates that are responsible for starting new colonies (hence new mounds) as well as the survivability of the new mounds formed. Knowing the chemical constituents of the termites allowed me to ascertain whether bioaccumulation is taking place.

The temperatures found within the mounds may also provide clues into possible impact of contaminated soils. The mounds of the *T. trinervoides* have been shown to keep temperatures within narrow limits of known temperatures at particular times of the year; therefore obtaining a nest temperature profile might provide insight into any disturbances caused by contamination (Field, 2008). For instance, if the temperatures within the nest are hotter or fluctuate more than usual, this will indicate the environment (once other explanatory factors have been ruled out) is causing abnormalities within the thermoregulatory mechanisms of the colony studied. In addition, a change in temperatures could lead to problems regarding the colony's survival as reproduction is highly dependent on temperature and hence temperature regulation (Jones and Oldroyd, 2007).

The investigation of the impact of mine tailings on the density and distribution of the termite mounds at the three sites are addressed in Chapter 2. The impact of mine tailings on the temperature profiles of the mounds are dealt with in Chapter 3. Chapter 4 describes an investigation into the chemical constituents of the mounds, the termites and soil surrounding the mounds. Chapter 5 is a general discussion of the entire dissertation and addresses whether *T. trinervoides* can be used as an effective bioindicator.

It is expected that the control site will have the highest termite density due to a more favourable environment when compared to the other sites. The control site is also expected to have the most constant temperatures in the centre of the mounds.

1.3. Study area

The study was conducted at three study sites located near the town of Orkney (26°58'50.92" S, 26°40'27.91"E) in the North-West province, South Africa. Two of the sites called West Complex and AEL were situated in the AngloGold Ashanti Vaal River complex. This Vaal River complex is comprised of four gold plants, one uranium plant and one sulphuric acid plant, each equipped with tailing storage facilities (or tailings dams). These experimental sites were selected based on their proximity to the tailings dams and the contamination plumes emitted by them (Figures 1.4 & 1.5). The West Complex site was situated on the border of a contamination plume and 1 200 m away from the nearest tailing storage facility while the AEL site was immersed in the plume and situated a mere 220 m from the nearest tailing storage facility. The control site was situated outside the complex on a field next to the town and was the furthest away from the contamination plume and tailing storage facilities. This was considered a site of low contamination. The control site could not be further from the mining activity as the soil type, vegetation and elevation needed to be the same at all three sites.

All sites were on Hutton and Mispah soils characterised by low clay content, well drained and aerated profiles (Viljoen, 2006). The vegetation of each site was characterised as grassland and sparse woodland and all the mounds in the sites were located in open grassland away from trees (Mucina *et al*., 2005) (Figure 1.6). The underlying bedrock geology of the sites is comprised of sediments of dolomite which were close to the soil surface (AngloGold Ashanti, 2007a). Annual rainfall of the area is 300 – 500 mm and all sites resided on a flat area at approximately 1320 m above sea level (AngloGold Ashanti, 2007a).

At each site, an area where there were termite mounds present was chosen and marked out. A 200 by 200 m area was plotted onto a Garmin GPS 60 device using MapSource version 6.10.2 (Garmin Ltd.). Using the GPS, the 200 by 200 m was paced out and steel droppers wrapped in danger tape were used to demarcate each corner.

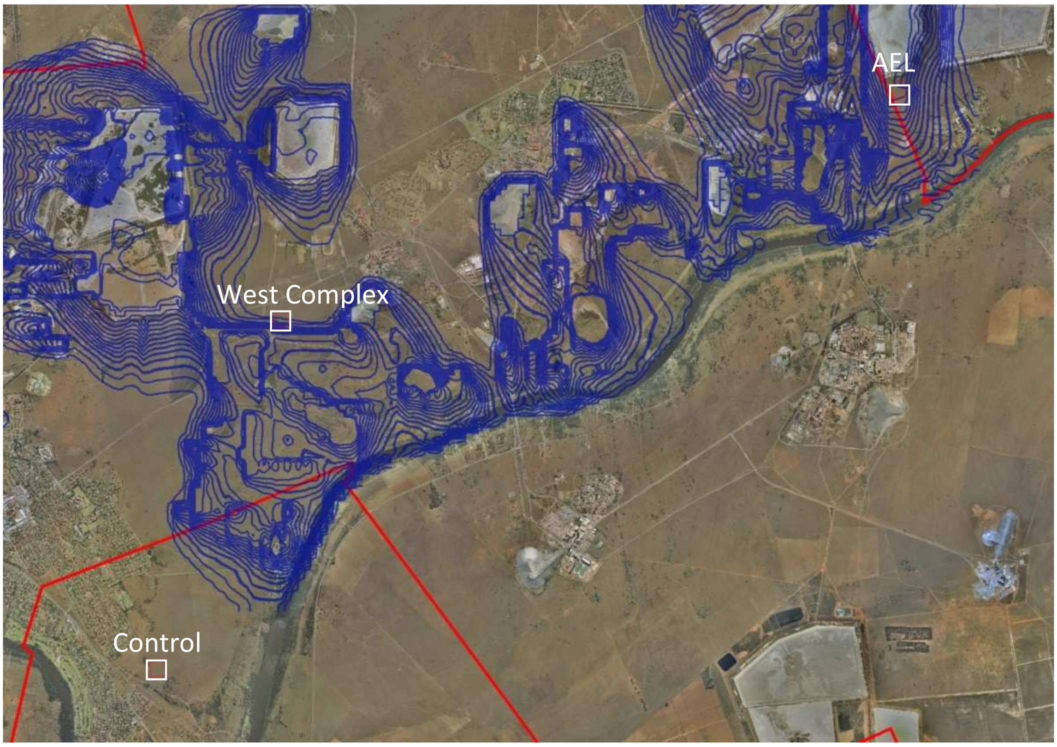


Figure 1.4. The locations of the three study sites (white blocks) and the associated contamination plumes (blue lines) (GCS, 2007).

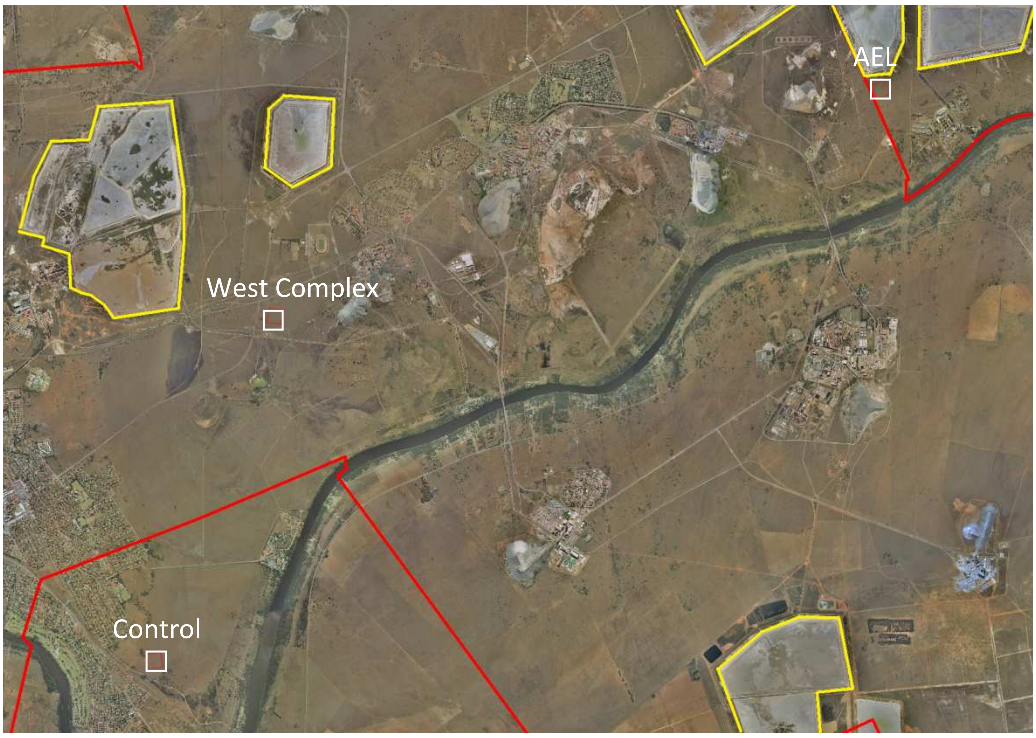


Figure 1.5. The proximity of the tailing storage facilities (highlighted in yellow) to each study site (white blocks) (GCS, 2007).

Figure 1.6. A termite mound at the AEL site. Note the cream-coloured tailings in the background.

1.4. Study species

The species *Trinervitermes trinervoides* (Sjostedt), a grass harvester termite that belongs to the subfamily Nasutitermitinae, were studied. Nasutitermitinae are characterised by the snout-like noses of the soldiers which allow for the secretion of a sticky odorous substance used in defence (Figure 1.7). *Trinervitermes trinervoides* is a widely distributed southern African species and is noted by its characteristic dome shaped mounds (Uys, 2002; Adam, 1993). In a typical snouted harvester termite colony there are different castes which perform different functions in the colony and reside in different parts of the nest (Abe *et al.*, 2000). The soldier caste is charged with the defence of the colony whereas the workers build and maintain the mound as well as forage for grass. The worker termites emerge during the night from foraging ports and gather dry grass (mainly litter) which are carried back and stored in special compartments along the periphery of the mound. Adam *et al.* (2008) found that a foraging party emerging from a single hole harvested over an area of approximately 0.78 m^2. In their study, foraging ceased during June, July and August. The reproductive caste consists of the king and queen and the alates. Each colony has a single king and queen which reside in the nest usually found in the centre at the base of the mound. The alates are the flying termites that swarm each year and go on to found new colonies.

Figure 1.7. *Trinervitermes trinervoides* soldiers

The mounds built by *T. trinervoides* have a hard outer crust consisting of soil and a softer, moist interior that is made up of multiple tunnels (Figure 1.8; Uys, 2002). The interior consists of carton, a combination of termite faeces and soil. These structures are kept together by the cement-like properties of the worker termites' saliva. Previous studies have found that most of the mound is subterranean where only a portion of it resides above the soil surface (Figure 1.8; Adam 1993; Abe *et al.*, 2000; Uys, 2002). However, this was not the case with the *T. trinervoides* mounds found at the Melville Koppies Nature Reserve in Johannesburg. These mounds were surface mounds and did not extend into the soil (Field, 2008).

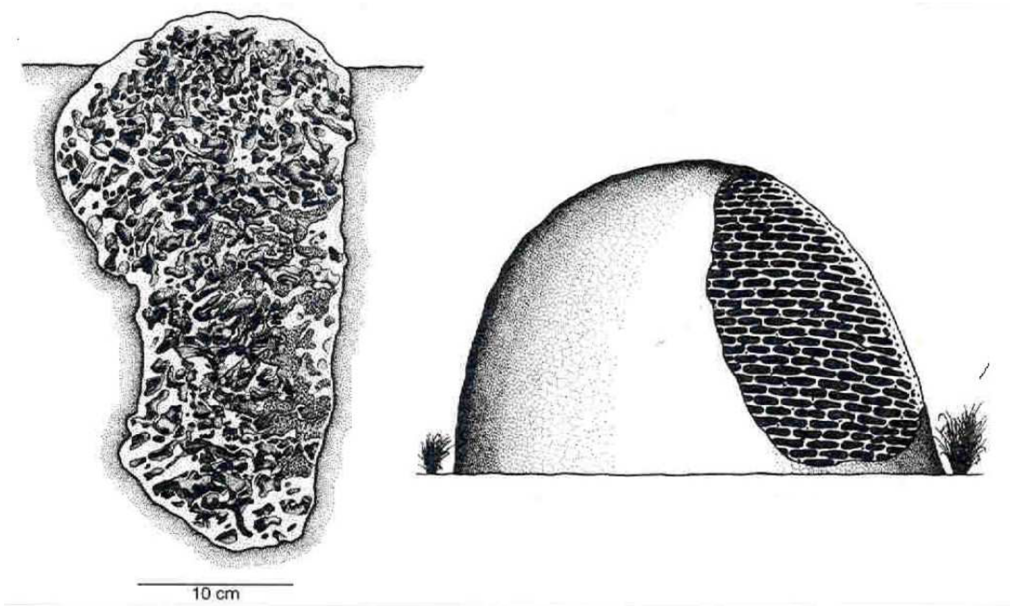


Figure 1.8. a profile of the mound (right) and nest (left) of the grass-harvester termite, *T. trinervoides* (Uys, 2002)

It has been shown that the mounds of fungus cultivating termites are used to maintain a constant temperature of 30 °C throughout the year (Korb and Linsenmair 1999, 2000a). These thermoregulatory properties of termite mounds were shown to a lesser extent in *T. trinervoides* mounds where particular temperatures were kept within narrow limits at different times of the year rather than a particular temperature throughout the year (Field, 2008).

CHAPTER 2 – THE IMPACT OF MINE TAILINGS ON THE DENSITY AND DISTRIBUTION OF THE TERMITE MOUNDS

2.1. Introduction

Termite mounds often form a conspicuous part of a landscape therefore their abundance and ubiquity has been noted by many authors (Sands, 1965). Due to the fact that termite mounds can be easily seen and are sessile, their distribution and density can be easily determined. Although there have been many studies on the density and distribution of mounds there has been no agreement on which factors affect mound distribution and density. According to Lee and Wood (1971) the main factors involved are climate, vegetation and soil which interact in their effects on the distribution and density of termites so that it is difficult to determine which variable is the most important. Pomeroy (1977) found that density and distribution of large termite mounds in Uganda were not obviously correlated with soil, climate or vegetation as these were similar throughout his study sites. He did however agree that it is difficult to single out particular environmental factor/s that influences termite distribution and density. Ferrar (1982) looked at the termite densities of *Cubitermes* of two savanna areas at Nylsvley. He found that small patches of land had greater densities but there were also substantial areas of apparently similar savanna in which no *Cubitermes* mounds could be found. He stated that there were obviously localised differences that were critical to *Cubitermes* distribution in African savanna but the “nature of these differences has so far eluded the human observer” (Ferrar, 1982).

Out of biotic factors such as food quality and quantity and intra- and interspecific competition for food, Korb and Linsenmair (2001) found that the most important determining factor of termite density and distribution was environmental temperature. *Macrotermes bellicosus* mounds occurred at markedly different densities in two thermally different habitats – the shrub savanna and the gallery forest. Termite mound densities were much higher in the warmer shrub savanna than in the cooler gallery forest and mounds only occurred in the open stands of the forests and never in dense forest. These termites are well known for keeping their nest temperatures at 30 °C which is the optimal temperature for both fungus cultivating and growth and development of the termites therefore they restricted mound construction only to those areas that were thermally suitable. San Jose *et al.* (1989) stressed the importance of moisture and humidity as a limiting factor of density and distribution. This was due to the fact that certain sites were not suitable for high termite numbers due to the temporary excess of water after heavy rains. To ensure the mounds avoided drowning, the mounds mainly occurred on ant hills and raised portions of large grass clumps. Picker *et al.* (2007) found that as the mean annual precipitation increased, the termite mound density increased. He suggested that this was because rainfall was positively correlated to vegetation cover, where more food can support higher densities of termites. Benzie (1986) determined termite mound densities of *Trinervitermes geminatus* and *T. oeconomus* in two habitats of the Guinea savanna. Although the analysis provided no evidence for a dominant role for any one environmental factor measured, grass composition did account for 12 % of the variation of densities of *T. geminatus*. This was confirmed by food preference tests where they found that *T. geminatus* preferred smaller finer grasses to tough resistant grasses. The habitat with the coarse resistant grass had lower densities of this termite when compared to habitats with finer grass.

To date no studies have been conducted on the impact of mine tailings on termite mound density and distribution. The aim of this study was to quantify the density and distribution of *T. trinervoides* in the three study sites (Section 1.3) and ascertain if soil contamination affects mound distribution and density. The study also looked at the proportion of dead mounds to live mounds at the three sites, the density and distribution of four mound size classes and the density of mounds in relation to soil depth.

2.2. Methods and Materials

2.2.1. Recording positions of mounds and calculating density

Within each study site, the number of mounds was counted and the position of the mounds was recorded using a Garmin GPS 60 device. The study sites were divided into small sections which were incrementally increased, the first section was 50 m by 50 m then it was expanded to 100 by 100 m then to 150 m by 150 m and lastly 200 m by 200 m. Each of these sections was cordoned off using string attached to steel droppers. This technique ensured that all mounds were counted. The coordinates of each mound were downloaded into ArcMap 9.3.1 (ESRI Inc.) and a distribution map of every mound at each site was drawn. Density was calculated by dividing the number of mounds by the area of each site.

2.2.2. Dead and live mounds

The status of the mounds, i.e. whether a mound was dead or alive was noted. A hole was drilled into each mound using a masonry drill. If no termite activity was observed after 2 minutes, the mound was presumed dead.

2.2.3. Dimensions of the mounds

The circumference and height of each counted mound was measured using a fabric and steel measuring tape respectively. The mounds were divided into four different size categories, based on mound height namely: incipient (< 10 cm), small (10 cm – 29 cm), medium (30 cm – 49 cm) and large (> 5). Using Adam's (1993) observation that it takes a termite colony 3 years to establish a mound 8 cm high, a rough estimate of the age of the termite mounds was determined.

2.2.4. Soil depth

Soil depth was determined by using an 8 cm diameter auger to drill to the bedrock layer at five points in each site (four points at each corner and one point in the middle of the sites). The depth of the hole was then measured using steel measuring tape and the average depth was calculated.

2.2.5. Data analysis

To determine the distribution of the mounds, a nearest neighbour analysis was conducted using *Microsoft*® Office *Excel*® 2007 and MapSource version 6.10.2 (Garmin Ltd.). This gives a numerical value (Rn) as a measure of a particular distribution pattern. The distribution patterns can have a tendency towards clustering ($Rn < 0.75$), random ($Rn = 0.75 – 1.25$) or a tendency toward a regular distribution ($Rn > 1.25$). The minimum of 30 mounds was used in the analysis to obtain statistically relevant results.

Pearson's chi-square test was conducted to determine whether there were significant differences among the different densities of the mounds. T-tests were used to compare the soil depths at each site.

2.3. Results

2.3.1. Density of the mounds

Comparison of all sites showed a greater total mound density in the AEL site than in the West Complex and Control site (Figure 2.1). There were significant differences in the density of mounds among the three sites ($\chi^2 = 222.354$; $p < 0.001$). Due to the high density of mounds at the AEL site, an area of only 150 x 100 m was surveyed.

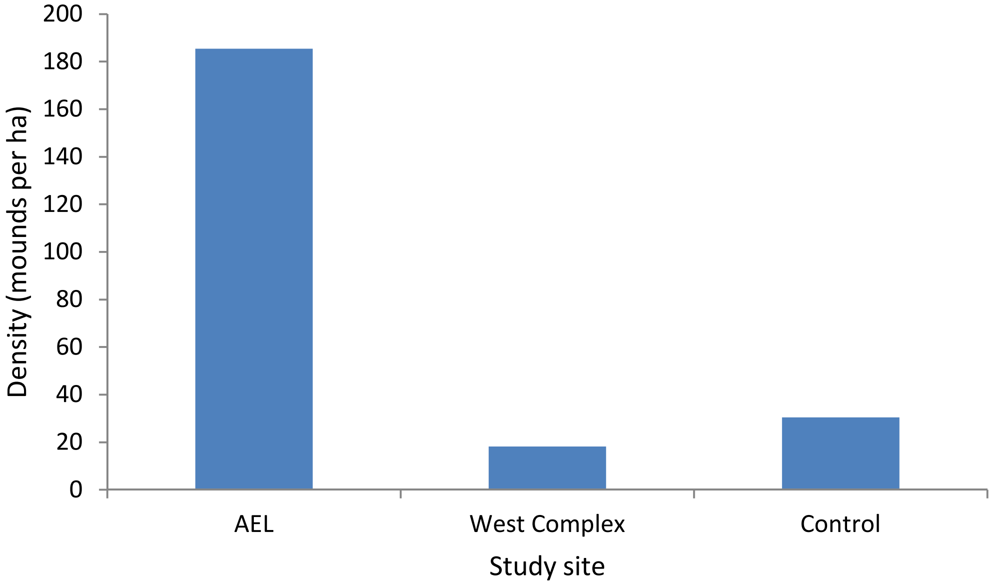


Figure 2.1. Density of all the *T. trinervoides* mounds at the three sites.

2.3.2. Size classes of all the mounds

The density of the different size classes was different in all three study sites (Table 2.1). The AEL site had the most mounds in all the size categories except for large mounds, in fact, the smallest number of large mounds was found in the AEL site when compared to the other sites. The West Complex site had the greatest number of large mounds. A markedly higher number of small mounds were found at the AEL with some 99.4 and 114.8 more mounds per ha than the Control site and the West Complex site respectively (Table 2.1). The AEL site also exhibited the most incipient mounds of 52 mounds per ha when compared to the other two sites which both have less than 2 incipient mounds per ha. Figures 2.2 – 2.4 illustrate an aerial view of the different size classes at each site.

Table 2.1. Mound densities (mounds per ha) of each size class at each of the sites.

	AEL	West complex	Control
Incipient (< 10 cm)	52	0.5	1.8
Small (10 cm - 30 cm)	117.3	2.5	17.9
Medium (31 cm - 50 cm)	14.7	10.8	7.9
Large (> 50 cm)	1.3	4.5	2.8
Total	185.3	18.3	30.4

Figure 2.2. Distribution of the different sized mounds in the AEL site. "+" = incipient mounds, "x" = small mounds, "▲ " = medium mounds and "★" = large mounds.

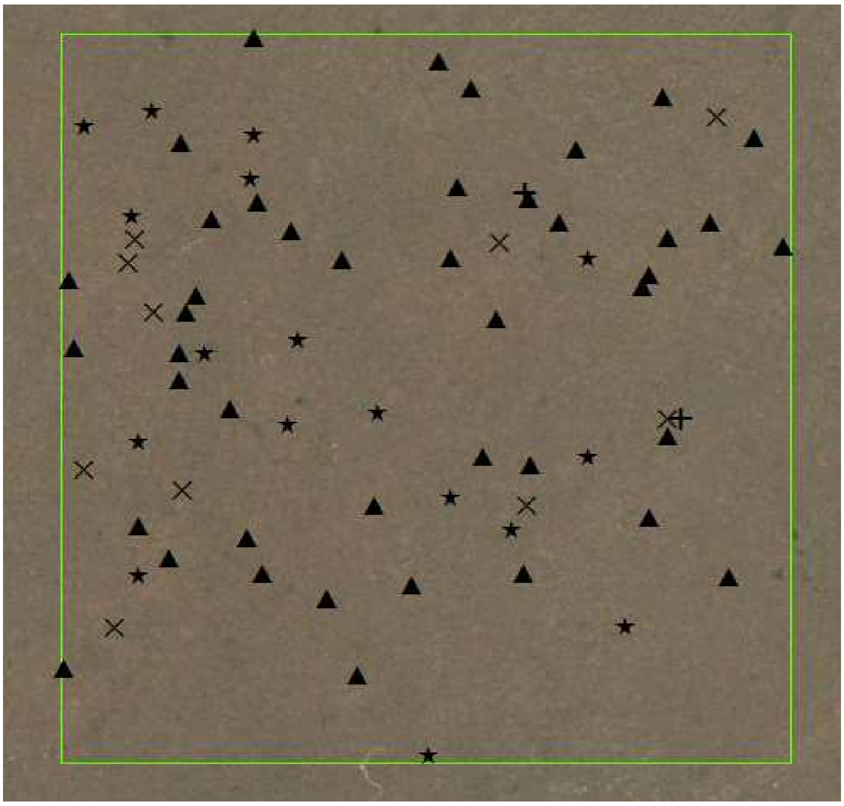


Figure 2.3. Distribution of the different sized mounds in the West Complex site. “+” = incipient mounds, “x” = small mounds, “ ▲” = medium mounds and “★” = large mounds.

Figure 2.4. Distribution of the different sized mounds in the Control site. “+” = incipient mounds, “x” = small mounds, “ ▲” = medium mounds and “ ★” = large mounds.

Out of all the size classes, the small mounds constituted the largest percentage of the mounds in the AEL and Control site whereas the medium mounds predominated in the West Complex site (Figure 2.5). The incipient mounds comprised the lowest percentage of mounds in the Control and West Complex site. Large mounds were the least prevalent in the AEL site.

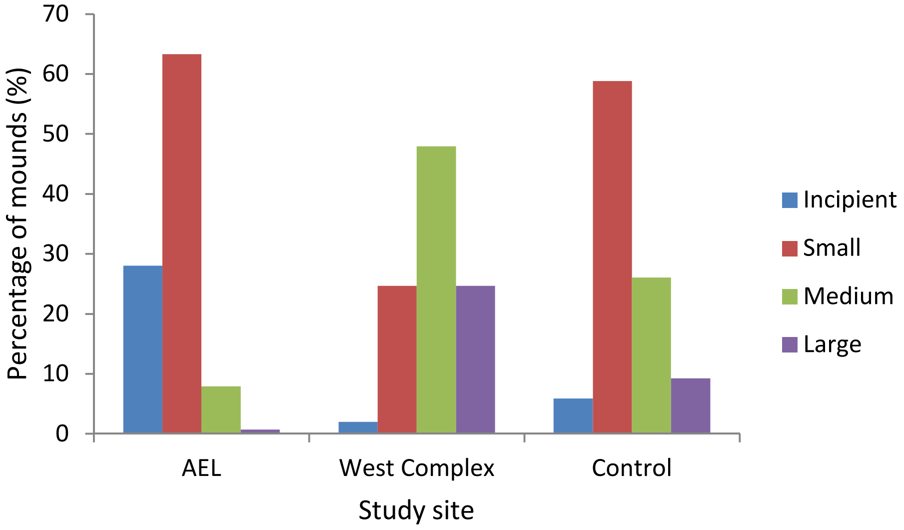


Figure 2.5. Percentage of mounds of each size class within each site.

2.3.3. Dead and live mounds

When the density of dead mounds was compared among the sites, the AEL site showed the greatest density of dead mounds (56.7 mounds per ha) whereas the control site had the least amount of dead mounds at 7.9 mounds per ha. The West Complex sites had 9 mounds per ha (Figure 2.6). The AEL site also had the greatest density of live mounds (128.7 mounds per ha) followed by the Control site (22.5 mounds per ha) and lastly, the West Complex site (9.3 mounds per ha) (Figure 2.6).

Taking proportion of dead and live mounds into account, the West Complex site had the greatest percentage of dead mounds (Figure 2.7). Almost 50 % of the mounds in the West Complex site were dead. The dead mounds at the AEL constituted 30% of the mound population whereas 26 % of the mounds at the Control site were dead. The live to dead mound ratios for each site were calculated as 1: 0.44 for the AEL site, 1: 0.97 for the West Complex site and 1: 0.35 for the Control site.

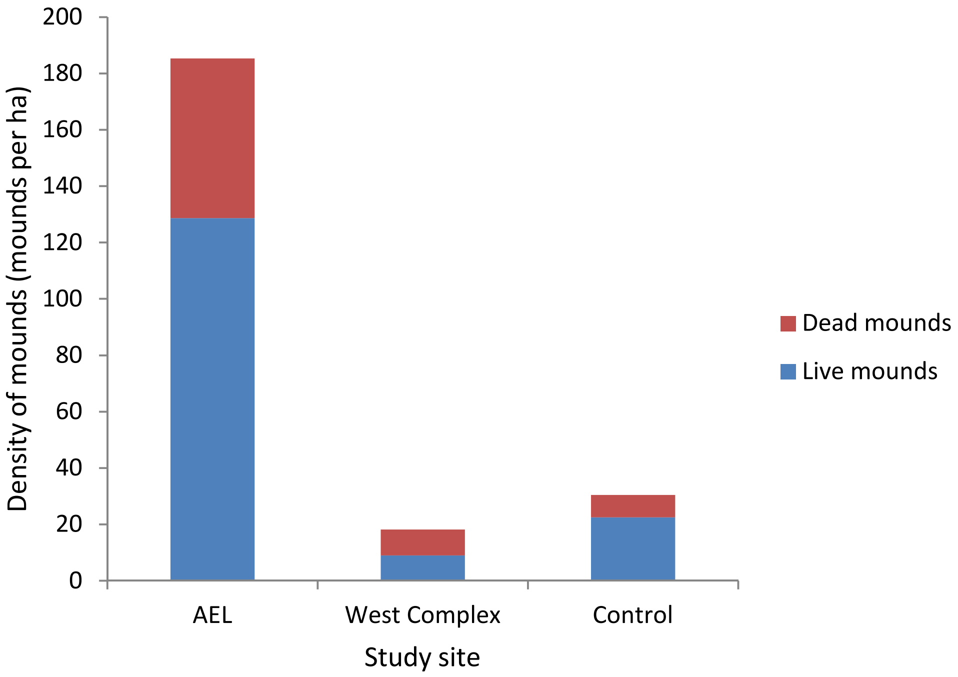


Figure 2.6. Mound densities of the live and dead mounds at the three sites.

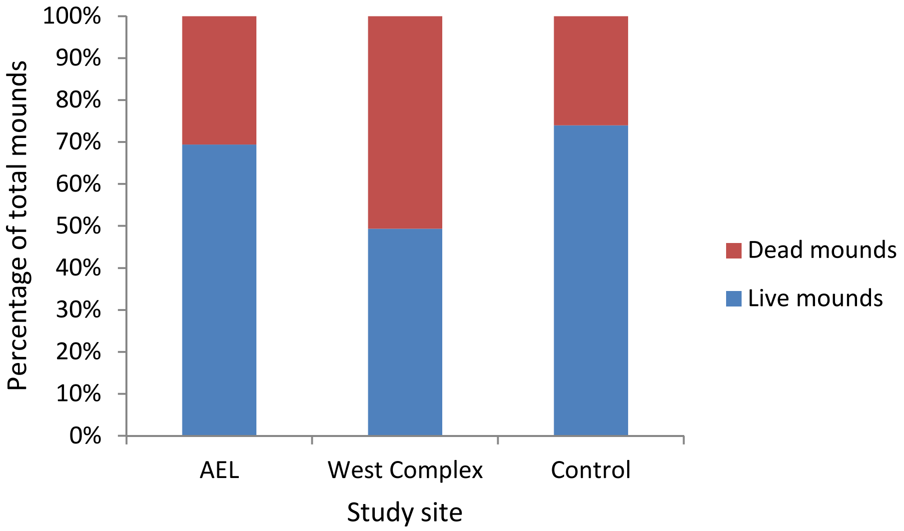


Figure 2.7. Percentage of the dead and live mounds at each site.

2.3.4. Size classes of dead and live mounds

In the Control and AEL site, the greatest density of dead mounds was comprised of the small mounds while most of the dead mounds in the West Complex site were medium mounds (Table 2.2). No incipient mounds were found dead at West Complex site and no large mounds were dead at the AEL site.

Table 2.2. Mound densities (mounds per ha) of the different size classes of the dead mounds.

	AEL	West Complex	Control
Incipient (< 10 cm)	16.0	0.0	0.5
Small (10 cm - 30 cm)	36.7	1.5	5.4
Medium (30 cm - 50 cm)	4.0	6.5	1.5
Large (> 50 cm)	0.0	1.3	0.5

When comparing the live mound classes in each site, the AEL site had the greatest number of small mounds per ha and the smallest number of large mounds per ha (Table 2.3). The small mounds at the Control site had the greatest density when compared to the other size classes whereas the medium mounds predominated in the West Complex site. West Complex site also had the least amount of live incipient mounds when compared to the other size classes as well as the other sites.

Table 2.3. The mound densities (mounds per ha) of the different size classes of the live mounds.

	AEL	West Complex	Control
Incipient (< 10 cm)	36.0	0.5	1.3
Small (10 cm - 30 cm)	80.7	1.0	12.5
Medium (30 cm - 50 cm)	10.7	4.3	6.4
Large (> 50 cm)	1.3	3.3	2.3

2.3.5. Soil Depth

The Control site had the deepest soil depth (0.78 ± 0.31 m) and was significantly different to the AEL (0.16 ± 0.10 m) and West complex site (0.16 ± 0.15 m) ($t = 4.33$ and $t = 4.08$ respectively, $p < 0.01$). The AEL and West complex site had similar depths and were not significantly different ($t = 0.037$, $p < 0.01$). The highest and lowest numbers of mounds were both found in shallow soil (Figure 2.8).

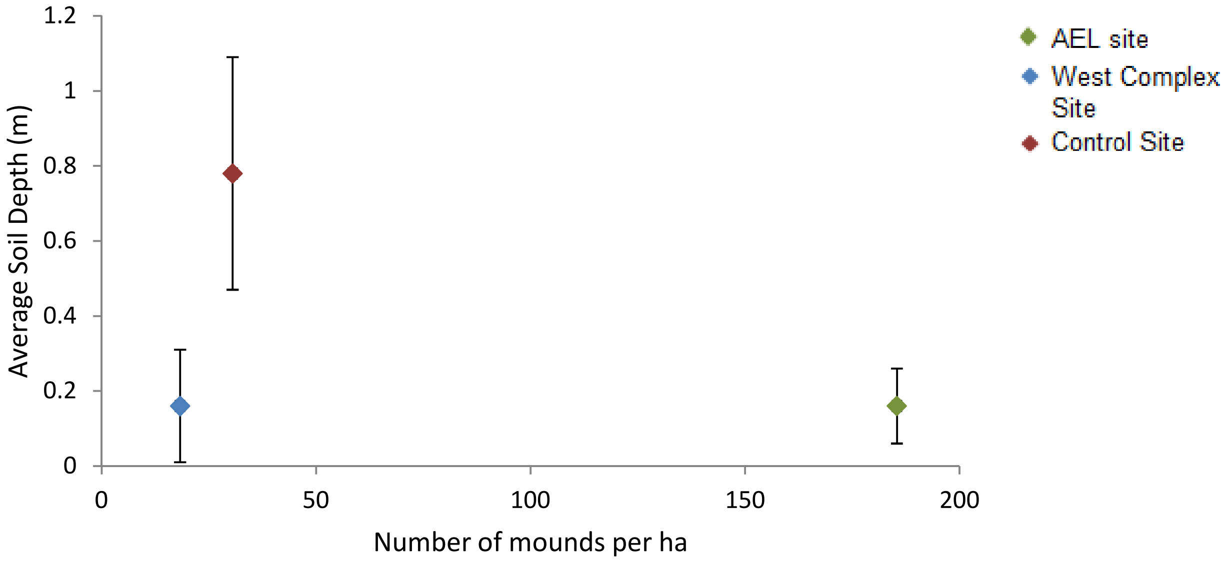


Figure 2.8. Average soil depth in relation to the density of *T. trinervoides* mounds at the three sites.

2.3.6. Distribution of the mounds

A nearest neighbour analysis was conducted to characterise the type of distribution of the mounds at the three sites. An analysis of the distribution of the mounds showed that the Control and the AEL site had a clustered mound distribution whereas the West Complex site had randomly spaced mounds (Table 2.4). This can be clearly seen in Figure 2.9. Table 2.5 shows the type of distribution of mounds depending on whether the mounds were dead or live. All the live mounds in the sites had a tendency towards clustering. The dead mounds represented the three different spatial patterns in each site as shown in Table 2.5.

Table 2.4. The nearest neighbour values and the type of distribution pattern of the mounds for each study site.

Site	Nearest neighbour value	Type of pattern
AEL	0.73	tendency towards clustering (n = 278)
West Complex	1.13	random (n = 73)
Control	0.81	tendency towards clustering (n = 119)

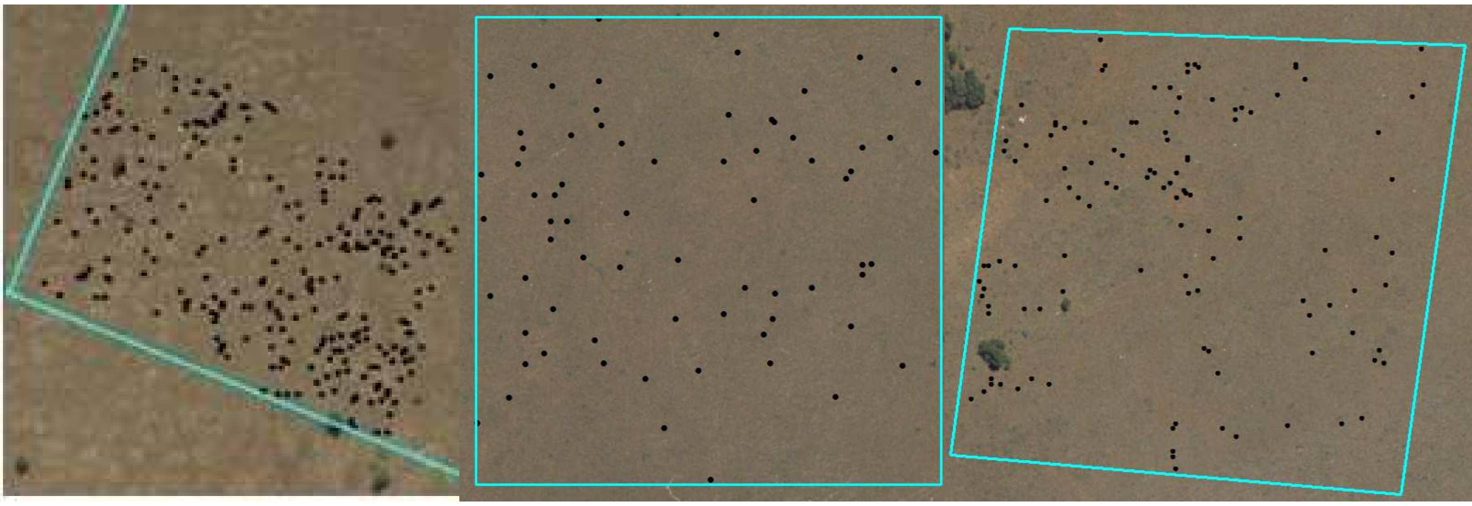


Figure 2.9. An aerial view of the distribution of the mounds at the AEL, the West Complex and Control site (left to right).

Table 2.5. The nearest neighbour values and the type of distribution pattern of the dead and live mounds for each study site.

	Dead		Live	
	Nearest neighbour value	Type of pattern	Nearest neighbour value	Type of pattern
AEL	0.84	tendency toward clustering spacing (n = 85)	0.67	tendency towards clustering (n = 193)
West Complex	1.23	tendency toward regular spacing (n = 36)	0.87	tendency towards clustering (n = 37)
Control	1.04	Random (n = 31)	0.76	tendency towards clustering (n = 88)

2.4. Discussion

Spatial distribution is an important aspect in the study of animal communities as this knowledge may help to understand important attributes of the community structure such as its carrying capacity and interactions with other organisms. It can be used as an indicator of the underlying mechanisms that regulate the organism's population dynamics. For the termite *M. bellicosus,* the apparent regular distribution of colonies has been interpreted as a consequence of intraspecific competition (Korb and Linsenmair, 2001). The distribution of the termite mounds over the West Complex study area is random, suggesting that intraspecific competition is not controlling their abundance. Other studies on *Macrotermes* species also found the mounds to be randomly distributed (Collins, 1981; Lepage, 1984; Schuurman and Dangerfield, 1997; Korb and Linsenmair, 2001). The AEL site and Control site both had a clustered distribution. This was also the case with *Trinervitermes ebenerianus* mounds in Northern Nigeria (Ohiagu, 1979).

The mound density results of this study were surprising as the purportedly most contaminated site, AEL, was expected to have the lowest mound density due to possible negative impacts of tailings on the environment (see Chapter 4). Yet the AEL site had the highest mound density when compared to the other less contaminated sites. The density of mounds at the AEL site (185.3 mounds per ha) is comparatively higher than most other studies on *T. trinervoides* mound densities. The densities of *T. trinervoides* recorded by Adam (1993) at four sites in Bulfontein were 66, 59, 25 and 20 mounds per ha. Coaton (1948) studied *T. trinervoides* at Koffiefontein and recorded a density of 100 mounds per ha which is considerably higher than Adam's (1993) results but still not as high as the AEL site. Nel and Malan (1974) recorded a mound density of 31 mounds per ha south of Bloemfontein. Only one other study conducted by Murray (1938) near Frankenwald, Gauteng, recorded a mound density higher than AEL site mound density, at 535 mounds per ha. The West Complex site (18.3 mounds per ha) and the Control site (30.4 mounds per ha) had densities which resembled Nel and Malan's (1974) and Adam's (1993) findings more closely. Table 2.6 summarises the data available on the densities of epigeal mounds of various other termite species that occur in savanna habitats. The variability of mound density is noticeable.

Table 2.6. The densities of mounds (mounds per ha) of various termite species in Savanna habitats.

Termite Species	Density	Habitat/ location	Author
Amitermes laurensis	Range: 28 - 210	Savanna woodland northern Australia	Lee and Wood (1971)
Amitermes vitiosus	Mean: 240	Savanna woodland in semi-arid northeastern Australia	Holt and Easey (1985)
Cubitermes curtatus	Mean: 72	Open grassland in northern Guinea	Benzie (1986)
Cubitermes pretorianus	Mean: 385 and 496	Nylsvley reserve, Limpopo Province	Ferrar (1982)
Cubitermes sp.	Mean: 0.33	Northern Kruger National Park	Meyer *et al.* (2000)
Macrotermes sp.	Range: 1 – 4	Subtropical savanna in Uganda	Pomeroy (1977)
Macrotermes sp.	Range: 3 – 10	Tropical Ethiopean savanna	Bouillon (1970)
Macrotermes bellicosus	Mean: 50	Subtropical savanna in Uganda	Pomeroy (1978)
Macrotermes bellicosus	Mean: 4	Northeast of Ivory Coast	Lepage (1984)
Macrotermes bellicosus	Range: 11.2 - 83.3	West guinea savanna	Korb and Linsenmair (2001)
Macrotermes michaelseni	Mean: 2.98	Moremi game reserve in the Okavango Delta	Schuurman and Dangerfield (1997)
Microhodotermes viator	Mean: 2.9 ± 1.2	Western and Northern Cape province of South Africa	Picker *et al.* (2007)

Nasutitermes sp.	Mean: 36 ± 50	*Trachypogon* savanna of Venezuele	San Jose *et al.* (1989)
Nasutitermes triodiae	Range: 3 – 7	Tree savanna, northern Australia	Lee and Wood (1971)
Trinervitermes geminatus	Mean: 501.41	Northern Guinea savanna	Sands (1965)
Trinervitermes geminatus	Mean: 22 ± 8	Open grassland in northern Guinea	Benzie (1986)
Trinervitermes geminatus	Range: 187 - 273	Southern Guinea savanna	Ohiagu (1979)
Trinervitermes oeconomus	Mean: 6 ± 3	Open grassland in northern Guinea	Benzie (1986)
Trinervitermes oesonomus	Mean: 7.41	Open grassland in Zaria, northern Nigeria	Sands (1965)
Trinervitermes togoensis	Mean: 2 ± 1	Open grassland in northern Guinea	Benzie (1986)
Trinervitermes togoensis	Mean: 9.88	Open grassland in Zaria, northern Nigeria	Sands (1965)
Trinervitermes trinervius	Mean: 12.35	Open grassland in Zaria, northern Nigeria	Sands (1965)
Velocitermes paucipilus	Mean: 202 ± 61	*Trachypogon* savanna of Venezuele	San Jose *et al.* (1989)

Adam (1993) attributed the differences in *T. trinervoides* termite mound densities among his sites to the influence of soil depth. In his sites the soil depth was limited by an underlying calcrete layer. He found a positive correlation between the total number of termite mounds and the average soil depth at the four sites. Mounds were not found on shallow soils and he hypothesised that the lack of building on shallow soils is a result of the termites avoiding possible water stress as shallow soils have poor drainage of water. The highest and lowest numbers of mounds were both found in shallow soil.

Trinervitermes trinervoides is a grass feeding termite therefore vegetation may be an important factor in determining its density. Adam (1993) showed that two of his study sites that supported the lowest termite mound density had vegetation that was considered at a pioneer stage of growth whereas the other sites had more climax stage grass species. He concluded that the grasses at the pioneer stage cannot support high termite numbers because of insufficient suitable grass for foraging and in turn supporting the potential colonies. Murray (1938) showed that the abundance of *T. trinervoides* mounds increased as secondary plant succession advanced from a two-year-old fallow to undisturbed veld. The constitution of soil is another important aspect when considering mound building termites as the correct proportions of sand, silt and clay are required to cement particles together during mound construction. Pomeroy (1978) showed that mound density in Macrotermitinae was directly proportional to the percentage clay content of the soil. Both vegetation and soil type could not account for the difference in mound density found in this study as these variables were the same at each study site. The sites were chosen for their similarities in grass species and soil types.

Termites are generally very susceptible to water loss and it is important for them to maintain a high humidity in the nest. If the humidity of the nest is too low, termites may tunnel down or outward to the water table, a perched water table or surface water to reach moist soil which they then carry back in their crops (Abe *et al.*, 2000). On several occasions when excavating a *T. trinervoides* mound, Adam (1993) discovered vertical shafts extending down below the sub-surface section of the mound. Although he was unable to follow these shafts to the water table he surmised that this is where the shafts terminated. The water table tends to follow surface topography therefore the tailings dam at the AEL site most likely decreases the depth of the water table, allowing termites to access water more easily than the termites at the other sites (Toth, 1962). This could account for a higher density of termite mounds at the AEL site.

The preponderance of small mounds at the Control site was similar to the size distributions found by Nel and Malan (1974) and Adam (1993). The AEL site had a very high density of incipient mounds (52 mounds per ha) which indicates that there is a high turnover of new mounds. This density was far higher than other recorded densities of incipient colonies of *T. trinervoides* - 12.3 and 0.5 mounds per ha for Nel and Malan (1974) and 10 mounds per ha for Adam (1993. The West Complex site had the largest percentage of medium mounds and a very low percentage of small and incipient mounds. This was consistent with other species of termites such as *Macrotermes jeanneli* where the survival rate of large nest was very high (93 % per year) while the survival rate for incipient colonies was low (Darlington *et al.*, 1992). In contrast, the AEL site had very few large mounds (1.3 mounds per ha) which indicates that although it has a high turnover of new mounds, the longevity of these mounds was low.

The high density of small and incipient mounds at the AEL site could also indicate that there is a relatively high output of alates from the few mature mounds on the site. The high rate of survival of these alates that go on to found new colonies could also be linked with reduced predation as a result of the impact of the tailings. Birds are a major predator of *T. trinervoides* alates as they are easy prey when undertaking their nuptial flight (Abe *et al.*, 2000). Birds that have died as a direct result of tailings have been documented particularly in the USA and Australia (Eisler, 1991; Henny *et al.*, 1994; Eisler and Weimeyer, 2004; Donato *et al.*, 2007). It is therefore possible that due to reduced predation, an increase in population density will result. However, there was no empirical evidence supporting the death of bird predators, i.e. no carcasses were found on the sites and for that reason an increase in mound density resulting from bird mortality is considered unlikely.

The three sites all had a high proportion of dead mounds, the highest being the West Complex site where almost half the population of mounds were dead. Once again this result was surprising as the AEL site was expected to have the highest proportion of dead mounds due to the possible impacts of the tailing storage facility. As expected, the Control site had the lowest mortality when compared to the other sites. At the West Complex site, the size class that had the highest mortality was the medium mounds. This is contrary to Adam's (1993) study where he found only 5 % of the medium mounds dead at his sites in Benfontein. The small mounds constituted the highest percentage of mounds dead (30 %) at his sites which is consistent with what was found at the AEL and Control site. Collins (1981) also had a similar finding when studying survivorship of *M. bellicosus* mounds and stated that it is generally accepted that mortality is high in the young stages of termite colonies. The results disagree however, with those of Nel and Malan (1974) who found that mortality in *T. trinervoides* occurred throughout the size class distribution of mounds and that small and large colonies generally had an equal chance of dying.

The reasons for the mound mortality are not clear and difficult to quantify. Adam (1993) suggested that small mounds have high mortality due to the inability of these colonies to forage and store enough grass for the winter months. Predation is another factor that could lead to the death of a mound (Adam, 1993). The small colonies could be especially susceptible as they have fewer soldier termites (essential for the defence of the colony) when compared to larger colonies. According to Collins (1981) a large colony of *M. bellicosus* withstood attacks by predatory ants but an attack on a small colony led to the death of the entire nest. Other factors such as overgrazing and unsuitable food sources could lead to the mortality of *T. trinervoides* colonies (Adam, 1993). In the study conducted by Adam (1993), there were indications of overgrazing at his fourth site (the site with the highest mortality) as the vegetation was in a pioneer stage, typical of overgrazing. The most common grass was a grass that was least preferred by *T. trinervoides*, suggesting that the mortality of the mounds at the fourth site was due to a scarcity of suitable grass and subsequent starvation. The high percentage of dead mounds at the West Complex site could be due to the potential contamination of the environment. However, the AEL site was expected to have the highest mound mortality as this site is the closest to the tailings dam and purportedly more contaminated therefore this hypothesis was not supported.

This study presented the first investigation into the possible impacts of tailing storage facilities on the density and distribution of termite mounds. It appears that the tailing storage facility did not have a negative impact on the density of the termite mounds as these were the highest at the AEL site. Variables such as climate, vegetation and soil type that could potentially explain the differences found at each site were eliminated as these were the same throughout the sites. Soil depth also did not have an effect on the density of the mounds as there was no correlation with the depth and the density of mounds. The tailing storage facility most likely decreases the depth of the water table therefore termites at the AEL site could possibly access water more easily than the termites at the other sites. This may account for the higher density of mounds at the AEL site. The size class distributions indicated that there was preponderance of small mounds at the Control site which was similar to those distributions found by Adam (1993) and Nel and Malan (1974). The AEL site had a very high density of incipient mounds but very few large mounds indicating that although it has a high turnover of new mounds, the longevity of these mounds was low. The West Complex site had the largest percentage of medium mounds and a very low percentage of small and incipient mounds which may indicate that although it is difficult for termites to start mounds, once they reach a certain size they persist for many years. The West Complex site had the highest percentage of dead mounds supporting the idea that the tailings dam affected termite density however this was not supported by the results from the AEL site.

CHAPTER 3 – THE IMPACT OF MINE TAILINGS ON THE TEMPERATURE PROFILES OF TERMITE MOUNDS

3.1. Introduction

Almost all insects are at the mercy of their environment, without any capability of regulating their internal temperature (Gullan and Cranston, 1994), however social insects have an advantage in that they live together in nests which provide an enclosed environment. Social insect ecology presents a novel way of illustrating homeostasis of a microclimatic system as social insects are able to regulate their nest environment at relatively constant temperatures with limited fluctuation. They achieve what Emerson (1956) called "social homeostasis". Mound building termites achieve social homeostasis by using their mounds, together with other behavioural and physiological mechanisms, for temperature control. The mounds termites build provide a means for termites to passively and actively regulate the internal nest temperatures (Jones and Oldroyd, 2007). Those remarkable, seemingly random piles of soil they build are like thermostats, regulating the temperature so that the internal nest temperature is maintained near a desired setpoint temperature. Keeping within this setpoint range as well as maintaining a humid nest environment is important as termites are soft bodied insects with a thin, delicate integument and are therefore compelled to live in a controlled environment without which they would shrivel and dry out (Uys, 2002). Unfavourable temperatures can also lead to other problems for the colony such as abnormalities in the brood and the denaturing of eggs which in turn leads to the lack of emergence of adults (Jones and Oldroyd, 2007).

Mound site selection, mound orientation and mound architecture are passive mechanisms that bring about the long-term control of the internal nest temperatures during a wide variety of environmental change (Jones and Oldroyd, 2007; Wilson, 1971). Active mechanisms are typically behavioural in nature and provide short-term control that serves to either heat or cool current nest temperature in response to ephemeral environmental perturbations (i.e. temporary heat fluxes). These mechanisms include brood reallocation to more favourable regions within the nest and the bringing in of water to promote evaporative cooling (Wilson, 1971; Jones and Oldroyd, 2007).

Termites live in a diverse array of nests ranging from simple galleries in wood to elaborate and sophisticated structures (Uys, 2002). These nests are either subterranean (completely below the ground level), epigeal (part of the nest protrudes in the form of a mound above the ground) or arboreal (above ground level usually in trees) (Uys, 2002). The mounds of epigeal nests consist of elaborate systems of tunnels. These tunnels are built using soil and are lined with faecal deposits containing large quantities of lignin and special salivary secretions that act as glue (Abe *et al.*, 2000). The complex architecture of the mound implies some physiological function, leading to the prevailing opinion that the mound functions to regulate the nest environment (Turner, 2001).

Mound architecture has been shown to influence internal nest temperatures of termite colonies (Wilson, 1971). Variations in wall thickness, mound surface design and general size of the mound serve to either increase or decrease heat exchange with the environment thus assisting in stabilizing nest temperatures (Jones and Oldroyd, 2007). Korb and Linsenmair (2000a; 2000b) showed that fungus growing termites, *Macrotermes bellicosus*, build complex mounds that help to maintain a central nest temperature of about 30 °C for optimum fungal growth all year round. Remarkably, the mounds of *M. bellicosus* differed greatly between two neighbouring habitats. Termites that lived in the shrub savanna built mounds that were cathedral shaped, thin walled and highly structured with many turrets and ridges (Figure 3.1A). In contrast, the mounds in the gallery forest habitat were dome shaped with thick walls and had few projecting structures (Figure 3.1B). Korb and Linsenmair (1999) showed that the differences in architecture of the mounds were due to the differences in ambient temperatures of the two habitats. The savanna habitat had higher ambient temperatures while the gallery forest had low sub optimal ambient temperatures. Heat that is produced in the nest by the metabolism of the termites and fungi, needs to either be expelled or contained depending on the thermal habitat the mounds inhabit. The high surface complexity and cathedral shape of the mounds in the savanna habitat appeared to be suited for the promotion of heat loss to the environment; conversely, the thick walls and dome shape of the mounds in the gallery forest habitat were suited to reduce loss of heat to the environment.

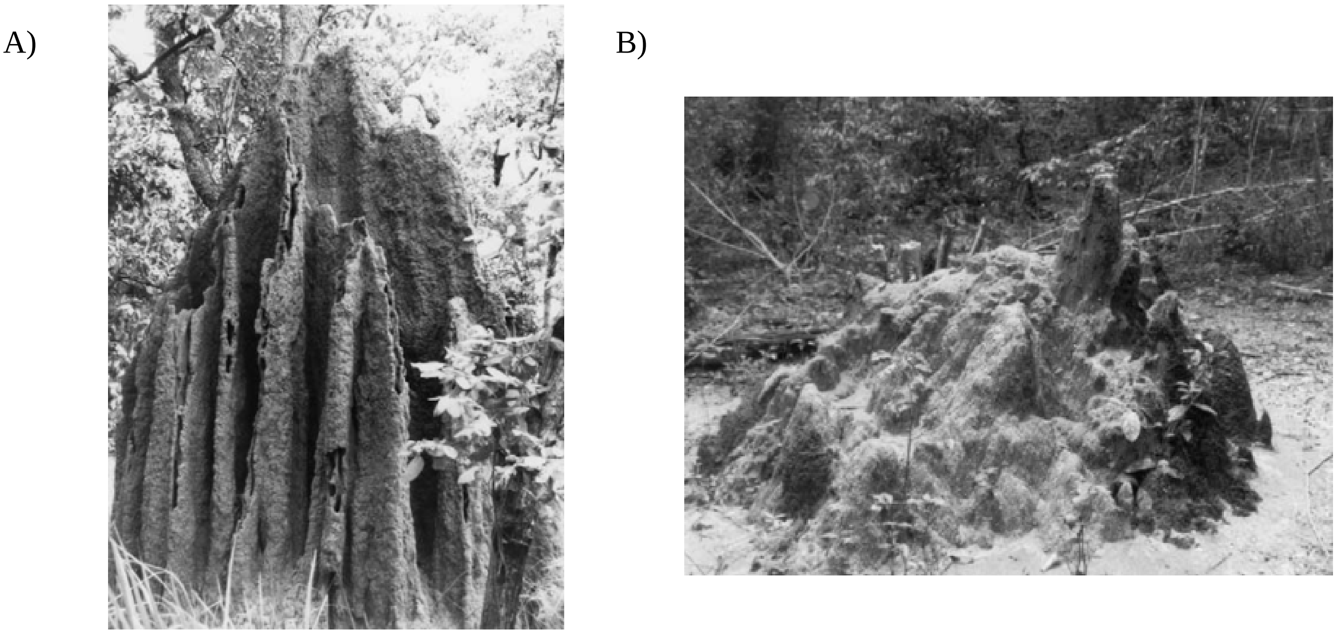


Figure 3.1. The mounds made by *M. bellicosus* in the A) savanna habitat and B) forest gallery habitat. (Korb, 2003).

Metabolic heat produced by termite colonies has important implications in the regulation of temperature and gases within the nest (Bristow and Holt, 1987). The heat generated by termite metabolism also allows for raised temperatures which is especially helpful during winter. During summer however, this metabolic heat may raise the temperatures above that of ambient temperatures and risk overheating the mound. Therefore active mechanisms in conjunction with the alteration of mound architecture are employed to expel this excess heat. By bringing in water to appropriate sites in the mound, evaporative cooling is permitted which results in the decrease of nest temperatures (Bristow and Holt, 1987; Abe *et al.*, 2000). Another active mechanism which allows termites to cope with excess heat includes the reallocation of brood (usually the most vulnerable individuals) to those regions in the nest or mound where the temperatures are at the optimum for brood development and growth (Jones and Oldroyd, 2007).

The termite, *Trinervitermes trinervoides*, builds dome-shaped mounds that have been shown to keep their temperatures within narrow limits of particular temperatures throughout the course of a year (Field, 2008). Their mound structure is similar to that of the well documented *M. bellicosus* mounds in having a closed ventilation system, meaning there are no external openings to the environment (Korb and Linsenmair 1999). However, an important difference between the two species is that *T. trinervoides* lack fungus gardens which are a major perturber of the nest atmosphere in *M. bellicosus* nests. The aim of this portion of the study was to obtain a monthly and seasonal profile of the temperatures in *T. trinervoides* mounds in the three sites (Section 1.3) to determine the thermoregulatory capabilities of the mounds with emphasis on the temperatures found in the centre of the mound where the queen, king and brood reside. This investigation will also clarify the possible influence contaminated soil has on nest temperature regulation.

3.2. Methods and Materials

The temperature profiles of 2 mounds as well as air temperatures at each site were obtained using iButtons (Maxim integrated products ©) which are small data loggers that allow for continuous and simultaneous measurement. The iButtons were programmed to record temperatures every hour and were left in allocated positions for 3 months. After 3 months, iButtons were then extracted and the temperature data were downloaded onto a computer. iButtons were then reprogrammed and reinserted back into the mounds where they were left for a further 2 months. The second week of each month was used in the analysis, giving a total of 5 weeks of temperature data.

3.2.1. Placement of the iButtons

Six iButtons were placed in each mound at various locations (Figure 3.2); two “Surface” iButtons 2 cm into the mound just below the hard outer crust, two “Internal” iButtons 15 cm into the mound, a “Top-Centre” iButton at the surface of the soil at the bottom of the mound and a “Bottom-Centre” iButton in the centre of the subterranean nest 20 cm below the soil surface. The mounds were found to be non-epigeous therefore the iButtons could not reach the “Bottom-Centre” so only “Top-Centre” was measured and was labelled “Centre” for the analysis. iButtons were mounted on a plastic holder to which wire was attached. This allowed the iButtons to be pulled out from the mound when the recording was completed. A masonry drill was used to drill a hole on either side of the mound that allowed for the insertion of the “Internal” and “Surface” iButton pairs. An 8 cm orga was used to make the hole for the insertion of the “Top-Centre” iButton. Ambient temperature was recorded over the same period using an iButton inside a canister bolted to a nearby tree in the shade.

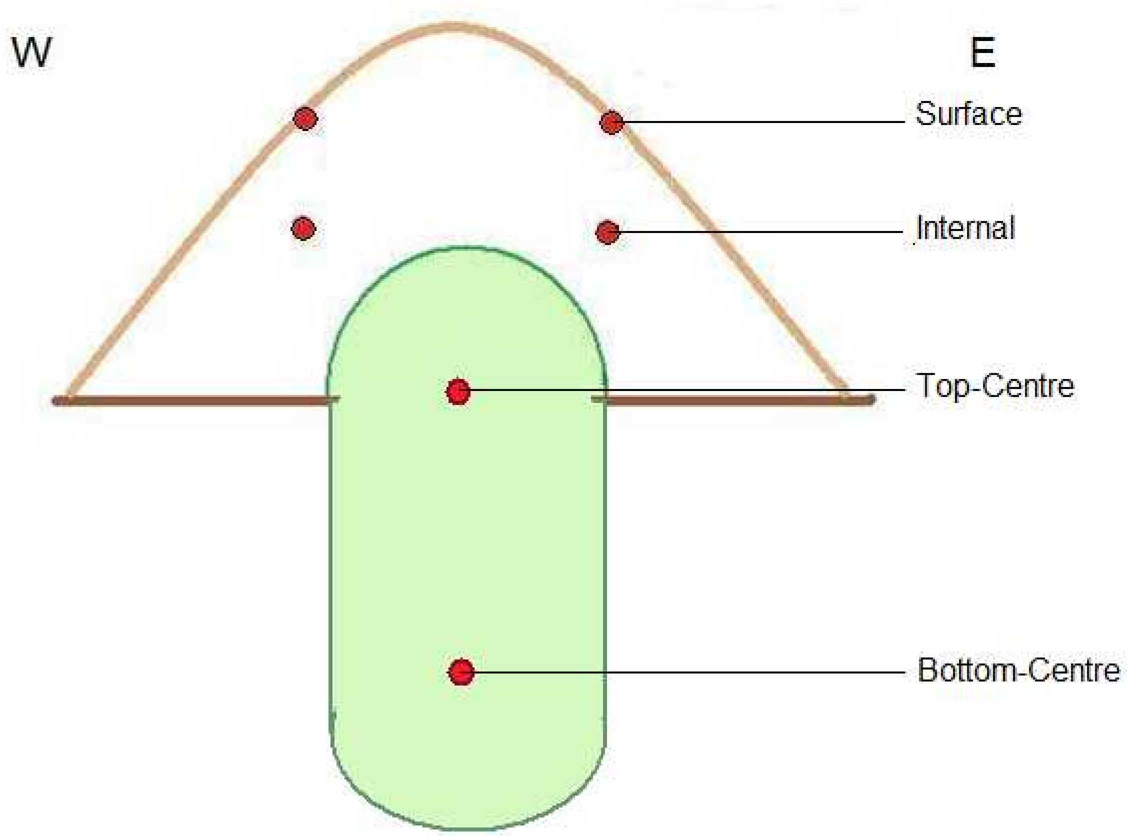


Figure 3.2. Schematic diagram of the *T. trinervoides* mound indicating relative positions of the iButtons.

3.2.2. Data analysis

The data analysis was conducted using Statistica version 6 (StatSoft, Inc.) and Microsoft® Office *Excel*® 2007. Repeated measures analysis of variance graphs (ANOVA) were used to compare daily temperatures of the mounds and ambient temperatures. Monthly averages of coefficients of variation (standard deviation/ mean) were calculated and used to compare the degree of variation of the temperatures in the centre regions of the mounds at the three sites. A regression analysis was used to investigate the correlation of ambient temperature with mound temperatures.

3.3. Results

3.3.1. Daily temperature profile

Figures 3.3, 3.4 and 3.5 show the mean daily temperatures of the centre, internal, surface and ambient temperature for one week each month in the mounds at the AEL, West Complex and Control site respectively. In all the mounds measured, the inside temperatures were greater than the ambient temperatures. The daily average ambient temperature fluctuated markedly throughout the weeks and during July it plummeted to 6.5 °C and got steadily warmer during August and September.

In most months, the centre temperatures were lower than either the surface temperatures or the internal temperatures but higher than the ambient temperatures. The centre temperatures were relatively constant each week however there was a significant drop from May to July and then increase from August to September. Average daily internal temperatures in the mounds gave the highest recorded temperatures for most months at all three sites and were considerably higher than ambient temperatures. Surface temperatures were higher than the ambient temperature and generally tracked it.

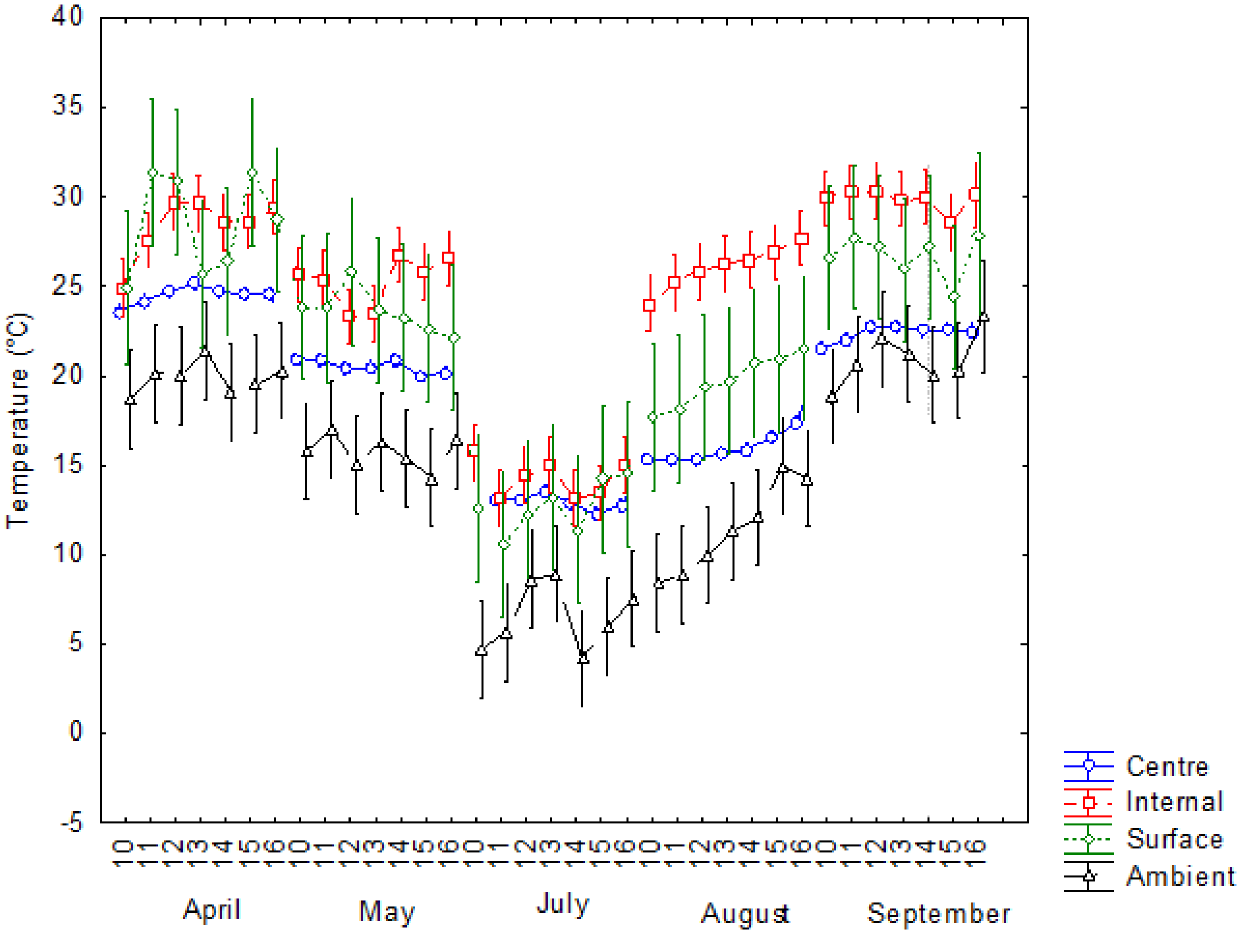


Figure 3.3. Mean ± S.D. daily temperatures of the three positions in the two mounds at the AEL site. Ambient temperature recorded at the site is also shown.

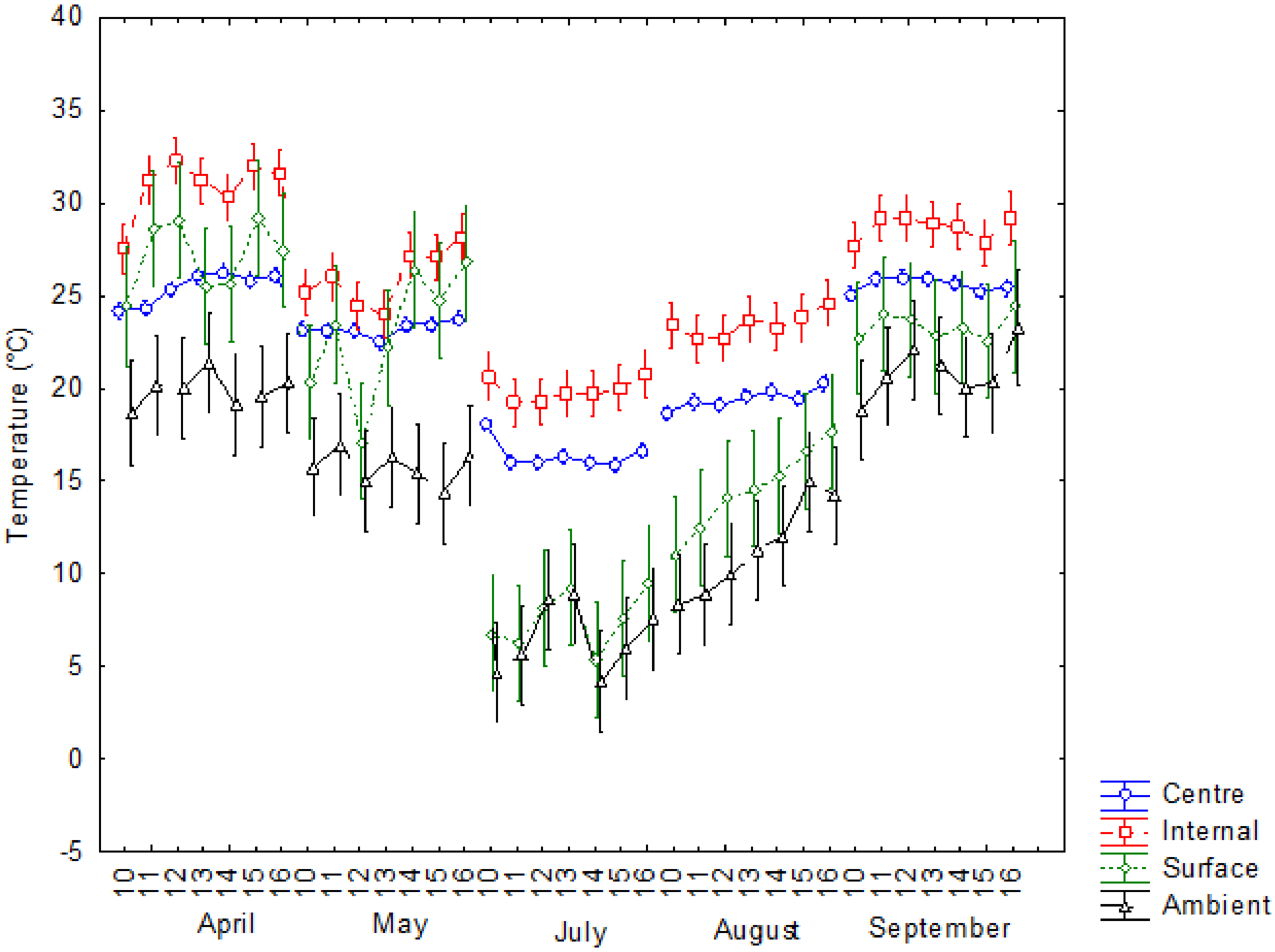


Figure 3.4. Mean ± S.D. daily temperatures of the three positions in the two mounds at the West Complex site. Ambient temperature recorded at the site is also shown.

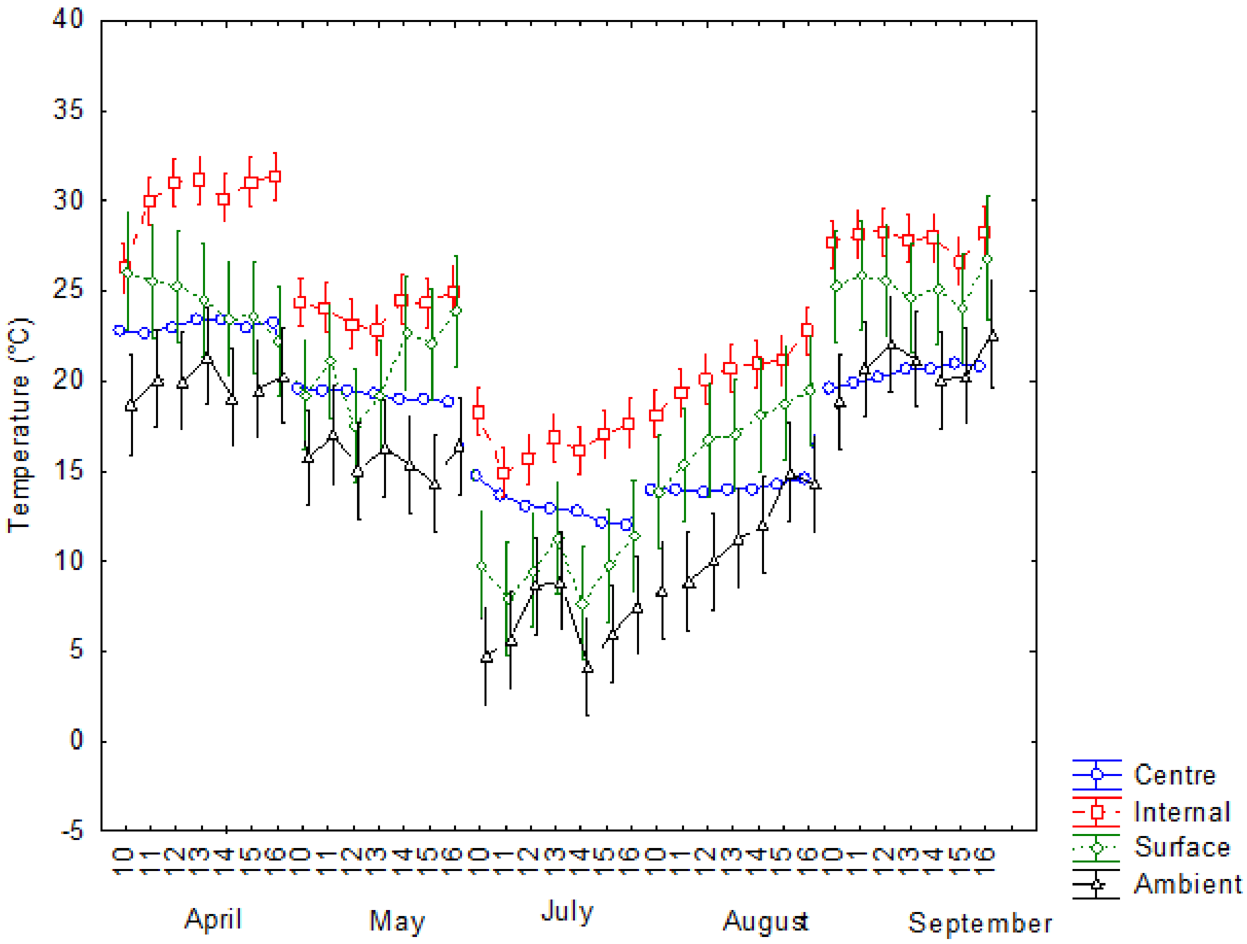
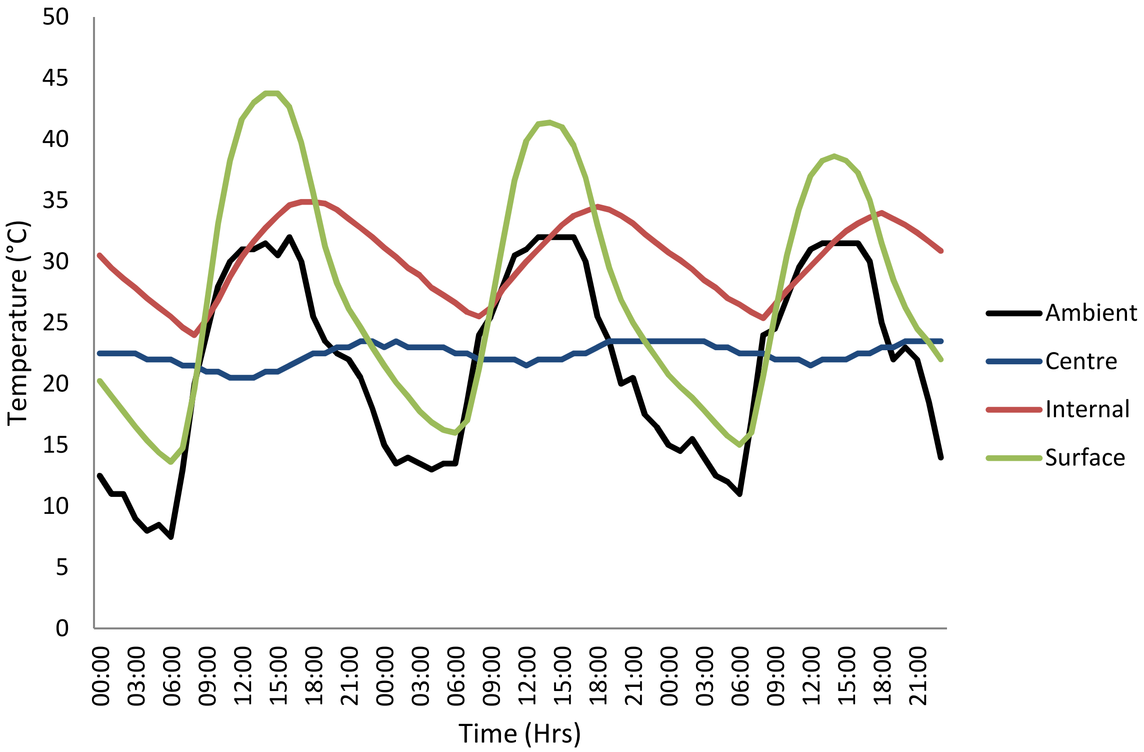


Figure 3.5. Mean ± S.D. daily temperatures of the three positions in the two mounds at the Control site. Ambient temperature recorded at the site is also shown.

Figures 3.6, 3.7 and 3.8 show the daily fluctuation of ambient and mound temperatures during the three hottest and coldest days in the middle of September and July respectively. Ambient temperatures during the day fluctuated with maxima in the afternoon (13:00 – 15:00) and minima in the morning (5:00 – 7:00). Despite these ambient temperature fluctuations, centre mound temperatures in the mounds were kept relatively constant in all three sites. These centre mound temperatures however varied according to the seasons. For instance, in the Control site, during summer the centre mound temperatures were kept at 20.39 ± 0.53 °C (Figure 3.8A) whereas during winter when ambient temperatures were colder, the centre mound temperatures dropped to 13.05 ± 0.85 °C (Figure 3.8B).

The other two sites, the AEL and West Complex, showed a similar trend although at both sites centre mound temperatures were not as consistent as the centre mound temperatures measured at the Control site (Figures 3.6 and 3.7). Surface mound temperatures of all three sites tracked the ambient temperature during both seasons and were generally higher than the ambient temperature. Internal mound temperatures fluctuated less than the surface mound temperatures but also appeared to track the ambient temperatures with a slight lag.

A)



B)

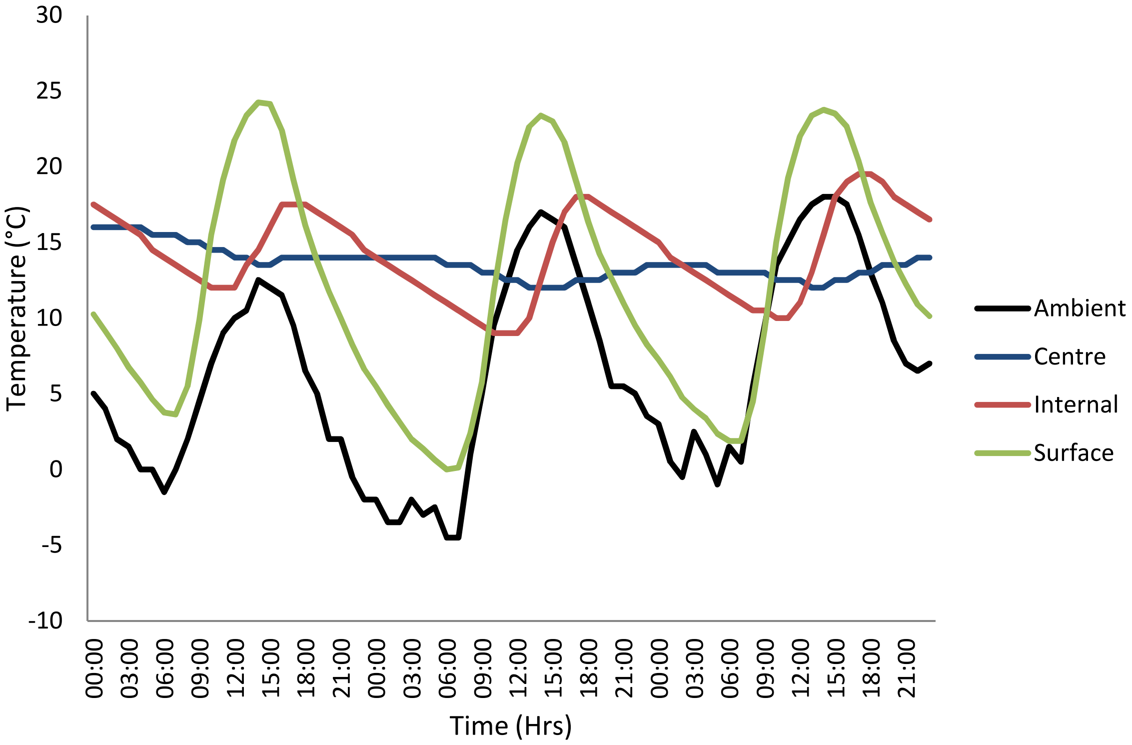


Figure 3.6. Ambient temperatures and mound temperatures over a period of three days during summer (A) and winter (B) at the AEL site.

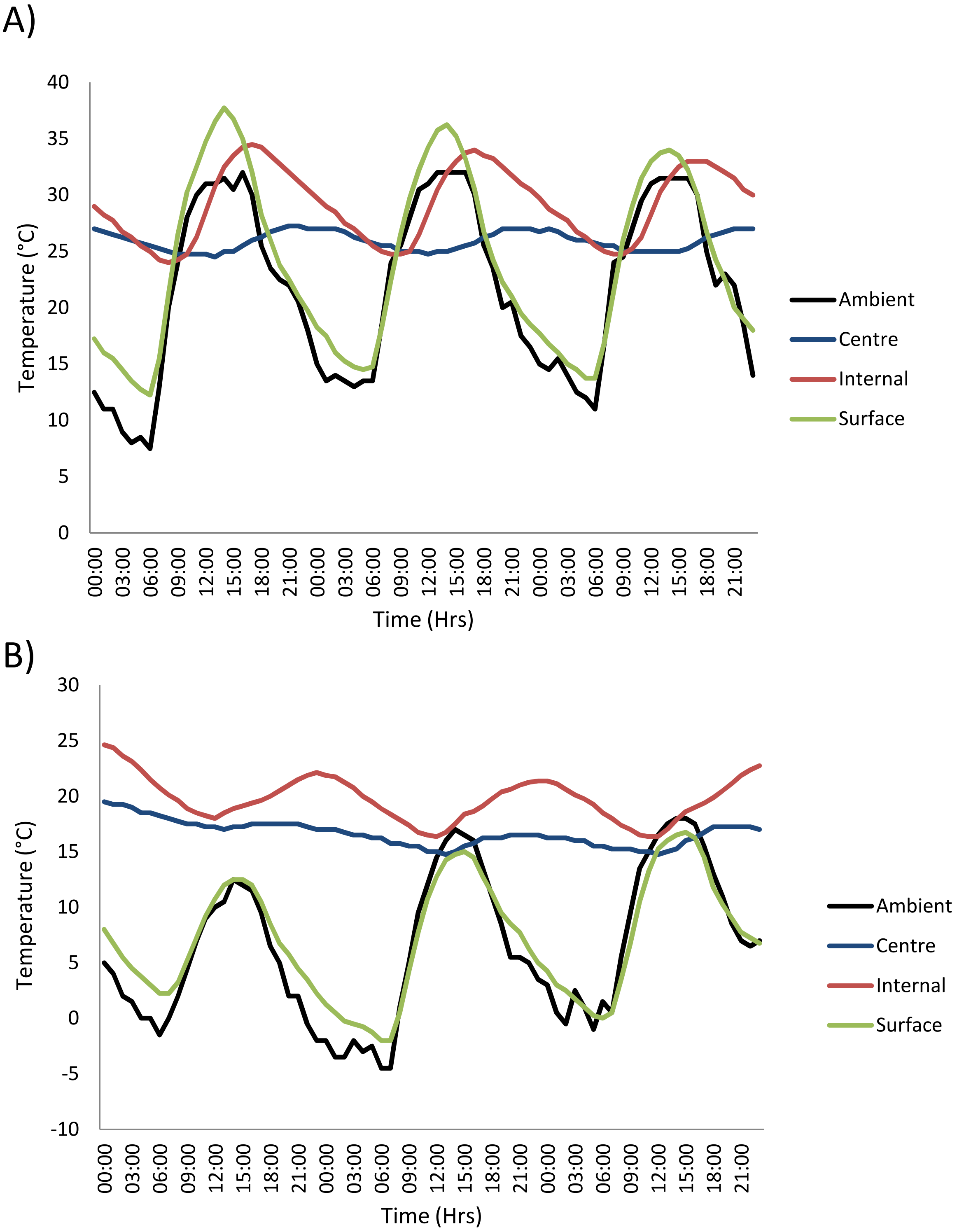


Figure 3.7. Ambient temperatures and mound temperatures over a period of three days during summer (A) and winter (B) at the West Complex site.

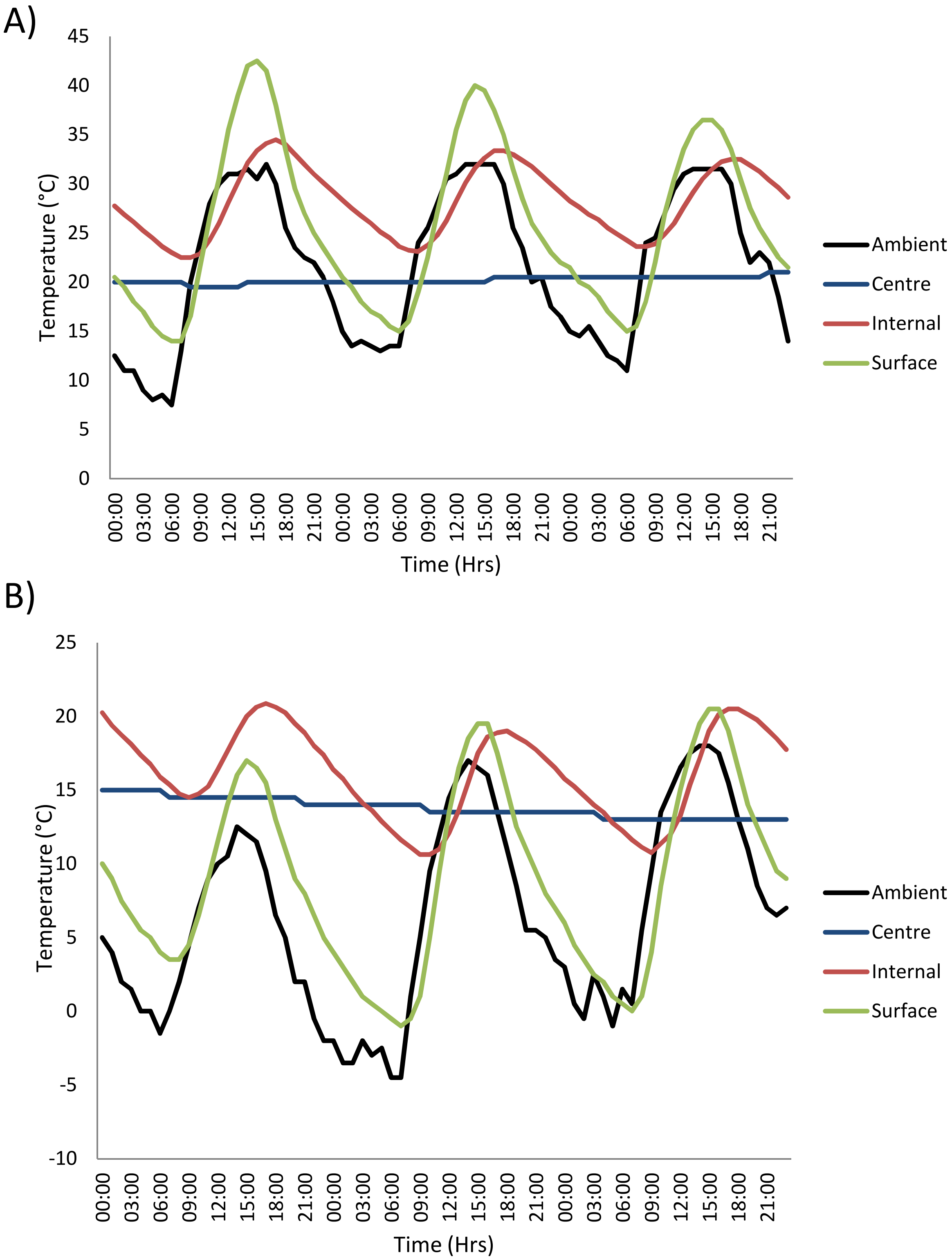


Figure 3.8. Ambient temperatures and mound temperatures over a period of three days during summer (A) and winter (B) at the Control site.

3.3.2. Variation of mound temperatures

The centre mound temperatures at the Control site showed greater temperature control than mounds at the other sites (Table 3.1). The internal mound temperatures varied more than the centre mound temperatures but not as much as the ambient and surface mound temperature. The surface mound temperatures had greater ranges than that of the ambient temperature with the highest ranges measured at the AEL site.

Table 3.1. Ranges of the ambient temperature and temperatures in the various positions of the mounds at the three sites.

	April	May	July	August	September
Air temperature					
	15.5	23	27	29.5	24.5
Centre mound temperature					
AEL	3.5	5	5	4.5	3
West Complex	3.5	5.5	5.5	5	3.5
Control	1	1	3	1.5	1.5
Internal mound temperature					
AEL	13	16	12.5	17.5	11.5
West Complex	15.5	16	9	10	12.5
Control	12.5	10.5	13	16	12.5
Surface mound temperature					
AEL	35	41.5	31.5	35	30
West Complex	29	37	22	26	26
Control	26.5	25.5	26	33.5	28.5

Coefficients of variation (CV) of the centre mound temperatures at each of the sites were calculated for each of the 7 days and averaged for each month (Figure 3.9). The centre mound temperatures at the Control site had the least amount of variation. The West Complex and AEL site centre mound temperatures were not significantly different to each other and had a much larger degree of variation when compared to the Control site.

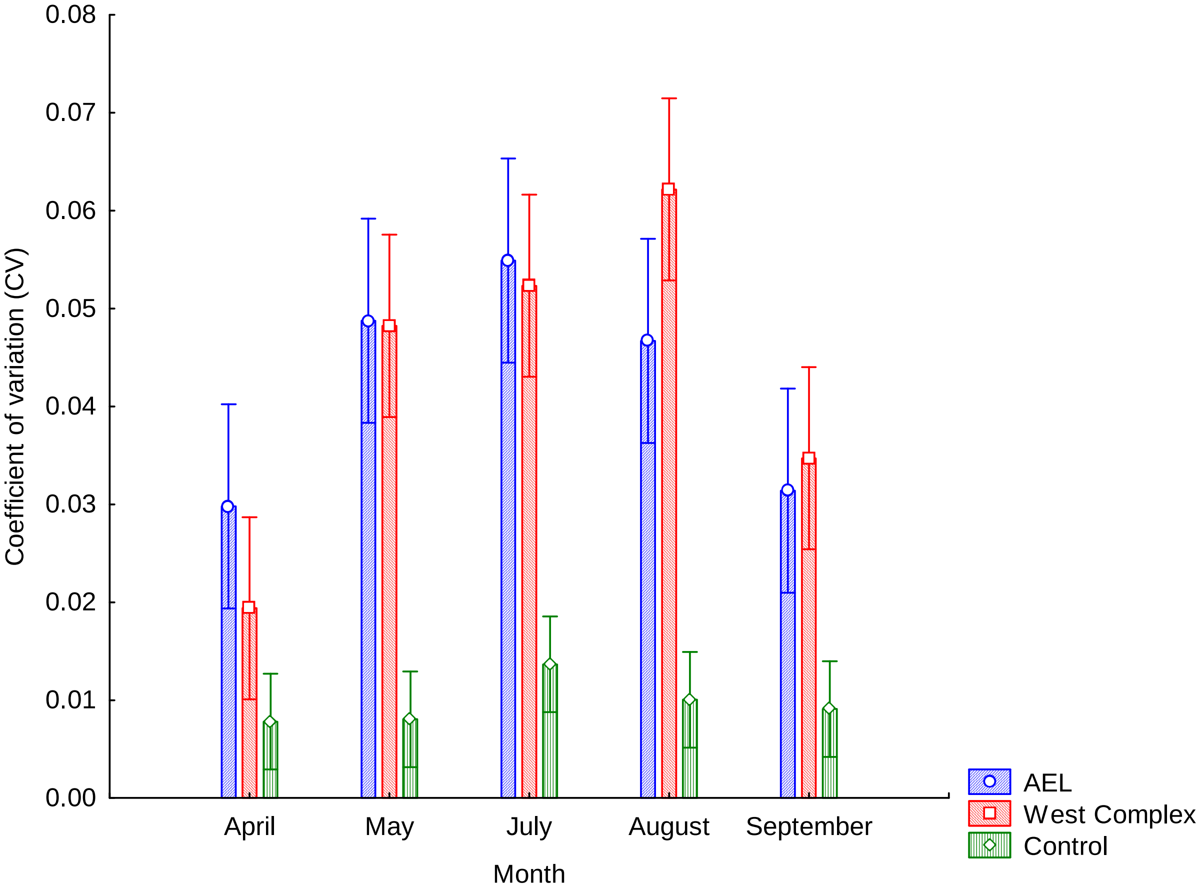


Figure 3.9. Average coefficient of variation (CV) of the centre mound temperatures at the Control, West Complex and AEL site per month. Vertical bars denote 0.95 confidence intervals.

3.3.3. Monthly profile of the centre mound temperatures

On a monthly basis, the average centre mound temperatures at each site varied in accordance to the ambient temperature i.e. when the average monthly ambient temperature dropped from 15.71 °C in May to 6.51 °C in July, the average monthly centre temperatures dropped from 7.34 °C, 6.82 °C and 6.18 °C at the AEL, West Complex and Control site respectively (Table 3.2). The West Complex site had the highest average centre mound temperatures reaching highs of 25.63 °C. All average monthly centre mound temperatures were kept within narrow limits, for example, during September the AEL site had a range of 3 °C, the West Complex site a range of 3.5 °C and the Control site a range of 1.5 °C (Table 3.2).

Table 3.2. Ambient temperature and centre temperatures of the three sites each month (mean ± SD, maxima and minima).

	April	May	July	August	September
Ambient temperature					
Mean	19.87 ± 3.99	15.71 ± 5.29	6.51 ± 7.12	11.476 ± 9.06	20.82 ± 7.28
Maximum	29	27.5	21.5	27.5	32
Minimum	13.5	4.5	-5.5	-2	7.5
Centre mound temperature					
AEL					
Mean	24.52 ± 0.85	20.52 ± 1.13	13.18 ± 1.02	15.929 ± 1.02	22.37 ± 0.83
Maximum	26.25	23.5	16	18.5	23.5
Minimum	22.5	18.5	11	14	20.5
West Complex					
Mean	25.50 ± 0.93	23.23 ± 1.22	16.41 ± 1.07	19.463 ± 1.27	25.63 ± 0.94
Maximum	27	26.25	19.5	21.75	27.25
Minimum	23.75	20.75	14.25	17	23.75
Control					
Mean	23.10 ± 0.34	19.23 ± 0.29	13.05 ± 0.85	14.131 ± 0.30	20.39 ± 0.53
Maximum	23.5	19.5	15	15	21
Minimum	22.5	18.5	12	13.5	19.5

3.3.4. Daily profile of the centre mound temperatures

A comparison of the daily average centre temperatures of the mounds at each site are shown in Figure 3.10. The centre temperatures at the West Complex site were the highest when compared to the AEL and Control sites. The Control site presented the lowest average daily temperatures followed by the AEL site. All the centre temperatures were warmer than the ambient temperatures each day and had little variation during each month as opposed to ambient temperatures that had marked fluctuation.

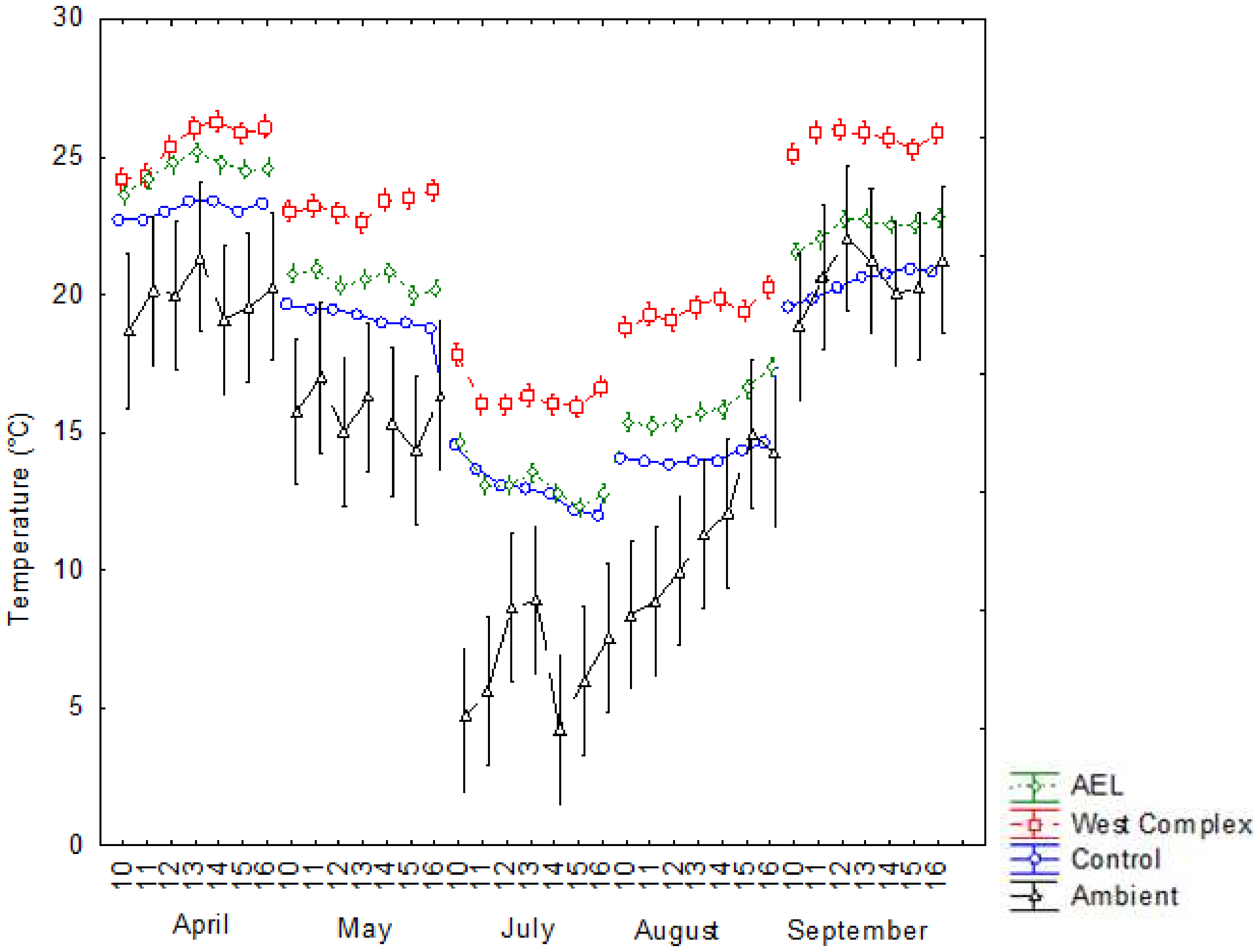


Figure 3.10. Mean ± S.D. daily temperatures of the centre mound temperatures for 2 mounds at the three sites. The ambient temperature is also shown.

3.3.5 Influence of ambient temperatures on centre mound temperature

A plot of the mean daily centre mound temperatures in relation to mean daily ambient temperature showed that centre temperature remained constant for each specific month and changed depending on the season (Figures 3.11 – 3.13). The Control site temperatures showed no tracking of the ambient temperature (Table 3.3). In two months at the AEL and West Complex sites there was a significant weak correlation between the ambient and centre mound temperature.

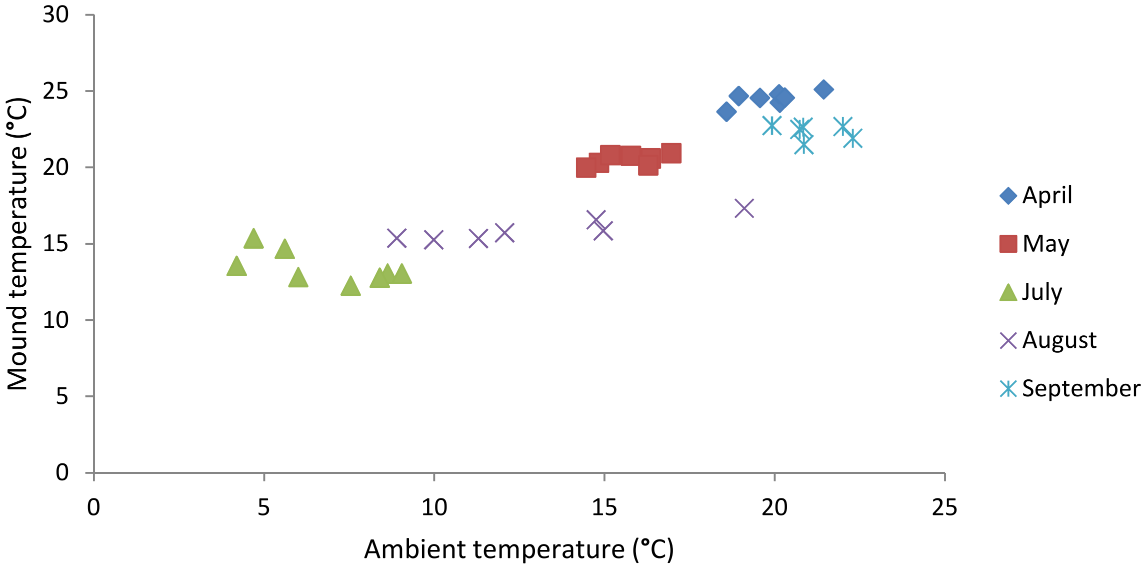


Figure 3.11. Average daily centre mound temperatures in relation to average daily ambient temperatures at the AEL site.

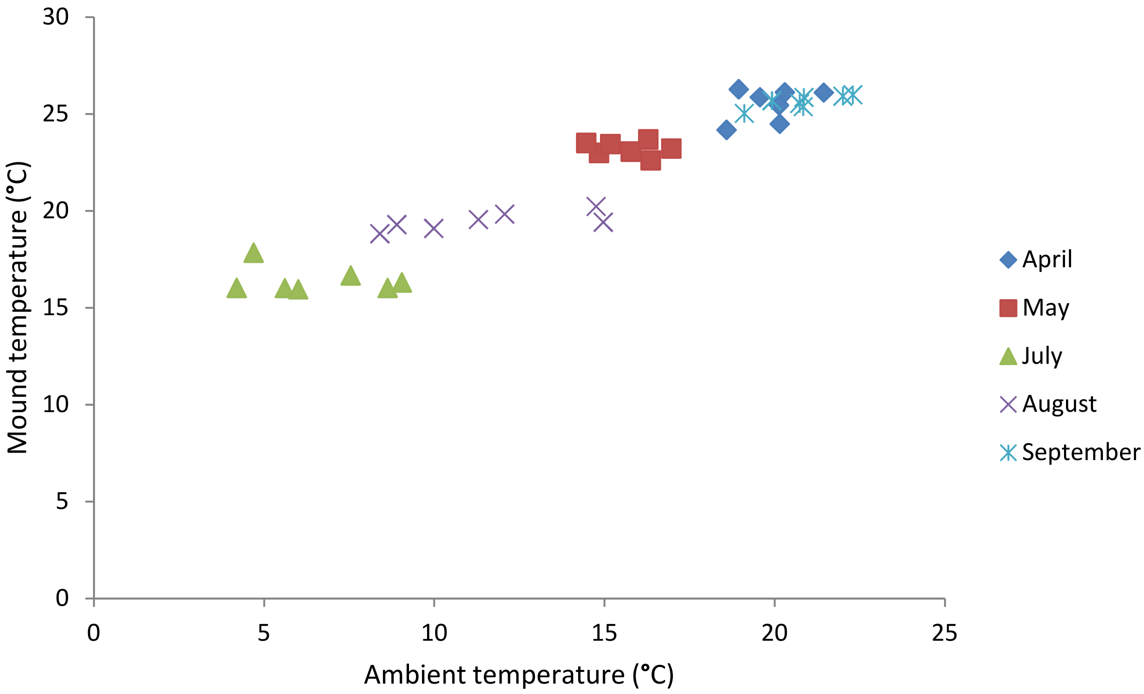


Figure 3.12. Average daily centre mound temperatures in relation to average daily ambient temperatures at the West Complex site.

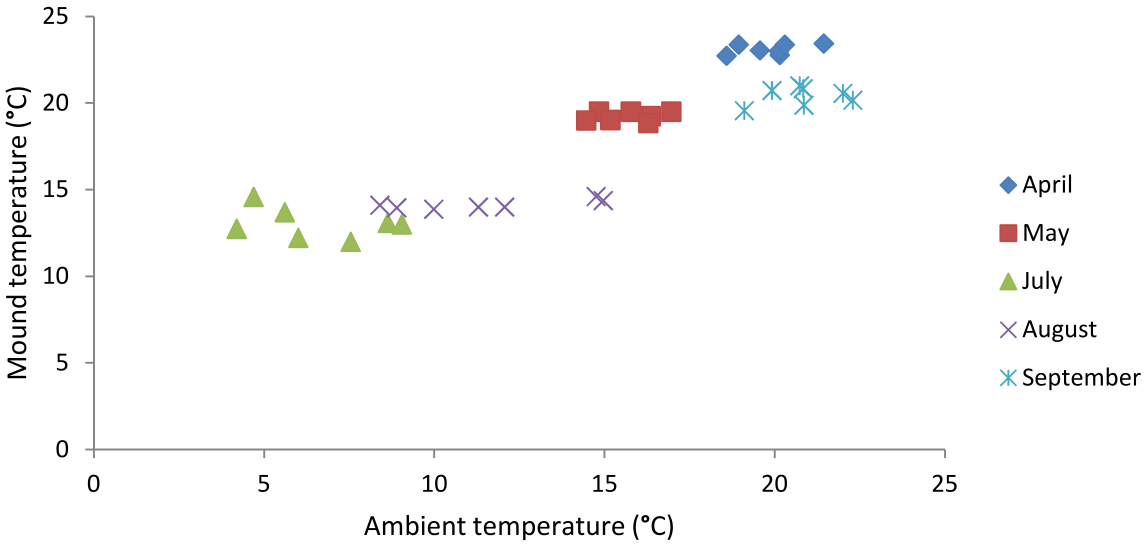


Figure 3.13. Average daily centre mound temperatures in relation to average daily ambient temperatures at the Control site.

Table 3.3. Regression analysis of the centre mound temperatures in relation to ambient temperature at the three sites.

AEL	r^2	Slope	p
April	0.336	0.051	0.00001
May	0.182	0.287	0.00001
July	0.005	-0.027	0.325
August	0.004	0.022	0.384
September	0.041	0.091	0.007
West Complex			
April	0.051	0.029	0.106
May	0.029	0.364	0.00001
July	0.003	0.024	0.471
August	0.001	0.014	0.655
September	0.156	0.233	0.00001
Control			
April	0.00003	0.006	0.934
May	0.001	-0.009	0.671
July	0.003	-0.023	0.42
August	0.00003	-0.001	0.94
September	0.0001	-0.006	0.877

3.4. Discussion

The mounds created by termites vary enormously among species in their ability to regulate temperature. *Amitermes evuncifer, Thoracotemres macrothorax* and *Microcerotermes edentatus* build dome shaped nests of simple structure that have thin walls and no thermoregulatory ability (Lüscher, 1961). The mushroom mounds built by the termites belonging to the large African genus, *Cubitermes*, have internal temperatures which follow, with a slight dampening, the fluctuations of the surrounding environment (Krishna and Weesner, 1970). Conversely, the large thick-walled mounds constructed by *Cephalotermes rectangularis* serve to keep nest temperatures within 2 °C of 30 °C (Krishna and Weesner, 1970). The most well-known and extensively studied example of thermoregulatory ability of termite mounds lies in the great mounds built by the *Macrotermes* genus which keep an extremely constant nest temperature of 30 °C (Korb and Linsenmair, 1999; 2000a; 2000b; 2003).

Studies on the thermoregulatory capabilities of mounds have been limited to wood or soil feeding termites; resulting in very little information on the thermoregulation in the mounds of grass eating (harvester) termites (Adam, 1993). Field (2008) presented the first detailed investigation of the thermoregulatory importance of the dome-shaped mounds built by the snouted harvester termite, *T. trinervoides* in Melville Koppies, Gauteng. The only other study was by Adam (1993) as part of his PhD in which he measured temperatures in one epigeous mound for 24 hours each month for a year.

The centre mound temperatures of the *T. trinervoides* mounds at the three study sites were kept relatively constant on a monthly basis despite fluctuations in the ambient temperature. Centre mound temperatures also had little daily fluctuation and did not vary more than 5 °C while the ambient temperature varied dramatically (up to 30 °C). A similar result was found by Field (2008) where centre mound temperatures also did not fluctuate by more than 5 °C despite ambient temperature fluctuations of 35°C. The constant temperatures were of significance since the brood, queen and king occupy this portion of the mound and a less variable temperature would be to their advantage (Adam, 1993; Jones and Oldroyd, 2007). Adam (1993) presented contrary findings: the centre mound temperatures in the mound he studied tended to track the ambient temperature and exhibited a large degree of variation (daily fluctuation of 13 °C as opposed to 5°C at these study sites). The centre mound temperatures in his study reached a high of 40 °C in July which was some 20 °C higher than recorded centre mound temperatures in this study. The average temperature in the centre of the mound was also always higher than the average temperatures in the rest of the mound which disagreed with results from this study where the internal temperatures were found to be the highest recorded temperatures. The centre mound temperatures in Adam's (1993) did present a 4 to 6 hour lag of temperature change, deeming them the most consistent temperatures throughout the mound as was also the case in this investigation. Adam’s (1993) results may be different to the results in this study as the mound he studied was epigeous. Internal temperatures varied more than the centre temperatures as the iButtons were closer to external environment. Surface temperatures varied the most and to the same extent as ambient temperature (in some cases, more than ambient temperature) which was expected as the iButtons were closest to the external environment.

Several studies have shown that the presence of termites tends to raise mound temperature due to the heat produced by termite metabolism (Krishna and Weesner, 1970; Bristow and Holt, 1987; Adam, 1993; Abe *et al.*, 2000; Turner, 2001). This could explain the high temperatures recorded within the centre of the *T. trinervoides* mounds which were up to 10 °C higher than the ambient temperatures. This result agrees with Field (2008) where centre temperatures were on average 4 °C to 10 °C higher than the ambient temperatures. Internal temperatures presented the highest recorded temperatures compared to the ambient, surface and centre temperatures. The high internal temperatures of the mound could be due to the combined effect of the heat absorbed by the mound from solar radiation and the heat generated by the termite metabolism. Soldiers and workers were always seen in the internal region of the mounds when inserting the iButtons. Solar radiation and the insulating properties of the mound probably accounts for the fact that surface temperatures are warmer than ambient temperatures.

Although centre temperatures were kept within narrow limits of particular temperatures each month, the centre temperatures were not kept constant throughout the study. This was highlighted by the distinct drop of temperature from May to July then an increase of temperatures from August to September. This change in centre mound temperatures throughout the months could be related to *T. trinervoides* ecology. Adam *et al.* (2008) showed that it was crucial for *T. trinervoides* workers to forage and store as much grass as possible during autumn so that the grass reserves subsist during the low winter temperatures, when foraging activity is restricted. The decrease of centre mound temperatures in this study could be a result of the colony decreasing in size to ensure the grass reserves within the mound are not depleted. Field (2008) also found an increase of centre mound temperatures from July to August of some 10 °C, reaching temperatures of 28 °C in September. In this study, temperatures only increased 3 °C from July to August and the highest recorded September temperature was 25 °C. This increase in temperature could be due to the commencement of foraging and thus the colony increasing in size. This resultant increase in nest temperatures could also be important for the development of the alates and ensured that they reach maturity in time for the first rains of spring (Abe *et al.*, 2000). Greaves (1964) noted that the presence of alates in *Coptotermes aninaciformis* colonies were accompanied by an elevation of nest temperatures. This was also noted by Holdaway and Gay (1948) in *Nasutitermes exitiosus* (as cited by Krishna and Weesner, 1970).

The centre mound temperatures were different at each site. West Complex centre mound temperatures were the highest followed by the AEL site and lastly, the Control site. Higher temperatures could be explained by higher numbers of termites which release metabolic heat and raise mound temperatures however this is unlikely as the mounds studied were the same size throughout the study sites. It has been shown that chronic exposure to toxic elements can lead to increases in metabolic rate in invertebrates therefore it is possible that the termites in the contaminated sites may be generating more heat than the termites in the Control site (see Chapter 4; Hopkin, 1989 as cited by Lagisz *et al.*, 2005).

At the Control site there was evidence of temperature regulation in the centre of the mound as no correlation between the ambient temperature and the centre temperature was found. Korb and Linsenmair (2000a) found that ambient temperature influenced the mean nest temperatures in small colonies of the fungus-cultivating, mound building termite *M. beliicosus* while in mounds that have reached a certain height, mean nest temperature did not correlate with ambient temperature. Large mounds kept their temperature at 30°C regardless of ambient temperature. A similar trend was found in this study as the mound temperatures during most months at the three sites were kept at particular temperatures regardless of the ambient temperatures. The mounds at the Control site appeared to be the most efficient at regulating temperatures as the mounds at the other two sites showed an internal weak correlation with the ambient temperature during some months. The fact that the mounds at the AEL and West Complex site did not appear to regulate temperature as efficiently as the mounds at the Control site may be due to the possible contamination of the sites.

Jones and Oldroyd (2007) hypothesised that the less fluctuation there is in the centre of a mound the more favourable it is to the colony. This is due to the fact that the queen, king and brood reside in this region and are particularly sensitive to changes in temperature, thus a more constant thermal environment would be to their benefit. The coefficient of variation of temperatures at each site showed that the contaminated sites presented more fluctuation of temperature when compared to the Control site. Korb and Linsenmair (2000a) showed that the structure of the mound is responsible for levelling out temperatures within the mound therefore it could be possible that the structure of the mounds at the West Complex and AEL site have been compromised due to possible contamination. The contamination may be affecting the workers ability to construct mounds with full thermoregulatory function.

This study demonstrated that the centre temperatures of the mounds of the snouted harvester termite, *T. trinervoides*, were kept constant on a monthly basis but fluctuated on a seasonal basis. This change in centre mound temperatures throughout the months could be related to *T. trinervoides* ecology. For example, the decrease of centre mound temperatures could be a result of the colony decreasing in size to ensure the grass reserves within the mound are not depleted. The centre mound temperatures at the West Complex site were hotter and more variable than at the AEL and Control site which may be a result of the impact of the mine tailings however this is considered unlikely as this is not supported by the results given by the mound at the AEL site.

CHAPTER 4 - HEAVY METAL ANALYSIS OF TERMITES, TERMITE MOUNDS AND SOILS FROM SITES OF DIFFERENT PROXIMITIES TO A TAILINGS DAM

4.1. Introduction

Mining activities are well known for their negative effects on the environment, due to the deposition of large volumes of wastes in the form of tailings on the soil. These tailings pose a significant risk to the environment as most mine tailings are not managed properly and as a result heavy metals migrate into the surrounding environment. This contributes to the contamination of soil substrates, destruction of soil texture, shortage of nutrients, destruction of ecological landscape, groundwater pollution and decrease in biological diversity (Rashed, 2010). Due to the fact that mine tailings are finely divided into small particles, there is a potential risk that such materials may find their way through the environment and food chain to animals (Conesa *et al.*, 2006). The negative impacts of gold mine tailings on various animal populations have been documented in many countries around the world, particularly the USA. Accidental spills of tailings into the local environment in the USA resulted in a decrease of wildlife especially that of waterfowl and bat populations (O'Shea *et al.*, 2001). Another study conducted in Arizona and California recorded 519 tailing related deaths of rodents and bats (Clark and Hothem, 1991). Between 1986 and 1991, cyanide in heap leach solutions and mill tailings ponds at gold mines in Nevada alone killed at least 9500 birds, mammals, reptiles, and amphibians (Henny *et al.*, 1994). Gold mine tailings in the Portovelo-Zaruma district in southern Ecuador have reduced biodiversity considerably as a result of a direct lethal effect on the biota close to the source (Tarras-Wahlberga *et al.*, 2001). In one case, mine effluents from a Canadian tailings pond released into a nearby creek killed more than 20 000 steelhead (*Oncorhynchus mykis*; Leduc, 1978). In 1995, the Australian

Northparkes Mine operated a carbon-in-pulp processing circuit that produced tailings high in soluble copper-cyanide complexes and resulted in the death of 2700 birds over four month period (Donato *et al.*, 2007)

To evaluate environmental concerns, scientists have measured the metal concentrations in the ecosystem around the mines (Rashed, 2010). Studies conducted by Rosner and van Schalkwyk (2000), Lee *et al.* (2001), Aucamp and van Schalkwyk (2003), Boularbah *et al.* (2006), Guo-li *et al.* (2008) and Antwi-Agyei *et al.* (2009) have shown that the soil at sites near mine tailings dams had higher levels of heavy metals when compared to control sites and all of the authors concluded that the tailings are a source of heavy metal contamination. Heavy metals are one of the most persistent pollutants in the environment (Khalil *et al.*, 2008). Unlike organic pollutants, they cannot be degraded but accumulate throughout the food chain, producing potential ecological disturbances. Bioaccumulation of heavy metals has been demonstrated in a wide variety of animals. For example, Bruce *et al.* (2003) showed that cattle grazing on grass growing on a tailings dam accumulated more than ten times the Zn and As concentration in their liver when compared to those grazing at a non-polluted site. Benthic invertebrates that inhabited a stream impacted by mining operations showed elevated concentrations of Cd, Cu and Zn in their tissue (Kiffney and Clements, 1993). Heikens *et al.* (2001) found that internal Pb, Cd and Cu concentrations in terrestrial invertebrates increased as Pb, Cd and Cu concentrations increased in their surrounding soil environment.

Some heavy metals such as Zn and Cu are essential for health, survival and production as they are part of vital physiological, structural, catalytic and regulatory processes in mammals (Reis *et al.*, 2010). However, if these heavy metals are ingested in excessive doses they may cause acute or chronic poisoning. Acute poisoning occurs soon after ingestion while chronic poisoning occurs when an animal constantly ingests toxic doses which are at lower concentrations than those that cause acute poisoning. Both types of poisoning can lead to the death of the organism (Reis *et al.*, 2010). Obviously the ingestion of toxic amounts of nonessential metals such as Pb and Cd can have serious deleterious effects too. These effects include subacute, acute or chronic poisoning which cause severe damage to various organs such as the liver and morphological and functional changes to the kidney, lung, nervous system and intestine in mammals (Reis *et al.*, 2010).

Heavy metals have been shown to adversely affect different aspects of the biology of insects. Augustyniak *et al.* (2009) showed that individuals of *Chorithippus brunneus* in heavy metal polluted sites laid significantly fewer eggs than insects from the control site. Xu *et al.* (2009a) studied the effects of $CaCl_2$ $CuCl_2$ $ZnCl_2$ and $PbCl_2$ on the development and hatching success of eggs of *Folsomia candida* and found that egg hatching success significantly decreased when concentrations of Cu, Pb and Zn reached 400, 1600 and 800 mg/kg dry soil respectively. When individuals of the ectoparasitic wasp *Nasonia vitripennis* were exposed to copper there were negative effects on parasitoid growth and development as well as fecundity. Copper exposure also inhibited vitellogenesis, a vital process involving yolk formation in the oocyte. This phenomenon was also found in *Oncopeltus fasciatus* females exposed to Cd (Cervera *et al.*, 2005). Xu *et al.* (2009b) showed that there was a reduction in adult survival and reproductive failure in the Collembolan, *Sinella curviseta* when exposed to soils contaminated with Cu and Zn. The adults that did survive exhibited a decrease in growth rate when compared to the control adults suggesting that metals affect *S. curviseta* metabolism. Due to the close interaction of termites with the soil, the likelihood of heavy metal accumulation from soils contaminated by mine tailings could be high. Termites are an important food source for many animals and contaminated termites could potentially impact the food chain.

The aim of this study was to evaluate the heavy metal content of the termites, termite mounds and soil profile at the two study sites (Section 1.3) near the tailing dams. A comparison of the metal content of termites in the surrounding area gave an indication as to whether bioaccumulation was taking place in the tissues of the termites. Snouted harvester termites form a vital part of the ecology of certain animals such as lizards and spiders, as they not only provide a source of nutrition but also shelter in the form of their abandoned mounds (Haddad and Dippenaar-Schoeman, 2002). Spiders and reptiles alike could be susceptible to contamination as the mound interior may be laden with contaminated faeces. Knowing the heavy metal levels of the mounds could also indicate whether the termites were bringing up heavy metals from the soil, hence making the contaminants more bioavailable to the environment. This phenomenon has been demonstrated in the *Macrotermes* spp. in northern and north-eastern Namibia where the authors suggested that the termites may be mining key micro-nutrients such as Mn, Co, Cu, Zn, Se and I for their fungus cultures (Mills *et al.*, 2009). This study presents the first investigation of the heavy metal content in the species *T. trinervoides* and their mounds near tailing storage facilities.

4.2. Methods and Materials

4.2.1. Collection of the mound, soil and termite samples

A medium mound was chosen at the AEL and West Complex sites for sampling. The soil profile next to the mound was sampled by using a Tractor-Loader-Backhoe (TLB) to dig a 2 m trench next to the chosen mound (Figure 4.1). The surface litter and soil samples were collected at 0 – 2 cm, 2 – 5 cm, 5 – 10 cm intervals then at 10 cm intervals until 120 cm below the surface. The mounds at each site were then lifted off the ground using the TLB, wrapped in plastic (to contain the termites) and transported to the University of the Witwatersrand. It was not possible to sample the Control site as this site resided on private land which had a fenced perimeter. The mounds taken from AEL and West Complex sites were then placed in plastic troughs where samples were taken using a plastic spade. Samples were taken from the hard outer crust then at 10 cm intervals to the bottom of the mound where the queen and brood reside. All soil and mound samples were double bagged and placed in a cold room. Once the samples of the mounds had been collected, the termites from each respective mound were collected using entomological forceps and placed in plastic containers. A week later a small mound was lifted by hand from the Control site, placed in a container and brought back to the University of the Witwatersrand where the termites were then collected as described above. Approximately 2 g of termites from each caste (workers, soldiers, nymphs and alates) were collected.

Figure 4.1. Photograph of the trench dug just next to the mound at the AEL site.

4.2.2. pH and conductivity

The pH and conductivity of each sample of the mound and soil profile was measured using a pH meter (WTW 330i) and conductivity meter (WTW Cond 3110). Before this was possible the meters had to be calibrated with standard solutions of known pH (pH 4 and 7) and conductivity (±100, ± 1500 and ± 5000 µs). The soil and mound samples were also placed in a pestle and mortar to ensure clumps of particles were removed. Ten grams of each sample were weighed out, added to 20 mls of water and mixed together. The electrodes of the meters were then placed in the solutions and once the meters had equilibrated, the pH and conductivity recorded.

4.2.3. Moisture content

To find out the moisture content of the soil and mound samples, a subsample was taken and placed in glass Petri dishes. These were labelled, weighed and placed in an oven at 50 ° C for 24 hours. Samples were taken out and weighed again. The difference between the samples before and after being oven dried was calculated.

4.2.4. Testing the samples for heavy metals and cyanide

Soil, mound and termite samples were prepared for the chemical analysis. The soil and mound samples were sieved and ground using an agate pestle and mortar and subsequently freeze dried. The termite samples were placed in a stirrer to remove any soil particles, freeze dried and ground in agate pestle and mortar. The concentration of heavy metals Mg, Fe, P, Li, Ti, V, Rb, Sr, Mo, Ba, La, W, Bi, Cd, As, Cu, Mn, Pb, Zn, U, Co, Cr and Ni for each sample were analysed. Cyanide content of the samples was unable to be analysed due to lack of time and available facilities that could conduct the analysis. Soil certified reference materials were analysed to ensure the accuracy of the results of the analysis of the soil and Plant certified reference materials were used for the termites. Samples were analysed by the Agricultural Research Council (ARC) using a nitric acid digestion and the ICP-MS method was used for most of the metals. The ICP-OES method was used to analyse Zn, Cu, Mg, Fe, P and Mn,

4.3. Results

4.3.1. Conductivity, pH and moisture content

The conductivity of the West Complex site showed a distinct trend of a steady decrease with increasing depth and then increased gradually from 40 cm. With a few exceptions (60 – 80 cm and 100 – 120 cm), the AEL site showed the same trend (Figure 4.2). The average conductivity of the soil between the two sites were not significantly different ($t = 1.07$, $p = 0.29$). When comparing the conductivity of the mounds between the two sites, the AEL mound had a higher average conductivity than the West Complex mound ($t = -2.35$, $p < 0.05$). The AEL mound also had a higher average conductivity than the AEL soil profile ($t = 2.33$,

$p < 0.05$) while the average conductivity of the West Complex mound did not differ significantly to the soil profile ($t = 0.017$, $p = 0.98$).

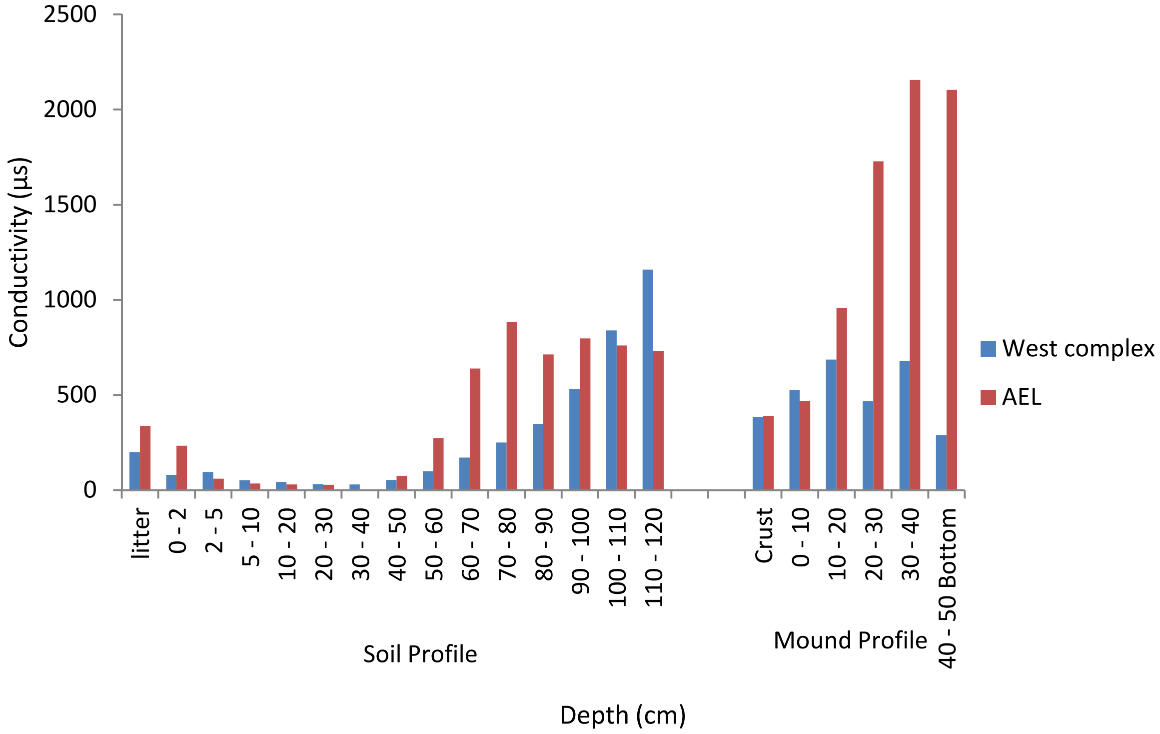


Figure 4.2. Conductivity of the soil and mound profile at the West Complex and AEL site.

The pH of the soil profile at both sites generally increased with increasing depth therefore the soils appear to be more alkaline at lower depths (Table 4.1). No patterns emerged when considering the distribution of pH throughout the mounds at the two sites (Table 4.2). The average pH content of the soils of the two sites did not differ significantly ($t = -0.70$, $p = 0.49$). The mounds however, were significantly different in that the AEL mound had a significantly higher average pH content than the West Complex mound ($t = -2.97$, $p < 0.05$). The pH content of the mounds at the AEL site did not differ significantly from the soil however the mounds at the West Complex site did have a significantly lower pH than the soil profile ($t = 4.01$, $p < 0.05$).

Table 4.1. pH values for the soil profiles at the two sites.

	AEL	West Complex
Litter	6.09	5.5
0 - 2	6.13	5.92
2 - 5	6.1	5.94
5 - 10	5.96	5.74
10 - 20	6.08	5.96
20 - 30	5.68	6.15
30 - 40	5.76	6.4
40 - 50	6.66	6.45
50 - 60	6.38	6.38
60 - 70	6.2	6.41
70 - 80	6.28	6.23
80 - 90	6.26	6.68
90 - 100	6.33	6.96
100 - 110	6.53	7.26
110 - 120	7.21	7.48
Mean ± SD	6.24 ± 0.37	6.36 ± 0.55

Table 4.2. pH values for the mound profiles at the two sites.

	AEL	West Complex
Crust	6.64	5.28
0 - 10	5.85	5.68
10 - 20	5.97	5.54
20 - 30	5.78	5.49
30 - 40	5.45	5.37
40 - 50 (bottom)	6	5.22
Mean ± SD	5.95 ± 0.39	5.43 ± 0.17

Generally, the soil profile appears to get moister as the depth increases evident by the small amount of water in the litter, then a gradual increase (with a few exceptions) and finally a large amount of water at the 80 cm depth (Figure 4.3).

The same can be said for the mounds. As expected the crust is much drier than the rest of the mound. Statistically, the moisture content of the soil and mound profile at the two sites did not differ (AEL: t = 0.36, p = 0.72; West Complex: t = 0.09, p = 0.93) however when the moisture content of the surface layer (litter – 20 cm) was compared to the mounds at the sites, the mounds had statistically more moisture content (AEL: t = - 4.13; West Complex: t = - 3.02, p < 0.05).

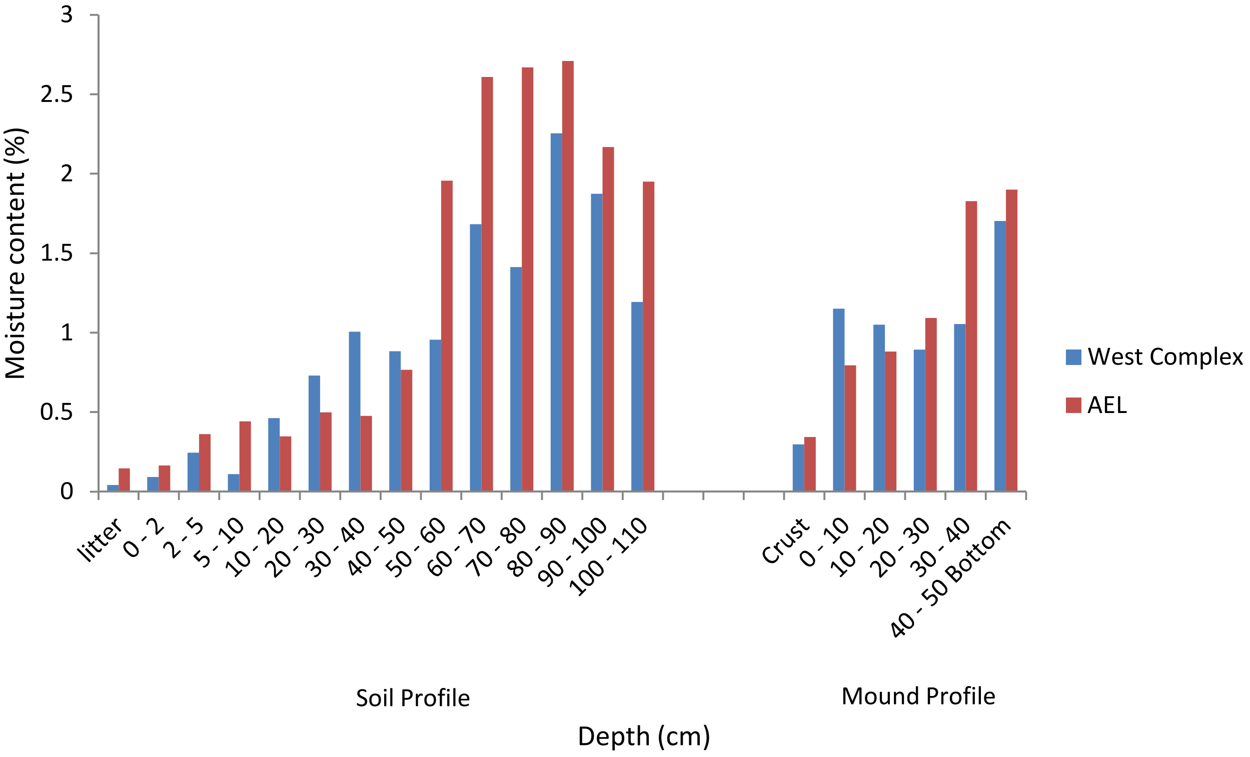


Figure 4.3. Moisture content of the soil and mound profile at the West Complex and AEL site.

4.3.2. Heavy metal analysis

The elements chosen to be presented in this study are Cu, Mn, Zn, Pb, U, As, Cd, Co, Cr and Ni as according to several studies (e.g. Rosner and van Schalkwyk, 2000; Kim and Jung, 2004; Guo-li *et al.*, 2008), these appear to be the most important heavy metals when considering contamination of soil and organisms. Due to the interference of Cl during the analysis of As, the accuracy of the results were substantially reduced and therefore excluded. All samples had less than 0.2 mg/kg of Cd. The concentrations of the other heavy metals are presented in the Appendix. The distribution of each heavy metal throughout the soil and mound profile at the two sites is presented to be able to compare the heavy metal concentrations between the soil and mound at the same site as well as between sites. Average heavy metal content in the mounds was compared to the surface layer (litter – 5cm) at both sites. Heavy metals in the termites were also compared with the heavy metals found in the soil and mound at the two sites.

4.3.2.1. Copper (Cu)

The soil profile at the West Complex site showed that Cu concentration was high at the surface, decreased with increasing depth and then increased again at 90 cm (Figure 4.4). The mounds at the West Complex site had significantly more average Cu content than the soil profile at the West Complex site (12.9 ± 1.8 and 9.6 ± 3.2 mg/kg respectively; $t = -2.31$, $p < 0.05$) however when the average mound content was compared to the surface (litter to 5 cm, 13.7 ± 1 mg/kg) there was no significant difference. The AEL site showed similar results (Figure 4.4). The Cu content of the soil profile at the AEL followed no specific pattern as the highest amount of Cu was found at 20 – 30 cm into the ground. The Cu content of the mounds at the AEL site however, increased with increasing depth. On average, the soil profile at the AEL site contained the statistically more Cu than the soil at the West Complex site (15.2 ± 2 and 9.6 ± 3.2 mg/kg respectively; $t = -2.22$, $p < 0.05$). This was also the case with the mounds at the AEL and West Complex site (16.1 ± 1.8 and 12.9 ± 1.9 mg/kg respectively; $t = -1.36$, $p < 0.05$).

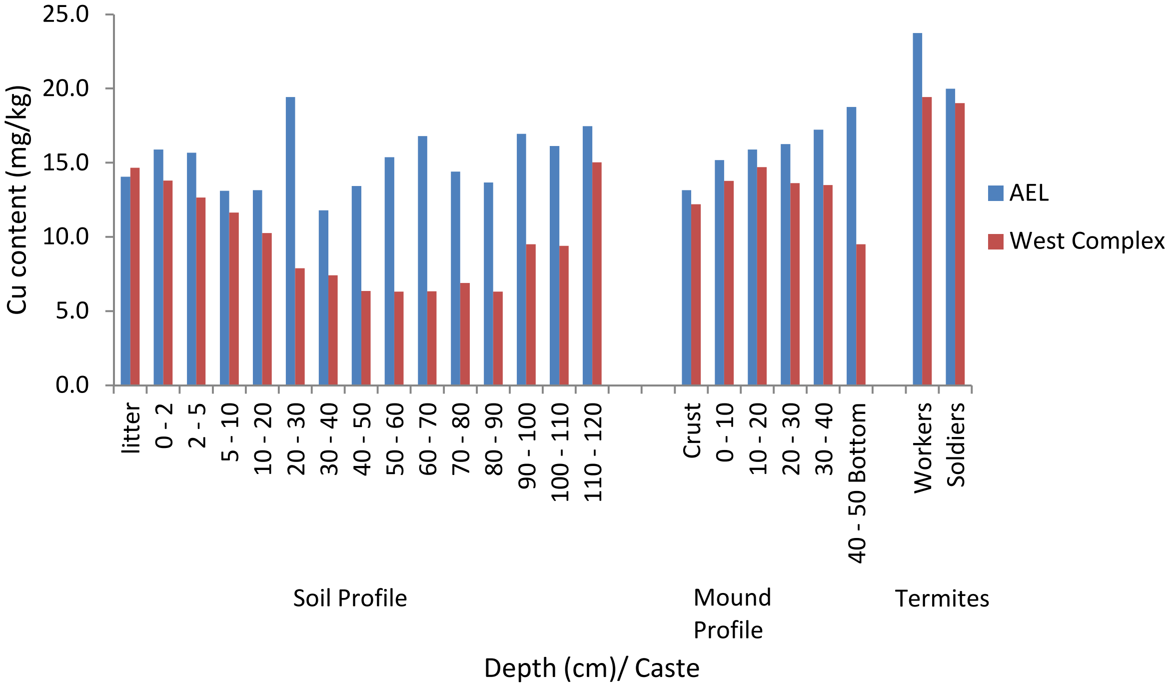


Figure 4.4. Copper (Cu) content of the soil and mound profile at the West Complex and AEL site. The Cu content of the worker and soldier termites collected at both sites shown for comparison.

4.3.2.2. Manganese (Mn)

There was a relatively large quantity of Mn at the 90 – 120 cm depth of the West Complex site (Figure 4.5). This large concentration did not occur in the mound. The average concentration of Mn in the soil (2575 ± 2249.7 mg/kg) did not differ significantly to the average concentration of Mn in the mound (2735 ± 644.3 mg/kg). This was not the case at the AEL site as the mound had on average more Mn than the soil (2454 ± 373 and 1838.2 ± 447.3 mg/kg respectively; $t = -2.82$, $p < 0.05$). At both sites the average Mn content of the mounds was the same to that of the surface layer (West Complex site: $t = -1.61$, $p = 0.15$; AEL site: $t = -0.68$, $p = 51$). The Mn content in both mounds did not show stratification and are more or less homogenous.

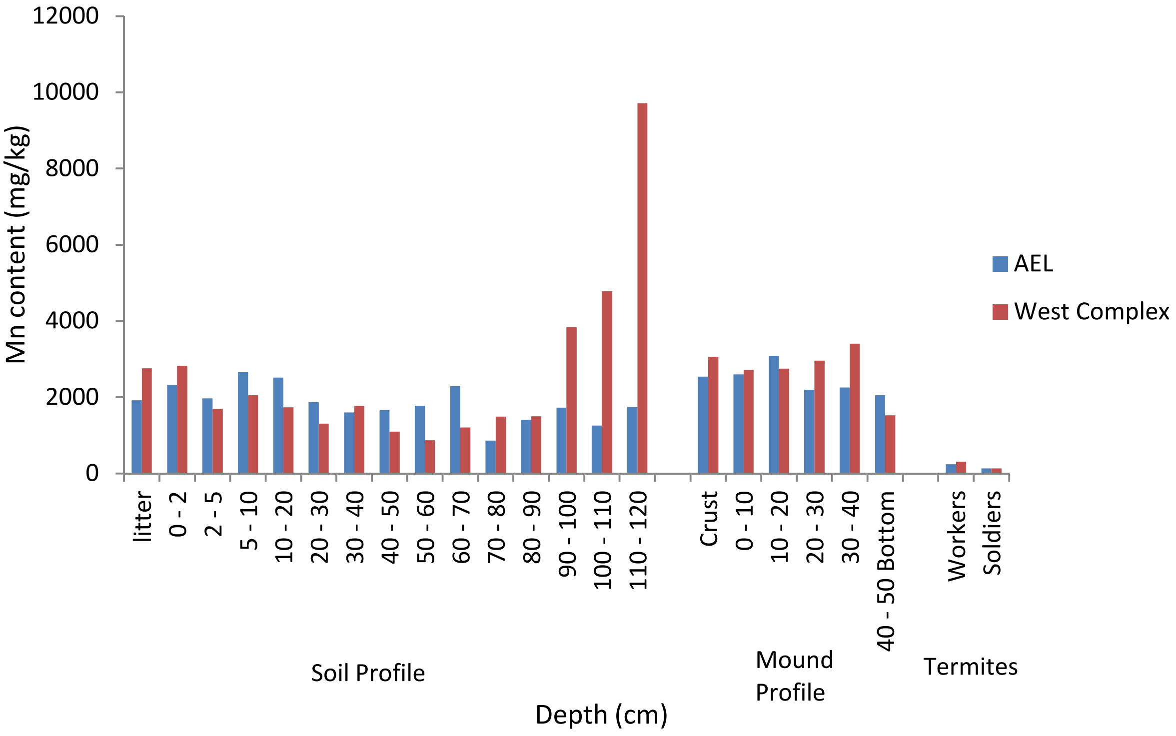


Figure 4.5. Manganese (Mn) content of the soil and mound profile at the West Complex and AEL site. The Mn content of the worker and soldier termites collected at both sites shown for comparison.

4.3.2.3. Zinc (Zn)

At both sites, Zn appears to be present in relatively high concentrations from the litter to 5 cm into the ground (Figure 4.6). The mound at the AEL site had statistically more Zn content than the whole soil profile at the site (37.1 ± 9.9 and 21.4 ± 9.8 respectively; $t = -3.29$, $p < 0.05$) however when it was compared to the surface (litter – 5 cm, 39.7 ± 1.3 mg/kg) it was found to not be statistically different ($t = 0.44$, $p = 0.67$). The average Zn content of the mound at the West Complex site (25.4 ± 2.6 mg/kg) was statistically the same to that of the soil profile (22.7 ± 12.5 mg/kg) but different to the surface layer (45.2 ± 10.6 mg/kg; $t = -2.03$, $p < 0.05$). The AEL mound had statistically more Zn than the West Complex mound (37.1 ± 9.9 and 25.4 ± 2.6 mg/kg respectively; $t = 2.77$, $p < 0.05$). The worker and soldier termites had an extremely high concentration of Zn. The concentration was so high that it had to be presented on a separate graph (Figure 4.7).

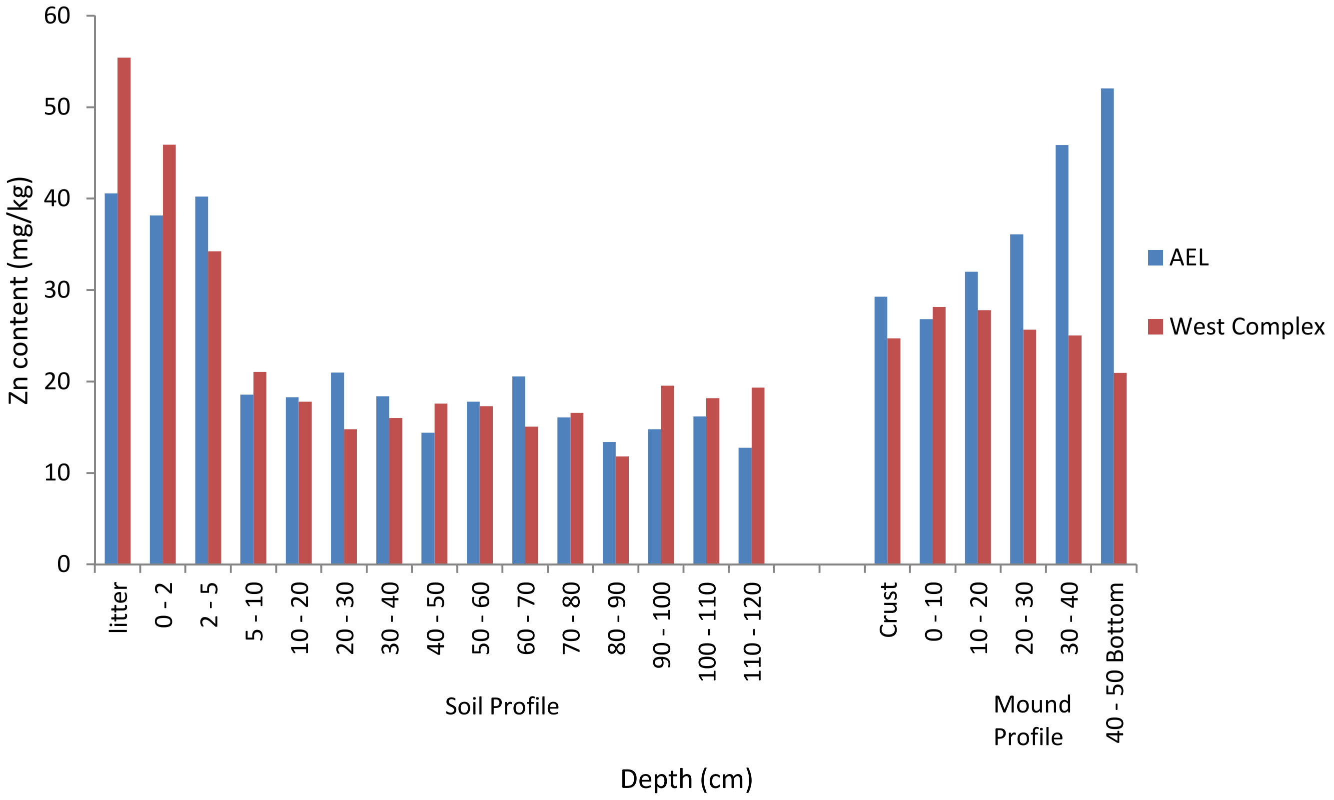


Figure 4.6. Zinc (Zn) content of the soil and mound profile at the West Complex and AEL site.

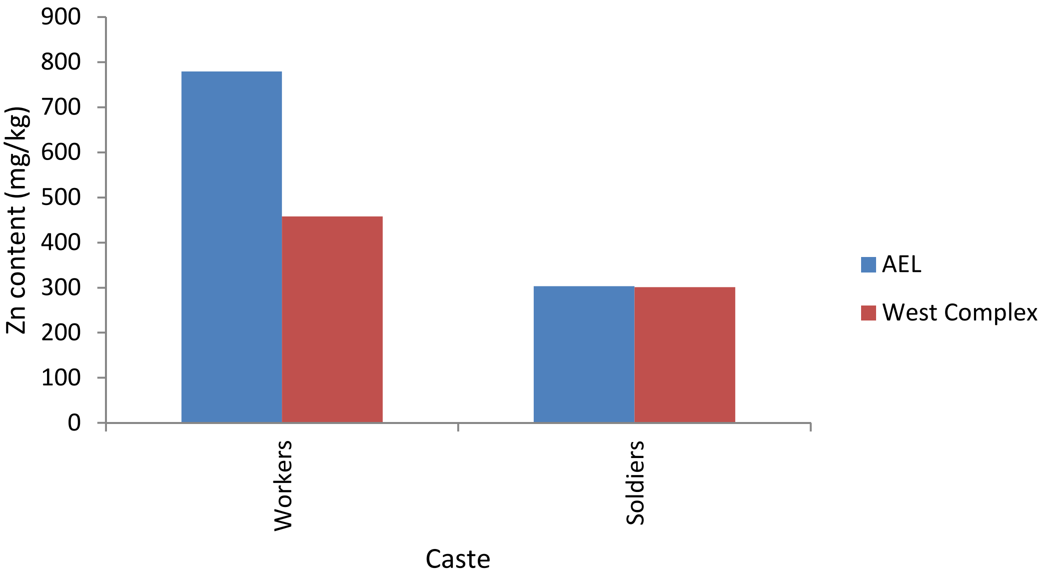


Figure 4.7. Zn content of the worker and soldier termites at the AEL and West Complex site.

4.3.2.4. Lead (Pb)

As with Zn, Pb appears to be present in relatively high concentrations from the litter to 5 cm into the ground at both the AEL and West Complex (Figure 4.8). The mound at the AEL site had statistically more Pb content than the soil profile at the site (20.5 ± 2.4 and 8.5 ± 5.7 mg/kg respectively; $t = -4.92$, $p < 0.05$) as well as statistically more Pb than the West Complex mound (11 ± 4.4 mg/kg; $t = -3.02$, $p < 0.05$). The average Pb content of the mound at both the AEL and West Complex site was not statistically different than the surface layer (AEL: $t = 0.90$, $p = 0.39$; West Complex: $t = 0.93$, $p = 0.38$). No patterns emerged when considering the Pb levels of the mounds.

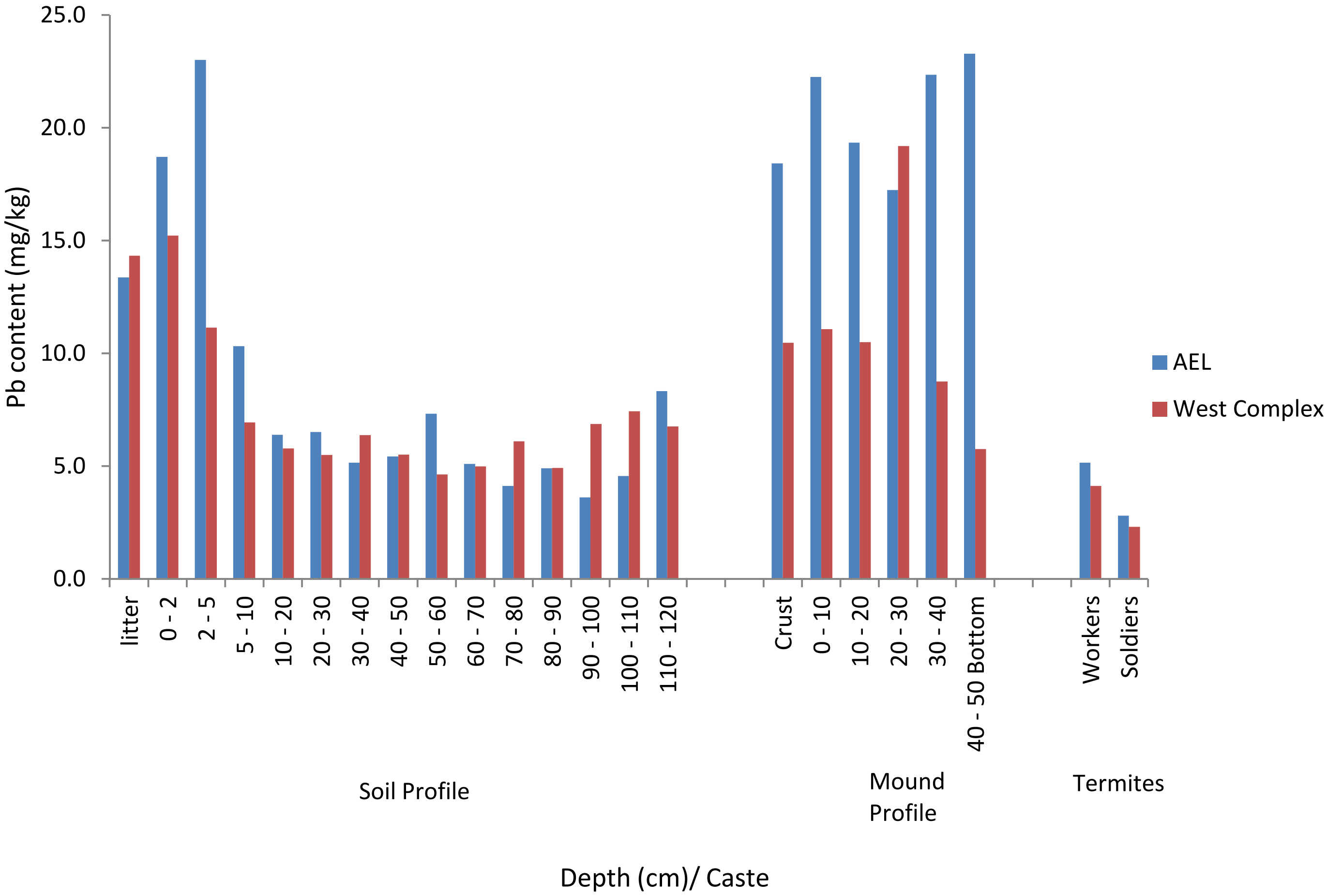


Figure 4.8. Lead (Pb) content of the soil and mound profile at the West Complex and AEL site. The Pb content of the worker and soldier termites collected at both sites shown for comparison.

4.3.2.5. Uranium (U)

Once again the trend of higher concentrations at the surface is also seen with the U content of the soil profile at both the sites (Figure 4.9). The U content of the mound at the AEL did not differ significantly from surface (3.08 ± 0.82 and 3.28 ± 0.46 respectively; $t = 0.36$, $p = 0.72$). The West Complex mound also had the same U content to that of the surface layer (1.91 ± 0.65 and 3.41 ± 1.67 respectively; $t = 2.01$, $p = 0.07$). Due to unforeseen problems with the ICP-MS, U content of the termites was unable to be analysed.

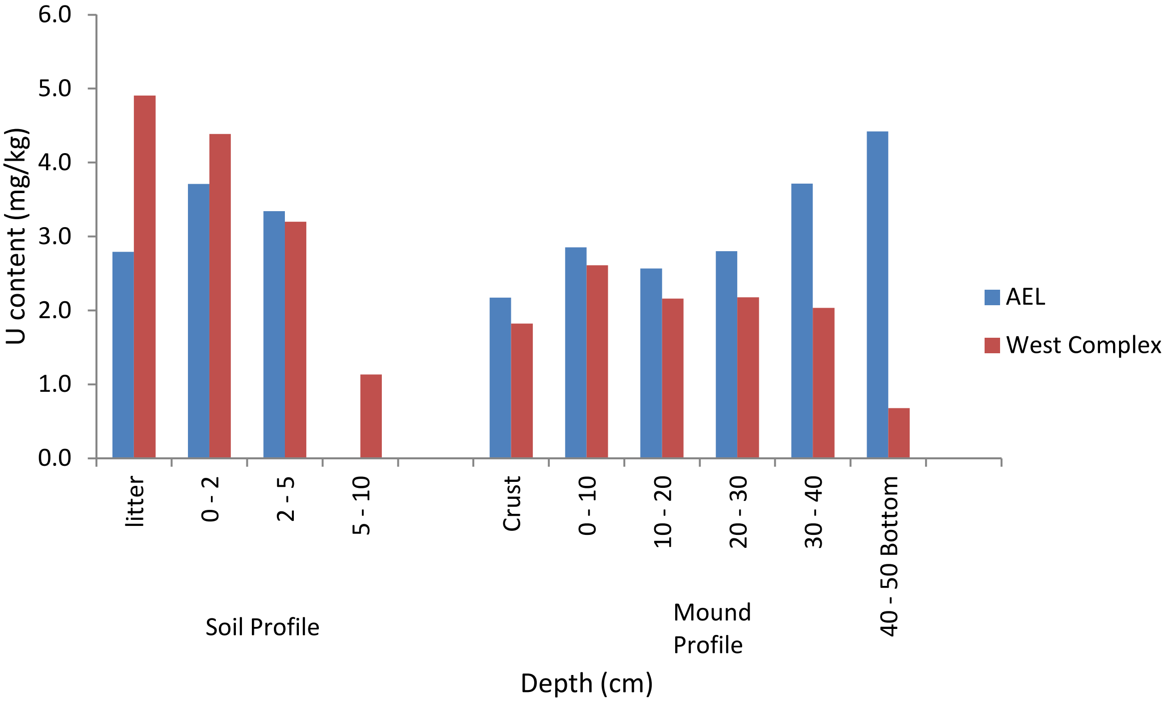


Figure 4.9. Uranium (U) content of the soil and mound profile at the West Complex and AEL site. Definite values were not given for the U content of the rest of the soil profile and these values were all < 1.

4.3.2.6. Cobalt (Co), Chromium (Cr) and Nickel (Ni)

The Co content of the soil profile at the West Complex site resembled that of the Cu content of the soil profile at the same site where Co content was high at the surface, decreased with increasing depth and then increased again at 90 cm (Figure 4.10). The Cr content of the soil profile at the West Complex site generally increased with increasing depth (Figure 4.11). As for the Co, Cr and Ni content of the soil profile of the AEL site and the Ni content of the West Complex site, no trends or patterns emerged (Figures 4.10 – 4.12). The same can be said for Co, Cr and Ni content of the mounds at the two sites. The soil profile at the AEL site had on average significantly more Co, Cr and Ni content than the soil profile at the West Complex site (Co: $t = 2.46$; Cr: $t = 2.07$ and Ni: $t = 2.41$, $p < 0.05$). The mound profile at the AEL had significantly more Co than the mound at the West Complex site (10.2 ± 1.1 and 7.5 ± 1.8 mg/kg respectively; $t = 3.24$, $p < 0.05$). The mounds at both sites had the same Co, Cr and Ni content to that of the surface.

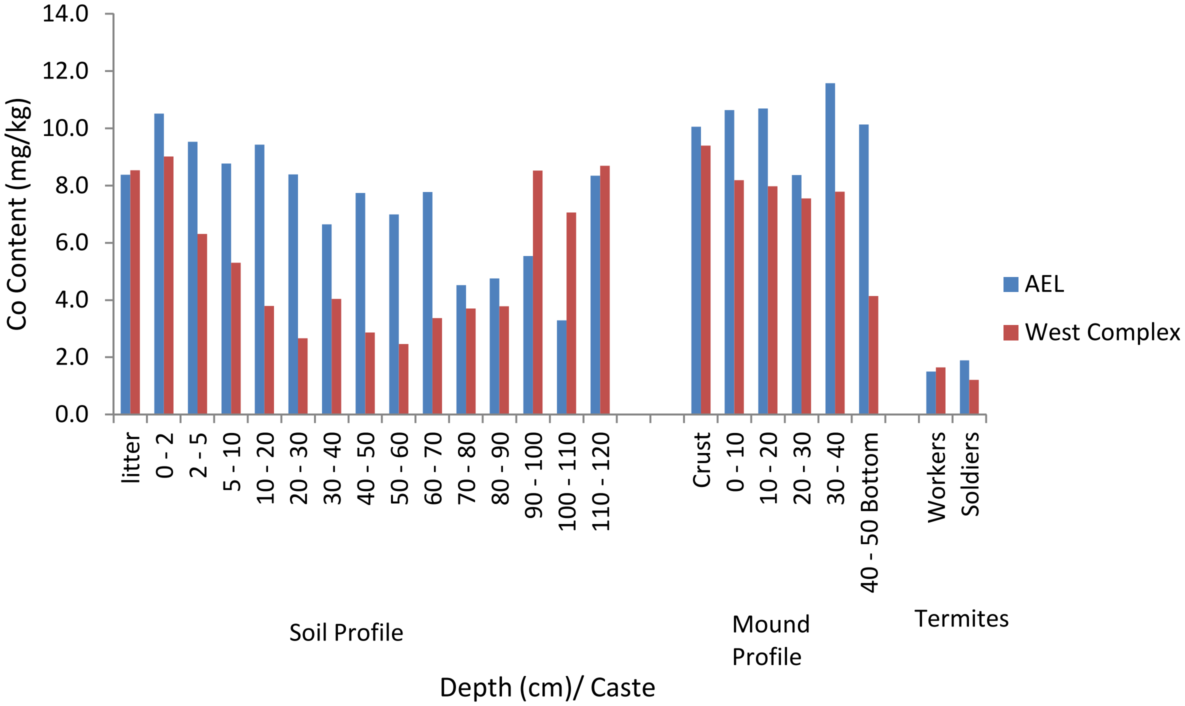


Figure 4.10. Cobalt (Co) content of the soil and mound profile at the West Complex and AEL site. The Co content of the worker and soldier termites collected at both sites shown for comparison.

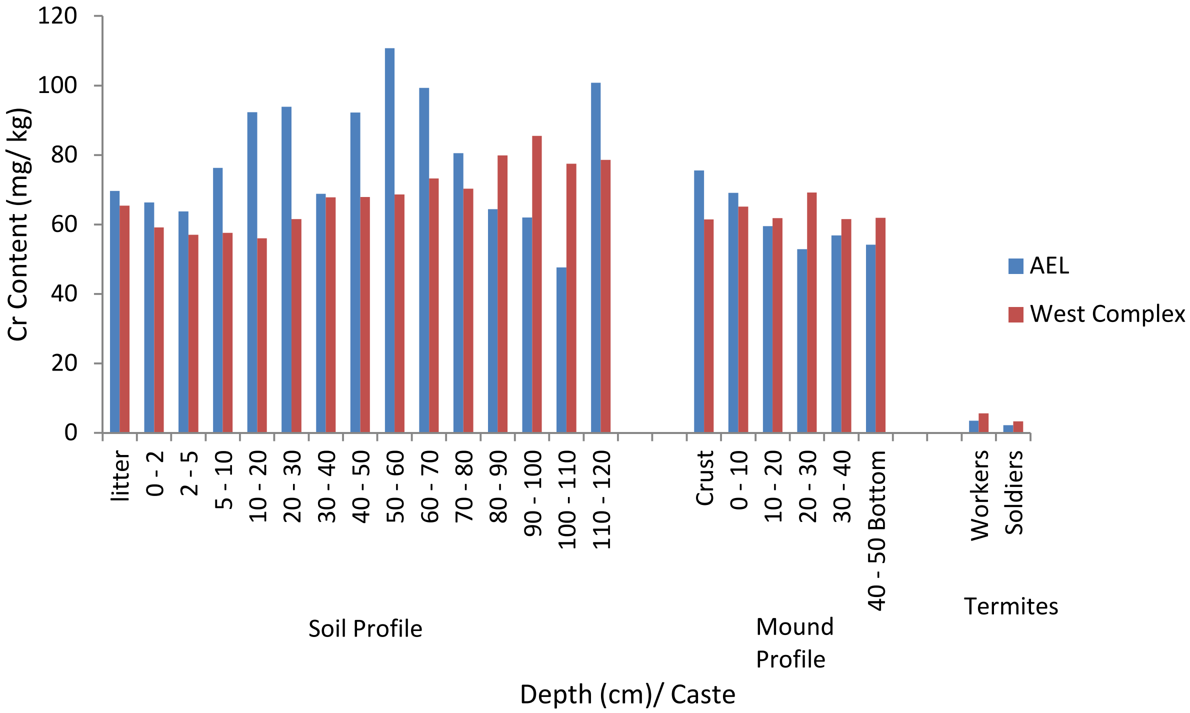


Figure 4.11. Chromium (Cr) content of the soil and mound profile at the West Complex and AEL site. The Cr content of the worker and soldier termites collected at both sites shown for comparison.

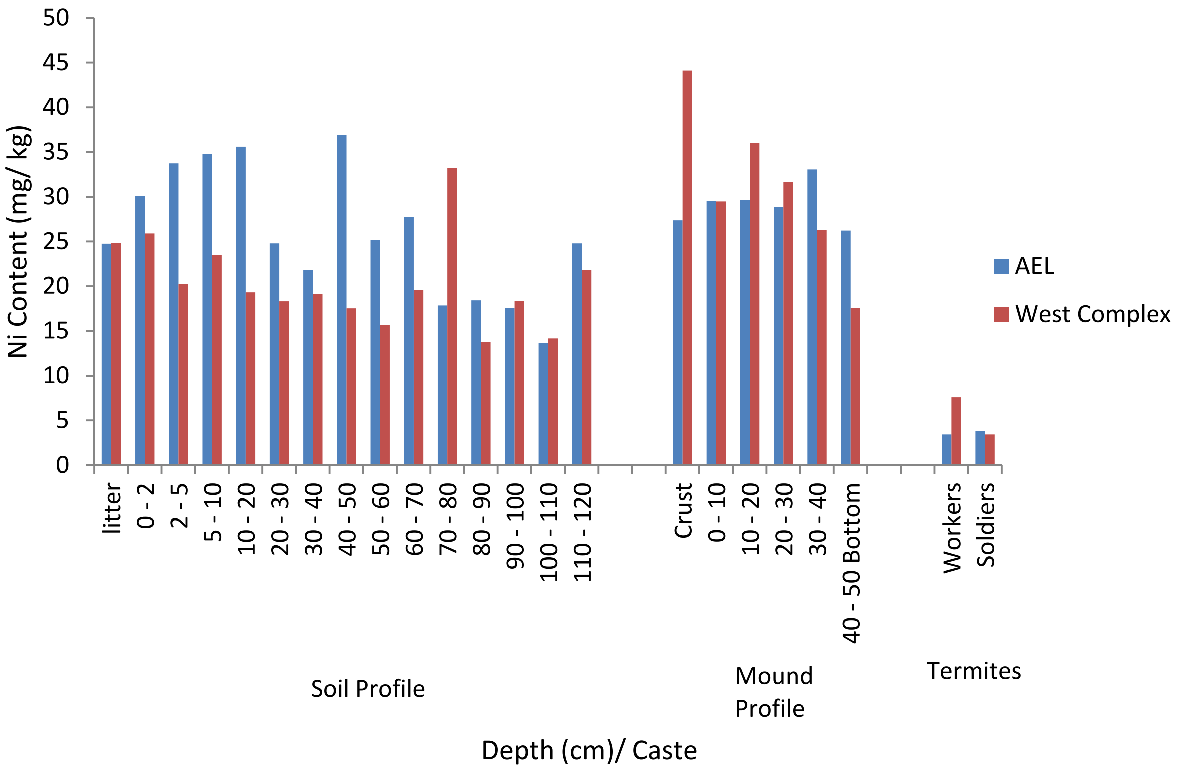


Figure 4.12. Nickel (Ni) content of the soil and mound profile at the West Complex and AEL site. The Ni content of the worker and soldier termites collected at both sites shown for comparison.

4.3.2.7. Termites

The termites had extremely high Zn concentrations when compared to the soil and mound Zn concentrations (Figures 4.6 and 4.7). Termites also had a higher Cu content than that of the soil and mounds (Figure 4.4). The rest of the heavy metals were present in low concentrations in the termites and on all occasions, were lower than the soil and the mound concentrations (Figures 4.5, 4.8, 4.10 – 4.12).

From Table 4.3 it can be seen that the worker termites generally had the largest quantity of heavy metals, followed by the soldiers then the alates with the least amount found in the nymphs. When comparing the sites, the workers at the AEL site had the highest Cu, Zn and Pb content followed by West Complex site and then the Control site. The same trend occurred with the soldiers. However, the workers collected had the most Co, Cr and Ni when compared to the AEL and

Control site. Surprisingly, the soldier and workers collected at the Control site had the highest Mn content.

Table 4.3. Heavy metal content of the different termite castes collected at the AEL, West Complex and Control site.

Element	Workers			Soldiers			Alates	Nymphs
	AEL	West Complex	Control	AEL	West Complex	Control	AEL	AEL
Cu	23.7	19.4	17.2	20.0	19.0	16.1	11.7	10.7
Mn	240.9	308.8	788.3	129.0	127.3	560.0	32	25
Zn	779.3	458.7	380.9	303.4	301.1	244.5	221.0	202.0
Pb	5.15	4.12	2.15	2.80	2.31	3.18	0.73	0.71
Co	1.503	1.651	1.125	1.893	1.206	1.030	0.309	0.166
Cr	3.5	5.6	3.8	2.2	3.4	4.3	1.4	1.1
Ni	3.5	7.6	3.3	3.8	3.5	3.5	1.3	0.9

4.4. Discussion

As expected, the site closest to the tailings (AEL site) had higher levels of certain heavy metals in the soil and mound profile when compared to the soil and mound profile at the West Complex site. In general, the distribution of the heavy metals throughout the soil profile did not follow a specific distribution pattern however Zn, U and Pb tended to be concentrated in the top layer of the soil. The average heavy metal content of the surface layer did not differ significantly from the average heavy metal content of the mounds therefore indicating that termites are not making heavy metals more bioavailable to the environment. However, the termites themselves appeared to be accumulating Zn and Cu as these elements were much higher in the termites than that found in the soil and the mounds. An interesting trend appeared where the termite workers tended to accumulate the highest levels of heavy metals followed by the soldiers, the alates and the nymphs.

As was the case in a study conducted by Mills *et al.* (2009) entailing an investigation of pH content and conductivity in *T. trinervoides* mounds, the termite mounds in the West Complex site had a lower pH content and a similar conductivity when compared to the soil. This was not the case in the AEL mound as it had a similar pH and higher conductivity when compared to the soil, a result which was similar to what Brossard *et al.* (2007) found when studying *Trinervitermes geminates* and *T. trinervius*. The AEL mound also had a higher conductivity than the West Complex mound indicating that there is a higher concentration of ions and therefore a higher soil salt content. Due to the fact that AEL mound had a higher conductivity than the soil, this could signify that the termites are bringing up salts to the surface and incorporating them into the mound. The electrical conductivity measures total solutes and does not differentiate among various elements therefore one cannot say whether these salts are harmful to the environment or not. According to Lee (2006), pH of soils generally increased with distance from mine tailings. This result was different to that found in this study as the pH was the same for the two sites even though the AEL site is closer to the tailings dams. The moisture content of both the mound and the soil at the two sites appeared to increase with increasing depth which can be expected due to the influence of gravity on water. The mounds had a higher moisture content than the surface layer indicating that water could be brought into the mound from deeper levels. Adam (1993), when excavating the mounds of *T. trinervoides* in the Free State, found vertical shafts extending 89 cm down into the soil where the soil was moister. Although no shafts were discovered in this study, it is possible that they were missed as according to Adam (1993) these shafts are very small (3 to 5 mm) and difficult to see.

Guo-li *et al.* (2008) showed a distinct relationship between heavy metal content and distance from tailings, where the closer the site was to the tailings the higher the heavy metal content of the soil. This same relationship was found in this study where the mounds at the AEL site had more Cu, Zn, Pb, U and Co and the soil at the AEL site had more Cu, Zn, Co, Cr and Ni than the mounds and the soil at the

West Complex site. The levels of heavy metals found in the soil at the AEL site however, were much lower than those reported in other studies (Table 4.4). They are also all lower than the Dutch B threshold concentration (DBTC) values which are used to establish whether total concentrations of certain heavy metals in soil samples exceed guideline concentrations (Table 4.4, Moen *et al.*, 1986 as cited by Aucamp and van Schalkwyk, 2003).

The metals of Zn, Pb and U levels were at their highest concentrations in the top layer of the soil (litter – 5 cm) at both the AEL and West Complex site. This particular distribution where metals tend to be concentrated in the uppermost soil layers was also found with Zn and Pb in a site located near a lead/ zinc smelter in Arnoldstein, Austria (Rabitsch, 1995) as well as with Cu and Cd in a site contaminated by a metal refinery in Merseyside, England (Hunter *et al.*, 1987). Rosner and van Schalkwyk (2000) showed that Zn levels in the soil profile near a gold mine tailings dam were the highest at the surface and sharply decreased with increasing depth. As for the other metals in this study, no particular distribution pattern according to depth was found. This lack of distinctive trend was also found by Kim and Jung (2004) who studied the Cu, Pb, Cr, Cu, Cd, Mn, As and Zn content of the soil profile in a paddy field 5 km from gold mine tailings located in Bongwha, Korea.

Table 4.4. The average heavy metal levels (mg/kg) found in the soil at sites near various mine tailings. The Dutch B threshold concentration (DBTC) values and depth at which the soil was sampled is also given.

Reference	Area	Type of mine	Depth (cm)	Zn	Pb	Cr	Cu	Co	Ni	Cd
Current Study	Orkney, South Africa	Gold	0 - 120	21.4	8.5	79.2	15.2	7.4	25.8	< 0.2
DBTC values				500	150	250	100	50	100	-
Rosner and van Schalkwyk, 2000	Johannesburg, South Africa	Gold	0 – 240	93.8	13.5	351	131.3	33	158	-
Antwi-Agyei *et al.* 2009	Obuasi, Ghana	Gold	0 - 12	72.64	24.22	-	39.64	-	-	-
Guo-li *et al.* 2008	Hunan province, China	Zinc-Lead	0 – 100	508.6	348.3	-	356	-	-	7.53
Boularbah *et al.* 2006	Southern Morocco	Polymetallic	0 – 15	8361	5756	-	554	-	-	31.5
Boularbah *et al.* 2006	Southern Morocco	Manganese	0 - 15	99	286	-	-	-	27.2	0.5
Kim and Jung, 2004	Bongwka, Korea	Gold	0 – 220	206	37	21.3	2	-	-	0.21

The results showed that the termites are not making the heavy metals found in the soil more bioavailable to the environment as the average mound content at both sites was not statistically different to the heavy metal content found in the surface layer of the soil. The termites are most likely getting the soil used for mound construction from the surface as none of the heavy metal concentrations of the deeper regions of the soil profile matched the heavy metal concentrations in the mound. This means that animals that inhabit abandoned termite mounds are not more of risk of contamination than if they burrowed into the soil in this area. This is contrary to what was found by Sako *et al.* (2009) in Namibia where the levels of the heavy metal Mn, Ni, Cu, Zn and Cd in the mound of a *Macrotermes* spp were greater than in the soil. They suggested that there was a possible external supply of enriched materials or accumulation of *in situ* weathering products of the underlying bedrock. Another study also conducted in Namibia showed a marked enrichment of Mn, Co and Cu in the mounds of *Macrotermes* spp when compared to the top soil (Mills *et al.*, 2009). They concluded that the termites are most likely mining these particular nutrients from the deeper soil profile.

In this study, it was found that there is a potential risk to termite predators as the termites themselves appear to be accumulating Cu and Zn. The average amount of Cu in the termites at the AEL site was 21.9 mg/kg which, according to Lopez-Alonso *et al.* (2006), is within the range 20 to 110 mg/kg where acute poisoning in mammals occurs. The levels of Zn were extremely high in the termites. The termite workers at the AEL site contained 779.3 mg/ kg, a value far higher than that of the soil. The likelihood of Zn poisoning occurring in a mammal predator is good as levels exceeding 700 mg of Zn/kg causes poisoning (Jenkins and Hidiroglou, 1991). However this poisoning would occur if the mammal was exclusively consuming worker termites only. A study conducted by Taylor *et al.* (2002) showed that the aardvark (one of the main predators of *T. trinervoides*) consumed more termite soldiers than termite workers (64 % soldiers and 36 % workers). Therefore due to the lower levels of heavy metals in the soldiers (303 mg of Zn/kg), these predators may not be at risk. As for the other metals, it is unlikely that Mn, Pb, Co, Cd, Cr and Cd poisoning would occur in predators as a result of termite consumption as these values were much lower than the heavy metal content found in the soil and are much lower than in the levels found in other invertebrates occupying areas contaminated by mining operations. For example, benthic invertebrates found in a stream impacted by discharged from mining operations accumulated approximately 3 mg/kg of Cd whereas in this case termites accumulated < 0.2 mg of Cd/kg (Kiffney and Clements, 1993). Earthworms found in soil contaminated by an abandoned mine in Spain had 190 mg of Pb/ kg (Ruiz *et al.*, 2009), much higher than the 4 mg/kg in the termites in this study. The termite alates are well known as a food source to bird predators as well as ground dwelling animals after the shedding of their wings (Abe *et al.*, 2000). These predators are not at risk as there were minute quantities of heavy metals in the alates, much lower than that found on the soil surface as well as that found in the other castes.

Out of the different castes of termites, the workers had the highest levels of heavy metals followed by the soldiers, the alates and the least amount of heavy metals was found in the nymphs. The same trend was found in a *Macrotermes* spp. studied by Mills *et al.* (2009) where the workers had more heavy metals than the soldiers. The differences concentrations between the castes may be a result of differences of feeding as according to Mills *et al.* (2009), worker termites ingest soil particles during mound construction. A reason why the nymphs have less heavy metal content than the alates could be a result of metal accumulation with increasing age, a conclusion which was drawn by Grzes (2010) when studying the metal concentrations of red wood ant workers and larvae.

To conclude, the termite mounds have the same levels of heavy metals as that in the surface layer of the soil therefore any contaminants at this soil level will enter the mounds. This shows that that termites are not making heavy metals more accessible to surface or shallow burrowing animals. Worker termites did accumulate high levels of Cu and Zn which could pose a potential risk to those predators that prey on them. However, the alates which are heavily predated upon by a broad spectrum of predators did not accumulate any heavy metals.

CHAPTER 5 – GENERAL DISCUSSION

5.1. Discussion

This study presented the first investigation of the impact of gold mine tailings on a termite species. The negative impacts of gold mine tailings on various animal populations have been documented in many countries around the world, particularly the USA. These studies have been mainly concerned with the detrimental effect of tailings on rodents (Clark and Hothem, 1991), birds (Henny *et al.*, 1994) and bats (O'Shea *et al.*, 2001). There have been only a few studies conducted on invertebrates (Besser and Rabeni, 1987; Ma *et al.*, 2002; Medina *et al.*, 2005) and no studies have been conducted on termites.

After an extensive investigation into certain aspects of the biology of the snouted harvester termites inhabiting the three sites, the results were unexpected. The West Complex site was presumed to be the most contaminated site as the mounds at the West Complex site had the hottest and most variable centre mound temperatures, a higher dead mound to live mound ratio and the lowest density of mounds. However, the soil chemical analysis confirmed that the AEL site was the more contaminated of the two sites due to higher levels of heavy metals. The AEL site was not a graveyard of dead termite mounds, in fact the termites appeared to be thriving in the most contaminated site. This could have been as a result of reduced predation as illustrated in another study on ants (Grzes, 2010) however this was unlikely due to the lack of evidence. This begs the question as to whether the tailings are having an impact on the termites or are these findings a result of other factors. Certain factors can be ruled out, for instance the lower density is not a result of soil type, texture or depth, vegetation composition, climate or rainfall as these were the same throughout the different study sites. Higher densities at the AEL site could be explained by the water table being closer to the surface, allowing the termites easier access to water and hence a more favourable environment within the mound. Termites are organisms with a thin integument and are prone to desiccation therefore it is of great importance that a moist microclimate is maintained (Nel, 1968; Adam, 1993). As for the differences in temperatures of the mounds, results from previous studies have shown that these temperatures do not adhere to a set point temperature as is the case in the fungus cultivating termites which keep their temperatures to 30 °C (Adam, 1993; Korb and Linsenmair, 2000a; Field, 2008). If *T. trinervoides* did keep their temperatures to a set point temperature, it would have been easier to pin point any physiological abnormalities were the temperatures to deviate from the set point. The variability of temperatures at the three sites could be due to basic biological variability and that this species can tolerate a range of temperatures provided the humidity of the nest is high (Nel, 1968).

One of the pertinent questions asked in this study was whether the termites were having an impact on the food chain through bioaccumulation of heavy metals and/or making the heavy metals more bioavailable to the environment by bringing heavy metals from the soil into their mounds. Snouted harvester termites play a significant role in the food chain as they provide a protein- and energy-rich food source to numerous predators. These predators include small rodents, mongooses, aardwolves, aardvarks, reptiles, birds and various invertebrates (Dean and Siegfried, 1991; Richardson and Levitan, 1994; Haddad and Dippenaar-Schoeman, 2002). This study found that the termite workers and to a lesser extent, soldiers, accumulated Cu and Zn. The concentrations of these elements within the termites were higher than that found in the surrounding soil and were also within the range that is known to cause acute poisoning in mammals (20 – 110 mg/kg for Cu and 700 – 1000 mg/kg for Zn; Jenkins and Hidiroglou, 1991, Lopez-Alonso *et al.*, 2006). The predators that are obligatory termite feeders (i.e. aardvarks and aardwolves) are at a particular risk due to the large volumes of termites they consume each day - an aardwolf has been known to eat more than 300 000 termites in a day (Richardson and Levitan, 1994). Termite alates are a good source of food for several animal species including birds, rodents and small mammals. Yet these animals are not at risk of heavy metal poisoning as this study has shown that alates do not accumulate any heavy metals. The snouted harvester termites may not only impact the food chain through bioaccumulation, their termite mounds also provide a shelter to other animals. These mounds may have been laden with heavy metals that have been brought up to the surface. This was not the case as the mounds had the same levels of heavy metals to that found in the top surface layer of the soil. It is unlikely that animals using the termitaria as a refuge are at any greater risk than burrowing into the soil.

Another consideration is the use of this termite species as a bioindicator. A bioindicator is a species or group of species that respond predictably, in ways that are readily observed and quantified, to environmental disturbance or a change in environmental state (McGeoch, 1998). Using this definition, this study found that on a population level the termites did not respond predictably as no trends appeared i.e. they did not increase or decrease in population size along a contaminant gradient as was the case in other studies conducted on insects that were used as bioindicators (Nahmani and Lavelle, 2002; Hobbelen *et al.*, 2006; Nummelin *et al.*, 2007). On a physiological level, the temperatures of the mounds were more variable at the contaminated sites. However as discussed above, it is difficult to say that this was a direct impact of contamination.

There were several limitations when conducting this study, the most prominent being the lack of contamination data for the Control site. Other limitations included the lack of particular site data that could account for the density and distribution of the termites such as vegetation cover which has been shown to influence termite density. From a statistical point of view, it would have been better to use at least 10 mounds when collecting temperature data however this would have been a very costly endeavour as the data loggers used to measure temperature (iButtons) are expensive.

5.2. Conclusion

Based on the density and distribution of the termite mounds as well as the temperature fluctuations within the mounds, the snouted harvester termites do not appear to be impacted by the tailings dams. Thus population status and temperature profile of the mound cannot be used as an indication of contamination. It is unlikely the termites are impacting the food chain in its entirety due to the fact that one of the main food sources to the predators (the alates) are not accumulating heavy metals. Obligator termite feeders may be at risk due to the fact that they ingest large quantities of worker and soldier termites which have been shown to accumulate Zn and Cu.

REFERENCES

Abe, T., Bignell, D. E. and Higashi, M. 2000. *Termites: evolution, sociality, symbioses and ecology.* Kluwer Academic Publishers, Amsterdam pp 121 – 140; pp 389 – 402.

Adam, R. A. 1993. *Aspects of the biology of the harvester termite* Trinervitermes trinervoides. Ph. D. Thesis, University of the Orange Free State, Bloemfontein.

Adam, R. A., Mitchell, J. D. and van der Westhuizen, M. C. 2008. Aspects of foraging in the harvester termite, *Trinervitermes trinervoides* (Sjostedt)(Termitidae: Nasutitermitinae). *African Entomology* 16: 153 – 161.

Alexandra, L. and de Bruyn, L. 1997. Soil mesofaunal responses to post-mining restoration treatments. *Ecological Economics* 23: 167 – 178.

Andres, P. and Mateos, E. 2006. Soil mesofaunal responses to post-mining restoration treatments. *Soil Ecology* 33: 67 – 78.

Anglogold Ashanti. 2005. Case Studies South Africa 4.3: Uranium mined as a by-product contributes to the bottom line *AngloGold Ashanti Annual Report -Report to Society* http://www.anglogold.com/subwebs/InformationForInvestors/ReportToSociety05/values_bus_principles/economic/ep_cs_sa_4_3.htm.

AngloGold Ashanti. 2006a. Review of Operations – South Africa *AngloGold Ashanti Annual Report - Annual Financial Statements* http://www.ashantigold.com/subwebs/InformationForInvestors/AnnualReport06/review/review-za.htm.

AngloGold Ashanti. 2006b. Case Studies: Biodiversity assessment in South Africa. *AngloGold Ashanti Annual Report – Report to Society* http://www.anglogold.co.za/subwebs/InformationForInvestors/ReportToSociety06/biodiversity.htm.

AngloGold Ashanti. 2007a. AngloGold Ashanti South Africa: Country Report: Vaal river operations. *AngloGold Ashanti – South Africa – Vaal river* pp 1 – 66.

AngloGold Ashanti. 2007b. Occupational safety and health *AngloGold Ashanti Report to Society* pp 24 – 54.

AngloGold Ashanti. 2008. *Mine Site Rehabilitation and Closure*. AngloGold Ashanti Annual Report -Report to Society p 127.

Antwi-Agyei, P., Hogarh, J. N. and Foli, G. 2009. Trace elements contamination of soils around gold mine tailings dams at Obuasi, Ghana. *African Journal of Environmental Science and Technology* 11: 353 - 359.

Aucamp, P. and van Schalkwyk, A. 2003. Trace element pollution of soils by abandoned gold mine tailings, near Potchefstroom, South Africa. *Bulletin of Engineering Geology and the Environment* 62: 123 – 134.

Augustyniak, M., Babczynska, A. and Augustyniak, M. 2009. Does the grasshopper *Chorthippus brunneus* adapt to metal polluted habitats? A study of glutathione-dependent enzymes in grasshopper nymphs. *Insect Science* 16: 33 - 42

Benzie, J. A. H. 1986. The Distribution, Abundance, and the Effects of Fire on Mound Building Termites (*Trinervitermes* and *Cubitermes spp*., Isoptera: Termitidae) in Northern Guinea Savanna West Africa. *Oecologia* 70: 559 - 567.

Besser, J. M. and Rabeni, C. F. 1987. Bioavailability and toxicity of metals leached from lead-mine tailings to aquatic invertebrates. Environmental Toxicology and Chemistry 6: 879 – 890.

Bhattacharya, R. and Flora, S. J. S. 2009. Cyanide toxicity and its treatment. *The Handbook of toxicology of chemical warfare agents 19: 255 – 271.*

Bouillon, A. 1970. *Termites of the Ethiopean Region. In: Biology of termites, Volume ii.* Eds. K Krishna and F. M. Weesner. Academic Press, New York. pp 153 - 280

Boularbah, A., Schwartz, C., Bitton, G and Morel, J. L. 2006. Heavy metal contamination from mining sites in South Morocco: 1. Use of a biotest to assess metal toxicity of tailings and soils. *Chemosphere* 63: 802 – 810.

Bristow, K. L. and Holt, J. A. 1987. Can termites create local energy sinks to regulate mound temperature? *Journal of Thermal Biology* 12: 19 – 21.

Brossard, M., Lopez-Hernandez, D., Lepage, M. and Leprun, J. C. 2007. Nutrient storage in soils and nest of mound-building *Trinervitermes* termites in Central Burkina Faso: consequences for soil fertility. *Biology and Fertility of Soils* 43: 437 - 447

Bruce, S. L., Noller, B. N., Grigg, A. H., Mullen, B. F., Mulligan, D. R., Ritchie, P. J., Currey, N. and Ng, J. C. 2003. A field study conducted at Kidston Gold Mine, to evaluate the impact of arsenic and zinc from mine tailing to grazing cattle. *Toxicology letters* 137: 23 – 34.

Cervera, A., Maymó, A. C., Martínez-Pardo, R. and Garcerá, M. 2005. Vitellogenesis inhibition in *Oncopeltus fasciatus* females (Heteroptera: Lygaeidae) exposed to cadmium. *Journal of Insect Physiology* 51: 895 - 911.

Clark, D. R., Jr., and Hothem, R. L. 1991. Mammal mortality at Arizona, California, and Nevada gold mines using cyanide extraction. *California Fish and Game* 77: 61 - 69.

Coaton, W. G. H. 1948. The snouted harvester termite. *Farming in South Africa* 23: 97 – 106.

Collins, N. M. 1981. Populations, Age Structure and Survivorship of Colonies of *Macrotermes bellicosus* (Isoptera: Macrotermitinae). *Journal of Animal Ecology* 50: 293 – 311.

Conesa H.M., Faz, A. and Arnaldos, R. 2006. Heavy metal accumulation and tolerance in plants from mine tailings of the semiarid Cartagena–La Unión mining district (SE Spain). *Science of the total environment* 366: 1 – 11.

Dangerfield, J. M., Mccarthy, T. S. and Ellery, W. N. 1998. The mound-building termite *Macrotermes michaelseni* as an ecosystem engineer. *Journal of Tropical Ecology* 14: 507 – 520.

Darlington, J. P. E. C., Zimmerman, P. R. and Wandiga, S. O. 1992. Populations in nests of the termite *Macrotermes jeanneli* in Kenya. *Journal of Tropical Ecology* 8: 73 – 85.

Dean, W. R. J. and Siegfried, R. W. 1991. Orientation of diggings of the Aardvark. *Journal of Mammalogy* 72: 823 – 824.

Donato, D. B., Nichols, O., Possingham, H., Moore, M., Ricci, P. F. and Noller, B. N. 2007. A critical review of the effects of gold cyanide-bearing tailings solutions on wildlife. *Environment Internal* 33: 974 – 984.

Eisler, R. 1991. Cyanide hazards to fish, wildlife and invertebrates: a synoptic review. *Biological report* 85: 1 – 58.

Eisler, R. and Weimeyer, S. N. 2004. Cyanide hazards to plants and animals from gold mining and related water issues. *Reviews of environmental contamination and toxicology* 183: 21 – 54.

Emerson, A. E. 1956. Regenerative behaviour and social homeostasis in termites. *Ecology* 37: 248 – 258.

Ferrar, P. 1982. Termites of a South African Savanna. II. Densities and Populations of Smaller Mounds, and Seasonality of Breeding. *Oecologia* 52: 133 - 138.

Field, M. A. 2008. Thermoregulation of the mounds of the harvester termites, *Trinervitermes trinervoides* (Sjostedt) (Isoptera: Termitidae) Unpublished Honours Report, University of the Witwatersrand, Johannesburg.

GCS. 2007. Vaal River Groundwater Assessment. *AngloGold Ashanti, Orkney.*

Gavrilescu, M., Pavel, L. V. and Cretescu, I. 2009. Characterization and remediation of soil contaminated with uranium. *Journal of Hazardous Materials* 163: 475 – 510.

Gongalsky, K. B. 2006. Bioaccumulation of metals by soil-dwelling insects in a uranium production area. *European Journal of Soil Biology* 42: 180 – 185.

Greaves, T. 1964. Temperature studies of termite colonies in living trees. *Australian Journal of Zoology* 12: 250 – 262.

Grzes, I. M. 2010. Ants and heavy metal pollution - A review. *European Journal of Soil Biology* 46: 350 – 355.

Gullan, P. J. and Cranston, P. S. 1994. *Insects: An Outline of Entomology, 1st edition.* Chapman and Hall, London, Glasgow, New York, Tokyo, Melbourne, Madras, p 299.

Guo-li, L., Da-xue, L. and Quan-ming, L. 2008. Heavy metals contamination characteristics in soil of different mining activity zones. *Transactions of Nonferrous Metals Society of China* 18: 207 – 211.

Haddad, C. R. and Dippenaar-Schoeman, A. S. 2002. The Influence of Mound Structure on the Diversity of Spiders (Araneae) Inhabiting the Abandoned Mounds of the Snouted Harvester Termite *Trinervitermes trinervoides. Journal of Arachnology* 30: 403 – 408.

Heikens, A., Peijnenburg, W. J. G. M. and Hendriks, A. J. 2001. Bioaccumulation of heavy metals in terrestrial invertebrates. *Environmental pollution* 113: 385 – 393.

Henny, C. J., Hallock, R. J. and Elwood, F. H. 1994. Cyanide and migratory birds at gold mines in Nevada, USA. *Ecotoxicology* 3: 45 – 58.

Hobbelen, P. H. F., van den Brink, P. J., Hobbelen, J. F. and van Gestel, C. A. M. 2006. Effects of heavy metals on the structure and functioning of detritivores communities in a contaminated floodplain area. *Soil Biology & Biochemistry* 38: 1596 – 1607.

Holdaway, F. G. and Gay, F. J. 1948. Temperature studies of the habitat of *Eutermes exitiosus* with special references to the temperatures within the mound. *Australian Journal of Science* 1: 464 – 493

Holt, J. A. and Easey, J. F. 1985. Polycalic colonies of some mound building termites (Isoptera: Termitidae) in northeastern Australia. *Insectes Sociaux* 32: 61 – 69.

Hopkin, S. P. 1989. *Ecophysiology of metals in terrestrial invertebrates*. Elsevier Applied Science, London, England.

Hull Sieg, C., Uresk, W. D., Hansen, R. M. 1987. Impact of Bentonite Mining on Selecting Arthropods. *Journal of Range management* 40: 128 – 131.

Hunter, B. A., Johnson, M. S. and Thompson, M. S. 1987. Ecotoxicology of Copper and Cadmium in a Contaminated Grassland Ecosystem. I. Soil and Vegetation Contamination. *Journal of Applied Ecology* 24: 573 - 586.

Jenkins, K. J., and Hidiroglou, M. 1991. Tolerance of the preruminant calf for excess manganese or zinc in milk repleacer. *Journal of Dairy Science* 74: 1047-1053.

Jones, J. C. and Oldroyd, B. P. 2007. Nest thermoregulation in social insects. *Advances in Insect Physiology* 33: 154 – 185.

Keith, S., Faroon, O. M. and Fowler, B. A. 2000. *Handbook on the Toxicology of Metals: URANIUM.* Academic Press, Inc. 12: 881 – 903.

Khalil, H. E., Hamiani, O. E., Bitton, G., Ouazzani, N. and Boularbah, A. 2008. Heavy metal contamination from mining sites in South Morocco: Monitoring metal content and toxicity of soil runoff and groundwater. *Environmental Monitoring Assessment* 136: 147 – 160.

Kiffney, P. M. and Clements, W. H. 1993. Bioaccumulation of heavy metals by benthic invertebrates at the Arkansas River, Colorado. *Environmental Toxicology and Chemistry* 12: 1507 - 1517.

Kim, M. J. and Jung, Y. 2004. Vertical Distribution and Mobility of Arsenic and Heavy Metals in and around Mine Tailings of an Abandoned Mine. *Journal of Environmental Science and Health* 39: 203 – 222.

Korb, J. and Linsenmair, K. E. 1999. The architecture of termite mounds: a result of trade-off between thermoregulation and gas exchange? *Behavioural Ecology* 3: 312 – 316.

Korb, J. and Linsenmair, K. E. 2000a. Thermoregulation of termite mounds: what role does ambient temperature and metabolism of the colony? *Insectes Sociaux* 47: 357 – 363.

Korb, J. and Linsenmair, K. E. 2000b. Ventilation of termite mounds: new results require a new model. *Behavioural Ecology* 5: 486 – 494.

Korb, J. and Linsenmair, K. E. 2001. The causes of spatial patterning of mounds of a fungus-cultivating termite: results from nearest-neighbour analysis and ecological studies. *Oecologia* 127: 324 – 333.

Korb, J. 2003. Thermoregulation and ventilation of termite mounds. *Naturwissenschaften* 90: 212 – 219.

Krishna, K. and Weesner, F. M. 1970. *The biology of termites*. Academic Press London pp 103 – 106.

Lagisz, M., Kramarz, P. and Niklinska, M. 2005. Metal kinetics and respiration rates in F1 generation of Carabid Beetles (*Pterostichus oblongopunctatus*) originating from metal-contaminated and reference areas. *Archives of Environmental contamination and toxicology* 48: 484 – 489.

Leduc, G. 1978. Deleterious effects of cyanide on early life stages of Atlantic salmon (*Salmo salar*). *Journal of the Fisheries Research Board of Canada* 35: 166 – 174.

Lee, S. 2006. Geochemistry and partitioning of trace metals in paddy soils affected by metal mine tailings in Korea. *Geoderma* 135: 26 – 37.

Lee, C. G., Chone, H. T. and Myung, C. J. 2001. Heavy metal contamination in the vicinity of the Daduk Au–Ag–Pb–Zn mine in Korea. *Applied Geochemistry* 16: 1377 – 1386.

Lee, K. E. and Wood, T. G. 1971. *Termites and soils*. Academic press, London pp 68 – 87.

Lepage, M. 1984. Distribution, Density and Evolution of *Macrotermes bellicosus* Nests (Isoptera: Macrotermitinae) in the North-East of Ivory Coast. *Journal of Animal Ecology* 53: 107 - 117.

Li, L., Xu, Z., Wu, J. and Tian, G. 2010. Bioaccumulation of heavy metals in the earthworm *Eisenia fetida* in relation to bioavailable metal concentrations in pig manure. *Bioresource Technology* 101: 3430 – 3436.

Liu, Y., Zhang, H. Z., Zeng, G. M., Huang, B. R. and Li, X. 2006. Heavy metal accumulation in plants on Mn mine tailings. *Pedosphere* 16: 131 – 136.

López-Alonso, M., Crespo, A., Miranda, M., Castilho, C., Hernández, J. and Benedito, J. L. 2006. Assessment of some blood parameters as potential markers of hepatic copper accumulation in cattle. *Journal of Veterinary Diagnostic Investigation* 18: 71 - 75.

Lüscher, M. 1961. Air-conditioned termite nests. *Scientific American* 205: 138-145

Ma, Y., Dickinson, N. and Wong, M. 2002. Toxicity of Pb/ Zn mine tailings to the earthworm *Pheretima* and the effects of burrowing on metal availability. *Biology and Fertility of Soils* 36: 79 – 86.

Macklin, M. G., Brewer, P. A., Balteanu, D., Coulthard, T. J., Driga, B., Howard, A. J. and Zaharia, S. 2003. The long term fate and environmental significance of contaminant metals released by the January and March 2000 mining tailings dam failures in Maramure County, upper Tisa Basin, Romania. *Applied Geochemistry* 18: 241 - 257.

Majer, J. D. 1983. Ants: Bio-lndicators of Minesite Rehabilitation, Land-Use and Land Conservation. *Environmental Management* 7: 375 - 383.

Markert, B. 2007. Definitions and principles for bioindication and biomonitoring of trace metals in the environment. *Journal of Trace Elements in Medicine and Biology* 21: 77 – 82.

McGeoch, M. A. 1998. The selection, testing and application of terrestrial insects as bioindicators. *Biological Review* 73: 181 – 201.

Medina, M., Andrade, S., Faugeron, S., Lagos, N., Mella, D. and Correa, J. A. 2005. Biodiversity of rocky intertidal benthic communities associated with copper mine tailing discharges in northern Chile. *Marine Pollution Bulletin* 50: 396 – 409

Meyer, V. W., Braack, L. E. O and Biggs, H. C. 2000. Distribution and density of *Cubitermes wasmann* mounds in the northern Kruger National Park. *Koedoe* 43: 57 – 65.

Mills, A. J., Milewski, A., Fey, M. V., Groengroeft, A. and Peterson, A. 2009. Fungus culturing, nutrient mining and geophagy: a geochemical investigation of *Macrotermes* and *Trinervitermes* mounds in southern Africa. *Journal of Zoology* 278: 24 – 35.

Moen, J. E. T, Cornet, J. P. and Evers, C. W. A. 1986. Soil protection and remedial action: criteria for decision making and standardization requirements. *In: Assink JW, Vanderbrink WJ (eds) Contaminated soil. Martinus Nijhoff, Dordrecht.*

Mucina, L., Rutherford, M. C. and Powrie, L. W. 2005. Vegetation map of South Africa, Lesotho and Swaziland *South African National biodiversity institute South Africa.*

Murray, J. M. 1938. An investigation of the interrelationships of the vegetation, soils and termites. *South African Journal of Science* 35: 288 – 297.

Nahmani, J. and Lavelle, P. 2002. Effects of heavy metal pollution on soil macrofauna in a grassland of Northern France. *European Journal of Soil Biology* 38: 297 – 300.

Naicker, K., Cukrowska, E., McCarthy, T.S. 2003. Acid mine drainage arising from gold mining activity in Johannesburg, South Africa and environs. *Environmental Pollution* 122: 29 – 40.

Nel, J. J. C. 1968. The influence of temperature and relative humidity on the weight loss and survival of the harvester termites *Hodotermes mossambicus* (Hagen) and *Trinervitermes trinervoides* (Sjostedt). *Suid Afrikaanse Tydskrif vir Landbouwet* 11: 389 – 402.

Nel, J. J. C. and Malan, E. M. 1974. The distribution of the mounds of *Trinervitermes trinervoides* in the central Orange Free State. *Journal of the Entomological Society of Southern Africa* 37: 251 – 256.

Nummelin, M., Lodenius, M., Tulisalo, E., Hirvonen, H. and Alanko, T. 2007. Predatory insects as bioindicators of heavy metal pollution. *Environmental Pollution* 145: 339 - 347.

Ohiagu, C. E. 1979. Nest and Soil Populations of *Trinervitermes* spp. with Particular Reference to *T. geminatus* (Wasmann), (Isoptera), in Southern Guinea Savanna near Mokwa, Nigeria. *Oecologia* 40: 167 - 178.

O’Shea, T., Clark, D. R. and Boyle, T. P. 2001. *Impacts of mine-related contaminants on bats and mining* a technical interactive forum, Office of surface mining, St. Louis, MO. pp 204 – 215.

Picker, M. D., Hoffman, M. T. and Leverton, B. 2007. Density of *Microhodotermes viator* (Hodotermitidae) mounds in southern Africa in relation to rainfall and vegetative productivity gradients. *Journal of Zoology* 271: 37 – 44.

Ping, Z., Huiling, Z. and Wensheng, S. 2009. Biotransfer of heavy metals along a soil-plant-insect-chicken food chain: Field study. *Journal of Environmental Sciences* 21: 849 – 853.

Pomeroy, D. E. 1977. The distribution and abundance of large termite mounds in Uganda. *Journal of Applied Ecology* 14: 465 - 475.

Pomeroy, D. E. 1978. The abundance of large termite mounds in Uganda in relation to their environment. *Journal of applied ecology* 15: 51 – 63.

Prasad, E. A. V., Jayarama, M. and Dunn, C. E. 1987. Significance of termite mounds in gold exploration. *Current Science* 56: 1219.

Rabitsch, W. B. 1995. Metal Accumulation in arthropods near a lead/zinc smelter in Arnoldstein, Austria. *Environmental Pollution* 90: 221 – 237.

Rashed, M. N. 2010. Monitoring of contaminated toxic and heavy metals, from mine tailings through age accumulation, in soil and some wild plants at Southeast Egypt. *Journal of hazardous materials* 178: 739 – 746.

Reis, L. S., Pardo, P. E., Camargos, A. S. and Oba, E. 2010. Mineral element and heavy metal poisoning in animals. *Journal of Medicine and Medical Sciences* 1: 560 - 579.

Richardson, P. and van Helten, J. J. 1984. The development of South African Gold-mining industry, 1895 – 1918. *The economic history review* 37: 319 – 340.

Richardson, P. K. and Levitan, C. D. 1994. Tolerance of Aardwolves to Defense Secretions of *Trinervitermes trinervoides. Journal of Mammalogy* 75: 84 – 91.

Ritcey, G. M. 2005. Tailings management in gold plants *Hydrometallurg*y 78: 3 – 20.

Rosner, T. and van Schalkwyk, A. 2000. The environmental impact of gold mine tailings footprints in the Johannesburg region, South Africa. *Bulletin of Engineering Geology and the Environment* 59 : 137–148.

Ruiz, E., Rodriguez, L. and Alonso-Azcarate, J. 2009. Effects of earthworms on metal uptake of heavy metals from polluted mine soils by different crop plants. *Chemosphere* 75: 1035 – 1041.

Sako, A., Mills, A. J. and Roychoudhury, A. N. 2009. Rare earth and trace element geochemistry of termite mounds in central and northeastern Namibia: Mechanisms for micro-nutrient accumulation. *Geoderma* 153: 217 – 230.

Sands, W. A. 1965. Termite Distribution in Man-Modified Habitats in West Africa, with Special Reference to species Segregation in the Genus *Trinervitermes* (Isoptera, Termitidae, Nasutitermitinae). *Journal of Animal Ecology* 34: 557 - 571.

San Jose, J. J., Montes, R., Stansly, P. A. and Bentley, B. L. 1989. Environmental Factors Related to the Occurrence of Mound-Building Nasute Termites in *Trachypogon* Savannas of the Orinoco Llanos. *Biotropica* 21: 353 - 358.

Schuurman, G. and Dangerfield, M. 1997. Dispersion and Abundance of *Macrotermes michaelseni* Colonies: A Limited Role for Intraspecific Competition. *Journal of Tropical Ecology* 13: 39 - 49.

Shehong, L, Baoshan, Z., Jianming, Z. and Xiaoying, Y. 2005. The distribution and natural degradation of cyanide in goldmine tailings and polluted soil in arid and semiarid areas. *Environmental Geology* 47: 1150 – 1154.

Solomon, E. P., Berg, L. R. and Martin, D. W. 2005. *Biology*. Thomson Learning Inc., Belmont, USA p 1045.

Tarras-Wahlberga, N. H., Flachier, A., Lanec, S. N. and Sagforsd, O. 2001. Environmental impacts and metal exposure of aquatic ecosystems in rivers contaminated by small scale gold mining: the Puyango River basin, southern Ecuador. *The Science of the Total Environment* 278: 239 – 261.

Taylor, W. A., Lindsey, P. A. and Skinner, J. D. 2004. The feeding ecology of the aardvark *Orycteropus afer*. *Journal of Arid Environments* 50: 135 – 152.

Toth, J. 1962. A theory of groundwater motion in small drainage basins in Central Alberta, Canada. *Journal of Geophysical Research* 67: 4375 – 4387.

Turner, J. S. 2001. On the mound of *Macrotermes michaelseni* as an organ of respiratory gas exchange. *Physiological and Biochemical Zoology* 74: 798 – 822.

Turusov, V., Rakitsky, V. and Tomatis, L. 2002. Dichlorodiphenyltrichloroethane (DDT): Ubiquity, Persistence, and Risks. *Blokhin Russian Cancer Research Centre* 110: 125 – 128.

Uys, V. 2002. *A guide to the termite genera of Southern Africa*. ARC – Plant Protection Research Institute, Pretoria pp. 107 – 109.

Van Niekerk, H.J. and Viljoen, M.J. 2005. Causes and consequences of the Merriespruit and other tailings–dam failures. *Land degradation and development* 16: 201 - 212.

Veiga, M. M., Hinton, J. and Lilly, C. 1999. Mercury in the Amazon: A Comprehensive Review with Special Emphasis on Bioaccumulation and Bioindicators. *National Institute for Minamata Disease Forum* pp 19 – 39.

Viljoen, C. J. 2006. Report on: Eskom kendal wetland delineation soil survey *(http://www.eskom.co.za/content/Part2.pdf) Accessed 3 June 2010.*

Way, J. L., Leung, P., Cannon, E., Morgan, R., Tamilinas, C., Leong-Way, J. Baxter, L., Nagi, A. and Chui, C. 1988. The mechanism of cyanide intoxification and it antagonism. *Annual Review Pharmaceutical Toxicology* 24: 451 – 81.

Wilson, E. O. 1971. *The Insect Societies*. Harvard University Press, Cambridge pp. 20 – 25.

Winde, F. and van der Walt, J. 2004. The significance of groundwater–stream interactions and fluctuating stream chemistry on waterborne uranium contamination of streams—a case study from a gold mining site in South Africa. *Journal of Hydrology* 287: 178 – 196.

Wonga, H. K. T., Gauthier, A. and Nriagu, J. O. 1999. Dispersion and toxicity of metals from abandoned gold mine tailings at Goldenville, Nova Scotia, Canada. *The Science of the Total Environment 228*: 47 – 60.

Xu, J., Wang, Y., Luo, Y., Song, J. and Ke, X. 2009a. Effects of copper, lead and zinc in soil on egg development and hatching of *Folsomia candida*. *Insect Science* 16: 51 – 55

Xu, J., Ke, X., Krogh, P. H., Wang, Y., Luo, Y. and Song, J. 2009b. Evaluation of growth and reproduction as indicators of soil metal toxicity to the collembolan, *Sinella curviseta*. *Insect Science* 16: 57 - 63

Yanqun, Z., Yuan, L., Schvartz, C., Langlade, L. and Liu, F. 2004. Accumulation of Pb, Cd, Cu and Zn in plants and hyperaccumulator choice in Lanping lead–zinc mine area, China. *Environment International* 30: 567 – 576.

APPENDIX

Table A1. Heavy metal content (mg/ kg) of the soil profile at the AEL site.

Depth (cm)	Mg	Fe (%)	P	Li	Be	Ti	V	Mn (%)	Rb	Sr	Mo	Cs	Ba	La	Bi
litter	405	1.310	216	8.36	0.35	59	26	0.192	9.0	2.7	0.3	0.45	179	6.4	0.12
0 - 2	403	1.430	225	9.36	0.36	62	28	0.232	9.4	3.2	0.3	0.47	258	7.7	0.12
2 - 5	427	1.45	234	8.10	0.41	66	29	0.197	12.1	3.1	0.3	0.72	248	10.8	0.15
5 - 10	347	1.62	207	9.55	0.45	63	30	0.266	12.6	3.1	0.2	0.54	289	9.9	0.10
10 - 20	306	1.62	192	10.70	0.48	63	32	0.252	11.8	2.6	0.2	0.55	212	8.2	0.09
20 - 30	273	1.63	183	8.88	0.50	62	35	0.187	11.1	2.6	0.2	0.61	165	8.4	0.13
30 - 40	276	1.57	186	8.04	0.45	51	30	0.160	9.9	1.9	0.2	0.54	135	7.6	0.09
40 - 50	306	1.75	210	9.74	0.59	59	34	0.166	9.9	2.0	0.1	0.87	198	8.1	0.10
50 - 60	319	1.93	207	9.03	0.59	62	38	0.178	8.9	3.9	0.1	0.71	466	8.5	0.11
60 - 70	355	1.97	198	8.07	0.61	69	37	0.229	8.5	4.3	0.2	0.71	633	8.3	0.11
70 - 80	333	1.72	153	5.18	0.45	48	33	0.086	5.8	2.1	0.1	0.49	203	4.6	0.10
80 - 90	396	1.49	146	5.66	0.47	48	31	0.141	6.6	3.1	0.1	0.52	331	5.3	0.11
90 - 100	417	1.70	149	5.26	0.51	46	32	0.172	6.5	3.5	0.1	0.59	346	5.8	0.12
100 - 110	435	1.89	145	3.68	0.37	35	26	0.126	5.0	2.6	0.1	0.55	124	4.7	0.10
110 - 120	476	2.21	166	7.10	0.85	72	61	0.174	10.6	4.9	0.2	1.13	233	12.4	0.21

Table A2. Heavy metal content (mg/ kg) of the soil profile at the West Complex site.

Depth (cm)	Mg	Fe (%)	P	Li	Be	Ti	V	Mn (%)	Rb	Sr	Mo	Cs	Ba	La	Bi
litter	578	1.30	368	6.55	0.33	71	34	0.275	9.9	7.4	0.4	0.52	148	8.5	0.13
0 - 2	434	1.36	252	7.16	0.34	45	33	0.283	9.2	5.6	0.3	0.46	123	7.8	0.13
2 - 5	400	1.14	233	5.14	0.33	38	28	0.169	9.7	2.6	0.2	0.45	45	6.9	0.10
5 - 10	318	1.16	222	5.07	0.35	34	28	0.205	9.9	3.1	0.3	0.47	76	6.9	0.07
10 - 20	310	1.18	195	5.75	0.29	29	24	0.174	8.1	3.7	0.1	0.40	55	5.7	0.06
20 - 30	298	1.15	196	4.55	0.34	28	25	0.130	8.2	4.2	0.1	0.47	39	6.0	0.06
30 - 40	315	1.24	163	6.45	0.36	26	28	0.177	7.4	4.3	0.2	0.50	72	6.7	0.11
40 - 50	356	1.09	154	5.35	0.39	24	25	0.109	7.3	2.7	0.2	0.59	43	6.4	0.07
50 - 60	378	1.10	163	5.13	0.39	26	26	0.087	6.7	2.0	0.1	0.60	43	5.9	0.07
60 - 70	364	1.33	150	4.47	0.44	30	32	0.120	6.1	1.8	0.1	0.61	35	6.1	0.09
70 - 80	347	1.45	146	3.55	0.44	39	34	0.149	5.4	2.3	0.1	0.55	64	6.4	0.08
80 - 90	379	1.50	144	3.58	0.47	44	38	0.150	6.4	2.5	0.1	0.66	53	6.9	0.08
90 - 100	517	1.96	151	5.35	0.48	55	43	0.384	6.0	3.4	0.1	0.61	65	9.3	0.08
100 - 110	669	1.83	149	4.22	0.44	56	41	0.478	6.2	3.8	0.1	0.62	46	8.5	0.08
110 - 120	3841	2.18	195	7.58	0.56	76	38	0.971	5.6	8.6	0.1	0.56	54	10.7	0.08

Table A3. Heavy metal content (mg/ kg) of the mound profile at the AEL site.

Depth (cm)	Mg	Fe (%)	P	Li	Be	Ti	V	Mn (%)	Rb	Sr	Mo	Cs	Ba	La	Bi
Crust	325	1.46	165	7.88	0.48	49	39	0.254	9.8	3.4	0.3	0.59	146	8.6	0.12
0 - 10	368	1.45	191	9.09	0.46	53	39	0.260	10.7	4.2	0.3	0.64	131	9.0	0.13
10 - 20	449	1.51	223	8.97	0.45	47	37	0.309	9.9	4.8	0.3	0.57	155	8.5	0.12
20 - 30	612	1.23	280	5.80	0.34	41	30	0.220	8.7	5.1	0.2	0.46	86	6.8	0.10
30 - 40	634	1.19	303	8.91	0.38	53	35	0.225	10.7	7.6	0.7	0.57	119	8.0	0.13
40 - 50 Bottom	746	1.18	392	7.30	0.37	60	33	0.205	11.6	8.3	0.3	0.63	108	8.1	0.13

Table A4. Heavy metal content (mg/ kg) of the mound profile at the West Complex site.

Depth (cm)	Mg	Fe (%)	P	Li	Be	Ti	V	Mn (%)	Rb	Sr	Mo	Cs	Ba	La	Bi
Crust	377	1.18	209	10.05	0.50	59	34	0.306	9.8	5.1	0.3	0.47	151	8.4	0.10
0 - 10	515	1.19	229	8.08	0.46	68	33	0.271	11.2	4.8	0.3	0.58	130	8.7	0.11
10 - 20	630	1.18	242	9.55	0.40	70	32	0.275	11.2	5.6	0.2	0.56	114	10.0	0.10
20 - 30	500	1.24	234	8.68	0.41	76	35	0.296	11.6	6.4	0.3	0.57	212	11.9	0.16
30 - 40	562	1.21	242	7.23	0.44	58	34	0.340	10.6	5.5	0.3	0.51	141	8.6	0.09
40 - 50 Bottom	375	0.99	199	5.58	0.33	49	29	0.152	10.5	4.6	0.2	0.51	69	6.7	0.07

Table A5. Heavy metal content (mg/ kg) of the termites at AEL, West complex and Control site.

Site	Caste	Mg	Fe	P (%)	Li	Ti	V	Rb	Sr	Mo	Ba	La	W	Bi
AEL														
	Workers	850	1110	0.506	0.730	16.3	1.6	1.6	3.4	0.58	9.1	0.74	0.19	< 0.1
	Soldiers	640	422	0.310	0.500	10.4	1.1	0.7	2.1	0.47	3.7	0.31	0.12	< 0.1
	Alates	565	124	0.379	0.105	11.6	0.5	0.9	0.9	0.32	0.76	< 0.2	0.06	< 0.1
	Nymphs	573	106	0.345	0.099	9.1	0.3	0.9	0.9	0.30	0.69	< 0.2	0.06	< 0.1
West Complex														
	Workers	839	1735	0.448	1.016	22.9	2.3	2.3	3.0	0.56	9.5	1.35	0.47	< 0.2
	Soldiers	671	695	0.342	0.407	13.1	1.1	1.0	2.0	0.43	3.83	0.51	0.27	< 0.1
Control														
	Workers	1069	1754	0.545	0.872	17.1	2.0	1.9	4.6	0.45	7.8	1.23	0.18	< 2
	Soldiers	1173	1469	0.613	0.567	17.2	1.9	1.5	4.0	0.40	6.8	1.04	0.32	< 2