

Assessing the ecotoxicity of goldmine tailings by utilising earthworms and soil mesofauna as bioindicators

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PREFACE

This study conducted an ecological risk assessment on an old abandoned gold mine, to determine the effects of gold mine tailing disposal facilities on the surrounding environment and the inhabiting organisms. The laboratory and field work was conducted following standard test procedures and used a common earthworm species (*Eisenia andrei*) as test organism. The arthropod diversity was also studied, to help understand how anthropogenic disturbances can influence the natural environment. The thesis was written following the format provided by the NWU.

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DECLARATION

The experimental work done and discussed in this dissertation for the degree Magister Scientiae in Environmental Sciences was carried out in the Unit for Environmental Sciences and Management, North-West University, Potchefstroom Campus, Potchefstroom, South Africa. This study was conducted under the supervision of Prof. P.D Theron and Prof. M.S. Maboeta.

The study represents original work undertaken by the author and has not been previously submitted for degree purpose to any other university. Appropriate acknowledgements have been made in the text where the use of work conducted by other researchers has been included.



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16 / 11 / 2018

Date

ABSTRACT

The mining industry plays a key role in the economic development of South Africa, which as a country, is one of the largest exporters of valuable resources such as, gold, platinum group metals (PGMs) and other metals and minerals. Unfortunately, mining produces large volumes of solid waste in the form of tailing disposal facilities (TDFs), which contain variety of heavy metals (e.g. Cr, Co, Ni, Cu, Zn, As, Pb and Cd) which are hazardous to the natural environment. Tailing disposal facilities are capable of altering the landscape, by taking up large areas of land and spreading of tailing material into the surrounding environment. Despite the negative effect associated with mining, the demand for valuable metals and minerals are still high. The aim of this study was to use soil metal analysis, earthworm bioassays, avoidance-behaviour tests and soil mesofaunal communities to assess the effects of a 78-year-old gold mine on the surrounding environment and inhabiting soil communities. Six soil samples were randomly taken from four different sites on a gold mine viz. two different TDFs and two grassy pasture sites. Sampling was done at four different times over the period of a year, to determine seasonal differences. The mesofauna were extracted and identified in order to determine the species diversity and abundance for each site. A list of mesofaunal species was constructed and divided into distinct functional groups based on the mesofauna feeding habits. Soil chemical analysis showed that the TDFs had the lowest pH levels and highest concentrations of heavy metals (especially chrome). Earthworms exposed to the TDF material showed significantly lower earthworm biomass than the control, with a very low cocoon production. Earthworms exposed to the two pasture sites had a higher biomass than the two TDF sites, while also being higher than the control. The northern pasture showed a lower cocoon production and juveniles per cocoon count than the control, while the southern pasture had a slightly higher cocoon production and juvenile per cocoon count than the control. Avoidance tests showed similar results, were earthworms generally preferred the pasture soils and control over the TDF material. Both the 100% BTDF and STDF exposures, had avoidance over the 80% threshold level. Mesofauna sampled from the TDFs site had the lowest species diversity and individual count compared to the two pasture site. Prostigmata, Cryptostigmata and Mesostigmata were the most dominant mite taxa within the TDFs, with Prostigmata being the most dominant group sampled. Prostigmatic mites might make good ecotoxicological bioindicators for future studies. Seasonal fluctuations influenced both the abundance and species with the highest individual count and species diversity recorded in Autumn. It can be concluded, that even though the gold mine was inactive for a long period of time, it still remains a highly contaminated area.

Keywords: bioassays, bioindicators, earthworms, soil, mesofauna heavy metals, tailing disposal facilities.

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CHAPTER 1

INTRODUCTION

1.1 General introduction and problem statement

Soil is a natural non-renewable resource (Bedano *et al.*, 2006), which is a combination of minerals, organic matter and degraded rock which takes a long period of time to form (Ashman & Puri, 2008). Soil provides several valuable ecological services, such as the regulation of water filtration and acts as growing medium for plants and mosses; it also provides habitats for organisms and supplies most known antibiotics. The rate of soil developing processes can be influenced by the amount and type of vegetation cover, as well as the organisms inhabiting the soil (Loots & Ryke, 1966). It is important to understand how anthropogenic activities influence and change soil faunal communities because they can lead to changes in the soil profile. Agriculture, mining and industries have shown to have profound effects on the soil chemical and physical properties (Bradshaw, 1997).

The mining industry plays a huge role in the economic wealth of most nations (Mbendi, 2017). The global demand for minerals and metals has increased over recent years (Maboeta *et al.*, 2018), furthering the exploitation of land and soil resources for mining. Mining is the largest industry in South Africa (Mbendi, 2017) and during 2010 South Africa had 1600 legally registered mines (Eijsackers *et al.*, 2014) producing 55 different minerals and exporting them to 87 countries (Mbendi, 2017; Chamber of Mines of South Africa, 2017). South Africa has the largest reserves of gold in the world and is ranked 7th worldwide in terms of gold production (Chamber of Mines of South Africa, 2017). Mining provides a multitude of benefits for the economy of nations globally, especially important for developing countries. But unfortunately, there are many environmental concerns associated with mining.

Tailing dam facilities (TDFs) are regarded as one of the biggest problems associated with mining. TDF consists of finely milled waste formed during the mining process (Aucamp & Van Schalkwyk, 2002). Tailing dams contain a variety of heavy metals, e.g. Cd, As, Cr, Co, Pb, Hg, Ni and Zn (Aucamp & Van Schalkwyk, 2002). A variety of factors may help these heavy metals to leach into the surrounding area (Fourie, 2009). Wind and water erosion spread the tailing material into the surrounding environment, distributing heavy metals to surrounding soil and water ecosystems (Bezuidenhout & Rousseau 2005; Yibas *et al.*, 2010). Consequently, TDFs fail at their designed purpose, namely to serve as permanent storage for mining waste (Fourie, 2009). This is mainly due to weak post-mining management and regulation, leading to these TDFs becoming abandoned for long periods of time, creating an environmental hazard (Rico *et*

al., 2008). Post-mining rehabilitation methods such as, vegetation, fertilisation and bioremediation, have been used to successfully reduce the impact of old mining sites (Edraki *et al.*, 2017). Active rehabilitation done on TDFs has shown promising results, such as reducing environmental risk, stabilisation of biological processes in soil and re-establishment of vegetation cover (Edraki *et al.*, 2017). Mining is also a major source of acid mine drainage (AMD), which is formed from a chemical reaction between water and rocks containing sulphur, creating a metal-rich water with an extremely low pH (Aucamp & Van Schalkwyk, 2002; Bezuidenhout & Rousseau, 2005). The AMD leachate increases the soil and groundwater systems acidity (lowering pH) (Aucamp & Van Schalkwyk, 2002), which increases the concentrations of salt in the environment, as well as the bioavailability of heavy metals (Bezuidenhout & Rousseau, 2005). Heavy metals accumulate within the top soil, which negatively influence any inhabiting fauna and flora (Otomo *et al.*, 2013).

It is important to assess current environmental problems, as well as predict the possible problems which can arise in the future because of unsustainable mining. Risk assessments (RAs) are regimes which can be used to evaluate the possible environmental risks presented by TDFs (Newman, 2015; Eijsackers *et al.*, 2017). Risk assessments are defined “*as the process by which one estimates the probability of some adverse effect(s) of an exciting or planned exposure to either human or ecological entities*” (Newman, 2015). Environmental risk assessments (ERA), can provide possible rehabilitation options for stakeholders and policy makers (Demidova & Cherp, 2005; Saunders *et al.*, 2011). Several countries have developed procedures for evaluating soil and ecological services they provide (Faber & Van Wensen, 2012; Eijsackers *et al.*, 2017). So far no large-scale ERA has been done on South African soils (Eijsackers *et al.*, 2017), but we do have policies with regards to soil screening values (South Africa, 2014). Soil screening values (SSV) can be used as standards for assessing contaminated environments (South Africa 2014), but SSV is not absolute (Eijackers *et al.*, 2014). Currently there are two SSVs; Soil Screening value 1 is defined as “*means soil quality values that are protective of both human and eco-toxicological risk for multi-exposure pathways, inclusive of contaminant migration of the water resource*”; Soil Screening Value 2 is defined as “*mean soil quality values that are protective of risk to human health in the absence of a water resource*” (DEAT, 2008).

Ecological risk assessment traditionally used physical and chemical analysis to screen for soil contamination (Landis *et al.*, 2003). However these methods cannot determine the effects of contaminants on biota and the chemical analysis only shows the number of elements available in the soil solution (Landis *et al.*, 2003). Bioindicators can be used in an ERA to determine the state of the environment as a whole. Bioindicators are defined as organisms, or a community of organisms which both qualitatively and/or quantitatively reflect the state of their environment

(Cortet *et al.*, 1999). Bioindication assesses the level of pollution by looking at the changes in organism response. For example, frequency, distribution, absence, presence and behaviour of chosen organisms (Vargha *et al.*, 2002). Before you can begin an ERA you must choose the appropriate bioindicators. They must have a tolerance to low levels of pollution, their response must be measurable and reproducible, must reflect at least one or more chemical and physical factors (Jamil, 2001; Crouau *et al.*, 2002; Markert *et al.*, 2003; Wahl, 2014). Soil mesofauna has been successfully used as bioindicators of soil pollution (Santos *et al.*, 2010). Mesofaunal communities consist of large populations with a variety of species, which interact with one another (McGeoch, 1998). Many of these soil organisms spend their entire life cycle in a few square meters of soil, which makes them great representatives of local conditions (Migliorini *et al.*, 2004). Comparing the mesofaunal communities of natural sites with anthropogenically disturbed sites can give an idea of the levels of pollution. Earthworms are acknowledged as good indicators of the condition of soil ecosystems (Cortet *et al.*, 1999), because they are abundant, play a large role in maintaining soil health, are sensitive to change in the soil ecosystems and are cheap to culture (Doran & Zeiss, 2000). Earthworm bioassays have been used in numerous ecotoxicological studies on platinum (Maboeta *et al.*, 2008; Jubileus *et al.*, 2012), chromium (Van Coller-Myburgh *et al.*, 2014) and gold (Van Coller-Myburgh *et al.*, 2015) mine tailing dams. Especially species from the genus *Eisenia* has largely been used in ecotoxicity studies, making it easy to compare available data to data from other studies (Maboeta & Fouché, 2014).

1.2 Aims and objectives

The aim of this study was to determine the effects of gold mine TDFs on the surrounding environment, utilising soil mesofaunal communities and earthworm bioassays (in terms of, growth, reproduction, mortality and avoidance behaviour).

The specific aims of this study were to:

- Determine the effects of gold mine tailings on soil mesofaunal communities, as well as the seasonal variations thereof.
- Assess the concentrations of selected metals (Cr, Co, Ni, Cu, Zn, As, Pb and Cd) at the different sampling sites.
- Utilise the earthworm reproduction test (OECD 222, 2004) to determine the effects of gold mine tailings on the growth and reproduction of *Eisenia andrei*.
- Investigate earthworm avoidance-behaviour (ISO 17512, 2007) towards different gold tailings and the pastures surrounding the tailings.

CHAPTER 2

LITERATURE REVIEW

2.1 Mining industry

Archaeological discoveries show evidence of mining during the Stone Age (or Neolithic Period) about 8000-2000 B.C, in soft chalk deposits in France (Clark *et al.*, 2017). Mined minerals were used for a variety of purposes such as the crafting of utensils (e.g. knives, scrapers and arrowheads) and as a form of currency. Over time mining has become common practice and the methods and techniques used to extract minerals have changed and improved significantly (Mbendi Information Services, 2016). Modern mining methods are divided into four broad categories viz. opencast-, underground-, borehole (mostly used to recover fuels, such as petroleum)- and deep ocean (dredging) mining (Yeboah, 2008). Currently, mining plays a crucial role in the economy of countries and has become a large and influential industry world-wide. The most commonly extracted minerals include coal, natural gasses, gold, bauxite, diamonds, limestone, iron, platinum, lead, nickel, phosphate, tin, molybdenum, uranium and rock salt (Yeboah, 2008). China, the United States of America (USA), Australia, South Africa, Canada and Chile are the leading mining countries globally (Mbendi Information Services, 2016).

South Africa is globally one of the leading producers of raw mineral resources and produces over 60 different minerals and metals (e.g. gold, PGMs, vanadium, manganese, uranium, diamonds, nickel, chromium, cobalt and bauxite) which are economically important (Mbendi Information Services, 2016). Most of South Africa's economic activities are centred on the mining industry that contributes ZAR304 billion; this accounts for 7.2% of the country's gross domestic product (GDP) in 2016 (Chamber of mines of South Africa, 2017). In 2005 it has been recorded that the country holds up to 75% of global platinum reserves (62% of produced platinum), 60% of cobalt, 40% of gold and 90% of PGMs (Mbendi Information Services, 2016). According to the South African Chamber of Mines of South Africa (2016), the following minerals make up the bulk of the SA's GDP percentage: coal (25%), PGM's (22%), gold (16%), other minerals (37%). The mining industry is also responsible for the employment of 455,109 individual workers (Chamber of Mines of South Africa, 2016).

2.2 Impacts of mining

Unfortunately, the mining industry is also responsible for negative environmental impacts e.g. acid mine drainage (Akciil & Koldas, 2006), land degradation, contamination of surface soil and groundwater (Schueler *et al.*, 2011), loss of surface vegetation, discharge of metal-rich tailings into the environment, mercury, cyanide, metal pollution and dust pollution (Tarras-Wahlberg *et al.*, 2001). This is a possibility due to the failure of establishing effective precautionary and rehabilitation measures, and the lack of an active enforcement policy for measures already in place (Aucamp & Van Schalkwyk, 2002; Van Coller-Myburgh *et al.*, 2015). For example, it is estimated that around 25% of the natural ecosystems in South Africa have been lost due to the impact of mining (DEAT, 1999). The discarding of equipment, tailings disposal facilities, rock dumps and polluted soil and water have left the mines and the areas surrounding them heavily degraded (Aucamp & Van Schalkwyk, 2002). One of the main contributors to both soil and water pollution is the creation of tailing disposal facilities (TDFs) (Wahl *et al.*, 2012; Eijsackers *et al.*, 2014). A large percentage of South African soils are arable soils, which are extremely susceptible to anthropogenic activities, such as mining (Eijsackers *et al.*, 2017). Tailing disposal facilities are storage facilities for the waste generated by mining and they may contain high concentrations of heavy metals. Ore-bearing rock is crushed and milled into grains about 0.5 mm in size, during the metallurgical phase minerals are extracted and the remaining residue forms a slurry (Aucamp & Van Schalkwyk, 2002; Fourie, 2009). At this stage, the slurry consists of large quantities of water and other dissolved mineral waste (Aucamp & Van Schalkwyk, 2002). The excess water is left to evaporate, stored for re-use in processing operations or allowed to drain into the soil (Fourie, 2009). The area where water is stored is referred to as the “pond”.

Figure 1 illustrates the basic structure of a TDF and indicates the oxidation and saturation zones within it. The sandy surface of the TDF is referred to as the “beach”, and just below the “beach” is the oxidation zone. It is in the oxidation zone where oxygen penetration occurs and then reacts with the sulphate minerals (Yibas *et al.*, 2010; Koch, 2014). Beneath the oxidation zone is the unsaturated zone, where oxygen fails to penetrate. When the deposit of oxygen and water stops, the saturation zone begins to sink and the unsaturation zone increases in size (Bezuidenhout & Rousseau, 2005; Yibas *et al.*, 2010; Koch, 2014).

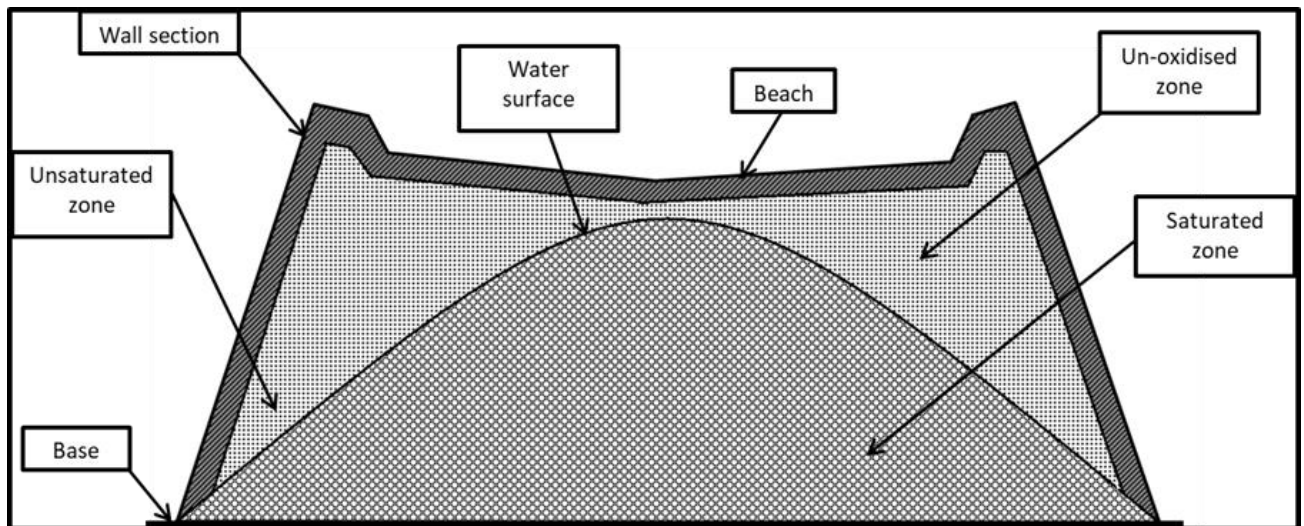


Figure 2-1: Illustration of a TDFs oxidation zone obtained from Bezuidenhout & Rousseau (2005)

The majority of TDFs are composed of 75% sand, 20% silt and 5% of clay fraction particles (Aucamp & Van Schalkwyk 2002; Bezuidenhout & Rousseau, 2005; Maboeta *et al.*, 2007). Tailing disposal facilities cover large areas of land. Fairbanks *et al.* (2000) estimated that mines and quarries cover about 175,421 hectares of South African soil. Unfortunately this is the most recent information available and is already out-dated by almost two decades. Around 270 gold mine tailings can be found near urban and agricultural land in South Africa (Aucamp & Van Schalkwyk 2002). Water and wind erosion further distribute the tailing material across the surrounding landscape. Over a long period of time, this can lead to the creation of small sand dunes, which can easily reach heights of one meter or more given enough time (Aucamp & Van Schalkwyk, 2002, and Jubileus *et al.*, 2012).

Tailing dams are created as control structures which provide safe and permanent storage for mining waste (Van Coller-Myburgh *et al.*, 2015), while minimising the impacts on the environment (Rossouw *et al.*, 2010). Unfortunately in some cases, the tailing dams are unable to achieve their designed purpose (Rossouw *et al.*, 2010; Van Coller-Myburgh *et al.*, 2015). Ensuring that tailing dams remain stable can prove to be challenging. The following reasons can lead to tailing dam failure: (1) quick construction of dam along with a sequential rise of dam height; (2) not following all of the regulations regarding the design criteria; (3) lack of continuous monitoring of tailing dam stability; (4) poor management of old or abandoned tailing dams (Rico *et al.*, 2008; Azam & Li, 2010). Tailing dams also contain large amounts of heavy metals such as, As, Co, Cr, Cu, Ni, Zn and Hg (Aucamp & Van Schalkwyk, 2002; Van Coller-Myburgh *et al.*, 2015). When these metals are present and are able to leach into the surrounding environment,

they can become hazardous and pose a great threat to the environment. Besides heavy metals, TDFs also contain high levels of sulphate minerals (such as, pyrite) (Akcil & Koldas, 2006). The oxidation of sulphate minerals lead to the generation of AMD, pyrite can come into contact with oxygen through rain water (Akcil & Koldas, 2006). Wind and water erosion can help the AMD to then leach into the surrounding environment. Acid mine drainage is also considered one of the major concerns linked to the mining industry (Akcil & Koldas, 2006; Koch, 2014).

Heavy metal pollution in terrestrial environments can increase due to anthropogenic activities, such as mining, smelting, military operations, industrial manufacturing, transportation and the application of a metal coating to fertilizer and pesticides used in agriculture (Gall *et al.*, 2015). Heavy metals (e.g. Zn, Cr, Ni, Cu, As, Se, Sr, Mo, Tc, Cd, Hg and Pb) are of particular environmental concern (Fent, 2004). Metal pollution has adverse effects on both terrestrial and aquatic environments, which leads to the obstruction of certain environmental functions (Fent, 2004). Even though elements such as, Zn, Cu, Mo and Fe are regarded as micro nutrients which are required for sustaining certain organisms as well as plant growth. Large concentrations of these elements may lead to toxicity within organisms (Niklinska *et al.*, 2006). Hao *et al.* (2004) stated that long-term exposure to these heavy metals and other contaminants found within TDFs will negatively affect the abundance, distribution and diversity of biota. In general, heavy metal contamination causes groundwater pollution, soil pollution, the deterioration of soil structure, ecological landscape destruction, nutrient deficiencies and reduction of biodiversity (Hao *et al.*, 2004). Unlike most organic pollutants heavy metals do not degrade over time and remains within the environment even after the source of pollution has been removed (Gall *et al.*, 2015).

The effects caused by metal pollution, or the degree of pollution, may vary depending on the following soil characteristics: particle size, organic matter content, hydrogen ion concentration and microbial activities. For example, the presence and/or mobility of metals increase within the soil with low pH values, whereas high phosphate concentrations can decrease the availability of some elements (Wong, 2003). Trace metals and other elements can also move through the food chain, by bioaccumulating within soil organisms (Newman, 2015). For instance, most terrestrial invertebrates accumulate trace elements through the consumption of their food (Dallinger & Rainbow, 1993; Newman, 2015), some invertebrates can also absorb certain trace elements through their skin (Dallinger & Rainbow, 1993). Accumulation of the substance occurs when the pollutants increase in concentration faster than the organism's ability to get rid of the pollutant, or when an organism containing the pollutants is ingested by another organism without the ability to fully excrete the pollutants (Zenker *et al.*, 2014; Newman, 2015). Bioaccumulation is defined by Newman (2015) as the "*net accumulation of a contaminant in (and in some cases on) an organism from all sources including water, air, and solid phases of*

environmental. Solid phases include food sources.” For instance, the bioaccumulation of metals such as, Pb, Zn and Cu decrease the rate at which cellulose degrades within microbial communities. This can restrict certain microbial activities required for the mineralisation of nitrogen (N) and reduce the decomposition of organic matter (Georgieva *et al.*, 2002; Wahl, 2007; Wahl *et al.*, 2012). Metal polluted soils can cause a decrease in plant abundance, a decrease in the growth rate or size of others, or in the worst case lead to the destruction of plant species within polluted areas (Wong, 2003).

Contaminants also move through a food chain through the processes of biomagnification, which is “*an increase in concentration from one trophic level (e.g. prey) to the next (e.g. predator) due to the accumulation of contaminants from food*” (Newman, 2015). Predatory species that prey on mites and pseudoscorpions, for example, will accumulate higher levels of toxicants in their bodies compared to other fauna that feeds on plants and other organic material (Jamil, 2001). According to Niklinska *et al.* (2006) metal pollution generally causes the reduction of productivity, diversity, community complexity and biomass of soil communities; but different types and concentrations of contaminants will produce different outcomes in soil communities.

Acid mine drainage is considered a major environmental impact caused in general through mining. Acid mine drainage generation is most common in old or abandoned tailing dams when the layers beneath the top layer of the dam are exposed to oxygen. For instance, oxidised water (rainwater) drains into the thick top layer of the dam, causing the sulphate minerals (mainly pyrite (FeS_2)) to undergo oxidation. This leads to the acidification of the tailing dam, lowering the overall pH (Naicker *et al.*, 2003 and Akcil & Koldas, 2006; Chandra & Gerson 2010). According to Koch (2014) pyrite is depleted in the oxidised zone of the TDFs. This zone usually has a low pH and high electrical conductivity - as the pyrite depletes the concentration of certain metals increases, which in turn increases the movement and leaching of metals. Oxidisation of pyrite usually occurs at a depth of 5 m. However, this can vary due to sand tailing dams being more permeable for water compared to black tailing dams, causing oxidation to take place deeper within sand tailing dams (Naicker *et al.*, 2003). Leaching of AMD into the surrounding environment leads to adverse ecological implications that include the contamination of soil and surface water, leading to the overall reduction of biodiversity (Naicker *et al.*, 2003; Akcil & Koldas, 2006).

Acid mine drainage is a multifactor pollutant which changes in terms of frequency, intensity and type from site to site (Akcil & Koldas, 2006). Temperature, soil salinity and types of metals present, the degree of water saturation, concentration of sulphide minerals, types of sulphides present, buffer capacity of the environment, rainfall and soil chemistry are all factors that have an influence on the degree at which AMD will affect the environment (Gray, 1997; Akcil &

Koldas, 2006). For example, in flowing water (lotic) systems, AMD can have either a direct or indirect impact on ecosystems, which removes a species and simplifies the food chain, decreasing the overall ecological stability (Gray, 1997).

2.3 Risk assessment

Risk assessment is defined “as the process by which one estimates the probability of chosen adverse effect(s) of an existing or planned exposure to either human or ecological entities” (Newman, 2015). Traditionally environmental risk assessment (ERA) would focus on estimating the risk of chemical contamination of a chosen site (Faber & Wensem, 2012). This risk assessment can be applied to assist in the decision-making process when faced with varied uncertain outcomes, due to varying conditions of doubt concerning the nature of the situation (Newman, 2015). Risk assessment involves the calculation of risk (e.g. ecological, health etc.) in affected areas and provides valuable information regarding feasible options (e.g. rehabilitation options) (Komnitsas & Modis, 2006). Risk assessment can be split into two broad categories, predictive- and retroactive risk assessment. Predictive risk assessment deals with a planned condition, for example determining the possible effects of contaminated groundwater, which will soon seep into a nearby drinking water well. While retroactive risk assessment is used to estimate the consequences of an existing condition, for example long-term effects related to a contaminated seepage basin (Newman, 2015).

Environmental risk assessment includes both human health risk assessment (effects on human health) and ecological risk assessment (effects on ecosystems). It mainly deals with the effects of pollutants (hazardous substances), which are present in a chosen environment (Lahr & Kooistra, 2010). The main goal of an ERA is to determine the likelihood of a specified adverse effect or ecological event due to a predetermined stressor or exposure (Newman, 2015). Environmental risk assessment should also include ecological entities such as, communities and metacommunities, composed of a variety of occupying species within a diverse environment (Saunders *et al.*, 2011).

There exists a variety of well-defined methods to perform an ecological risk assessment. For example, the species sensitivity distribution (SDD) model is used to provide an ecological perspective by determining target and intervention values for pollutants by assessing the effects of these pollutants on chosen species communities. The model uses available ecotoxicological data to provide the range of effects of a single contaminate on reproduction, growth, or survival of a specie(s) (Faber & van Wensem, 2012). Biomarkers have been used in past ERA studies in order to assess the health soil environments (Asensio *et al.*, 2013). Biomarkers are used to

determine the quantifiable changes within biochemical, physiological and behavioural states within organisms as a result of specific stimuli (Saunders *et al.*, 2011). Risk assessment can also utilise bioassays to estimate the bioavailability of toxicants toward organisms.

Risk assessment is thus required to measure and evaluate the possible environmental risks presented by heavily contaminated sites (e.g. tailing disposal facilities) over a period of time, as well as the effects on the organisms associated with the contaminated soil. Risk assessment can be described generally as the calculation of risk (e.g. ecological) within a chosen area, which can provide information on possible rehabilitation options for stakeholders and policy makers (Saunders *et al.*, 2011).

2.4 Soil environment

The soil is a fundamental but limited resource (Aspetti *et al.*, 2010), that forms a basis for both aquatic and terrestrial life on earth (Blanco-Canqui & Lal, 2010), while also being responsible for most of the ecological services provided by terrestrial ecosystems (Janion-Scheepers *et al.*, 2016). For instance, the soil is a vital medium for plant growth, biological sustainability, water flow regulation and also acts as an environmental buffer (Aspetti *et al.*, 2010). Verhoef (2004) defined soil as a living system where plants (roots), microorganisms and soil fauna are able to thrive. While it is difficult to define soil quality, many see it as the ability of soil to sustain biological productivity, promote the well-being of human, plant and animal health and to maintain the overall quality of the environment (Blair *et al.*, 1996). The soil is a heterogeneous ecosystem, which consists of both living and non-living components, this includes assemblages of soil biota and the products they deliver (Blair *et al.*, 1996). The terrestrial environment consists of several biotic components which include microbes, plants and soil invertebrates (Blair *et al.*, 1996). These biotic components interact with one another, which influences both the soils environment and soil characteristics (Blair *et al.*, 1996). According to Blanco-Canqui & Lal (2010), soil ecosystems consist of a variety of physical and chemical factors such as water, organic matter, gases, plant roots and other solids. Soil systems provide ecological services - soil can be a variety of habitats for different organisms and also serves as a source of nutrition (Bardgett, 2005). Soil communities are the main drivers of vital functions such as litter decomposition, nutrient cycling, carbon cycling, regulation of above ground vegetation processes and support for soil biota (Janion-Scheepers *et al.*, 2016). Geological processes and organisms within the soil create, change and maintain these soil ecosystems (Janion-Scheepers *et al.*, 2016). Soil ecosystem studies have become a major focal point for scientific studies (Avidano *et al.*, 2005). Assessment studies on soil ecosystems may lead to further development of ways to identify and classify soil pollution, as well as a better understanding of soil biota abundance and distribution (Ou *et al.*, 2005). Fluctuations of soil quality are one of the major

criteria when assessing the long-term sustainability of soil ecosystems (Aspetti *et al.*, 2010). According to Jubileus *et al.* (2012), most biological activity occurs in the top layer of soil (around 10 cm). Biological processes can occur across different scales within the soil and influence one another. For example, microbial activities responsible for the mineralisation of certain elements can be directly influenced by the predation of microbes by certain nematodes.

The soil is largely important for industries such as agriculture and forestry and also for infrastructure (Blanco-Canqui & Lal, 2010). Soil can also provide ecological service of great benefit for human well-being and higher quality can lead to an increase in microarthropod biodiversity (Aspetti *et al.*, 2010). Rehabilitation of degraded soils can prove to be challenging and expensive and in some cases it may be impossible to restore or renew heavily degraded areas (Blanco-Canqui & Lal, 2010).

2.5 Bioindicators

Over the last two decades, the importance of soil has shifted scientific studies to investigate soil health, determining the effects of metal polluted soil on organisms and the tolerance of soil communities to metal pollution (Avidano *et al.*, 2005; Piotrowska-Seget *et al.*, 2005). Various methods, which can be used to determine the health and quality of an ecosystem already exist; for instance, chemical analysis, physical analysis and the use of biological indicators (Lavelle *et al.*, 2006). However, chemical analysis requires knowledge of the pollution classes, to analyse the different types of pollutants, this data gives little to no information about the bioavailability of pollutants (Crouau *et al.*, 2002). It is highly recommended to complement the chemical analysis data with bioindicator data (Schloter *et al.*, 2003). Bioindicators are typically described as a single organism, or a group of organisms, which are able to reflect and characterise the current state of an ecosystem (McGeoch, 1998; Jamil, 2001; Markert *et al.*, 2003), bioindicators must play a role in the ecological function of the environment, as well as show an observable reaction to changes within the environment (Cortet *et al.*, 1999). Bioindicators are used to assess the effects of disturbances by observing the absence, presence, distribution and abundance of organisms within a chosen environment under stress (Markert *et al.*, 2003). Currently, there are a wide variety of soil organisms, which are used in ecological assessments such as, microbial organisms, molluscs, enchytraeids, earthworms and microarthropods, such as springtails, mites and insects (Crouau *et al.*, 2002; Van Coller-Myburgh, 2015). The organism or organisms used as bioindicators are chosen based on the study objective. The test species used in bioindication must be able to tolerate low levels of pollution, while showing measurable responses to drastic changes within their environment (Jamil, 2001; Markert *et al.*, 2003). Bioindicators can be associated with one or more chemical or physical factors, for example, pollution levels,

temperature and the availability of moisture in the environment (Markert *et al.*, 2003). Before an organism can be chosen to represent the state of the environment for an ERA study, certain criteria must be established to help determine the best candidate. Criteria can include the following: rapid response to environmental change; relative sensitivity to environmental change; species commonly found in abundance in the study area (avoiding specialist species); feeding preferences; habitat preferences; amount of time spent on soil; movement of organisms and size of organisms (Cenci & Jones, 2009).

2.5.1 Soil mesofauna as bioindicators

South Africa is considered to be a biodiversity hotspot for large groups of soil organisms where mammals, amphibians, reptiles and birds are well documented (Janion-Scheepers *et al.*, 2016). Most studies on biological measurement focus on microbial populations, but recently there has been an increase in awareness of the importance of terrestrial invertebrates as components within soil ecosystems (Blair *et al.*, 1996). Studies have documented the potential use of invertebrates as indicators of soil health (Blair *et al.*, 1996), as well as the importance of soil as an environmental component (Aspetti *et al.*, 2010).

Soil biota can be defined as individuals that live in a soil environment, influence the soil system and are also influenced by the processes within the system (Wallwork, 1970; Blair *et al.*, 1996). The documentation of invertebrate species diversity remain few and far in between, the current number of described insect species are only a third of the total estimated number (Janion-Scheepers *et al.*, 2016). Most of the soil phyla used as representatives, spend at least a part of their life cycle within the soil (Wallwork, 1970). Soil mesofauna is defined by Lavelle *et al.* (2006) as organisms which range from, 0.2 to 2mm in length. However, there are a few exceptions; for example, the body length of the majority of mites is less than 1mm (Ryke, 1959), while some Collembola species are known to be larger than 2 mm. The bulk of soil mesofauna consist of a diverse group of Enchytraeidae (Annelida) and microarthropods (Blair *et al.*, 1996; Janion-Scheepers *et al.*, 2016). Microarthropods refer to the general grouping of small arthropods that includes, mites (Acari), Collembola (Springtails), Protura, Diplura, Pauropoda and other small insects from various orders (Blair *et al.*, 1996). Mites and Collembola make up 90-95 percent of the total microarthropods found in soil (Blair *et al.*, 1996). Studies on the diversity and abundance of soil invertebrates have been done in some natural and managed ecosystems. Although actual numbers are not known, they clearly represent a large portion of belowground food webs within these ecosystems (Blair *et al.*, 1996). At present it is impossible to identify all of the members in soil invertebrate communities; researchers must make use of schemes designed to group soil organisms according to functional similarities or taxonomic relationships

(Blair *et al.*, 1996; Janion-Scheepers *et al.*, 2016). For example, organisms can be grouped according to the types of food they ingest. The most appealing schemes group organisms according to how they interact with the soil environment or group the major taxa (macro-, meso- and microfauna) according to their body size (Wallwork, 1970; Blair *et al.*, 1996). Figure 2-2 illustrates the classification of soil invertebrates based on size. Mesofauna are classified as any organism which is anywhere from 100 μm to 2 mm in body length (Blair *et al.*, 1996). Other studies look at fluctuations in mesofaunal communities, fluctuations can be the cause of migration, reproduction and mortality (Olivier & Ryke, 1965). However, to fully explain the fluctuations, knowledge on the different species' reproduction, development periods, lifespan and abiotic factors are required. Unfortunately, there is still a lack of knowledge of the ecology of these different species (Olivier & Ryke, 1965). Studies will typically make use of environmental factors such as, rainfall, temperature, moisture and wind to help explain changes within communities (Olivier & Ryke, 1965).

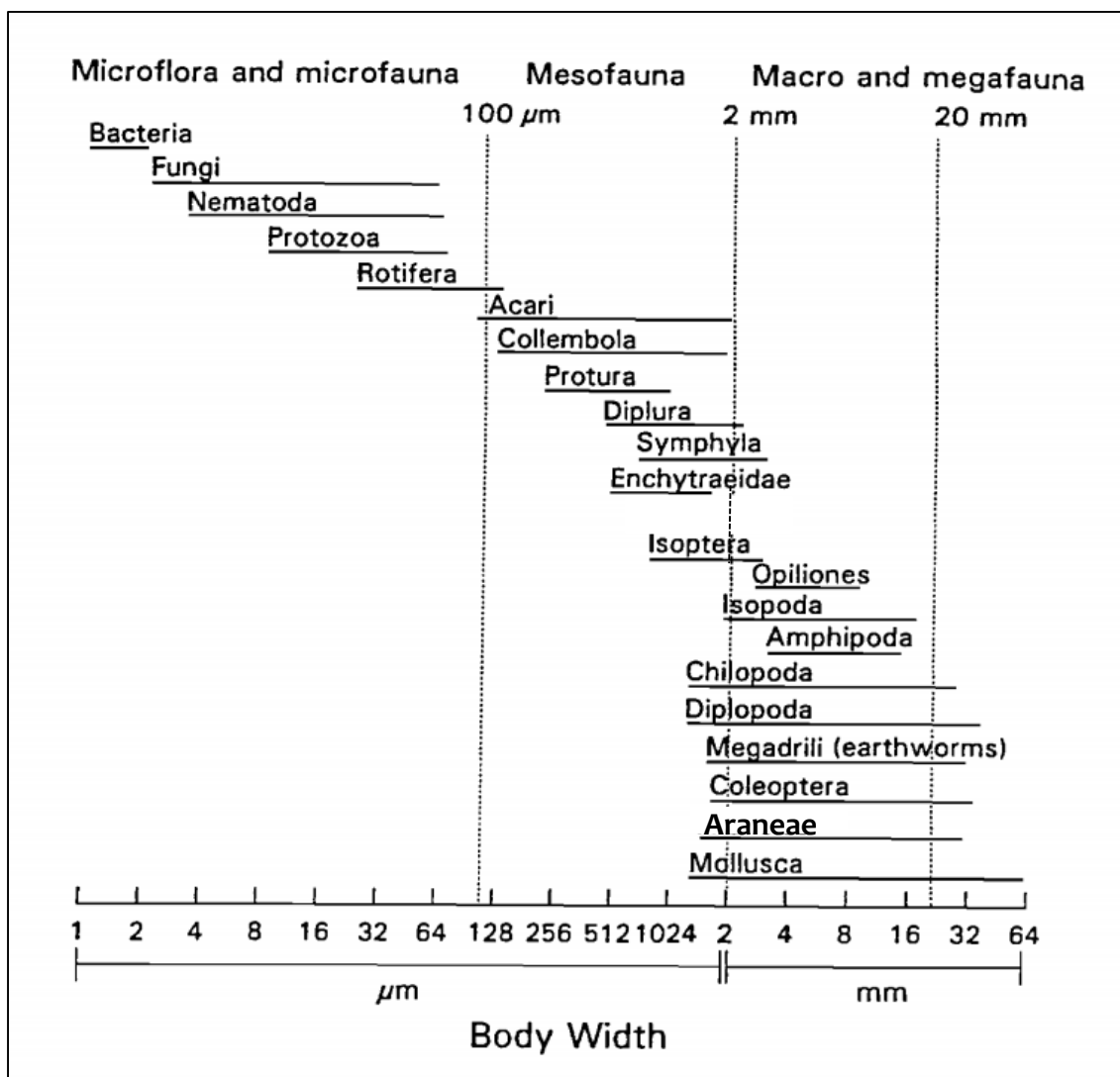


Figure 2-2: A graph showing soil invertebrates based on size (Blair *et al.*, 1996)

Soil invertebrates make the ideal bioindicators of the soil environment, because they can reflect changes in the presence of physical and chemical disturbances (Blair *et al.*, 1996; Longcore, 2003). Soil invertebrates have the ability to affect their surrounding environment, for example they can change soil structures, microbial patterns, alter the organic matter dynamics and influence nutrient cycling (Blair *et al.*, 1996; Janion-Scheepers *et al.*, 2016). But soil invertebrates are also affected by any drastic change within the soil environment and are unable to escape from the disturbance. Soil invertebrates tend to spend most of their life cycle, if not their entire life, within the soil (Wallwork, 1970), which makes them sensitive to any drastic changes in their environment. Thus they are useful in assessing the quality of soil and can also be used in the monitoring of rehabilitation sites, making them a great indicator of an area's conditions (Blair *et al.*, 1996; Longcore, 2003). Two ecological studies were done in 2012 by Wahl *et al.* (2012) and Jubileus *et al.* (2012) to assess platinum mine tailing by using soil Mesofauna and earthworms as biological indicators. These studies found the Prostigmata, Cryptostigmata and Mesostigmata to be the most abundant among the mite taxa, on platinum mine tailing dams. The number and diversity of species found in samples increased with the distance from the platinum tailing dams (Wahl *et al.*, 2012). Knowledge of soil communities can be vital for a variety of practices, including agriculture, food security and bioremediation (Janion-Scheepers *et al.*, 2016). Basic information on soil biota and their interaction with their environment can help to avoid poor decision making in natural resource management where mismanagement of natural resources can lead to reduced soil functionality, reduction in ecological services and in worse cases cause permanent damage to ecosystems (Janion-Scheepers *et al.*, 2016).

2.5.2 Sampling and extraction of soil mesofauna

Sampling of microarthropods (such as mites) can be difficult, due to their small size and they are often invisible to the human eye (Baker & Wharton, 1964). A variety of techniques and methods have been developed for sampling of soil invertebrates, for instance, the top layer of soil is usually collected because top soil contains a large variety of free-living soil microarthropods (Baker & Wharton, 1964). Olivier & Ryke (1965) stated that if the top layer of soil is decimated or dried, it is best to sample the top 5 cm to a 10 cm layer of soil. Because soil organisms tend to migrate downward in order to escape the dry heat of the sun, or because of the reduced moisture in the top soil (Olivier & Ryke 1965). Another commonly used method is surface or sub-surface pitfalls to catch species which are active on the soil surface (Smith *et al.*, 2008). When collecting soil samples, it is important to note the date and time of collection, description of habitat, temperature and humidity. Otherwise, the collection will have little value

(Baker & Wharton, 1964). Efficient extraction of edaphic invertebrates from soil remains a difficult challenge, most extraction methods can only help to obtain an estimation of the soil population (Smith *et al.*, 2008). Extraction methods usually involve the physical removal of organisms from soil, either by removing them through hand sorting, washing the soil through a fine sieve with water (Smith *et al.*, 2008). Or using a dynamic method (e.g. Berlese-Tullgren Funnel extraction technique) which relies on behavioural responses of organisms to a certain stimulus or stimuli (e.g. temperature, humidity) (Baker & Wharton, 1964; Smith *et al.*, 2008).

The Berlese-Tullgren funnel method is commonly used in the extraction of small arthropods (such as, mites) from soil and litter (Baker & Wharton, 1964; Smith *et al.*, 2008). The method was designed in 1905 by Italian acarologist, Antonio Berlese. The contraption consists of a large funnel, filter mesh placed on top of the funnel, sampling bottle or collecting pot containing ethanol underneath the funnel and a heat source (Antonio Berlese originally used a water jacket). Most recent versions use a light bulb as a heat source (Capinera, 2008). Figure 2-3 is a basic representation of how this method works – sample is placed on top of the mesh, the light bulb is used as a heat stimulus, which causes the organisms in the sample to move downward through the mesh to escape from the stimulus and fall through the funnel into the collection bottle (Smith *et al.*, 2008). The Berlese-Tullgren funnel is an effective extraction method, proven to extract approximately 80% of mesofauna from the sample (Smith *et al.*, 2008; Capinera, 2008).

2.5.3 Earthworms as bioindicators

Earthworms are cosmopolitan, found in most common of soils, and play a vital role in the maintenance of soil structure, fertility and function. Earthworms consume large quantities of plant material and soil, and normally stay in constant contact with the soil, thus reacting quickly to any natural or anthropogenic induced changes within their environment, making them a good choice for bioindicators of soil fertility and land use in ecotoxicological studies (Antunes *et al.*, 2008; Jubileus *et al.*, 2012). For example, the exposure of *Eisenia fetida* to soils with high concentrations of metals such as Cd and Pb, showed a loss of weight in juveniles, an extended time to reach sexual maturity and reduced production of earthworm cocoons (Zaltauskaite & Sodiene, 2014).

Earthworms belong to the phylum Annelida, subphylum Clitellata and thus the class Oligochaeta. Charles Darwin considered earthworms to play a vital role in the creation of soil structures, to increase soil fertility (Reinecke, 1992). Earthworms keep soil systems stable by altering soil structure through mixing faecal matter and decaying organic matter with the soil

(Reinecke, 1992; Blair *et al.*, 1996), they also influence the drainage, moisture holding capacity and aeration of soil (Reinecke, 1992). Due to this ability of earthworms to regulate organic matter and nutrient cycling and to change soil structure and influence microbial diversity, they are considered to be a vital part of soil formation (Enami *et al.*, 2001). It is of great importance to keep the earthworm population healthy in order to avoid severe soil degradation (Reinecke, 1992). Earthworms consume large quantities of plant material and soil and stay in constant contact within the soil. Thus earthworms are not just sensitive to the chemicals in their surrounding environment, but also accumulate a variety of chemicals within their tissues (Reinecke, 1992; Jongmans *et al.*, 2003). As a result, they react quickly to either natural or anthropogenic stressors within their surrounding environment, making earthworms an ideal early warning system of possible adverse changes (Antunes *et al.*, 2008). Earthworms are cosmopolitan and are commonly found in most soils (such as, garden soil), thus they are fairly easy to obtain (Reinecke, 1992). Earthworms are an ideal laboratory organism because they are both easy to breed and handle, making them exceptional test organisms to use in bioindication of chemicals in terrestrial environmental studies (Reinecke, 1992; Maboeta *et al.*, 2003; Van Gestel *et al.*, 2009).

Ecotoxicological tests are usually designed to determine the concentrations at which a selected pollutant becomes harmful, or triggers a negative response from the test organism (Reinecke, 1992). But it should be noted that even though a concentrated chemical may not kill the test earthworms, it could still drastically affect organisms higher up the food chain (Reinecke, 1992). Acute toxicology tests mainly focus on the mortality end point, because a clear correlation can be found between earthworm's mortality and environmental pollution (Kokta, 1992). Reproduction and growth are used as sub-lethal endpoints in acute toxicity; these end points are used in the test to measure the effects of pollution on population dynamics (Kokta, 1992). These sub-lethal endpoints tend to be sensitive, making them ideal for determining the effects of pollutants on populations (Kokta, 1992).

Reproduction is regarded as an important factor in assessing population dynamics, where cocoon production, hatching rate and juvenile survival are used as parameters to investigate levels of stress (Kokta, 1992). Contaminants such as, heavy metals and pesticides can accumulate within earthworm tissues and other organisms by moving through the food chain (Van den Brink, 2004). Earthworms are very efficient when it comes to accumulating metals, making them perfect for ecological studies on metals in soil (Morgan *et al.*, 1992). For instance, one can estimate the different types of metals within a chosen study area and compare it to the quantity of these metals which accumulated within a chosen earthworm species. Determining the bioaccumulation factor can give an idea of the degree of environmental pollution and the

concentration of pollutants which may accumulate within organisms (Morgan *et al.*, 1992; Svendsen *et al.*, 1996).

The Organisation for Economic Co-operation and Development (OECD) guideline comprises of ecotoxicological tests that can be used to determine at what concentrations a specific chemical becomes harmful to the environment, by exposing a chosen test organism to continuous exposures (OECD 222 Guideline, 2004). These tests provide successfully reproducible results because the same amount and species of test organisms are used (Reinecke, 1992). *Eisenia* species (such as, *E. andrei* and *E. fetida*) is a well-known earthworm species, which is found in most soils (such as garden and compost soils) and makes a popular choice for use in ecological risk assessment (ERA) studies. It is mainly due to their sensitivity to changes in soil ecosystems, that they make a good choice for indicators of soil quality and health (Paoletti, 1999; cited by Jubileus *et al.*, 2012). *Eisenia andrei* is also commonly used in ecological studies due to its ability to tolerate a wide range of temperature and moisture changes within the soil.

The avoidance behaviour test is a rapid screening method which can also be used to evaluate the functioning of soil environments and the influence of pollutants (such as, chemicals, metals, etc.) on the environment, by measuring earthworm behaviour (ISO, 2007). Unlike the reproductive test, the avoidance test requires less incubation time and is less labour intensive, while testing at a similar level of sensitivity than the reproduction test. However, the ISO (2007) guideline states that the avoidance test should not be used as a replacement for the reproduction test, but can be used along with a reproduction test.

CHAPTER 3

MATERIALS AND METHODS

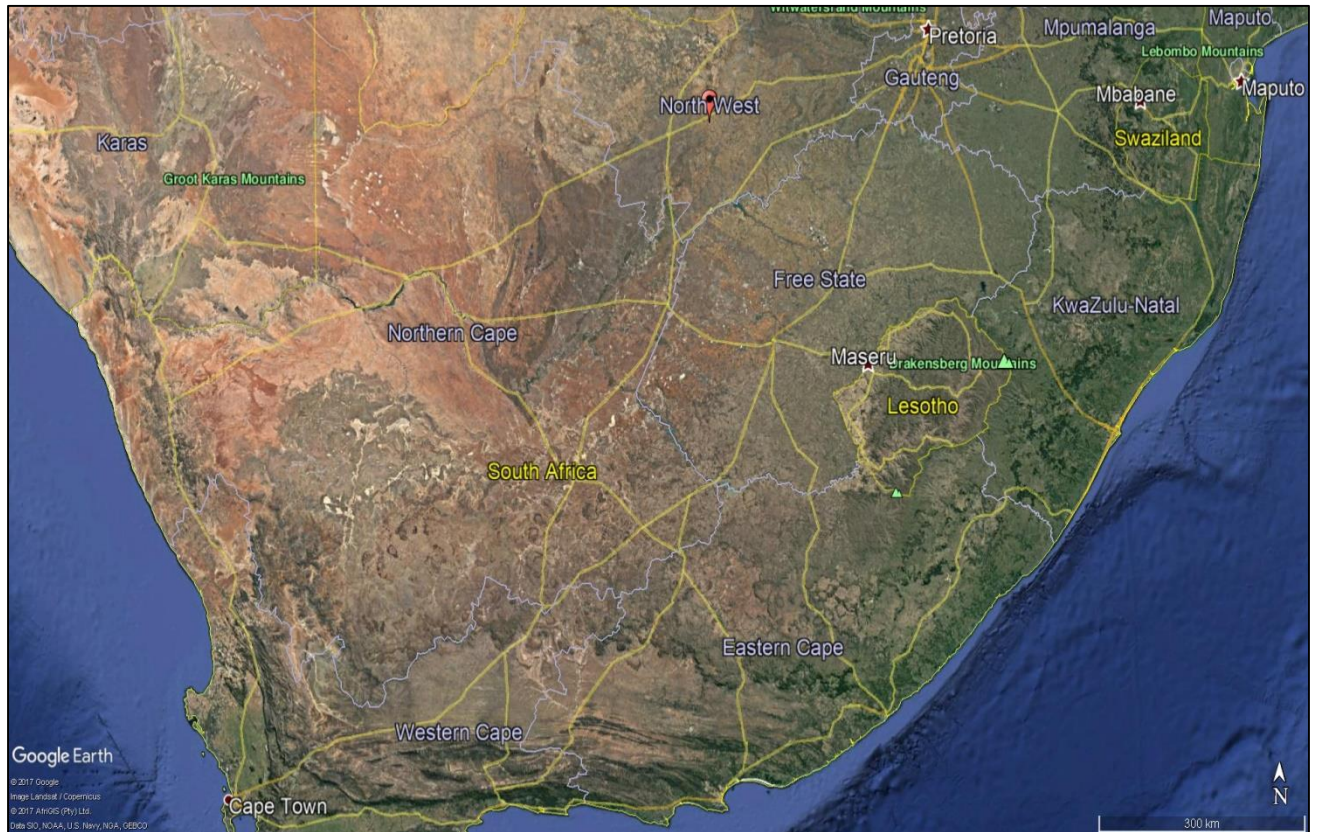


Figure 3-1: A map of South Africa showing the North West province (Google Earth, 2017)



Figure 3-2: Locality of the gold mine site (Google Earth, 2017).

The study site chosen for this study was an old abandoned gold mine. The mine is located within the North West Province, 22 km west-northwest of Potchefstroom and 24 km north of Stilfontein, at the following coordinates: 26°40'12.27"S 26°52'12.25"E. Kromdraai Spruit passes through the mining site and connects to the Koekemoer Spruit. The gold mine site falls under the KOSH gold mining region, which includes Klerksdorp, Orkney, Stilfontein and Hartebeestfontein (Koch, 2014).

3.1 History of the gold mine

The gold mine was opened in 1904 by local farmers and was mostly mined part-time until 1930. In 1940 the mine was officially closed down due to the lack of ability to cost-effectively extract gold (Aucamp & Van Schalkwyk, 2002). In 2009, the Department of Land Affairs donated the mine to the Tlokwe City Council. From 1904 to 1940 approximately 5200 kg of gold was mined (King *et al.*, 2007). After the closure of the mine a total of five tailing dam disposal facilities were constructed, a total of 2.5 million tons of TDF material was left behind without any form of management. Since the closure in 1940, the mine has been left undisturbed for 77 years. According to King *et al.* (2007), the mine was scheduled to reopen in 2003, but due to issues concerning the surface mining rights, the reopening was cancelled. During the last 77 years, the tailings dams became heavily eroded due to wind and water disturbance. Wind erosion led to

the dispersal of sheetwash and aeolian sowed slope into the surrounding environment (Aucamp & Van Schalkwyk, 2002). Tailing dam material was distributed into the surrounding environment, most likely by means of wind distribution, which led to the creation of small sand dunes. The TDF material has already spread over 1.1 km² to the southeast into the surrounding environment (Aucamp & Van Schalkwyk, 2002). In 2016, the mines were purchased by a mining company and re-opened. They are currently re-mining the tailing disposal facilities (started early in 2017).

3.2 Description of study site

For the purpose of this study four sites were selected at the discarded gold mine. Figure 3-3 is an aerial photograph of the mine showing the study sites. The sites consisted of two TDF sites and two pasture reference sites.

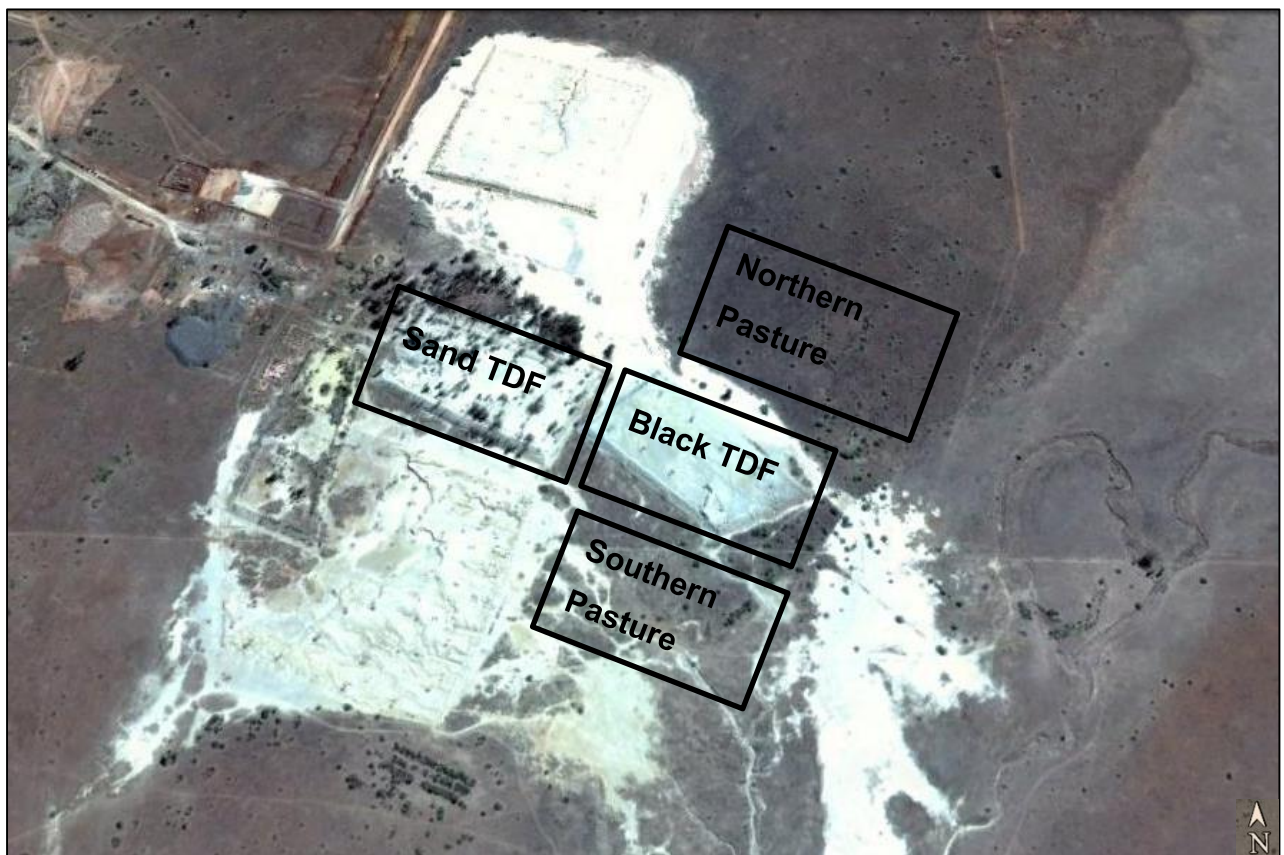


Figure 3-3: Aerial photograph from GoogleEarth (2016) of the gold mine complex, showing the four study sites.



Figure 3-4: Photograph of the black TDF.

The first TDF (TDF1 figure 3-4) has a greyish colour and hard cracked surface. There is no visible vegetation on the tailing's surface. Koch (2014) stated that the tailing dam contains high carbon content compared to the other tailings. This results in a darker or blackish appearance compared to the sand tailing and consists of 99% quartz (Koch, 2014). All pH readings of the study site were recorded in a laboratory. The pH of the top soil was 3.4.



Figure 3-5: Photograph of the sandy TDF.

The second TDF (TDF 2 figure 3-5) can be characterised by the beige colour of soil, the top texture is mostly sand-like and soft, but with certain areas being hard and cracked. *Eucalyptus* sp. trees can be found growing on top of the TDF 2 and on the slopes of the tailing. Reasonable amounts of organic matter were found beneath the *Eucalyptus* trees. A variety of grass species was also found growing on the sides of the tailing. The grass does not seem to grow on the tailing, but rather on a layer of sand which was deposited by the wind. Average top soil pH recorded of the tailing was around 3.5.



Figure 3-6: Photograph of the Northern Pasture.

Even though the pastures are used as representations of natural environments, the pastures are highly disturbed due to the wind distribution of tailing material. The northern pasture (figure 3-6) has a dense vegetation cover, but most of the grass species present are known to grow mainly in stressed environments. Vegetation consists mainly of *Cynodon dactylon*, *Seriphium plumosum* (formerly known as *Stoebe vulgarus*) and *Vachellia* species (found in low abundance). Both *Cynodon dactylon* (Couch grass) and *Seriphium plumosum* (Bankrupt bush) are well known for appearing in disturbed areas, especially in overgrazed areas. The average pH recording of the top soil was 4.6.



Figure 3-7: Photograph of the southern pasture.

The Southern pasture (figure 3-7) is more degraded than the northern pasture. Some of the tailing material was found scattered across this pasture, creating small sand deposits. Tailing material was distributed by the wind and over long periods of time created these dunes. Vegetation found mainly consisted of *Cynodon dactylon*, *Cymbopogon* sp. and *Cortaderia selloana*. Top soil pH recorded was 3.3.



Figure 3-8: Photograph of the area surrounding the mine disposal facilities.

Figure 3-8 is a photograph of the surrounding area, small sand structures can be seen in the photo and the patches of grass growing on them.

Vegetation found in the study area is mainly comprised of *Cymbopogon* and *Themeda* species. *Eucalyptus* trees dominate the surrounding area and are also found to grow near and on the Sand TDFs. The only indigenous trees found are of the *Rhus* species and *Vachellia karroo* (formally known as *Acacia karroo*) are present. *Cynodon dactylon* (Couch grass) grass is common to and in abundance on the study site. The grass is found growing in the two pasture sites and on the Sand tailing slope. The grass is regarded as a pioneer species which is relatively adaptive to environmental change; this species is also considered a “weed”. *Seriphium plumosum* was mainly present in the northern pasture. *Cortaderia selloana* (Pampas grass) is an invasive species, occurring in both dry and damp areas and also found growing in disturbed areas.

3.3 Climate

Worldweather (2013) was used to obtain a graph indicating the average rainfall, temperature (of each season) and annual wind distribution of Potchefstroom for 2013.

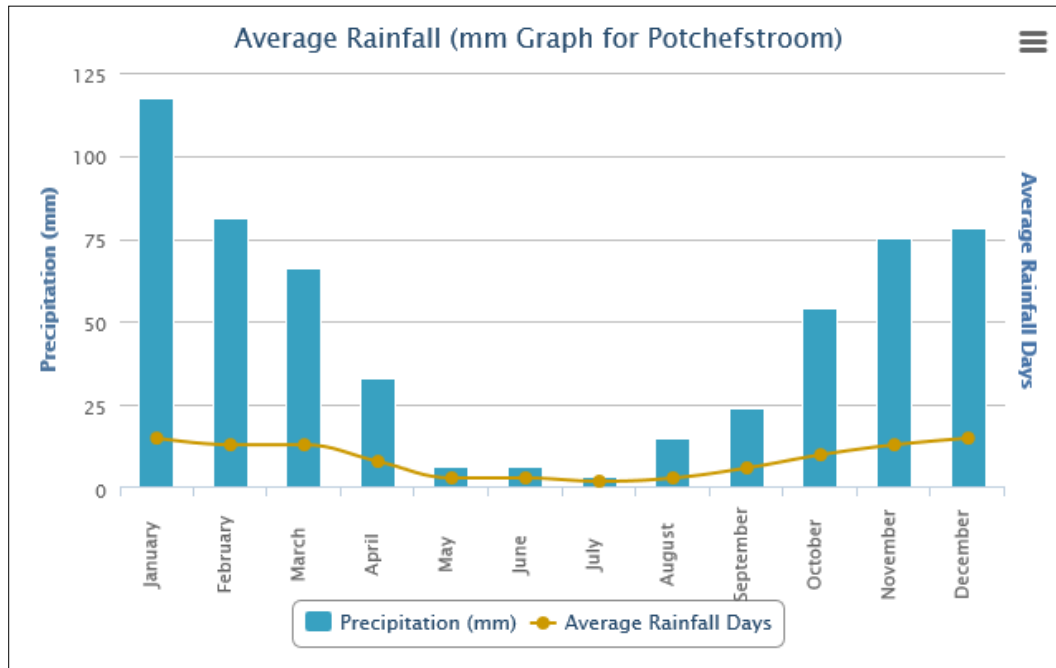


Figure 3-9: Graphical representation of the average rainfall per annum in Potchefstroom (2013). The graph was obtained from Worldweather (2013).

Figure 3-9 indicates that the average precipitation at the study site was 81 mm; the highest rainfall was recorded during the summer (January, February, March, October, November, December), while the lowest rainfall occurs during the winter (May, June, July, August). Figure 3-10 of the average temperature per annum, shows similar information where the average temperature ranges from 14.4°C to 27°C.

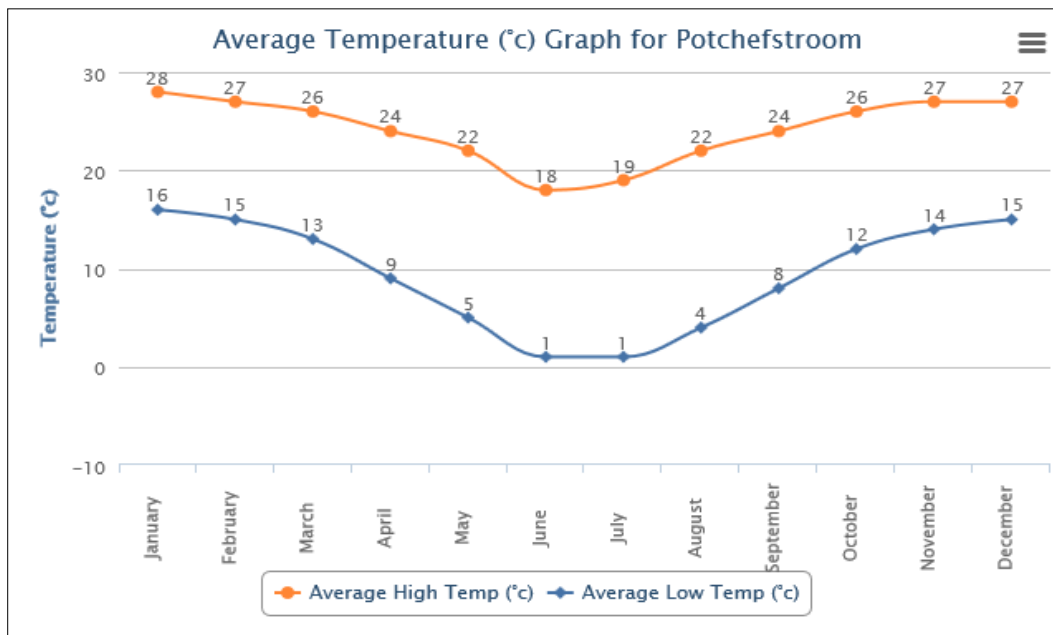


Figure 3-10: Graph depicting the average annual temperature of Potchefstroom in 2013. Obtained from Worldweather (2013).

During the summer the minimum average temperature was 16°C, with a maximum average of 28°C. In winter the minimum average temperature was 1°C, with a maximum average at 19°C.

The wind is on average distributed in a north-northeast and northeast direction at an average wind speed of 12 km per hour; wind speed rarely exceeds 6 km per hour in the western and southern directions.

3.4 Soil mesofauna

3.4.1 Preparation of soil material sampled

Samples were obtained from four different sampling sites, namely two from tailing storage facilities and the two from surrounding pastures. The latter were used as natural representations of the environment. At each, site six random samples were taken as follows: Each sampling site was divided into three plots (north, middle and south); two samples were taken in each of these three plots, giving a total of 24 sample plots. Only the top 10 cm of material was obtained by collecting 1000g of material per plot. Samples were placed into plastic bags and sealed using a thin nylon rope. Four total sample sets were taken, each representing a different season, autumn (March 2013), winter (July 2013), spring (October 2013) and summer (January 2014).

3.4.2 Extraction and identification of soil mesofauna from samples

Mesofauna was extracted from the soil using the Berlese-Tullgren funnel method, Figure 3-10 is a basic representation of how this apparatus is generally used. Soil material from each site was placed on top of a mesh, natural sunlight was used as a heat source to help drive the organisms downward. This slowly forced the mesofauna through the sieve to the bottom of the funnel. A plastic bottle (350 ml) containing 75% ethanol was placed underneath the funnel, collecting the organisms which fell through the funnel. Extraction was done indoors to prevent large changes in temperature, moisture and prevent wind disturbances. The duration of the extraction was seven days. Organisms collected were stored within the ethanol-containing bottles for identification and counting.



Figure 3-11: Berlese-Tullgren funnels used for extracting terrestrial mesofauna.

Each collection bottle content was poured into a petri-dish and viewed under a stereomicroscope, organisms were then counted and identified.. Organisms found were recorded into species lists and classified according to their order, family and species. Species lists were later divided into different functional groups according to their feeding habits.

3.5 Earthworm reproduction test

The earthworm reproduction test was done by following the provided OECD guideline 222 (OECD, 2004). The main purpose of the test is to determine the effects of certain pollutants

(such as heavy metals) within the soil, by investigating the sub-lethal endpoints. Sub-lethal endpoint includes the growth of the earthworms over a period of time, as well as their reproductive success and mortality.

Soil samples for the earthworm reproduction and avoidance test were taken from the same sites on the gold mine as those used for the soil mesofaunal extraction test. Soil material collected was air-dried and then sifted through a 2 mm sieve to ensure that the soil was evenly distributed. A total of three replicates per site and concentration were prepared with three controls. *Eisenia andrei* was the chosen test species for the purpose of this study.

The control soil was made from the artificial material according to the OECD guideline 222 (2004). The artificial soil consisted of 70% industrial silica sand (350 g), 20% kaolin clay (100 g), and 10% dried sphagnum peat (50 g) and were mixed together. If necessary up to 1% calcium carbonate can be added to adjust the soil pH as close to 5.5 to 6.0 as possible. Samples from each study site were divided into the four different concentrations (25%, 50%, 75% and 100%), of test material mixed with the artificial soil. Each of these concentrations consisted of three replicates per site. Each plastic container was filled with 500 g of soil material; distilled water was mixed into the soil ensuring a 60% maximum water holding capacity. Ten earthworms were placed into a plastic container; the worms were pre-weighed to ensure an average weight between 2.5 and 3 grams. The earthworms were stored for 28 days within an environmental chamber at a constant temperature. Every seven days each earthworm were removed from their respected containers and cleaned by washing them in a petri-dish and dried on paper towels. Cleaned earthworms were weighted before being returned to their container and fed dried horse manure (5 g per container).

At the end of the 28-day period, the mature earthworms were removed from the container, leaving only the juveniles and cocoons behind. At the end of every seven days, any unresponsive worms were prodded with a needle on the anterior side of the body. Worms that did not respond to the stimulus were counted as dead. Any worms missing from the containers will also be counted as dead. All of the earthworms were removed from their containers and sealed in marked Ziploc bags, the Ziploc bag were then placed into a deep freeze. Containers were returned to the environmental chamber and left for 56 days. After the 56-day waiting period the hatched and unhatched cocoons, as well as the juveniles were counted in order to determine the reproductive success.

3.6 Metal analysis

Metal analysis was done on substrate and earthworms taken from each of the exposures according to the EPA 3050b, protocols by the USEPA. Substrate from the control, two pasture sites and each of the different concentrations of the two TDFs, were oven dried at a temperature of 40°C. Two grams of dried substrate was weighed and added to a tube containing 10mL 65% distilled nitric acid (HNO₃). The earthworms were left to defrost, each worm was weighed. One whole earthworm was placed into a tube, 7mL 65% distilled nitric acid and 1 mL 30% hydrogen peroxide (H₂O₂) was added. Digestion of both the substrate and earthworms was done in the Maxi-44 ETHOS EASY, advanced microwave digestion system [Milestone s.r.l., Sorisole (BG), Italy]. The tube containing the samples were removed and left to cool, the solutions were then poured over into Falcon tubes and 1% of nitric acid was added to the volume up to 50mL. Each sample tube was filtered through a cellulose nitrate filtration paper and poured back into their respective Falcon tube. The heavy metal concentrations of each samples was measured by means of Inductively 7700X Coupled Plasma Mass Spectrometry (ICP/MS), (Agilent, CA, USA). The heavy metals that were analysed were Chromium (Cr), Cobalt (Co), Nickel (Ni), Copper (Cu), Zinc (Zn), Arsenic (As), Lead (Pb) and Cadmium (Cd). Afterwards the Bioconcentration factor (BCF) was determined by dividing the metal concentration in the earthworm with the metal concentration in the substrate. When the BCF is higher than one (>1), then bioaccumulation of the metal occurred in the earthworm tissue.

3.7 Avoidance test (ISO/FDIS°17512-1:2007)

As in the case of the reproduction test, the same species of earthworms (*Eisenia andrei*) were used, as well as the same soil material sampled at the mine. For the control, artificial soil was also prepared using the recipe provided by the OECD 222 guideline.

The avoidance test was done according to the ISO guideline (2007). Each container was divided into two equal sections by placing a plastic divider in the middle. One side of the container holds 250 grams of test material and the other contains 250 grams of artificial soil. Once the soil was added, the divider is removed and ten worms are placed per container. The worms were placed in the middle of the container on the dividing line. Containers with worms were placed within an environmental chamber for 48 hours, at a temperature between 19 °C and 20 °C. At the end of the 48 hour period, the test and control soil were separated with a plastic divider. Earthworms found on both sides of the containers were counted and recorded. The earthworms that were divided by the insertion of the divider are counted as half a worm (0.5); this is independent of the length of the remaining body. Dead and missing worms were recorded as escaped.

The soil material from each of the four test sites was diluted by mixing artificial soil with test soil in concentrations of 25%, 50%, 75% and 100%. The study consisted of five replicates per concentration.

3.8 Statistical analysis

Earthworm bioassay data were analysed by using the SigmaStat® program (SigmaStat for Windows Version 3.11, 2004 Systat Software Inc.). Differences between treatment groups were determined through one-way analysis of variance, using GraphPad Prism 5 Windows Version 5.00, (2005). The treatment groups data was rated to determine the error rate of $P < 0.05$. All the data were represented by the mean $\pm$ SD value. Mortality was determined by comparing the number of surviving worms with the number of dead worms at the end of the 28-day period.

All the soil invertebrates found were identified and sorted according to their order, family, genus and species (when possible). Statistical analysis was done by using Microsoft Excel 2003 and GraphPad Prism 5, to create most graphs and tables. Each of the four different sites was compared with one another. The total number of organisms of each order found in the entire study area was also calculated. The calculated number for each site was then converted into average and standard deviation (SD). The Shannon-Weaver-index was also calculated using for each sites using the collected numeral data. Four different seasons of mesofaunal data were compiled in a table and compared to each other.

CHAPTER 4

RESULTS

4.1 Soil pH

Table 4-1 shows the pH of each sampled site, including the control, as well as those of the black- and sand TDF at four different concentrations (25%, 50%, 75% and 100%). It is evident that the pH at the two TDFs (100%) was lower (more acidic) than the two natural pasture soils. The Black TDF material was overall the most acidic site, especially when compare to the other sites at the 100% concentration.

Table 4-1: The pH levels of the soil substrates collected from the black tailing disposal facility (BTDF), sand tailing disposal facility (STDF), pasture north (PN), pasture south (PS) and control.

Soil pH at different concentrations				
Site	25%	50%	75%	100%
BTDF	4.43	3.96	3.9	3.65
STDF	5.22	4.98	4.19	3.83
PS	-	-	-	4.10
PN	-	-	-	3.97
Control	-	-	-	5.91

4.2 Metal analysis

Table 4-2 summarises the results of the total metal concentrations of each site and control, earthworm tissue metal concentrations and the bioconcentration factor (BCF). The bioconcentration factor is the concentration of a contaminant (e.g. Chromium) divided by the total concentration of the contaminant in the soil. The BCF was calculated to determine the amount of heavy metal accumulation within the earthworm's tissues.

All of the earthworm metal analysis results from the control, reference sites and the two TDF sites were compared to one another. The metal analysis results were also measured against the benchmarks used by Efroymson *et al.* (1997). These benchmarks represent the metal concentrations that are toxic to earthworms, the benchmark is shown in the table as E/W. Soil microorganisms (SMO) and microbial processes (MP) are both benchmarks of soil toxicity (Efroymson *et al.*, 1997). According to Efroymson *et al.* (1997), the E/W benchmark is composed of numerous acute toxicity tests, which benchmarked the concentrations of heavy metals and chemicals that are harmful to earthworms. The SMO and MP benchmarks were created by measuring the effects of contaminants on the enzyme activities, C-Mineralisation and N-Transformation on microorganisms and their activities (Efroymson *et al.*, 1997). The total metal concentrations of all the substrates were compared to the South African Soil Screening Values (SSV). These values represent the protective standard for both human health and ecotoxicological risk. The soil screening values provide a minimum standard for assessing necessary environmental protection. However, these values are not necessarily the absolute minimum values or the default remediation levels (Eijsackers *et al.*, 2014). The total metal concentrations of each exposure were compared to the SSV1 benchmark, in order to determine whether the soil is contaminated or not.

Table 4-2: Soil metal concentrations ($\mu\text{g g}^{-1}$) for control, the two different reference sites, pastures north (PN) and south (PS), black tailing dam (BTDF) and sand tailing dam (STDF). Reference and tailing material were all measured against the following benchmarks: TMT, TIL, SSV1. Earthworm tissue metal concentrations were compared to E/W values, SMO and MP (Efroymsen *et al.*, 1997). Earthworm BCF was measured in $\mu\text{g g}^{-1}$ e.g. (i.e. $\text{BCF} = [\text{contaminant in biota}] / [\text{total material concentration}]$).

		Cr	Co	Ni	Cu	Zn	As	Pb	Cd
E/W SMO and MP SSV1		0.4	-	200	50	200	60	500	20
		10	1000	90	100	100	100	900	20
		6.5	300	91	16	240	5.8	20	7.5
Control	Substrate	2.82±0.34 ^a	0.54±0.04 ^a	1.29±0.02 ^a	1.65±0.08 ^a	2.43±0.31 ^a	0.28±0.01 ^a	0.02±0.01 ^a	0.01±0.00 ^a
	EW	0.08±0.028 ^a	0.02±0.00 ^a	0.05±0.02 ^a	0.61±0.2 ^a	0.51±0.07 ^a	0.08±0.02 ^a	0.00±0.00 ^a	0.07±0.02 ^a
	BCF	0.03	0.04	0.03	0.37	0.21	0.30	0.00	7.30
PS	Substrate	3.43±0.2.87 ^{ac}	0.20±0.15 ^a	0.60±0.43 ^a	1.08±0.81 ^a	1.38±0.91 ^a	1.94±1.32 ^a	0.01±0.00 ^a	0.01±0.00 ^a
	EW	0.07±0.05 ^a	0.04±0.01 ^a	0.09±0.07 ^a	1.24±1.11 ^a	0.59±0.39 ^a	0.15±0.04 ^a	0.00±0.00 ^a	0.21±0.18 ^a
	BCF	0.02	0.43	0.17	0.92	0.51	0.13	0.01	19.84
PN	Substrate	11.59±2.75 ^b	0.79±0.08 ^a	1.66±0.19 ^a	1.67±0.16 ^a	2.09±0.04 ^a	1.35±0.01 ^a	0.01±0.00 ^a	0.01±0.00 ^{ac}
	EW	0.08±0.03 ^a	0.02±0.00 ^a	0.05±0.01 ^a	0.65±0.24 ^a	0.55±0.19 ^a	0.14±0.05 ^a	0.00±0.00 ^a	0.01±0.04 ^a
	BCF	0.01	0.03	0.03	0.40	0.26	0.10	0.01	9.92
BTDF (25%)	Substrate	4.64±0.60 ^b	0.49±0.03 ^a	1.12±0.04 ^a	1.51±0.07 ^a	1.94±0.18 ^a	1.09±0.02 ^a	0.01±0.00 ^a	0.01±0.00 ^a
	EW	0.09±0.04 ^a	0.02±0.00 ^a	0.05±0.02 ^a	1.12±0.35 ^a	0.44±0.09 ^a	0.11±0.06 ^a	0.00±0.00 ^a	0.18±0.06 ^a
	BCF	0.02	0.04	0.05	0.74	0.23	0.10	0.00	25.07
BTDF (50%)	Substrate	5.71±3.46 ^{ad}	0.38±0.16 ^a	0.98±0.39 ^a	1.53±0.77 ^a	1.74±0.66 ^a	3.20±2.93 ^a	0.01±0.01 ^a	0.02±0.01 ^b
	EW	0.10±0.01 ^a	0.03±0.00 ^a	0.05±0.02 ^a	1.25±0.18 ^a	0.44±0.07 ^a	0.09±0.01 ^a	0.00±0.00 ^a	0.19±0.01 ^a
	BCF	0.02	0.08	0.07	1.00	0.30	0.05	0.02	18.73

BTDF (75%)	Substrate	4.78±1.34 ^{ad}	0.35±0.04 ^a	1.18±0.13 ^a	1.52±0.25 ^c	1.76±0.18 ^a	2.24±0.08 ^a	0.01±0.00 ^a	0.01±0.00 ^a
	EW	0.10±0.03 ^a	0.03±0.00 ^a	0.06±0.03 ^a	0.88±0.33 ^a	0.42±0.09 ^a	0.08±0.01 ^a	0.00±0.00 ^a	0.39±0.37 ^a
	BCF	0.02	0.07	0.05	0.62	0.24	0.04	0.01	49.56
BTDF (100%)	Substrate	8.11±1.34 ^{ad}	0.35±0.04 ^a	1.11±0.03 ^a	1.45±0.19 ^a	1.66±0.15 ^a	2.69±0.16 ^a	0.01±0.00 ^a	0.01±0.00 ^a
	EW	0.09±0.10 ^a	0.04±0.01 ^a	0.16±0.23 ^a	7.84±13.42 ^b	3.611±5.72 ^a	0.17±0.07 ^a	0.00±0.01 ^a	7.23±12.51 ^b
	BCF	0.01	0.11	0.14	4.69	1.99	0.06	0.03	1061.55
STDF (25%)	Substrate	4.66±1.16 ^{ad}	0.49±0.01 ^a	1.17±0.07 ^a	1.64±0.04 ^a	1.86±0.25 ^a	1.00±0.02 ^a	0.02±0.00 ^a	0.01±0.00 ^a
	EW	0.11±0.10 ^a	0.02±0.00 ^a	0.05±0.04 ^a	1.87±2.90 ^a	1.21±1.47 ^a	0.08±0.00 ^a	0.00±0.00 ^a	2.26±3.45 ^a
	BCF	0.03	0.03	0.04	1.17	0.73	0.08	0.00	369.18
STDF (50%)	Substrate	3.5±0.35 ^c	0.41±0.03 ^a	1.10±0.06 ^a	1.61±0.16 ^a	1.72±0.09 ^a	1.46±0.09 ^a	0.01±0.00 ^a	0.01±0.00 ^a
	EW	0.09±0.10 ^a	0.02±0.00 ^a	0.03±0.02 ^a	0.66±0.53 ^a	0.45±0.20 ^a	0.11±0.02 ^a	0.00±0.00 ^a	0.42±0.39 ^a
	BCF	0.02	0.05	0.02	0.40	0.26	0.08	0.00	39.67
STDF (75%)	Substrate	5.65±1.90 ^{ad}	0.41±0.01 ^a	1.15±0.03 ^a	1.57±0.04 ^a	1.71±0.06 ^a	2.29±0.23 ^a	0.01±0.00 ^a	0.01±0.00 ^a
	EW	0.01±0.00 ^a	0.01±0.00 ^a	0.01±0.00 ^a	0.06±0.01 ^{ad}	0.21±0.00 ^a	0.09±0.01 ^a	0.00±0.00 ^a	0.00±0.00 ^a
	BCF	0.00	0.03	0.01	0.04	0.12	0.04	0.00	0.22
STDF (100%)	Substrate	5.20±1.24 ^{ad}	0.42±0.07 ^a	1.40±0.17 ^a	1.47±0.12 ^a	1.88±0.21 ^a	2.82±0.19 ^a	0.01±0.0 ^a	0.01±0.00 ^a
	EW	0.02±0.00 ^a	0.02±0.00 ^a	0.01±0.00 ^a	0.08±0.03 ^a	0.21±0.00 ^a	0.15±0.03 ^a	0.00±0.00 ^a	0.02±0.00 ^a
	BCF	0.00	0.04	0.01	0.05	0.11	0.05	0.00	0.28

(a-d): Values sharing the same letter within columns in superscript were not statistically different (P>0.05)

Total concentrations of Cr exceeded the SSV1 benchmark in both the PN and BTDF (100%) exposures, while the rest of the tested heavy metals were all below their individual SSV1 benchmark values. The total Cr concentrations exceeded the E/W benchmark in the control, PS, PN, BTDF (25%, 50%, 75% and 100%) exposures, while Co, Ni, Cu, Zn, As, Pb and Cd did not exceed the E/W benchmark in any of the exposures. The total concentrations of all the tested heavy metals did not exceed the SMO and MP benchmarks in any of the exposures and control.

The total measured Cr concentration in PN (11.59 ± 2.75), BTDF 25% (4.64 ± 0.60) and STDF 50% (3.5 ± 0.35) were significantly higher ($p < 0.05$) than that in the control (2.82 ± 0.34). The total concentration of Cr measured in PS site was significantly lower ($p < 0.05$) compared to the PN (11.59 ± 2.75), BTDF 25% (4.64 ± 0.60), 50% (5.71 ± 3.46), 75% (4.78 ± 1.34), 100% (8.11 ± 1.34), STDF 25% (4.66 ± 1.16), 75% (5.65 ± 1.90) and 100% (5.20 ± 1.24) exposures. The total amount of Cr in PN was significantly higher ($p < 0.05$) than the BTDF 50%, 75%, 100%, STDF 25%, 50%, 75% and 100% exposures. The total Cr in the BTDF 25% exposure was significantly lower ($p < 0.05$) than in the BTDF 50%, 75%, 100%, 25%, 75%, 100% exposures and significantly higher ($p < 0.05$) than the STDF 50% exposure. The STDF 50% had a significantly lower ($p < 0.05$) concentration of Cr when compared to BTDF 75%, 100%, STDF 25%, 50% and 100% exposures. The concentration of Cd in the BTDF 50% (0.02 ± 0.01) exposure was significantly higher when compared to the control (0.01 ± 0.00), PN (0.01 ± 0.00), PS (0.01 ± 0.00), BTDF 25% (0.01 ± 0.00), 75% (0.01 ± 0.00), 100% (0.01 ± 0.00), STDF 25% (0.01 ± 0.00), 50% (0.01 ± 0.00), 75% (0.01 ± 0.00), 100% (0.01 ± 0.00). The following heavy metals; Co, Ni, Cu, Zn, As and Pb showed no significant difference ($p > 0.05$) when compared to their specific control and other exposures.

Based on the total metal concentrations found in each exposure the following trend was determined, showing the metals from the highest to the lowest concentration: $\text{Cr} > \text{As} > \text{Zn} > \text{Cu} > \text{Ni} > \text{Co} > \text{Pb} > \text{Cd}$. The trend was based on the total concentration of all the metals in each exposure: $\text{PN} > \text{BTDF (100\%)} > \text{BTDF (50\%)} > \text{STDF (100\%)} > \text{STDF (75\%)} > \text{BTDF (75\%)} > \text{STDF (25\%)} > \text{BTDF (25\%)} > \text{STDF (50\%)} > \text{Control} > \text{PS}$.

The average Cu concentration within the earthworm tissues measured in the BTDF 100% (7.84 ± 13.42) was significantly higher ($p < 0.05$) compared to the control (0.61 ± 0.2), PN (0.65 ± 0.24), PS (1.24 ± 1.11), STDF 25% (1.87 ± 2.90), 50% (0.66 ± 0.53), 75% (0.06 ± 0.01), 100% (0.08 ± 0.03), BTDF 25% (1.12 ± 0.35), 50% (1.25 ± 0.19) and 75% (0.88 ± 0.33). Cd measured within the earthworm tissue was significantly higher ($p < 0.05$) in the BTDF 100% (7.23 ± 12.51) exposure compared to the control (0.07 ± 0.02), PN (0.01 ± 0.04), PS (0.21 ± 0.18), BTDF 25% (0.18 ± 0.06), 50% (0.19 ± 0.01), 75% (0.39 ± 0.37), STDF 25% (2.26 ± 3.45), 50% (0.42 ± 0.39), 75% (0.00 ± 0.00) and 100% (0.02 ± 0.00). The following metals; Co, Ni, Cu, Zn, As, Pb, showed no significant difference ($p > 0.05$) when compared to the TDF and the two pasture exposures to the

control earthworms. Based on the total metal concentrations found in the test soils the following trend from high to low was determined: Cd>Cu>Zn>As>Cr>Ni>Co>Pb. The trend was based on the total concentration of all the metals in earthworm tissue of each exposure: BTDF(100%)>STDF(25%)>PS>BTDF(50%)>BTDF(25%)>BTDF(75%)>STDF (50%)>PN>Control>STDF (100%)>STDF (75%).

4.3 Earthworm bioassays

4.3.1 Earthworm growth over time

Table 4-3 shows the biomass (g) of earthworms over a 28-day exposure period and compares the average weights of earthworms from each of the different exposures. The average weight of the earthworms was recorded each week and compared against each other within each group.

Table 4-3: Measured weight of *Eisenia andrei* over a 28 day period exposure in two contaminated soil samples, control and two reference sites. Averages, standard deviation ($\pm$ SD) and the relative growth rate (RGR) of *Eisenia andrei* exposed to the control material, the reference site material pasture north (PN) and south (PS), and the black (BTDF) and sand TDF (STDF) are used in the table below. Black TDF and Sand TDF were tested at different concentrations (25%, 50%, 75% and 100%) of contaminated soil to the artificial soil.

Growth (g)						Relative growth rate (%)
Day 1	Day 7	Day 14	Day 21	Day 28		
25%						
Control	0.43±0.10 ^a	0.43±0.09 ^a	0.43±0.07 ^a	0.41±0.07 ^b	0.38±0.07 ^b	-13.82*
PN	0.44±0.10 ^a	0.48±0.11 ^a	0.46±0.10 ^b	0.43±0.09 ^b	0.41±0.09 ^b	-7.79
PS	0.43±0.08 ^a	0.44±0.09 ^a	0.44±0.09 ^{ab}	0.42±0.09 ^{ab}	0.39±0.09 ^b	-6.43
B-TDF	0.44±0.10 ^a	0.46±0.09 ^a	0.42±0.09 ^{ab}	0.40±0.08 ^a	0.37±0.08 ^b	-15.67*
S-TDF	0.40±0.14 ^a	0.42±0.12 ^a	0.40±0.11 ^a	0.40±0.10 ^a	0.35±0.08 ^a	-12.66*
50%						
Control	0.43±0.10 ^a	0.43±0.09 ^a	0.43±0.07 ^a	0.41±0.07 ^a	0.38±0.07 ^a	-13.82*
PN	0.44±0.10 ^a	0.48±0.11 ^a	0.46±0.10 ^b	0.43±0.09 ^b	0.41±0.09 ^b	-7.79
PS	0.43±0.08 ^a	0.44±0.09 ^a	0.44±0.09 ^{ab}	0.42±0.09 ^{ab}	0.39±0.09 ^b	-6.43
B-TDF	0.45±0.09 ^a	0.46±0.08 ^a	0.44±0.08 ^{ab}	0.41±0.08 ^a	0.40±0.08 ^b	-9.81
S-TDE	0.32±0.08 ^b	0.37±0.09 ^a	0.35±0.06 ^a	0.38±0.08 ^a	0.37±0.06 ^b	+14.69

75%						
Control	0.43±0.10 ^a	0.43±0.09 ^a	0.43±0.07 ^a	0.41±0.07 ^a	0.38±0.07 ^a	-13.82*
PN	0.44±0.10 ^a	0.48±0.11 ^a	0.46±0.10 ^b	0.43±0.09 ^b	0.41±0.09 ^b	-7.79
PS	0.43±0.08 ^a	0.44±0.09 ^a	0.44±0.09 ^{ab}	0.42±0.09 ^{ab}	0.39±0.09 ^b	-6.43
B-TDF	0.41±0.11 ^a	0.40±0.11 ^a	0.39±0.12 ^a	0.37±0.10 ^a	0.36±0.09 ^a	-12.64
S-TDF	0.30±0.03 ^b	0.36±0.05 ^c	0.35±0.05 ^b	0.37±0.05 ^b	0.36±0.05 ^b	+17.67*
100%						
Control	0.43±0.10 ^a	0.43±0.09 ^a	0.43±0.07 ^a	0.41±0.07 ^a	0.38±0.07 ^a	-13.82*
PN	0.44±0.10 ^a	0.48±0.11 ^a	0.46±0.10 ^b	0.43±0.09 ^b	0.41±0.09 ^b	-7.79
PS	0.43±0.08 ^a	0.44±0.09 ^a	0.44±0.09 ^{ab}	0.42±0.09 ^{ab}	0.39±0.09 ^b	-6.43
B-TDF	0.30±0.10 ^b	0.30±0.11 ^b	0.28±0.10 ^a	0.26±0.09 ^a	0.25±0.10 ^a	-17.34*
S-TDF	0.30±0.09 ^b	0.29±0.11 ^b	0.29±0.11 ^a	0.28±0.10 ^a	0.28±0.10 ^a	-7.36

(a-b): Values sharing the same letter within columns in superscript were not statistically different ($P>0.05$). The RGR values with an asterisk (*) were statistically different ($P<0.05$) from the initial body weight (day 1)

On day 1 only BTDF 75% (0.41±0.11), 100% (0.30±0.10) and STDF 100% (0.30±0.09) was significantly lower ($p<0.05$) than the control (0.43±0.10). Day 7 BTDF 75% (0.40±0.11), 100% (0.30±0.11) and STDF 100% (0.29±0.11) was significantly lower ($p<0.05$) than the control (0.43±0.09). The STDF 100% (0.29±0.11) was significantly lower ($p<0.05$) compared to the BTDF 75% (0.40±0.11) and 100% (0.30±0.11) exposures. Day 14 the PS (0.44±0.09), PN (0.46±0.10) and BTDF 50% (0.44±0.08) exposures were significantly higher ($p<0.05$) than the control (0.43±0.07). PN and PS are also significantly different from each other ($p<0.05$). Day 21 PN (0.43±0.09) and PS (0.42±0.09) exposures were significantly higher ($p<0.05$) than the control (0.41±0.07). The PN and PS exposures were also significantly different from each other ($p<0.05$). Day 28 PN (0.41±0.09), PS (0.39±0.09), BTDF 25% (0.37±0.08), exposures were significantly higher ($p<0.05$) than the control (0.38±0.07). While the BTDF 50% (0.40±0.08), STDF 50% (0.37±0.06) and 75% (0.36±0.05) exposures were significantly lower ($p<0.05$) than the control (0.38±0.07).

The relative growth rate was determined to see whether there was a significant increase or decrease in total body weight. The relative growth rate was determined for each exposure, by calculating the difference between earthworm weight on the 28th day with the initial earthworm weight. Starting at the 25% concentration exposures, the control (-3.82%), BTDF (-15.67%) and STDF (-12.66%) showed a significant difference ($p<0.05$) when compared to the initial weight. With the 50% concentration exposures, only the control (-13.82%) showed a significant decrease ($p<0.05$) in weight. The 75% concentration exposures showed a significant decrease in earthworm weight at the control (-13.82%), while the STDF (+17.67%) had a significant increase ($p<0.05$) in total body weight. The 100% concentration exposures showed a significant decrease in body weight between the control (-13.82%) and BTDF (-17.34%). Based on the final weight of the earthworms on day 28 the following trend was determined: BTDF

(100%)>STDF (100%)>STDF (25%)>BTDF (75%)>STDF (75%)>STDF (50%)>BTDF (25%)>PS>Control>BTDF (50%)>PN. The trend starts with the exposure that did the worst (i.e. earthworms with the lowest average weight).

4.3.2 Earthworm reproduction

The results of the total counted cocoons, juveniles per cocoons and the percentage (%) of hatched cocoons are shown in table 4-4. The data are represented as the mean and standard deviation ($\pm$ SD).

Table 4-4: Measured reproduction success of earthworm placed in gold mine tailing material (black TDF and sand TDF), as well as two reference sites (pasture north and south) and an artificial control soil. The two TDFs impacts on reproduction were measured at four different concentrations (25%, 50%, 75% and 100%). The table compares the number of cocoons produced and juveniles hatched.

	Mean number of cocoons per exposure	Percentage of hatched cocoons (%)	Mean number of juveniles per cocoon
Control	20.33 $\pm$ 4.04 ^a	89.68 $\pm$ 5.40 ^a	5.44 $\pm$ 0.14 ^a
PS	14.00 $\pm$ 2.00 ^a	100 $\pm$ 0.00 ^b	4.48 $\pm$ 1.04 ^b
PN	25.33 $\pm$ 9.29 ^a	99.07 $\pm$ 1.60 ^a	5.18 $\pm$ 0.87 ^a
BTDF (25%)	23.00 $\pm$ 9.00 ^a	96.02 $\pm$ 3.45 ^a	3.89 $\pm$ 1.32 ^a
BTDF (50%)	15.00 $\pm$ 6.10 ^a	93.33 $\pm$ 11.55 ^a	3.23 $\pm$ 0.84 ^b
BTDF (75%)	1.00 $\pm$ 1.00 ^b	83.33 $\pm$ 28.87 ^a	0.83 $\pm$ 0.76 ^a
BTDF (100%)	0.33 $\pm$ 0.58 ^b	66.67 $\pm$ 57.74 ^a	0.00 $\pm$ 0.00 ^a
STDF (25%)	11.67 $\pm$ 1.16 ^a	96.97 $\pm$ 5.25 ^a	5.51 $\pm$ 1.74 ^b
STDF (50%)	4.67 $\pm$ 0.58 ^a	100 $\pm$ 0.00 ^b	4.07 $\pm$ 0.31 ^a
STDF (75%)	0.67 $\pm$ 0.58 ^b	100 $\pm$ 0.00 ^b	1.67 $\pm$ 1.53 ^a
STDF (100%)	0.67 $\pm$ 0.58 ^b	100 $\pm$ 0.00 ^b	1.00 $\pm$ 1.00 ^a

(a-b): Values sharing the same letter in superscript were not statistically different ($P>0.05$)

The mean number of cocoons produced in the control (20.33 $\pm$ 4.04), PS (14.00 $\pm$ 2.00), PN (25.33 $\pm$ 9.29), BTDF 25% (23.00 $\pm$ 9.00), 50% (15.00 $\pm$ 6.10), STDF 25% (11.67 $\pm$ 1.16), and 50% (4.67 $\pm$ 0.58) exposures were significantly ($p<0.05$) higher than the number of cocoons found in the BTDF 75% (1.00 $\pm$ 1.00), 100% (0.33 $\pm$ 0.58), STDF 75% (0.67 $\pm$ 0.58) and 100% (0.67 $\pm$ 0.58) exposures. No significant differences ($p>0.05$) were found when comparing the control to the BTDF 25%, 50%, STDF 25%, 50% and two pasture sites (PS and PN). The percentage of

hatched cocoons in the control (89.68 ± 5.40), PN (99.07 ± 1.60), BTDF 25% (96.02 ± 3.45), 50% (93.33 ± 11.55), 75% (83.33 ± 28.87) and 100% (66.67 ± 57.74) exposures were all significantly ($p < 0.05$) lower than the percentage of hatched cocoons in the PS (100 ± 0.00), STDF 25% (100 ± 0.00), 50% (100 ± 0.00), 75% (100 ± 0.00) and 100% (100 ± 0.00) exposures. It should be noted that even though the STDF 50%, 75% and 100% exposures had a 100% hatch percentage, they had a very low cocoon and juvenile count. The BTDF 100% exposure shows an average 66.67% hatchability, only one of the three replicates had one unhatched cocoon. The mean number of juveniles per cocoon in the control (5.44 ± 0.14) were significantly ($p < 0.05$) higher than the PS (4.48 ± 1.04) and BTDF 50% (3.23 ± 0.84) exposures, while significantly lower ($p < 0.05$) than the STDF 25% (5.51 ± 1.74) exposure. The PN, BTDF 25%, 75%, 100%, STDF 50%, 75% and 100% exposures were not significantly ($p > 0.05$) different from the control. The following trend was determined by comparing the juveniles that hatched per cocoon in each exposure, the exposures are ordered from high to low: STDF (25%)>Control>PN>PS>STDF (50%)>BTDF (25%)>BTDF (50%)>STDF (75%)>STDF (100%)>BTDF (75%)>BTDF (100%).

4.3.3 Earthworm mortality

The mortality percentage of the earthworms was recorded each week up to the 28th day. The mortality of earthworms on day 7 was 3%, but this early in the experiment the earthworm most likely went missing. No mortality was recorded in soils taken from the two natural reference sites (PS and PN). Earthworms exposed to the BTDF material showed a mortality of 6% on day 21 in the 25% exposure, 3% mortality in the 50% exposure on day 28, 13% mortality in the 75% exposure between day 21 and 28 and a mortality of 3% in the 100% exposure on day 14. Earthworms exposed to STDF material showed no mortality in the 25 % exposure, 50% exposure had a 3% mortality on day 14, mortality was 3% in the 75% exposure on day 21 and 100% exposure had a 3% mortality on day 14. Based on the mortality percentages the following trend was determined: BTDF>STDF>Control>PS=PN.

4.3.4 Avoidance assay

Figure 4-1 shows the result of the two-week avoidance test done with *Eisenia andrei*. After two weeks the numbers of earthworms present in both the control and test material side of the container were compared, each concentration was compared separately. Values are presented in a graph as means and SD.

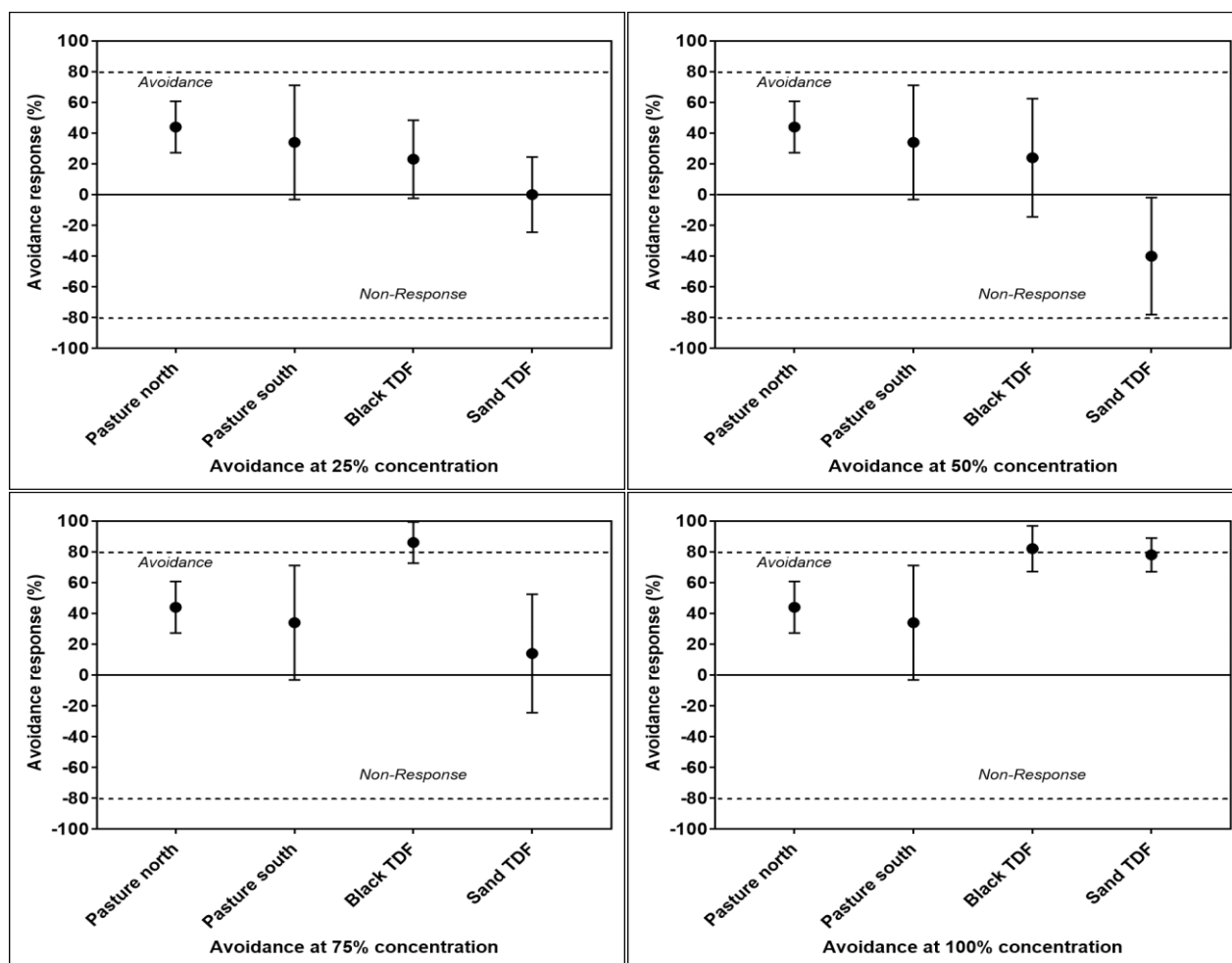


Figure 4-1: Results of the avoidance test performed with *Eisenia andrei*, when exposed to gold mine tailing material (black- and sand TDF were tested at four different concentrations), two reference sites (pasture-, north and south) and a control. Tailing material is compared to both the reference sites (at 100% concentration) and control.

At the 25% concentration, the earthworms showed avoidance behaviour in all of the exposures, except in the sand TDF where the earthworms were almost equally distributed in both sides. At the 50% concentration, all exposures showed a slight avoidance response, except for in the sand TDF where most of the earthworms were found in the test side. The 75% concentration showed avoidance response in all of the exposures, black TDF had the largest avoidance percentage which was above the 80% threshold. In the 100% concentration, all the exposures showed avoidance, both the black- and sand TDF showed an avoidance response above the 80% threshold.

4.4 Mesofauna

4.4.1 Mesofaunal count and diversity

Table 4-5 and 4-6 compare the average numbers and standard deviation of each order present at each sampled site, while also comparing the samples taken in the four different seasons. Starting with autumn the amount Prostigmata found in the BTDF (48.50 ± 28.82) were significantly higher ($p < 0.05$) from the Prostigmata found in the STDF (30.67 ± 19.99), PN (98.50 ± 45.55) and PS (99.17 ± 37.09) sites. While STDF was significantly higher ($p < 0.05$) compared to the BTDF, PN and PS, the PN and PS exposures were not statistically different ($p > 0.05$) from each other. When comparing the Astigmata none of the sites was statistically different ($p < 0.05$) from each other, the BTDF (0.67 ± 1.63) and STDF (3.83 ± 4.40) were not statistically different ($p > 0.05$) from each other, while both of them were significantly lower ($p < 0.05$) than PN (21.33 ± 23.06) and PS (31.83 ± 9.11). The sites PS and PN were not statistically different ($p > 0.05$) from each other, while both sites were statistically different ($p < 0.05$) from both BTDF and STDF. Comparing the Collembola at each of the sites only PS (20.00 ± 30.80) was significantly higher ($p < 0.05$) from the other three sites, while BTDF (0.00 ± 0.00), STDF (0.00 ± 0.00) and PN (4.00 ± 5.18) were not statistically different ($p > 0.05$) from one another. The numbers of Cryptostigmata, Mesostigmata, Metastigmata, Araneae, Coleoptera, Psocoptera, Hymenoptera, Hemiptera, Isoptera, Diptera, Lepidoptera, Blattodea, showed no significant difference ($p > 0.05$) between the samples taken during the autumn.

From the samples taken during the winter, the numbers of Prostigmata from the BTDF (21.33 ± 13.57) sites showed statistical difference ($p < 0.05$) when compared to STDF (28.50 ± 14.38), PN (7.50 ± 14.38) and PS (70.50 ± 21.00), while STDF was statistically different ($p < 0.05$) from PN, PS and BTDF. The PN and PS samples were not statistically different ($p > 0.05$) from each other, while both were statistically different ($p < 0.05$) from the BTDF and STDF sites. The Cryptostigmata samples from the BTDF (0.00 ± 0.00) sites were not statistically different ($p > 0.05$) from the STDF (0.00 ± 0.00) sites, while both the BTDF and STDF site were statistically different ($p < 0.05$) from PN (11.83 ± 9.24) and PS (14.50 ± 6.86) sites. The PN and PS sites were statistically not different ($p > 0.05$) from each other. When comparing the Collembola samples, both the PN (12.33 ± 11.50) and PS (16.33 ± 9.29) sites were statistically different ($p < 0.05$) from the BTDF (0.00 ± 0.00) and STDF (0.00 ± 0.00) sites, while the BTDF and STDF were not statistically different ($p > 0.05$) from each other, as well as the PN and PS sites not being statistically different ($p > 0.05$) from each other. The following orders showed no statistical difference ($p > 0.05$) between the sample sites: Mesostigmata, Astigmata, Metastigmata, Araneae, Coleoptera, Psocoptera, Hymenoptera, Hemiptera, Isoptera, Diptera, Lepidoptera and Blattodea.

Comparing the Prostigmata samples taken during the spring the BDF (14.67 ± 5.35) sites showed to be statistically different ($p < 0.05$) from the PN (68.17 ± 13.38) and PS (34.67 ± 11.48) sites, while not statistically different ($p > 0.05$) from STDF (15.17 ± 4.54) sites. The PN and PS sites were statistically different ($p < 0.05$) from each other, while also statistically different ($p < 0.05$) from both the BTDF and STDF sites. The Cryptostigmata were statistically different ($p < 0.05$) when comparing the BTDF (0.00 ± 0.00) and STDF (0.00 ± 0.00) sites with the PN (8.00 ± 10.56) and PS (10.67 ± 9.05), while PN and PS were statistically different ($p < 0.05$) from each other. After comparing the Mesostigmata samples the BTDF (1.50 ± 0.55) and STDF (1.17 ± 2.04) were both statistically different ($p < 0.05$) from both the PN (20.67 ± 5.50) and PS (3.67 ± 2.88) sites, while BTDF and STDF were not statistically different ($p > 0.05$) from one another. PN and PS were not statistically different ($p > 0.05$) from each other. Astigmata samples taken from the PS (4.17 ± 1.17) sites were statistically different ($p < 0.05$) from BTDF (4.50 ± 1.38), STDF (7.83 ± 6.55) and PN (3.50 ± 2.07), while no statistical difference ($p > 0.05$) was found between BTDF, STDF and PN. After comparing the Diptera samples, only the samples taken from the PS (2.00 ± 1.26) sites showed any statistical difference ($p < 0.05$). From the Collembola samples taken from the four sites, only the PS (3.17 ± 4.31) site showed statistical difference ($P < 0.05$) when compared to the other three sites. The Metastigmata, Araneae, Coleoptera, Psocoptera, Hymenoptera, Hemiptera, Isoptera, Lepidoptera and Blattodea showed no statistical difference ($p > 0.05$) between the samples taken from the four sample sites.

When comparing the Prostigmata sampled during the summer at the four different sites, the BTDF (12.50 ± 4.81) and STDF (10.67 ± 5.99) sites were not statistically different ($p > 0.05$) from each other, while both sites were statistically different ($p < 0.05$) from the PN (129.17 ± 16.65) and PS (53.00 ± 14.28) sample sites. The PN and PS samples sites were also statistically different ($P < 0.05$) from one another. After comparing the Cryptostigmata samples, the BTDF (0.83 ± 0.98) and STDF (3.00 ± 1.79) samples sites were not statistically different ($p > 0.05$), while the PN (26.83 ± 18.88) and PS (15.50 ± 3.02) sites were statistically different ($p < 0.05$) from both the BTDF and STDF sites. Mesostigmata samples taken from the BTDF (1.67 ± 1.63), STDF (1.33 ± 1.03) and PN (19.33 ± 12.44) sites were not statistically different ($p > 0.05$) from each other, while PS (10.33 ± 4.59) sites were statistically different ($p < 0.05$) from BTDF, STDF and PN sites. Collembola samples taken from BTDF (0.00 ± 0.00), STDF (1.00 ± 1.67) and PN (29.17 ± 18.74) sites were not statistically different ($p > 0.05$) from each other, while all three were statistically different ($p < 0.05$) from the PS (10.50 ± 9.56) sites. Astigmata, Metastigmata, Araneae, Coleoptera, Psocoptera, Hymenoptera, Hemiptera, Isoptera, Diptera, Lepidoptera, Blattodea, showed no statistical difference ($p > 0.05$) between the four samples sites.

The results of the soil mesofauna studies conducted on four different seasons (summer, winter, autumn and spring) are shown in table 4-5 and 4-6 below, as well as in figures 4-2 to 4-6 below.

See the appendix for the full seasonal tables of mesofauna data which were identified at species and genus level (where possible). Black TDF site showed a total of 437 individuals in the autumn samples, 130 individuals in the winter, 124 in the spring and 199 samples in the summer. Shannon-Weaver-index calculated for the BTDF showed an index value of 2.95 for autumn samples, 2.20 for winter, 2.52 for spring and an index of 3.31 for summer. Summer had the highest diversity index value followed by autumn, spring and then winter.

The sand TDF site showed a total of 360 counted individuals in autumn samples, 175 in winter, 148 in spring and 202 in summer samples. The Shannon-Weaver index value of 3.13 for the autumn samples was 2.43 for winter, 2.55 for spring and an index value of 3.50 for summer samples. Sand TDF showed the same trend as black TDF where summer had the highest diversity index value followed by autumn, spring and winter.

The pasture north site had a total of 1137 individuals in the samples taken in autumn, 663 in winter, 632 in spring and 377 in the summer samples. The calculated Shannon-Weaver index value for autumn is 3.89, 3.66 for winter, 3.61 for spring and an index of 3.98 for summer samples. Summer had the highest index value followed by autumn, winter and spring.

The pasture south site showed a total of 1298 counted individuals in the autumn site, 763 in the winter, 1515 in spring and 642 in the summer. Shannon-Weaver index showed a diversity value of 3.70 for the autumn samples, 3.74 for winter, 4.11 for spring and an index of 4.28 for summer. Summer showed to have the highest diversity index followed by spring, winter and autumn. After comparing the four sample sites, the pasture south had the highest total of counted individuals followed by pasture north, BTDF and STDF.

Table 4-5: Mean numbers of individuals of different orders, recorded in the autumn and winter samples taken from the black TDF, sand TDF, pasture north and south. Shannon-Weaver index calculated per site and per season The Means and standard deviation ($\pm$ SD) of individuals collected were determined.

		Autumn				Winter			
		BTDF	STDF	PN	PS	BTDF	STDF	PN	PS
Class	Order								
Arachnida	Prostigmata	48.50 $\pm$ 28.82 ^a	30.67 $\pm$ 19.99 ^b	98.50 $\pm$ 45.55 ^c	99.17 $\pm$ 37.09 ^c	21.33 $\pm$ 13.57 ^a	28.50 $\pm$ 14.38 ^a	67.50 $\pm$ 18.14 ^b	70.50 $\pm$ 21.00 ^b
	Cryptostigmata	11.83 $\pm$ 7.14 ^a	13.00 $\pm$ 2.83 ^a	28.50 $\pm$ 16.32 ^a	27.83 $\pm$ 13.45 ^a	0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a	11.83 $\pm$ 9.24 ^b	14.50 $\pm$ 14.02 ^b
	Mesostigmata	11.00 $\pm$ 6.51 ^a	10.83 $\pm$ 6.79 ^a	23.50 $\pm$ 13.55 ^a	18.67 $\pm$ 9.95 ^a	0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a	5.50 $\pm$ 1.38 ^a	8.67 $\pm$ 11.09 ^a
	Astigmata	0.67 $\pm$ 1.63 ^a	3.83 $\pm$ 4.40 ^a	21.33 $\pm$ 23.06 ^b	31.83 $\pm$ 9.11 ^b	0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a	5.67 $\pm$ 8.91 ^a	4.50 $\pm$ 6.86 ^a
	Metastigmata	0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a	0.50 $\pm$ 0.55 ^a	0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a
	Araneae	0.33 $\pm$ 0.82 ^a	0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a	4.00 $\pm$ 6.07 ^a	0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a	0.67 $\pm$ 0.82 ^a	1.00 $\pm$ 1.55 ^a
Insecta	Coleoptera	0.00 $\pm$ 0.00 ^a	0.33 $\pm$ 0.82 ^a	5.67 $\pm$ 10.23 ^a	2.00 $\pm$ 1.90 ^a	0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a	0.33 $\pm$ 0.82 ^a	0.00 $\pm$ 0.00 ^a
	Psocoptera	0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a	2.00 $\pm$ 2.28 ^a	0.33 $\pm$ 0.83 ^a	0.67 $\pm$ 1.63 ^a	0.00 $\pm$ 0.00 ^a	0.67 $\pm$ 1.21 ^a
	Hymenoptera	0.50 $\pm$ 1.22 ^a	1.33 $\pm$ 1.75 ^a	8.00 $\pm$ 10.30 ^a	6.33 $\pm$ 12.03 ^a	0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a	6.50 $\pm$ 5.24 ^a	4.33 $\pm$ 2.58 ^a
	Hemiptera	0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a	0.33 $\pm$ 0.82 ^a	2.17 $\pm$ 5.31 ^a
	Isoptera	0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a	1.83 $\pm$ 2.99 ^a	0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a	4.67 $\pm$ 5.50 ^a	3.00 $\pm$ 6.42 ^a
	Diptera	0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a	0.17 $\pm$ 0.41 ^a	0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a	2.33 $\pm$ 2.58 ^a	2.00 $\pm$ 2.61 ^a
	Lepidoptera	0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a	0.33 $\pm$ 0.82 ^a	0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a
	Collembola	0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a	4.00 $\pm$ 5.18 ^a	20.00 $\pm$ 30.80 ^b	0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a	12.33 $\pm$ 11.50 ^b	16.33 $\pm$ 9.29 ^b
	Blattodea	0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a	1.67 $\pm$ 4.08 ^a	0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a	0.50 $\pm$ 1.22 ^a
Shannon Weaver Index		2.95	3.13	3.89	3.70	2.20	2.43	3.66	3.74

a–c: Values within a row sharing the same letter in superscript were not statistically different from each other ($P>0.05$).

Table 4-6: Mean numbers of individuals of different orders, recorded in the spring and summer samples taken from the black TDF, sand TDF, pasture north and south. Shannon-Weaver index calculated per site and per season. The Means and standard deviation ($\pm$ SD) of individuals collected were determined.

		Spring				Summer			
		BTDF	STDF	PN	PS	BTDF	STDF	PN	PS
Class	Order								
Arachnida	Prostigmata	14.67 $\pm$ 5.35 ^a	15.17 $\pm$ 4.54 ^a	68.17 $\pm$ 13.38 ^b	34.67 $\pm$ 11.48 ^c	12.50 $\pm$ 4.81 ^a	10.67 $\pm$ 5.99 ^a	129.17 $\pm$ 16.65 ^b	53.00 $\pm$ 14.28 ^c
	Cryptostigmata	0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a	8.00 $\pm$ 10.56 ^a	10.67 $\pm$ 9.05 ^b	0.83 $\pm$ 0.98 ^a	3.00 $\pm$ 1.79 ^a	26.83 $\pm$ 18.88 ^b	15.50 $\pm$ 3.02 ^b
	Mesostigmata	1.50 $\pm$ 0.55 ^a	1.17 $\pm$ 2.04 ^a	20.67 $\pm$ 5.50 ^b	3.67 $\pm$ 2.88 ^b	1.67 $\pm$ 1.63 ^a	1.33 $\pm$ 1.03 ^a	19.33 $\pm$ 12.44 ^a	10.33 $\pm$ 4.59 ^b
	Astigmata	4.50 $\pm$ 1.38 ^a	7.83 $\pm$ 6.55 ^a	3.50 $\pm$ 2.07 ^a	4.17 $\pm$ 1.17 ^b	6.67 $\pm$ 1.86 ^a	7.33 $\pm$ 5.72 ^a	18.67 $\pm$ 8.12 ^a	4.50 $\pm$ 1.38 ^a
	Metastigmata	0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a	0.83 $\pm$ 0.98 ^a	0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a	0.83 $\pm$ 1.17 ^a	0.67 $\pm$ 0.82 ^a
	Araneae	0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a	0.33 $\pm$ 0.82 ^a	0.17 $\pm$ 0.41 ^a	0.33 $\pm$ 0.82 ^a	0.00 $\pm$ 0.00 ^a	2.17 $\pm$ 0.98 ^a	0.67 $\pm$ 1.21 ^a
Insecta	Coleoptera	0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a	3.83 $\pm$ 3.25 ^a	0.67 $\pm$ 1.21 ^a	2.83 $\pm$ 1.47 ^a	0.50 $\pm$ 0.55 ^a	4.50 $\pm$ 2.17 ^a	0.5 $\pm$ 1.22 ^a
	Psocoptera	0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a	0.33 $\pm$ 0.52 ^a	0.00 $\pm$ 0.00 ^a	0.50 $\pm$ 0.55 ^a	0.33 $\pm$ 0.82 ^a	0.00 $\pm$ 0.00 ^a
	Hymenoptera	0.00 $\pm$ 0.00 ^a	0.33 $\pm$ 0.82 ^a	0.00 $\pm$ 0.00 ^a	1.33 $\pm$ 1.51 ^a	6.67 $\pm$ 2.80 ^a	2.50 $\pm$ 3.89 ^a	7.17 $\pm$ 3.97 ^a	4.50 $\pm$ 6.47 ^a
	Hemiptera	0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a	0.17 $\pm$ 0.41 ^a	0.83 $\pm$ 0.98 ^a	1.33 $\pm$ 0.82 ^a	1.00 $\pm$ 0.63 ^a	0.33 $\pm$ 0.52 ^a
	Isoptera	0.00 $\pm$ 0.00 ^a	0.10 $\pm$ 0.41 ^a	0.00 $\pm$ 0.00 ^a	0.50 $\pm$ 0.84 ^a	0.83 $\pm$ 1.33 ^a	2.33 $\pm$ 2.88 ^a	0.83 $\pm$ 0.75 ^a	1.50 $\pm$ 1.52 ^a
	Diptera	0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a	2.00 $\pm$ 1.26 ^b	0.00 $\pm$ 0.00 ^a	3.00 $\pm$ 1.79 ^a	11.17 $\pm$ 12.19 ^a	4.17 $\pm$ 1.94 ^a
	Lepidoptera	0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a	0.17 $\pm$ 0.41 ^a	0.00 $\pm$ 0.00 ^a	0.17 $\pm$ 0.41 ^a	0.50 $\pm$ 0.84 ^a	0.50 $\pm$ 0.84 ^a
	Collembola	0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a	3.17 $\pm$ 4.31 ^b	0.00 $\pm$ 0.00 ^a	1.00 $\pm$ 2.45 ^a	29.17 $\pm$ 18.74 ^a	10.50 $\pm$ 9.65 ^b
	Blattodea	0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a	0.50 $\pm$ 0.84 ^a	0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a	1.00 $\pm$ 1.67 ^a	0.50 $\pm$ 0.55 ^a
Shannon Weaver Index		2.52	2.55	3.61	3.98	3.31	3.50	4.11	4.28

a–c: Values within a row sharing the same letter in superscript were not statistically significantly different from each other ($P>0.05$).

4.4.2 Mesofauna collected during different seasons

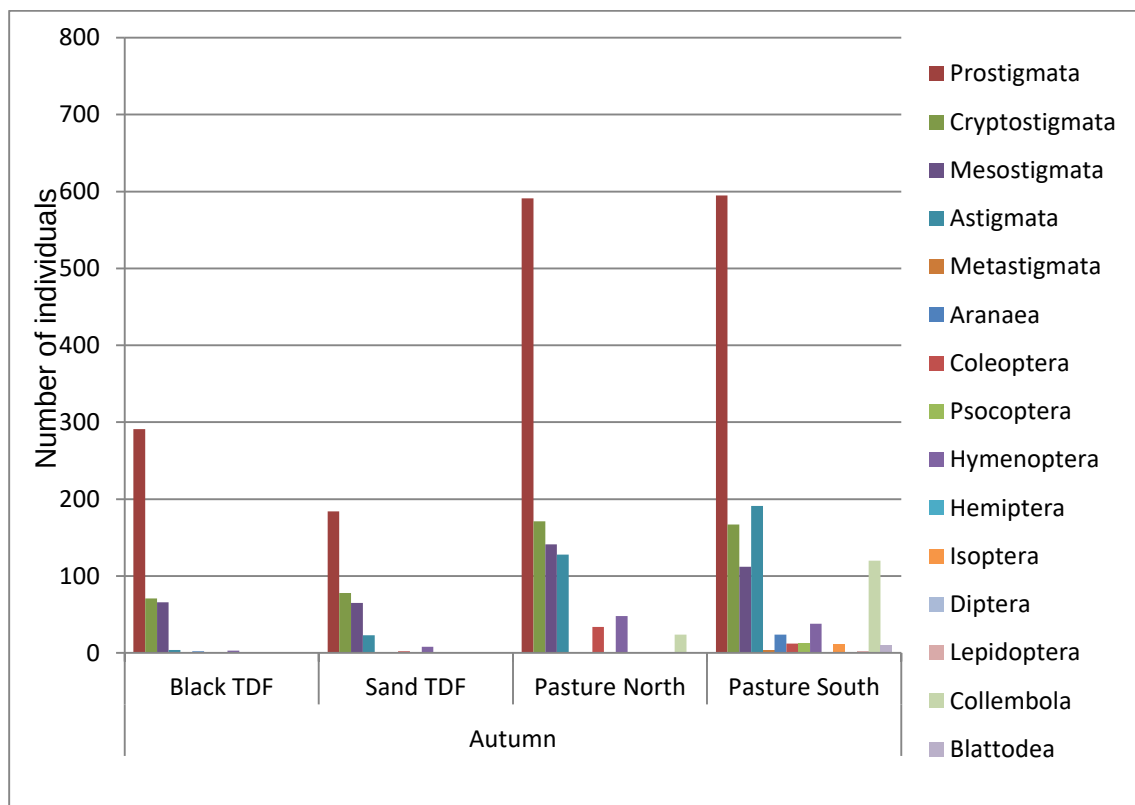


Figure 4-2: Comparison of the total number of individuals at the order level, sampled at each study site during the autumn.

Figure 4-2 compares the total number of counted individuals per order in the autumn. The black TDF and sand TDF have the lowest number of different orders when compared to the two pasture sites, pasture south has the most orders. Between all of the orders, Prostigmata had the highest total number of individuals. Within Prostigmata, the family Pediculochelidae, *Pediculocheles* sp. had the most individuals compared to the rest of the species found. Family Nanorchestidae, *Speleorchestes meyeri*, family Tydeidae, *Coccotydaeolus* sp., *Coccotydaeus* sp. and *Pronematus ubiquitous*, and family Micropsammidae, *Micropsammus* sp. also had high individual counts in the same range as that for *Pediculocheles* species.

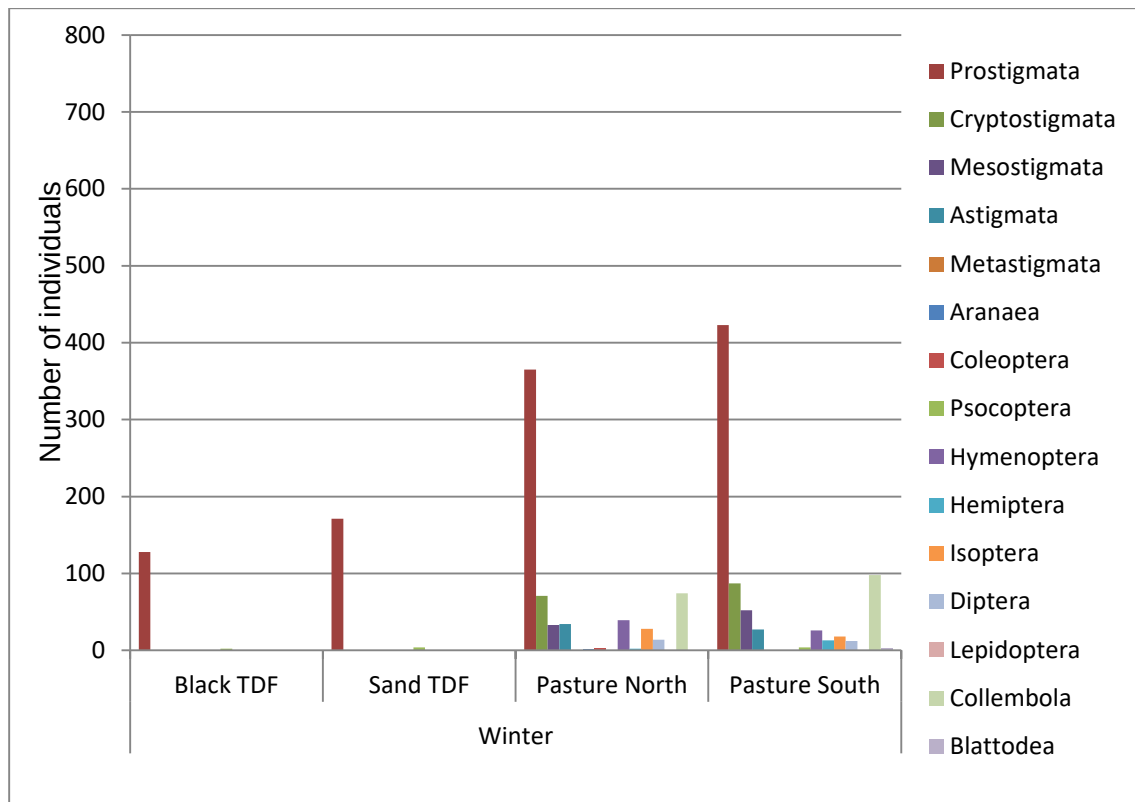


Figure 4-3: Comparison of the total number of individuals at the order level, sampled at each study site during the winter.

Figure 4-3 shows all of the orders present in the study area during the winter and the total counted individuals per order. The black TDF and sand TDF have the lowest number of counted individuals compared to the two pasture sites; pasture south had the highest number of counted individuals, with pasture north having the second highest individual count. Pasture north and south have the same number of orders present, while the sand- and black TDF have the lowest number of orders. Prostigmata is the order with the highest abundance of individuals. When comparing the individual count of species of the Prostigmata, within the family Nanorchestidae, *Speleorchestes potchensis* and *S. meyeri* had the highest individual counts.

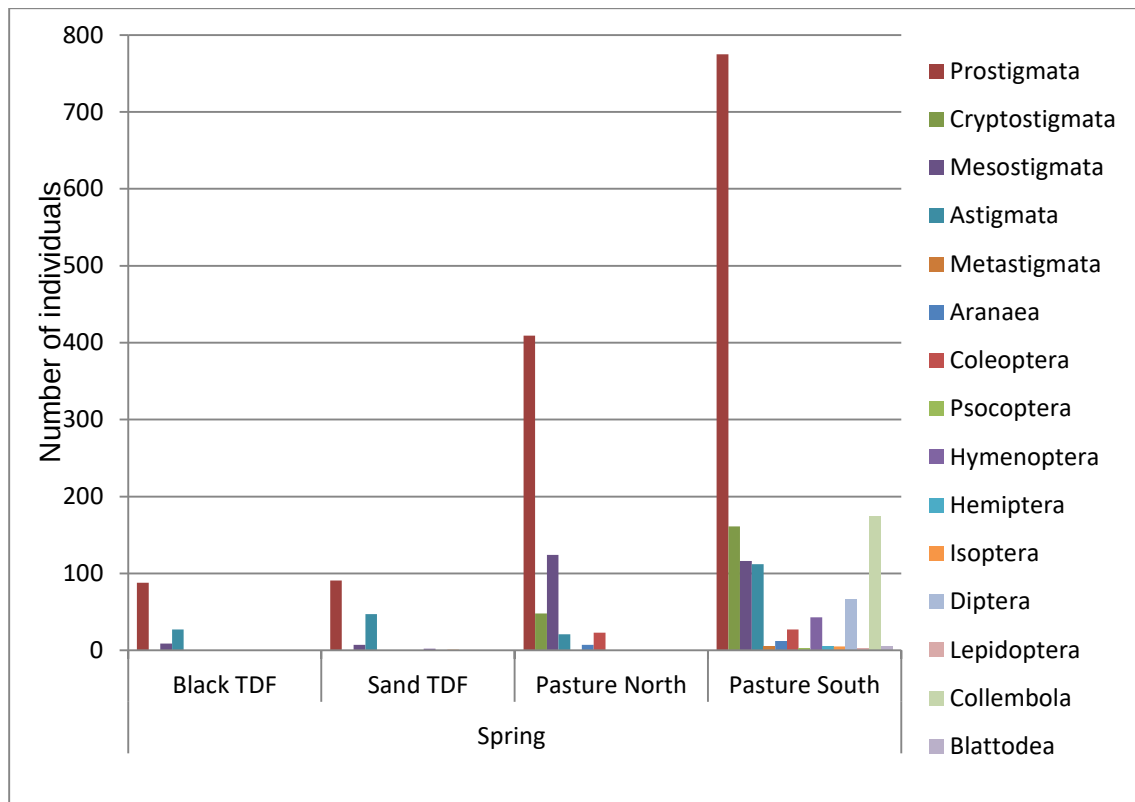


Figure 4-4: Comparison of the total number of individuals at the order level, sampled at each study site during the spring.

The total number of individuals counted from the samples taken during the spring at the different study sites are compared in figure 4-4. Pasture south has the highest total number of individuals followed by pasture north, black TDF and sand TDF. Pasture south also has the most orders present followed by pasture north, sand TDF and black TDF. The order with the highest total number of individuals is the Prostigmata. Between the different species of Prostigmata present, *Coccorhagia* sp., *Micropsammus* sp., *Coccotydaeolus* sp., *Speleorchestes meyeri* and *Pediculocheles* sp. had the highest individual count.

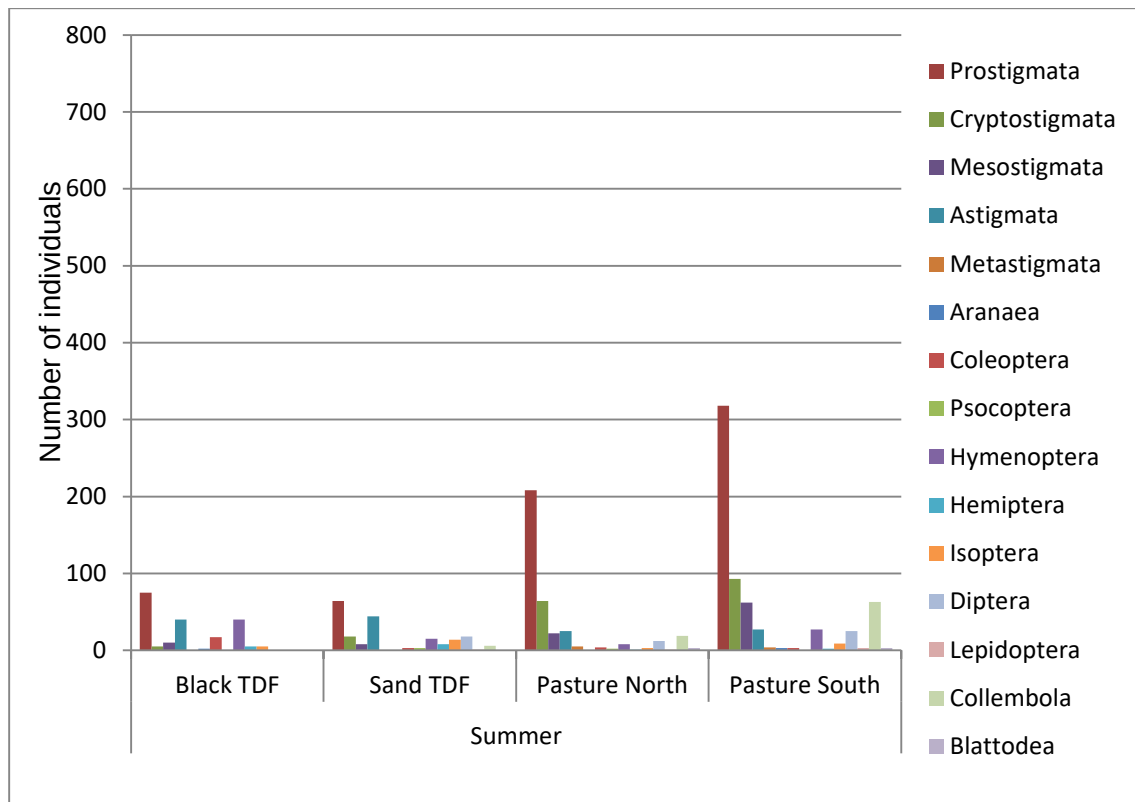


Figure 4-5: Comparison of the total number of individuals at the order level, sampled at each study site during the summer.

All of the orders found at each study site during the summer are compared in figure 4-5 by looking at the total number of individuals sampled. Pasture south has the highest individual count followed by pasture north, sand TDF and black TDF, with the pasture south and pasture north having the same number of orders present. Black TDF followed by sand TDF had the lowest number of different orders present. During the summer *Pediculocheles* sp. showed the highest abundance when compared to other Prostigmata species found.

Comparing the figures for each of the seasons it is clear that autumn had the highest overall abundance of individuals, followed by spring, winter and summer. Clearly, when comparing each site and season the Prostigmata were not only present at each site, they also had the highest total individual count when compared to the other orders. A common trend could be seen between the different study sites, pasture south had the highest individual count followed by pasture north, sand TDF and the black TDF.

4.4.3 Feeding habits of sampled mesofauna

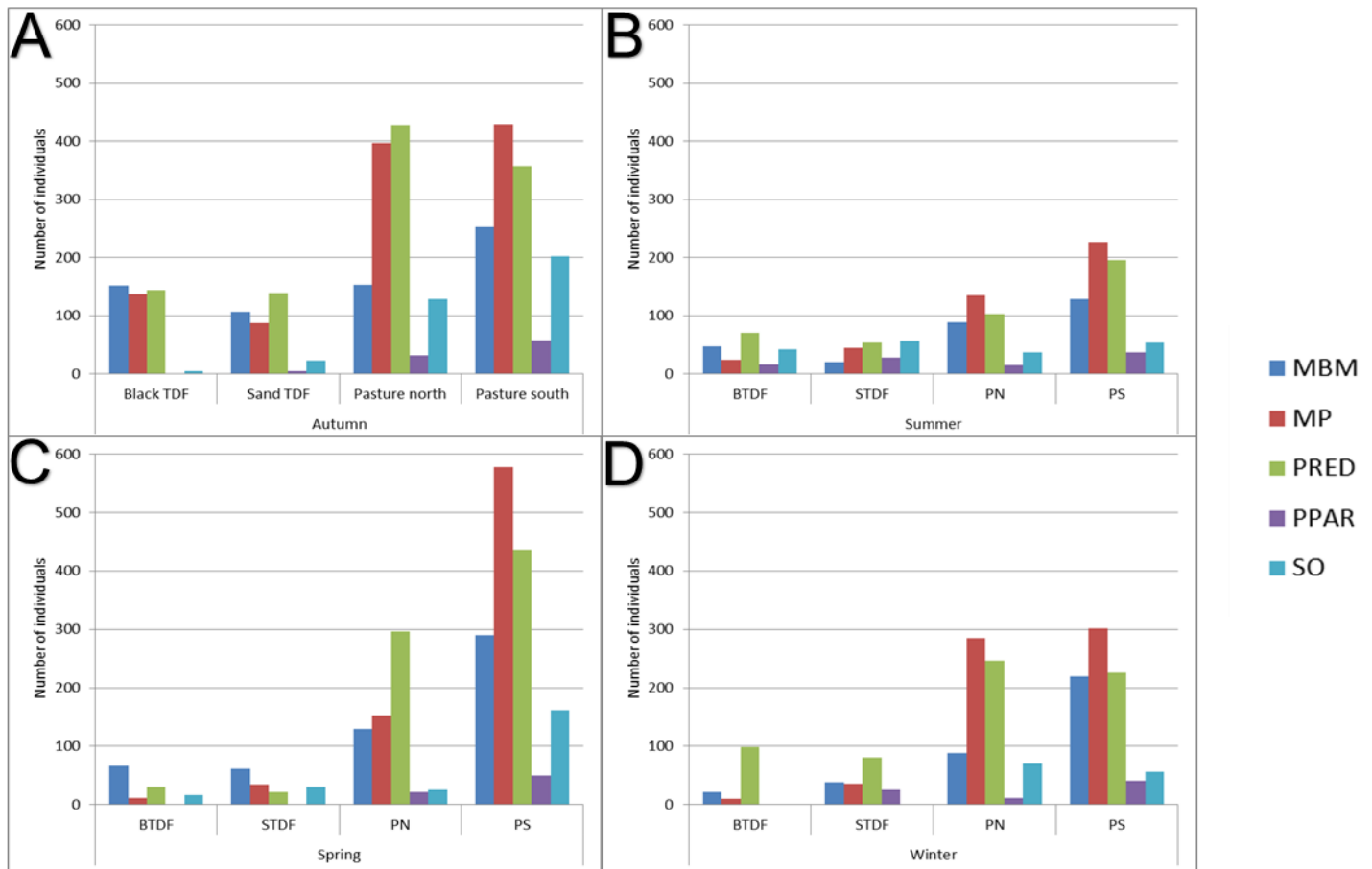


Figure 4-6: Functional groups (based on primary feeding habits) of mesofauna sampled at the black-, sand TDF, pasture south and –north. A: autumn samples; B: summer samples; C: spring samples; D: winter samples.

Figure 4-6 contains a histogram for each of the four seasons, comparing the functional groups found at each site. Mesofauna were grouped according to five different feeding functional groups namely: Mycophagous (MP) which mainly feeds on fungi; Predators (PRED) which mainly feeds on other living organisms; saprophagous and omnivorous (SO) which feed on dead or decaying animal and plant material; bacteriophagous and micro-algivorious (MBM) mainly feed on bacteria, fungi and algae; and finally plant parasites (PPAR) which feed on living plant material.

Graph A compares the number of individuals counted per functional group in the autumn. PRED had the highest count of individuals followed by MP, MBM, SO and PPAR. Functional group PPAR was absent in the BTDF site and barely present in the STDF site.

Graph B shows the total number of individuals for each functional group sampled during the summer. The functional group MP had the highest individual count followed by PRED, MBM, SO and PPAR. No functional groups were absent in the summer samples.

Graph C is a comparison of the total number of counted individuals of each functional groups of samples taken in spring. Functional group PRED had the highest number of individual followed by MP, MBM, SO and PPAR. The functional group PPAR was absent in the BTDF sites and barely present within the STDF site.

Graph D compares the individuals counted of each functional group from samples taken in the winter. Where the functional group PRED had the highest number of individuals followed by MP, MBM, SO and PPAR. Functional group PPAR and SO was absent in the BTDF site, while only SO is absent in the STDF site.

When comparing the samples taken at each season the functional group MP and PRED had the highest abundance of organisms present, with PPAR having the least. PPAR and SO was also completely absent in some of the sample site.

CHAPTER 5

DISCUSSION

Tailing disposal facilities are created as control structures with the purpose of providing safe and permanent storage for the residues produced during the mining process (Aucamp & Van Schalkwyk, 2002). However these tailing disposal facilities tend to fail in their designed purpose as a long-term storage solution (Rossouw *et al.*, 2010; Van Coller-Myburgh *et al.*, 2015). It is known that poor management of disposal facilities can lead to the escape of seepage which have negative impacts on the surrounding environment and affect water and soil quality, which may lead to the death of wildlife (e.g. birds, fish, etc.) (Rosner & Van Schalkwyk, 2000). Unfortunately, there are few long-term ecotoxicological studies on the effects of TDFs on the surrounding environment, as well as the sustainability of current rehabilitation techniques (Jubileus *et al.*, 2012).

Determining the physical and chemical composition of the tailing dam material might give an indication of the effects of tailing dam material on the surrounding environment. According to Bezuidenhout & Rousseau (2005), gold tailings usually consist of quartz, white mica, chlorite and pyrophyllite. The gold mine tailing dams at the study site were constructed on very shallow dolomitic soils (Koch, 2014), most of the older mines were constructed on dolomitic landscapes due to the rapid drainage of fluids away from the TDFs (Bezuidenhout & Rousseau, 2005; Yibas *et al.*, 2010). Unlike natural soil, mining material is mostly uniform in size. Most of the material collected from the TDFs was consistent with sand-like particles. Material from the TDFs are extremely permeable and has a low water- and nutrient holding capacity (Aucamp & Van Schalkwyk, 2002) and due to the TDF material consisting of fine particles it can be very susceptible to wind erosion (especially the top soil layer). This leads to the spreading of material across the surrounding environment, further contributing to dust pollution. The low vegetation cover also makes it easier for wind distribution. The pH of the material decreases deeper in the TDF (Rosner & Van Schalkwyk, 2000), but the top soil of the TDFs can be fairly acidic due to the low organic matter content present on the TDF (Aucamp & Van Schalkwyk, 2002; Vega *et al.*, 2004).

It is well known that acid mine drainage is one of the major problems associated with TDFs. Low pH levels can increase the bioavailability of certain metals as well as, increase the mobility of these metals within the environment (Aucamp & Van Schalkwyk, 2002; Vega *et al.*, 2004). In

table 4-1 the overall pH of all the sites was indicated to be very low, especially in the black TDF 100% exposure which had a pH of 3.65. The low pH readings of TDFs can be due to acid mine drainage, which is produced through the oxidation sulphides (such as, pyrite) Even in the two reference samples taken around the TDFs had a fairly low pH value. The northern pasture had a pH of 4.1 and the southern pasture a pH value 3.97. The low pH of the surrounding environment can be due to the distribution of tailing dam material via wind and water erosion. Furthermore, the low vegetation cover and fine uniform material of the TDF makes it easy for contaminants to leach into the surrounding environment, increasing the contaminated areas acidity (Vega *et al.*, 2004). Acidic material and soil are shown not to be ideal for most soil organisms (Gray, 1997), low pH may be part of the reason earthworm reproduction was low in the TDF material compared to the control soil.

For this ecological risk assessment on mine tailings dams, the screening for contaminants was done on old gold mining tailings and the soil surrounding the tailing dams. From all of the metals found in the screening only Cr, Co, Ni, Cu, Zn, As, Pd and Cd were chosen, because these metals are typically found in most local mining sites and in the surrounding ecosystems (Otomo *et al.*, 2013; Jubileus *et al.*, 2012). The bioconcentration factor of earthworms was determined by dividing the concentration of metals within the earthworm with the concentration of metals in the soil. Various benchmarks were used to determine whether the heavy metal concentrations measured in the test soils were above or below the toxicological threshold of the South African Screening Values (SSV). However, it should be noted that these values are not scientifically concrete and only provide a general standard for environmental assessment and should not be regarded as the absolute minimum values of metal toxicity (Eijsackers *et al.*, 2014). The concentration of heavy metals that accumulated within the earthworm tissues were compared to the earthworm (EW) and soil microorganisms (SMO) toxicity and microbial processes (MP) benchmarks, which were used by Efroyman *et al.* (1997).

None of the heavy metals recorded in the earthworm tissues exceeded the EW, SMO and MP benchmarks. However the Cr concentrations recorded in the test soil and the control, exceeded the SSV values in both the PN (11.59 ppm) and BTDF (100%) (5.20 ppm) exposures, while none of the other heavy metal concentrations recorded in each exposure came close to exceeding the SSV benchmark. The bioconcentration factor (BCF) was calculated by comparing the ratio of heavy metals in the earthworm tissue to the metal concentration in the material. The following heavy metals showed a very low bioconcentration factor at each exposure: Cr, Co, Ni, As and Pb. Copper (Cu) showed notable accumulation at the BTDF (50%), BTDF (100%) and STDF (100%). Zinc (Zn) had a very low bioaccumulation in all the exposures except in the BTDF (100%) exposure where some bioaccumulation occurred. The recorded BCF of Cd was

higher than the soil concentration, making the data invalid. Therefore, the Cd BCF values will not be considered in the discussion or conclusions of the results. From all eight of the studied heavy metals, chromium, copper and zinc had the highest recorded concentrations. The metals were all looked at individually, due to the fact that most of these metals were present in a mixture within the test material, however it can be rather difficult to interpret the effects they have on the environment and organisms (Maboeta *et al.*, 2007 & Van Coller-Myburgh *et al.*, 2015). However, it is possible to compare the TDF material to natural or control substrates in order to estimate the relative effects of the gold mine TDF on the environment and organisms.

5.1 Earthworm bioassay

Multiple studies from the past ten years (Jubileus *et al.*, 2012; Wahl *et al.*, 2012; Van Coller-Myburgh *et al.*, 2015; Maboeta *et al.*, 2018) have shown that earthworm bioassays have been successfully used to assess and determine the effects of pollution on the environment and its components. The result from this ecological risk study showed a definite negative impact on test organisms (*Eisenia andrei*). The Cr soil concentration exceeded the SSV1 benchmark for soil quality in the 100% BTDF and northern pasture, with the 100% STDF exposure just being below the SSV1 benchmark. High concentrations of Cd, Zn and Cu accumulated within all of the earthworm tissues.

After comparing the averages of the earthworms' weight after the first 7 days, most of the exposures showed an increase in the average weight of the earthworms, except for the 75% BTDF and 100% STDF exposures, which had a slight decrease in overall earthworm weight. This increase in earthworm weight was most likely due to it being the first week of the test. The earthworms were not exposed long enough to the test substrate to accumulate enough heavy metals to influence their health. From day 14 to day 28 showed a clear decrease in the average worm weight in all of the exposures. The relative growth rate was determined by comparing the earthworm weight from day 1 to day 28, where only the control (-13.82), BTDF 25% (-15.67), 100% (-17.34) and STDF 25% (-12.66) exposures showed a significant ($p < 0.05$) decrease in weight. Except for the 50% STDF and 75% STDF exposures, they showed an increase in weight on day 21. The STDF 75% exposures had a significant increase over their 28-day period, having a relative growth rate (%) of +17.34. Comparing the final average weights of the earthworms the northern pasture seems to be the most favourable to the earthworm, followed by the 50% BTDF and the control. The soil used from the two pasture sites had a decent amount of organic matter present, especially the northern pasture. Organic matter may have played a role in making it more preferable to the earthworms than even the control.

When comparing the 100% concentrations of the BTDF and STDF to the control and two pasture sites, both the 100% TDF exposures showed a significant difference ($p < 0.05$) in earthworm weight, with 100% BTDF having the lowest recorded earthworm weight. This could be due to a variety of factors which can negatively impact the earthworms. For instance, both of the TDFs had extremely low pH values, with BTDF having the overall lowest value. Low soil pH has been proven to inhibit the growth of earthworms (Morgan *et al.*, 1992), especially when the pH value is below 4. Previous studies have shown that heavy metals such as Cd and Cr can slow down the growth of earthworms and in high concentrations can lead to weight loss, as well as reduce their reproductive output. For example, Zaltauskaite and Sodiene (2014) exposed *Eisenia fetida* to soil with high concentrations of Cd and Pb, resulting in a decrease of weight in juveniles, reduction in cocoon production and an increase in the duration for worms reach maturity. While Zn was also found in high concentrations in most of the earthworm tissues, it was still below the E/W benchmark. Zinc is a known micro nutrient which promotes an organism's vitality and growth. The effect of the tailing dam material was also viewed when weighing and counting the earthworms near the end of the 28-day period. All of the earthworm exposed to the 100% black TDF material, were found on top of the tailing material or in the horse manure. When counting the earthworms in the 100% BTDF exposure they were mostly motionless, the earthworms seemed to avoid the tailing material.

The results from the reproduction test reported in Chapter 3 showed a significant difference ($p < 0.05$) between the control and both the 75% and 100% exposures for the BTDF and STDF cocoon production. While a significant difference ($p < 0.05$) was also seen between the two pasture sites and the BTDF and STDF sites at the 75% and 100% concentrations. There was also a large overall difference between the number of juveniles per cocoon; where the control and pasture site will average 5 juveniles per cocoon, the high concentration exposures (75% and 100%) averaged between 3 and 1 juvenile worms per cocoon respectively. The black TDF had the worst result where no juveniles were present after the 28 days. The two pasture sites, control and black TDF had the highest cocoon production and juvenile yield. Previous studies stated that the average of juveniles per cocoon should be around 6. When the hatchability success percentage was compared between all of the sites, the northern pasture, control, black TDF (25% to 100%) and sand TDF (25%) exposures were significantly different ($p < 0.05$) from the southern pasture and sand TDF (50% to 100%). It should however be noted that the 100% BTDF exposure had the highest percentage of unhatched individuals, where the 75% and 100% STDF exposures only had approximately one cocoon each with a low juvenile yield of between 1 and 2 per cocoon. Percentage-wise the STDF seems to have a higher hatchability rate, while it

also had low cocoon yield. The 100% BTDF seems to have the highest impact on the earthworm's ability to reproduce, where only one of the three containers had one unhatched cocoon present. The total number of cocoons and juveniles produced per cocoon can be used as a sensitive endpoint for assessing the impact of mine tailings on organisms in the surrounding environments. The percentage of hatched cocoons of each exposure cannot be used as a sensitive endpoint, because of the low number of cocoons produced in the tailing dam material exposures. With such a low number of cocoons found in the high concentrations of tailing material, it is difficult to interpret the number of juveniles per cocoon. For example, the 100% STDF exposure showed an average cocoon production of 0.67, with a juvenile yield of 1, the formula used to determine showed a hatchability percentage of 0%, the number of cocoons was lower than the total juvenile yield, making the result unusable.

The recorded mortality after the 28 days showed a 3% mortality on day 7 in the control. Throughout the experiment, the two pasture sites (PN and PS) showed no mortality. On day 21 there was a 6% mortality recorded in the 50% BTDF and 13% mortality in the 75% BTDF exposure. Mortality was 25% in the 25% STDF exposure, 3% mortality in both the 50% and 100% exposure on day 14. Three percent mortality was recorded in the 75% STDF exposure on day 21. The 3% mortality recorded on day 7 in the control was most likely due to a single earthworm that went missing; the chances of an earthworm dying this early on in the experiment are fairly slim. There were no significant differences ($p < 0.05$) when comparing the control to both the BTDF and STDF exposures. There was also no significant difference between the BTDF and STDF exposures when compared to the southern pasture and northern pasture exposures.

5.2 Avoidance test

The avoidance test was done following the ISO[®]17512:1 (2007) guideline. The experiment fell within the guidelines criteria where the earthworms showed at least some avoidance of 40% to 60%. During the two-day testing period no earthworms were recorded missing or dead. Similar to the reproduction test the material taken from the gold mine and material taken from the surrounding environment was compared to a control made from artificial soil (OECD guideline), the tailing material was divided into four different exposure (25%, 50%, 75% and 100%) concentrations. The ISO avoidance test is used as a rapid screening method and takes less time and resources than the OECD reproduction test, but should not be used as a replacement test. It should be noted that the northern and southern pasture sites were only compared at a 100% concentration to serve as a natural representative of soil.

The 25% exposure showed no significant statistical difference ($p < 0.05$) when comparing the STDF and BTDF to the control and the two pasture sites. The earthworms showed no real preference for any of the material and were almost equally distributed between the control and test material. In the 50% exposures, no statistical differences ($p < 0.05$) were observed. Similar to the 25% exposures the earthworms showed no preference for a particular soil and was distributed randomly. Except for the STDF exposure where the earthworms were mostly found in the test soil side of the container. The 75% exposures showed slight avoidance when comparing the control material to the two pasture sites and the SDTF exposures. Only the BTDF exposure was statistically different from the control ($p < 0.05$) where the average of earthworms that avoided the test material was above the avoidance threshold (80%). The final 100% exposures had the largest avoidance response, where both the BTDF and STDF was statistically different ($p < 0.05$) from the control. On average, the STDF and BTDF are just above the 80% avoidance threshold, while both the northern and southern pastures stayed below the threshold. After comparing the results from the four different concentrations it is clear that notable avoidance response occurs in the 75% and 100% exposures, with 100% exposures showing the most avoidance from the tailing dam material, while the 25% to the 75% concentrations the earthworms showed no preference for any particular soil, the earthworms were almost equally distributed between the two sides of the container. Similar to the reproduction test significant response to the tailing material was also observed at the 100% concentration exposures. The contaminated material consists of a variety of heavy metals and is difficult to determine whether the earthworms are avoiding a certain metal or a combination of metals. But the relative toxicity and effects of the gold mine tailings can be compared to the responses of the earthworms to the contaminated soil. One factor that can play a role in earthworm worm avoidance is the low pH of the tailing material, especially the BTDF material. *Eisenia andrei* is known to tolerate pH levels as low as 4, both the STDF (3.83 pH) and BTDF (3.65 pH) had relatively low pH values. It is also known that low pH levels in soils can increase both the bioavailability and mobility of certain metals. Thus a multitude of factors can play a role in the avoidance response of earthworms to the contaminated soil. For example, an avoidance study done on three different earthworms species, comparing their responses to lead and zinc contaminated soils, showed that each species of earthworms had its own tolerance to a certain element (Lukkari & Haimi, 2005).

5.3 Mesofauna

One of the major components of this study was the compilation of species data collected. The species list was composed of the total number of individually collected species at each sample

site during four different seasons. Samples collected were identified up to species level (when possible) and then divided into functional groups. The organisms were placed into a functional group based on their diet, but some of the species have not been extensively researched or may have more than one type of preferred food. For example, there are predatory mite species which feed on pollen when their primary source of food becomes scarce (McMurtry & Croft, 1997). Some species have multiple sources of food and, in some cases, mites' dietary needs change with the different stages of their life cycle (Blair *et al.*, 1996; Walter, 1987). The functional groups used in this study are based on two other similar studies done on mesofauna and earthworms (Wahl, 2012; Jubileus, 2012). Mesofauna collected were identified and placed accordingly into five different functional groups. It should be noted that other reports may use different functional groupings than this study. The functional groups used, were chosen to complement this specific study.

5.3.1 Dominant groups

The dominant orders were determined by comparing the total number of counted individuals from each order group. The orders Prostigmata, Cryptostigmata and Mesostigmata were found to be the three most dominant orders, after comparing the total individual count across the four different seasons. The order Prostigmata was found in high abundance within all of the samples taken from each of the different sites. Previous studies done on platinum mines (Wahl *et al.*, 2012; Jubileus *et al.*, 2012), showed similar results when comparing the orders, but some of the species found and their individual count varied. According to Osler *et al.* (2000), the Prostigmata are known to be an opportunistic group which can be found even within the harshest of environments containing low organic matter (Loots & Ryke, 1966). Prostigmata covered all of the functional groups, for example, Nanorchestidae mostly consisting of bacteriophagous and micro-algivororous individuals. The family Tydeidae had a variety of species which fell into different feeding groups. For example, they consisted of predatory, bacteriophagous and micro-algivororous mite species. Nanorchestidae is a family of cosmopolitan mites and they are abundant in both hot and cold deserts (Rounsevell & Greenslade, 1988). These mites inhabit both the soil and soil surface, they are also known to be the dominant mite group in arid regions (Rounsevell & Greenslade, 1988), while the Mesostigmata only consisted of predatory mites and the Cryptostigmata mainly fed on fungi. Thus the Prostigmatic mites make a decent indicator species due to their ability to rapidly reproduce and survive in the harsh dry conditions of the tailing dams (Behan-Pelletier, 1999; Holmstrup *et al.*, 2007). Previous studies have explored the idea that the presence of Prostigmata can be used to indicate that a certain environment is in a state of poor health.

The class Insecta was mainly present in the surrounding pasture sites, but very few individuals were found in the soil samples taken from the two tailing dam sites. The presence of insects in and on the TDFs most likely results from larva being brought there by the wind or insects that landed on the TDFs while looking for food. For example, ants were seen roaming on top of the TDFs, but there was no indication of ant nests on the TDF surface. The Collembola were the most dominant insect group found within the two pasture sites, while being completely absent in the collected TDF material. Collembola has been used in multiple European studies as an indicator species, due to their tolerance to most toxicants. According to Blakely *et al.* (2002), Collembola occur in disturbed sites as long as minimum vegetation or organic matter is present. However, their number is greatly reduced near heavy metal contaminated sites. This is most likely due to their bodies having a thin cuticula, allowing metals to accumulate through the skin (Blakely *et al.*, 2002), thus making the Collembola the third most dominant group when only looking at the two pasture sites, with Prostigmata being the most dominant group followed by Mesostigmata.

5.3.2 Seasonal fluctuations and functional groups

The total number of individuals collected and species diversity was used to determine which sites are disturbed and also to estimate the level of disturbance. Previous studies (Skubala and Kafel, 2004; Jubileus, 2012; Wahl, 2012) have shown that samples taken from the TDFs are generally the most disturbed and contain the lowest number of species when compared to the surrounding environment. After comparing the results from the samples taken during the four seasons, the tailing dam facilities showed the overall lowest total individual count and diversity index values, with the BTDF having the lowest number of organisms present, compared to the STDF. The BTDF had no visible vegetation cover on the surface, with the top soil being almost uniform in composition. Thus the BTDF has a very limited food supply available, which may be inadequate for most populations. All of the functional groups present showed a low diversity pattern, especially the plant saprophagous and omnivorous groups were found in low abundance, while the plant parasites were completely absent in the soil samples taken during the winter, autumn and spring seasons.

The STDF had a slightly higher species diversity and individual count than the BTDF, while also having a larger variety of functional groups present. The only exception was the absence of plant parasites in the spring, winter and autumn seasons, while the saprophagous and omnivorous group was only absent in the winter samples. The STDF had a higher abundance of organisms due to the higher concentration of organic matter present in the top soil and the

presence of Eucalyptus trees on the surface which could provide a habitat for some organisms, for example by providing shade during the summer for organisms with a lower tolerance to heat. Nevertheless, the STDF is still regarded as a highly degraded site, consisting of a very sandy and uniform top soil that is not very hospitable for most organisms.

Both the BTDF and STDF sites consisted of soil with a combination of heavy metals present in high concentrations and low pH levels which can increase both the concentrations and mobility of certain metals, such as lead (Aucamp & Van Schalkwyk, 2002; Van Coller-Myburgh *et al.*, 2015). A previous study, done on arthropod communities as bioindicators for soil health, demonstrated how different soil pH can have both a positive and negative effect on soil preference of arthropods (Van Straalen, 1996). The average of arthropods struggled to survive in the soil with a pH of 3 or lower. Thus the low pH and high heavy metal concentrations might be the cause of the low organisms count compared to the two pasture sites. According to Newman (2015), when certain metals begin to bioaccumulate within the organism and exceed their ability to excrete or absorb these metals, it could affect their biology, for example by reducing their size or inhibit their ability to reproduce successfully, thus resulting in the reduction of soil communities' overall ability to survive. Soil mesofauna, such as the mites are known to spend a large portion of their life cycles within the soil, only able to move a few centimetres within their life time (Lavelle *et al.*, 2006). Most of the species found within the tailing dam facilities seem to have the ability to tolerate the low pH and have the ability to neutralise most metals within their bodies (Lavelle *et al.*, 2006), in order to survive with these harsh environments.

The northern and southern pasture sites had a significantly higher species diversity and total individual count than both the BTDF and STDF sites. All of the functional groups were present within both of the pasture sites; mycophagous, predators, bacteriophagous and micro-algivoracious groups were found in high abundance, while the plant parasites, saprophagous and omnivorous groups were found to be in lower abundance. However, the saprophagous and omnivorous groups did show a decent increase in the autumn and spring samples. Even though the two pasture sites represented natural reference soils for this study, they are still relatively disturbed when compared to normal soil standards, most likely due to wind and water erosion which distributed the tailing material throughout the two surrounding pasture sites, which may also have led to the possible increase in heavy metal concentrations within the soil. Acid mine drainage that formed in the tailing dams and leaches into the surrounding environment along with the heavy metals can over time reduce the overall pH (Rösner & Van Schalkwyk, 2000; Aucamp & Van Schalkwyk, 2002). Between the two pasture sites, the northern pasture had a lower total individual count and species diversity than the southern pasture. There was a

difference between the individuals sampled from the two pasture sites for example, members from the orders Psocoptera, Lepidoptera and Blattodea, were found in low abundance or absent from samples. This could be due to the difference in vegetation cover of the two pasture sites, the southern pasture was mainly dominated by *Cynodon dactylon*, *Cortaderia sellona* and *Cybopogon* sp. while plant species such as, *Seriphuim plumosum*, *Vachellia* sp. and *Cynodon dactylon* are mostly present in the northern pasture. Both *Seriphuim plumosum* and *Cynodon dactylon* are known to occur in overgrazed or disturbed areas. The northern pasture also had a higher concentration of heavy metals such as, Cr, Co, Ni, Cu and Zn present than the southern pasture.

CHAPTER 6

CONCLUSION AND RECOMMENDATIONS

This study was conducted to examine the potential effects of tailing disposal facilities on both the environment and the inhabiting organisms. It is already well-known that TDFs are a source of heavy metal pollution. The tailing material has a low pH which affects the bioavailability and mobility of certain metals. Tailing material is composed of fine and uniform sand-like particles. Because of the sand-like nature of the material, it is easily spread via wind into the surrounding environment. The dust pollution caused by TDFs may even have negative effects on the soil populations, for example reducing their reproductive success.

The first ecotoxicological test exposed earthworms (*Eisenia andrei*) to different concentrations of the TDF material collected. All of the TDF exposures were compared to a control and the two pastures sites. The collected TDF material was screened for eight chosen heavy metals (Cr, Co, Ni, Cu, Zn, As, Pb and Cd); these metals are common in gold mining sites. From all of the tested metals, only Cr exceeded the SSV1 benchmark. Even though most of these metals were not above the SSV1 benchmark, all eight of the chosen metals were present within all the pasture and TDF exposures. Most of the exposures above the 75% concentration showed a decrease in average earthworm weight. Especially the 100% black TDF exposures showed the lowest overall earthworm weight at the end of the 28 days of exposure. This can be due to the black TDF having no visible vegetation on the surface, as well as the lowest recorded pH and highest concentration of heavy metals. The earthworms exposed to the sand TDF and two pasture material were less affected and showed a higher average in earthworm weight, than those exposed to the black TDF. In some exposures, the sand TDF showed an increase in earthworm biomass. This may be due to *Eucalyptus* trees growing near and on top of the sand TDF, thus causing a higher amount of organic matter than in the black TDF. Comparing the earthworm reproduction results, the sand and black TDFs at the 75 and 100% concentrations had the lowest quantity of cocoons present, having an average of around one cocoon per exposure compared to the control with twenty cocoons on average. The northern pasture had the highest number of cocoons present, even higher than the southern pasture exposure. The control, northern pasture and southern pasture exposures also had the highest average number of juveniles per cocoon, compared to the sand and black TDFs. Both the black and sand TDFs had low pH values and higher heavy metal concentrations than the control and two pasture sites, which can explain why the TDFs have the lowest number of juveniles and cocoons present. For instance, the earthworms exposed to the black TDF 100% showed the highest bioaccumulation of Cd. Earthworm mortality was low for the duration of the experiment, with the

75% black TDF exposure having the highest mortality of 13% on the 28th day of the exposure. But it should be noted that in both the 100% concentration of the sand and black TDFs, the earthworms were almost lifeless and only found in the top layer of horse manure. Thus, the earthworms were clearly avoiding the tailing material.

An avoidance behaviour test was done to establish at which ratio of tailing material to the artificial soil, the earthworms would start preferring the control over the test substrate. At both the 25% and 50% exposures the earthworms were found randomly distributed between the test substrate and control, at all four of the sites. At 75% and 100% exposures most of the earthworms were avoiding the test substrate, with avoidance being over the 80% threshold, for both the black and sand TDF at a 100% concentration. A similar reaction was seen in the earthworm bioassay, where the earthworms tried to avoid the 100% black and sand TDF material. This can be due to the low organic content of the TDF material as well as the low pH and high metal concentrations of the material. The fine and uniform structure of the TDF material could also be undesirable for earthworms. Earthworms have been successfully used as bioindicators of disturbance in multiple studies, over the last decade. Avoidance test can be used as a quick screening method, where avoidance behaviour could indicate disturbance. Earthworm bioassays can be used afterward to provide accurate data on the effects of contaminated soils.

Most mesofaunal studies focus on a single species or taxa to estimate the ecological impacts of anthropogenic disturbances. While this approach can provide accurate results based on reproductive and physiological data, it provides limited data on how the structure of mesofaunal communities are affected. This study attempted to determine the effects of different sites of a gold mine on mesofaunal communities, by looking at total sampled individuals and species diversity. Samples were taken from two different TDFs and two pasture sites that were near the TDFs. The northern and southern pastures sites represent the natural environment. Samples were also taken during all four different seasons, to determine the effects of seasonal variation on soil communities.

There was a significant difference between the two TDFs sites and pastures sites, as seen by just comparing the total number of counted individuals. Both pastures had approximately twice the number of individuals of the TDF sites. In order to interpret the fluctuations of soil mesofauna biomass, knowledge on their life cycle, reproduction and various abiotic factors are required. However, little is still known about the ecology of many species. It is therefore easier

to correlate the fluctuations in diversity and abundance with physical factors such as rainfall, temperature and water holding capacity of the soil. Species composition and abundance can give an indication of the level of disturbance. Both the sand and black TDFs had low organic matter, acidic soil and the highest average heavy metal concentrations, which can explain low species abundance compared to the surrounding soils. Another factor which influences the number of species found within the soil is seasonal change. Autumn had the highest number of individuals per order, followed by spring, winter and then summer. The autumn samples were taken in March, when the temperature average between 13°C and 26°C; with decent rainfall. The presence of rain and the temperature not being too high or low, could explain why autumn had the highest abundance of species. The Prostigmata, Cryptostigmata, Mesostigmata and Collembola were the most dominant of the arthropod taxa, sampled from the four different sites, with the Prostigmata being the most dominant group overall while the Collembola were only present within the two pasture sites. The Prostigmata are well known to survive in dry and disturbed areas, Prostigmata also boasts a high metal tolerance and may have a mechanism for either neutralising or removing metals from their bodies (Osler *et al.*, 2000).

The species list was divided into five different functional groups based on their feeding preferences. Functional groups could help to provide insight into the structure of the soil communities present. The functional groups used were mycophagous (MP), predators (PRED), saprophagous and omnivorous (SO), and bacteriophagous and micro-algivoracious (MBM). The MBM, MP and PRED functional groups were the most abundant in all of the sample sites. While SO and PPAR groups were either in low number or absent in the TDFs samples. This can be mainly due to the low amount of organic matter and sparse vegetation cover on the TDFs.

Soil mesofauna can be used successfully as bioindicators of environmental health, by reflecting the effects of contaminated soils through community structure and number of individual species present. Even though this study was conducted on an old gold mine (78 years old), both the earthworm bioassays and mesofauna study indicated that the TDFs are still highly contaminated.

Recommendations

After the four weeks, earthworm reproduction test showed significant decreases in both earthworm weight and reproduction from the 75% TDF exposures. Future studies might want to use a higher ratio of test to control substrate, while increasing the percentage at smaller intervals to the 100% concentration. This could help estimate at which concentrations of contaminated soil the earthworms start being negatively affected by the TDFs.

This study focused on the number of earthworm cocoons produced in contaminated soils and the number of juveniles that hatched afterward. Other studies recommend focusing on viability of cocoon production. Because it is known that cocoon viability, is also affected by contaminated soils, not just the production of cocoons. When determining cocoon viability it is recommended to remove the cocoon after production, to avoid the death of juveniles because of metal contamination.

Seasonal fluctuations did affect the amount of mesofauna sampled at different times of the year. For the earthworm bioassay, the different seasons were not taken into account. It is recommended to determine the concentrations of heavy metals and the pH values of the TDFs at different seasons, doing bioassays for each season in order to determine how reproduction, bioaccumulation and weight of earthworms are affected by seasonal fluctuations.

The soil mesofauna was successfully utilised as bioindicators to assess environmental contamination when focusing on the number of individual and different species. Future studies could attempt to determine the bioavailability of certain metals, to better understand how metals can accumulate within certain organisms.

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ANNEXURES A

Complete species lists of individuals collected from tailing disposal facilities and two surrounding pastures at a gold mine.

Table A-1: Species list showing the amount of individuals sampled at a sand TDF, black TDF and two surrounding pasture sites, in the autumn.

Order	Family	Genera	Black TDF						Sand TDF						Pasture North						Pasture South					
			S1	S2	M1	M2	N1	N2	S1	S2	M1	M2	N1	N2	S1	S2	M1	M2	N1	N2	S1	S2	M1	M2	N1	N2
Prostigmata	Pediculocheilidae	<i>Pediculocheles sp.</i>		11		13	9	7		8	19	9			13	1		24		5	13	7	4	17	6	29
	Nanorchestidae	<i>Speleorchestes meyeri</i>	3	4	10	9				8	19	8	1		13	5		3			13		4	6		7
		<i>Speleorchestes potchensis</i>		7		8	2								18	9		7				37				
		<i>Nanorchestes globosus</i>																					2			
		<i>Nanorchestes usitatus</i>													11											
	Alicorhagiidae	<i>Alicorhagia usitata</i>													9	4		3		13		13		3		4
		<i>Stigmalychus veretrum</i>													13		16					23				
	Alycidae	<i>Alycus acaciae</i>														6			4							1
		<i>Bimichaelia sp.</i>															2				4					3
		<i>Petrallycus longicornis</i>																								
	Terpnacaridae	<i>Terpnacarus glebulentus</i>																								
		<i>Alycosmesis sp.</i>															19							2		
	Tydeidae	<i>Coccotydaeolus sp.</i>	12								2		12		9	7			6		19	1	3		37	
		<i>Coccotydaeus sp.</i>	10		13	24		9	7				10				7		1		16		24		7	
		<i>Tydeus grabouwii</i>	4		3							13						6				3	3	7		

	<i>Tydeus munsteri</i>		4							17				5	9	6					5				
	<i>Brachytydeus sp.</i>											7							7		19				
	<i>Pronematus ubiquitus</i>	6		4	8	1								17		7	9				8	13	20	9	
Paratydeidae	<i>Tanytydeus sp.</i>		4	8	3				6						24	20									
	<i>Sacotydeus sp.</i>			2					6		5	4			16		6		9						
Eupodidae	<i>Cocceupodes sp.</i>								4																
	<i>Eupodes parafusifer</i>													7	7										
Rhagidiidae	<i>Rhagidia sp.</i>													3				3		17		6			5
	<i>Coccorhagia sp</i>	3		4		5	7	2	2	4				7	6	9		4			16				
Anystidae	<i>Anystis baccharum</i>													4	9				3		3			1	
Erythraeidea	<i>Abrolophus sp.</i>														7					3	4			6	
Scutacaridae	<i>Scutacarus sp.</i>													3	4		7			7		6	4		
Tetranychidae	<i>Eotetranychus sp.</i>														3	7	1								
Stigmaeidae	<i>Eustigmaeus sp.</i>																1								
Nematalycidae	<i>Gordyaliscus sp.</i>								4						1	1		6	10	4	4	9	7	16	
Micropsammidae	<i>Micropsammus sp.</i>		7											46		18	7	16		34	14	9			
Bdellidae	<i>Cyta sp.</i>																							3	
	<i>Spinibdella thori</i>														6	4									
Pseudoscheylidae	<i>Tarsocheylus sp.</i>																								
Pygmephoridae	<i>Bakerdania sp.</i>	7	4	14	33		2				3	4			2										

	Rapignathidae	<i>Genus indet.</i>	7														3								
	Cheyletidae	<i>Chylertia sp.</i>												6		3	1	3	4		2	7		2	
	Pyemotidae	<i>Pyemotes sp.</i>																			4				
Cryptostigmata	Oppiidae	<i>Multioppia sp.</i>																		4	4				
	Oribatulidae	<i>Zygoribatula sp.</i>																		5	5				
	Schelobatidae	<i>Scheloribates sp.</i>	7	6	3	5	3	1	4		6	9	11	1											
	Belbidae	<i>Belba sp.</i>							1	7					2							3	1	4	
	Camisiidae	<i>Acronothrus sp.</i>					3								1	4	6	1		2	1	2			
	Tectocepheidae	<i>Tectocepheus sp.</i>						14						14			10			1	1			14	
	Ceratozetidae	<i>Hypozetes sp.</i>												7		2		1		4		1	2	3	
	Gymnodamaeidae	<i>Jacotella sp.</i>															1	2			4	1			
		<i>Gymnodameus sp.</i>													6	9	1	3	7	6			1	1	4
	Cosmochthoniidae	<i>Cosmochthonius sp.</i>					6	7		3								17	6	3		6	9		13
		<i>Brachythionius sp.</i>				1	1			1	4	7		9		6		14			7		9	6	3
	Galumnidae	<i>Galumna sp.</i>							4	1		2			7	1	5	4	7	6	7	4	14	12	
	Microzetidae	<i>Microzetes sp.</i>													7	4	1					7		2	
	Hypochothoniidae	<i>Cosmochthonius sp.</i>	4		1		2		4		1		3												
	Epilohmanniidae	<i>Epilohmannia sp.</i>	4		1		2																		
Mesostigmata	Laelapidae	<i>Cosmolaelaps sp.</i>													6			7			3		3		
		<i>Leptolaelaps sp.</i>													7		12	4							

		<i>Pachylaelaps sp.</i>																						10		
		<i>Hypoaspis sp.</i>															9				4					
	Rhodac aridae	<i>Rhodacarus sp.</i>	7						7														6	6		7
		<i>Gamasellus sp.</i>	6		8	7	1	9													7	6		4		
		<i>Rhodacarellus sp.</i>		1		1			7		9				14											
	Ascidae	<i>Protogamasellus sp.</i>	7	3	7	4	1	1						10								7		3		
		<i>Proctolaelaps sp.</i>												6												
		<i>Asca sp</i>							2		3				12	3		5		3	4		1	6	1	
		<i>Lasioseius sp.</i>														11	7		9							
	Digama sellidae	<i>Digamasellus sp.</i>					1												6		4	4		1		
	Macroch elidae	<i>Macrocheles sp.</i>						1	1		1		1		2		2									
	Phytosei idae	<i>Typhlodromus sp.</i>						1	6	4		4	4		3		2	1	4	3		1	3		6	
	Parastid ae	<i>Pergamasus sp.</i>								6			3		1		4			2		1				
		<i>Parasitus sp.</i>						7																		
Astigm ata	Acarida e	<i>Caloglyphus sp.</i>					4						7	1	6	9	11	3								
		<i>Rhizoglyphus echinopus</i>						6		7		10			35	16			14	16		20	6			
		<i>Tyrophagus putrescentiae</i>													18	16			18	23	44	10	16	21		
		<i>Bakerdania sp.</i>												6								3				
Metasti gmata	Ixodidae	<i>Amblyomma sp.</i>																		1			1	1		
Aranae a	Lycosid ae	<i>Lycosa sp.</i>																	16	4	2		2			
	Salticida e	<i>Salticus sp.</i>				2																				
Coleopt era	Tenebri onidae															6									3	
	Bupresti dae																		4			1				

	Anthicidae										2							4							
	Staphylinidae																	3						2	
	Elateridae																	19						2	
	Curculionidae													2											
Psocoptera	Psocidae																		3			5		4	
Hymenoptera	Formicidae	<i>Pheidole sp1.</i>																				30			
		<i>Pheidole sp2.</i>																			4				
		<i>Anaplolepis sp.</i>																							
		<i>Plagiolepis sp.</i>							1												4				
		<i>Tetramonium sp.</i>			3					1															
		<i>Monomonium sp.</i>																1	7						
		<i>Technomyrmex sp.</i>												17											
		<i>Cerapachys sp.</i>								2	4							16							
		<i>Crematogaster sp.</i>																7							
		<i>Myrmecaria sp.</i>																							
		<i>Tetraoponera sp.</i>																							
Hemiptera	Coccidae																								
Isoptera	Hodotermitidae	Hodotermes sp.																		4			7		
Diptera	Brachycera																			1					
	Nematocera																								
	Muscidae																								
	Cecido																								

	myiidae																									
Lepidoptera	Geometridae																				2					
Collembola	Poduridae															10					70			7		
	Isotomidae																				11	10			9	
	Entomobryidae															11		3								
	Sminthuridae																							13		
Blattodea																									10	

Table A-2: Species list showing the amount of individuals sampled at a sand TDF, black TDF and two surrounding pasture sites, in the winter.

Order	Family	Genera	Black TDF						Sand TDF						Pasture North						Pasture South					
			S1	S2	M1	M2	N1	N2	S1	S2	M1	M2	N1	N2	S1	S2	M1	M2	N1	N2	S1	S2	M1	M2	N1	N2
Prostigmata	Pediculocheilidae	<i>Pediculocheles sp.</i>																								
	Nanorchestidae	<i>Speleorchestes meyeri</i>		7			16	3	1	5	7		2	3	17	4	3	5	5			10	4		17	
		<i>Speleorchestes potchensis</i>	2		10	9					7		1			5	14		7			3		10		17
		<i>Nanorchestes globosus</i>														2	1	1		3						
		<i>Nanorchestes usitatus</i>															16	3	2							
	Alicorhagiidae	<i>Alicorhagia usitata</i>													17	21		8		2		4	3	2		3
		<i>Stigmalychus veretrum</i>													1		7		6	3		3	5	1		1
	Alycidae	<i>Alycus acaciae</i>														6	7		4			4		14		1
		<i>Bimichaelia sp.</i>																					2			4
		<i>Petralyclus longicornis</i>													2											
	Terpnacaridae	<i>Terpnacarus glebulentus</i>													7		4	16		3		4	16	6	10	
		<i>Alycosmesis sp.</i>																				4		3		
	Tydeidae	<i>Coccotydaeolus sp.</i>							1	3		1	1								26	6		7		4
		<i>Coccotydaeus sp.</i>																				17	9	6		4
		<i>Tydeus grabouwii</i>														1		1	4	5	7	4				
		<i>Tydeus munsteri</i>								3		7										6	6		1	
		<i>Brachytydeus sp.</i>			4			1																		
		<i>Pronematus ubiquitus</i>		3	4		6									13		8	7		4		7	11	10	2
	Paratydeidae	<i>Tanytydeus sp.</i>	12		7	13			12		7	13	4													
		<i>Sacotydeus sp.</i>		3	4											6	3		6			3				
	Eupodidae	<i>Cocceupodes sp.</i>			6					3			6					4	3			2				
		<i>Eupodes parafusifer</i>		2						9																

	Rhagididae	<i>Rhagidia sp.</i>								1	4				7		3			1		6	1	
		<i>Coccorhagia sp.</i>		4	1		2										3	3						
	Anystidae	<i>Anystis baccharum</i>												4	3		2			1	4	1		
	Erythraeidae	<i>Abrolophus sp.</i>									2	1	1						2	2	6			
	Scutacaridae	<i>Scutacarus sp.</i>																			4		7	7
	Tetranychidae	<i>Eotetranychus sp.</i>												3		4			2		7	1		3
	Stigmaeidae	<i>Eustigmaeus sp.</i>																						
	Nematalycidae	<i>Gordyaliscus sp.</i>								14		3	9						1	6		4	4	
	Micropsammidae	<i>Micropsammus sp.</i>														6	6		4			4	11	7
	Bdellidae	<i>Cyta sp.</i>									12						6		4		1		1	1
		<i>Spinibdella thori</i>	2	4		8				5	6	3						5	4			2	3	1
	Pseudoscheyletidae	<i>Tarsocheylus sp.</i>	3	5	1		7										2	1	1					
	Pygmephoridae	<i>Bakerdania sp.</i>							3		4	7		4	10	5	7	10	11		3	4	3	12
	Rapignathidae	<i>Genus indet.</i>												3	1		4							
	Cheyletidae													3		3		2				4		3
	Pyemotidae	<i>Pyemotes sp.</i>		1	4										2		6							
Cryptosigmata	Oppiidae	<i>Multioppia sp.</i>												3	1	1		4				2	2	3
	Oribatulidae	<i>Zygoribatula sp.</i>												1		2	3				1	1		2
	Schelori batidae	<i>Scheloribates sp.</i>															14				4		16	

	Belbidae	<i>Belba sp.</i>													3	1	4		1								
	Camisiae	<i>Acronothrus sp.</i>															4		1								
	Tectocephidae	<i>Tectocephus sp.</i>																									
	Ceratozetidae	<i>Hypozetes sp</i>																			2		1				
	Gymnodamaeidae	<i>Jacotella sp.</i>																									
		<i>Gymnodameus sp.</i>																									
	Cosmochthoniidae	<i>Cosmochthonius sp.</i>													1	4			3			10		7			
		<i>Brachythionius sp.</i>																							17		
	Galumnidae	<i>Galumna sp.</i>																					5				
	Microzetidae	<i>Microzetes sp.</i>																									
	Hypochothoniidae	<i>Cosmochthonius sp.</i>														17			1		2		1		11		2
Epilohmanniidae	<i>Epilohmannia sp.</i>																										
Mesostigmata	Laelapidae	<i>Cosmolaelaps sp.</i>																									
		<i>Leptolaelaps sp.</i>																									
		<i>Pachylaelaps sp.</i>																	4		7						
		<i>Hypoaspis sp.</i>																									
	Rhodacaridae	<i>Rhodacarus sp.</i>														7		1		4			18		1		2
		<i>Gamasellus sp.</i>																									
		<i>Rhodacarellus sp.</i>																									
	Ascidae	<i>Protogamasellus sp.</i>																			1		3			1	
		<i>Proctolaelaps sp.</i>																									
		<i>Asca sp</i>																									
		<i>Lasioseius sp.</i>																									

[illegible]

		<i>Anaplolepis</i> sp.															6								
		<i>Plagiolepis</i> sp.													7				1				3		4
		<i>Tetramonium</i> sp.												6			4								
		<i>Monomonium</i> sp.														5		2	1	6			1		1
		<i>Technomyrmex</i> sp.																							
		<i>Cerapachys</i> sp.																	6		2			1	
		<i>Crematogaster</i> sp.																							
		<i>Myrmicaria</i> sp.																							
		<i>Tetraponeta</i> sp.																							
Hemiptera	Coccidae													2						13					
Isoptera	Hodotermitidae	Hodotermes sp.											6		14		1	7	16				2		
Diptera	Brachycera													2		1		1							
	Nematocera																		2	1					
	Muscidae															4								7	1
	Cecidomyiidae													2				4							1
Lepidoptera	Geometridae																								
Collembola	Poduridae																		12		9			4	
	Isotomidae												11	4	13		2		7		16	5	3	1	
	Entomobryidae												18	7	11	2	2	4				2			
	Sminthuridae																		8	17		11			3
Blattodea																				3					

Table A-3: Species list showing the amount of individuals sampled at a sand TDF, black TDF and two surrounding pasture sites, in the spring.

Order	Family	Genera	Black TDF						Sand TDF						Pasture North						Pasture South					
			S1	S2	M1	M2	N1	N2	S1	S2	M1	M2	N1	N2	S1	S2	M1	M2	N1	N2	S1	S2	M1	M2	N1	N2
Prostigmata	Pediculocheilidae	Pediculocheles sp.	4		2	11	1	1	4	1		3	1	3			5	5	1	1	11			3	21	4
	Nanorchestidae	Speleorchestes meyeri	3		3			1	7		9	1	1	3		10		1				4		17		20
		Speleorchestes potchensis	4		6	4			7	2	1	4	1		7			6	3	2	1	7	7	4		4
		Nanorchestes globosus															1		16			10		12		6
		Nanorchestes usitatus													6			4		4	4		3		2	
	Alicorhagiidae	Alicorhagia usitata														9	1	1	1		10			6		
		Stigmalychus veretrum														4	6	4				20		1	16	
	Alycidae	Alycus acaciae																	3					3		1
		Bimichaelia sp.																			4		5			
		Petrallycus longicornis																			4					4
	Terpnacaridae	Terpnacarus glebulentus																				3		4		6
		Alycosmesis sp.																								
	Tydeidae	Coccotydaeolus sp.		10		3							8		6	7		13	8			25		16		11
		Coccotydaeus sp.		1	6		4		1		1			3				6		9			17			
		Tydeus grabouwi						3									3				17		6	13		6
		Tydeus munsteri				2														6		6				
		Brachytydeus sp.															1	1	5	4	6			1		3
		Pronematus ubiquitus			1		4	7				2	1		7		9	3		5		16	6		1	
	Paratydeidae	Tanytydeus sp.												2		2					4		2			3
		Sacotydeus sp.		1		4																	1		1	6
	Eupodid	Cocceupodes sp.		1			1									3					26		2		20	20

	ae	Eupodes parafusifer															6				1		8	4		
	Rhagidii dae	Rhagidia sp.															6			7		7	3	4	7	
		Coccorhagia sp												11	12	2	11	6	6	20	4	6		16	18	
	Anystida e	Anystis baccharum											1	6	1	6	1	1	7		3	1	7			
	Erythrae idea	Abrolophus sp.											6		9	8		3	3			7		1		
	Scutaca ridae	Scutacarus sp.											1		1					10	16					
	Tetrany chidae	Eotetranychus sp.													3											
	Stigmae idae	Eustigmaeus sp.											4								2					
	Nematal ycidae	Gordyalicus sp.													3			3		6		7		12		
	Microps ammida e	Micropsammus sp.												4		7	14	16	14	17	13	20		2		
	Bdellida e	Cyta sp.							1				4								2		9	2		
		Spinibdella thori								1		1		3		6		4	2		6	3			1	
	Pseudo cheylida e	Tarsocheylus sp.													2		3		1	3				3	1	
	Pygmep horidae	Bakerdania sp.							2	4	4	2	4	2	1		20	1	6	2		9	6		7	1
	Rapigna thidae	Genus indet.																					3			
	Cheyleti dae	Chyletia sp.																			2				1	
Pyemoti dae	Pyemotes sp.																		1	1						
Cryptos tigmata	Oppiida e	Multioppia sp.																		1					1	
	Oribatuli dae	Zygoribatula sp.																		4	3	1	18		3	

	Schelori batidae	Scheloribates sp.															22	13							1
	Belbida e	Belba sp.																8			4	1	7		1
	Camisiid ae	Acronothrus sp.																		4	2				1
	Tectoce pheidae	Tectocephus sp.																		3	2				1
	Ceratoz etidae	Hypozetes sp																				2	1		1
	Gymnod amaeida e	Jacotella sp.																	7		3				
		Gymnodameus sp.																							
	Cosmoc hthoniid ae	Cosmochthonius sp.																		4		2			
		Brachythionius sp.																	7		1				1
	Galumni dae	Galumna sp.										1		1											
	Microzet idae	Microzetes sp.												3											
	Hypocht honiidae	Cosmochthonius sp.																		30		9			20
	Epilohm anniidae	Epilohmannia sp.																		9			6		
Mesosti gmata	Laelapid ae	Cosmolaelaps sp.						1												1			1		
		Leptolaelaps sp.																					6		
		Pachylaelaps sp.												1		1				6					7
		Hypoaspis sp.																	3	4	6	5			
	Rhodac aridae	Rhodacarus sp.						1		1			10		7	6	6	3		2	4	1	4		
		Gamasellus sp.										6							1		1				
		Rhodacarellus sp.										7	6	3	1		1	4	1	4					7
	Ascidae	Protogamasellus sp.											9	7			1					3			
		Proctolaelaps sp.											1			3	7								

		Asca sp												4		4	3		6		9						
		Lasioseius sp.														6		1	1			4					
	Digama sellidae	Digamasellus sp.												1					1								
	Macroch elidae	Macrocheles sp.												1							7						
	Phytosei idae	Typhlodromus sp.									4								2		3						
	Parastid ae	Pergamasus sp.												1		4			1	1							
		Parasitus sp.	1	2	2	1	2	1							1					1		7	6	7		1	
Astigm ata	Acarida e	Caloglyphus sp.		1		1					3		4	9	3					2			3	2	4		
		Rhizoglyphus echinopus	1	2	1			2													13		4		7	1	
		Tyrophagus putrescentiae	1	2	2	1	1	1	1	4	8				1	1	2	4	1	7	1		7	13	9	6	7
		Bakerdania sp.	1	1	2	2	2	3				7	3	4	3								10	11	8	7	
Metasti gmata	Ixodidae	Amblyomma sp.																			1	3				1	
Aranae a	Lycosid ae	Lycosa sp.																			4		2		1		
	Salticida e	Salticus sp.													2							2		2		2	
Coleopt era	Tenebri onidae														2			3			1	1	4				
	Bupresti dae														3		1							3		1	
	Anthicid ae														2								2				
	Staphyli nidae														2						4		1		3		
	Elaterid ae															1											
	Curculio nidae															4	2	2	1		1	2	1	1	1	1	
Psocop	Psocida																				2						

tera	e																							
Hymenoptera	Formicidae	Pheidole sp1.																		1				
		Pheidole sp2.																		1				
		Anaplolepis sp.																		1				
		Plagiolepis sp.																		1	1	1		
		Tetramonium sp.																				1	1	
		Monomorium sp.																				1	1	
		Technomyrmex sp.																			2		2	
		Cerapachys sp.																	2	1				
		Crematogaster sp.								1									4	4	2	2	2	
		Myrmecaria sp.								1									1	2	1	1	1	
		Tetraoponera sp.																	1	1	1	1	2	
Hemiptera	Coccidae																		1	1	1	1	2	
Isoptera	Hodotermitidae	Hodotermes sp.								1										1	1	1	2	
Diptera	Brachycera																			1		1	2	
	Nematocera																			3		21		7
	Muscidae																		4	14		11		
	Cecidomyiidae																				3			
Lepidoptera	Geometridae																		1	2				
Collembola	Poduridae																			44		6	10	
	Isotomidae																		7		11		7	8
	Entomobryidae																			19		17		

	Sminthuridae																			12		1	14		19
Blattodea																						2			4

Table A-4: Species list showing the amount of individuals sampled at a sand TDF, black TDF and two surrounding pasture sites, in the summer.

Order	Family	Genera	Black TDF						Sand TDF						Pasture North						Pasture South					
			S1	S2	M1	M2	N1	N2	S1	S2	M1	M2	N1	N2	S1	S2	M1	M2	N1	N2	S1	S2	M1	M2	N1	N2
Prostigmata	Pediculocheilidae	<i>Pediculocheles</i> sp.	7	7	6	6			4	7	4	1			7	3		1	1		7	4	7	7	6	2
	Nanorchestidae	<i>Speleorchestes meyeri</i>	2	7	2	5	1								4	6	1	7		1	3	4	3	2		0
		<i>Speleorchestes potchensis</i>		2											6	6	7	6	3		9	10	4	3	1	1
		<i>Nanorchestes globosus</i>													3	4	3		1	3		4	4			4
		<i>Nanorchestes usitatus</i>											4		4	2	1			2	1		1		4	1
	Alicorhagiidae	<i>Alicorhagia usitata</i>													4	4	2	1		2	1	4	2	3	2	
		<i>Stigmalychus veretrum</i>															2				2			2	1	1
	Alycidae	<i>Alycus acaciae</i>													1	2					2		3			
		<i>Bimichaelia</i> sp.													2	1					4	1		3	1	2
		<i>Petralytus longicornis</i>															1					4	1			1
	Terpnacaridae	<i>Terpnacarus glebulentus</i>													1	2	2	1			2		2	1		2
		<i>Alycosmesis</i> sp.																				2	1			
	Tydeidae	<i>Coccotydaeolus</i> sp.															1				1	4	2	1	1	1
		<i>Coccotydaeus</i> sp.															1				2		1			
		<i>Tydeus grabouwi</i>														2				2	1	1				1
		<i>Tydeus munsteri</i>															1				1					
		<i>Brachytydeus</i> sp.																	2		1	2	6	5	1	
		<i>Pronematus ubiquitous</i>			4		1	4	1			2	2	1		1							1	4		1
	Paratydeidae	<i>Tanytydeus</i> sp.	1		4				3									2			2	1				
		<i>Sacotydeus</i> sp.		2					2	2	1	3			2	3	1		5	4		1	4	3		1
	Eupodidae	<i>Cocceupodes</i> sp.	1	1												1					5	3	4	3	2	1
		<i>Eupodes parafusifer</i>													2	1	1					2	1		3	
	Rhagidiidae	<i>Rhagidia</i> sp.															4	1	2	1	3	3				
		<i>Coccorhagia</i> sp.													1	1						3	1	4	3	7
	Anystidae	<i>Anystis baccharum</i>														2	1			1	2	1		2		1

	Erythraeidea	<i>Abrolophus sp.</i>					1	3	1	4							1	2	1		1					
	Scutacaridae	<i>Scutacarus sp.</i>				1				2							1	1			1			1		
	Tetranychidae	<i>Eotetranychus sp.</i>				1									1		2	1		1				1		
	Stigmaeidae	<i>Eustigmaeus sp.</i>													1				1	1			2	1		
	Nematalycidae	<i>Gordyalicus sp.</i>											2	1	2	1		1		2	2	7	10			
	Micropsammidae	<i>Micropsammus sp.</i>								1	1		1	4	1		3		2	1	7	1	1		1	1
	Bdellidae	<i>Cyta sp.</i>					1		1	1			1	2		1		9		1	1	2	1		2	1
		<i>Spinibdella thori</i>					1	2						2	1				1	2		2			1	
	Pseudocheyleidae	<i>Tarsocheylus sp.</i>													1								2		1	
	Pygmephoridae	<i>Bakerdania sp.</i>												1	2	1	3				1		1		1	
	Rapignathidae	<i>Genus indet.</i>												1	3	2					1	1		2		1
	Cheyletidae	<i>Chyletia sp.</i>						1							1						2		2		1	1
	Pyemotidae	<i>Pyemotes sp.</i>						1	2						1								1		2	

Cryptosigmata	Oppiidae	<i>Multioppia sp.</i>																		1		2			1	
	Oribatulidae	<i>Zygoribatula sp.</i>																							1	
	Scheloribatidae	<i>Scheloribates sp.</i>						1			1	2		2		4		1		1	3	4	1	4	1	1
	Belbidae	<i>Belba sp.</i>								2				1					1	2	1	1	4	2	1	
	Camisiidae	<i>Acronothrus sp.</i>												3				3	3			1	4	1		
	Tectocepheidae	<i>Tectocepheus sp.</i>																			4	2		4	1	
	Ceratozetidae	<i>Hypozetes sp.</i>																	1		1					
	Gymnodamaeidae	<i>Jacotella sp.</i>													1						2		1	4		
		<i>Gymnodameus sp.</i>													1						1		3	1		1
	Cosmochthoniidae	<i>Cosmochthonius sp.</i>				1	2	1	2	2	1				11						4					2
		<i>Brachythionius sp.</i>							1	2						4		1	2	1			1	1	2	
	Galumnidae	<i>Galumna sp.</i>													1	1	2		2		1	1	2	1	2	
	Microzetidae	<i>Microzetes sp.</i>																		1						1

	Hypochthoniid ae	<i>Cosmochthonius sp.</i>											1	1	11		2		3		1	1	2	1	2	2
	Epilohmanniid ae	<i>Epilohmannia sp.</i>																1	1	2	1	1	1			1
Mesostigmata	Laelapidae	<i>Cosmolaelaps sp.</i>															1	3							1	1
		<i>Leptolaelaps sp.</i>													1	2										1
		<i>Pachylaelaps sp.</i>														2	1							1	2	1
		<i>Hypoaspis sp.</i>															1	1						2	1	1
	Rhodacaridae	<i>Rhodacarus sp.</i>																						1	2	1
		<i>Gamasellus sp.</i>																						1		1
		<i>Rhodacarellus sp.</i>																		2				1	1	1
	Ascidae	<i>Protogamasellus sp.</i>													4	2	2	1			2	1	1	1		1
		<i>Proctolaelaps sp.</i>															1					2	1	1	2	1
		<i>Asca sp.</i>																				1	1	1		1
		<i>Lasioseius sp.</i>																				1	1	1	2	1
	Digamasellidae	<i>Digamasellus sp.</i>					1		2	1												1	1	1		
	Macrochelidae	<i>Macrocheles sp.</i>			1			1														1	1	1	2	
	Phytoseiidae	<i>Typhlodromus sp.</i>						2			1											1	1			
	Parastidae	<i>Pergamasus sp.</i>						1	1					1									1	1	2	
		<i>Parasitus sp.</i>				2	2						2												2	1
Astigmata	Acaridae	<i>Caloglyphus sp.</i>	2	1		1	1	1	2	4		2	2	1	2	2	2				1	2	1	2	1	
		<i>Rhizoglyphus echinopus</i>	2	2		1	1	1	2	2		2	1	1	2	1	1				1	1	2	2	2	2
		<i>Tyrophagus putrescentiae</i>	2	2	1	2	2	1	12	1	1	1	1	1	2	1	1	1	1	3	2			1	1	2
		<i>Bakerdania sp.</i>	4	2	4	1	2	4	2	2	1	1	1	1				2	2	2				1	1	2
Metastigmata	Ixodidae	<i>Amblyomma sp.</i>													2				2	1	2	1	1			
Araneae	Lycosidae	<i>Lycosa sp.</i>	1															1			1					
	Salticidae	<i>Salticus sp.</i>	1																		2	1				
Coleopt	Tenebrionidae		2	2	1	1	2	1																		

era	Buprestidae		1				2	1																			
	Anthicidae						1												1	1							
	Staphylinidae																		1	1							
	Elateridae					2													1	1							
	Curculionidae		1						1	1					1	1											
Psocoptera	Psocidae								1	1				1	1					1							
Hymenoptera	Formicidae	<i>Pheidole sp1.</i>	1	1		1	2	1						1					1	2					1		
		<i>Pheidole sp2.</i>	1	1	1	1	2	1						1					1	2					1		
		<i>Anaplolepis sp.</i>	1	2																2							
		<i>Plagiolepis sp.</i>																		1							
		<i>Tetramonium sp.</i>	1					1						1					1	1					1		
		<i>Monomorium sp.</i>																		2	1						
		<i>Technomyrmex sp.</i>												1						2	1	1					
		<i>Cerapachys sp.</i>					1	2	1	1										1					1		
		<i>Crematogaster sp.</i>					1	2	1	2					1	1				1					1		
		<i>Myrmicaria sp.</i>		1			2		1	4					1	1				1					1		
		<i>Tetraponera sp.</i>	2	1	1	1	1	1	4	1					1	1			2		2				1		
Hemiptera	Coccidae		2	2	1				2	2	1	2		1	1					1				1			
Isoptera	Hodotermitidae	Hodotermes sp.	3	2					2	2	8	1		1	1				2		2			4	2	1	
Diptera	Brachycera								1	1	1	1	2	1	1				1		1			2	2		
	Nematocera									2	1				2	1	1				2	1	2	2	2	1	
	Muscidae									3	2	1	1				2	2	1	1			1	1	1	2	
	Cecidomyiidae														1								1	1	1	2	
Lepidoptera	Geometridae													1	1									1	2		
Collembola	Poduridae														5	5	5	2						2	1	1	1
	Isotomidae														1	1								1	2	1	2
	Entomobryidae															2	1								14	10	12

	Sminthuridae														2	1								1	10	1	2
Blattodea															2	1								1	1	1	

ANNEXURES B

Table B-1: Abbreviations for each of the functional groups

Group	Abbrev.
Mycophagous organisms	MP
Micro-algivororous & bacteriophagous	MBM
Predators	PRED
Plant parasitic & herbivorous	PPAR
Saprophagous and omnivorous organisms	SO

Table B-2: A list showing each species functional group.

Order	Family	Genera	Functional Group
Prostigmata	Pediculocheilidae	<i>Pediculocheles sp.</i>	MBM
	Nanorchestidae	<i>Speleorchestes meyeri</i>	MBM
		<i>Speleorchestes potchensis</i>	MBM
		<i>Nanorchestes globosus</i>	MBM
		<i>Nanorchestes usitatus</i>	MBM
	Alicorhagiidae	<i>Alicorhagia usitata</i>	MP
		<i>Stigmalychus veretrum</i>	MP
	Alycidae	<i>Alycus acaciae</i>	MP
		<i>Bimichaelia sp.</i>	MP
		<i>Petralyus longicornis</i>	MP
	Terpnacaridae	<i>Terpnacarus glebulentus</i>	MP
		<i>Alycosmesis sp.</i>	MP
	Tydeidae	<i>Coccotydaeolus sp.</i>	MBM
		<i>Coccotydaeus sp.</i>	MBM
		<i>Tydeus grabouwi</i>	PRED
		<i>Tydeus munsteri</i>	PRED
		<i>Brachytydeus sp.</i>	PRED
		<i>Pronematus ubiquitous</i>	PRED
	Paratydeidae	<i>Tanytydeus sp.</i>	PRED
		<i>Sacotydeus sp.</i>	PRED
	Eupodidae	<i>Cocceupodes sp.</i>	MBM
		<i>Eupodes parafusifer</i>	MBM
	Rhagidiidae	<i>Rhagidia sp.</i>	PRED
		<i>Coccorhagia sp</i>	PRED
	Anystidae	<i>Anystis baccarum</i>	PRED
	Erythraeidea	<i>Abrolophus sp.</i>	PRED

	Scutacaridae	<i>Scutacarus sp.</i>	MP
	Tetranychidae	<i>Eotetranychus sp.</i>	PPAR
	Stigmaeidae	<i>Eustigmaeus sp.</i>	PRED
	Nematolycidae	<i>Gordyalicus sp.</i>	PPAR
	Micropsammidae	<i>Micropsammus sp.</i>	MP
	Bdellidae	<i>Cyta sp.</i>	PRED
		<i>Spinibdella thori</i>	PRED
	Pseudocheylidae	<i>Tarsocheylus sp.</i>	PRED
	Pygmephoridae	<i>Bakerdania sp.</i>	MP
	Rapignathidae	<i>Genus indet.</i>	PRED
	Cheyletidae	<i>Chyletia sp.</i>	PRED
	Pyemotidae	<i>Pyemotes sp.</i>	PRED
Cryptostigmata	Ooppiidae	<i>Multioppia sp.</i>	MP
	Oribatulidae	<i>Zygoribatula sp.</i>	MP
	Scheloribatidae	<i>Scheloribates sp.</i>	MP
	Belbidae	<i>Belba sp.</i>	MP
	Camisiidae	<i>Acronothrus sp.</i>	MP
	Tectocepheidae	<i>Tectocepheus sp.</i>	MP
	Ceratozetidae	<i>Hypozetes sp.</i>	MP
	Gymnodamaeidae	<i>Jacotella sp.</i>	MP
		<i>Gymnodameus sp.</i>	MP
	Cosmochthoniidae	<i>Cosmochthonius sp.</i>	MP
		<i>Brachythionius sp.</i>	MP
	Galumnidae	<i>Galumna sp.</i>	MP
	Microzetidae	<i>Microzetes sp.</i>	MP
	Hypochthoniidae	<i>Cosmochthonius sp.</i>	MP
	Epilohmanniidae	<i>Epilohmannia sp.</i>	MP
Mesostigmata	Laelapidae	<i>Cosmolaelaps sp.</i>	PRED
		<i>Leptolaelaps sp.</i>	PRED
		<i>Pachylaelaps sp.</i>	PRED
		<i>Hypoaspis sp.</i>	PRED
	Rhodacaridae	<i>Rhodacarus sp.</i>	PRED
		<i>Gamasellus sp.</i>	PRED
		<i>Rhodacarellus sp.</i>	PRED
	Ascidae	<i>Protogamasellus sp.</i>	PRED
		<i>Proctolaelaps sp.</i>	PRED
		<i>Asca sp.</i>	PRED
		<i>Lasioseius sp.</i>	PRED
	Digamasellidae	<i>Digamasellus sp.</i>	PRED
	Macrochelidae	<i>Macrocheles sp.</i>	PRED
	Phytoseiidae	<i>Typhlodromus sp.</i>	PRED
	Parastidae	<i>Pergamasus sp.</i>	PRED
		<i>Parasitus sp.</i>	PRED

Astigmata	Acaridae	<i>Caloglyphus sp.</i>	SO
		<i>Rhizoglyphus echinopus</i>	SO
		<i>Tyrophagus putrescentiae</i>	SO
		<i>Bakerdania sp.</i>	MP
Metastigmata	Ixodidae	<i>Amblyomma sp.</i>	PRED
Aranaea	Lycosidae	<i>Lycosa sp.</i>	PRED
	Salticidae	<i>Salticus sp.</i>	PRED
Coleoptera	Tenebrionidae		SO
	Buprestidae		PPAR
	Anthicidae		MP/PRED
	Staphylinidae		PRED
	Elateridae		PRED
	Curculionidae		PPAR
Psocoptera	Psocidae		MP
Hymenoptera	Formicidae	<i>Pheidole sp1.</i>	PRED
		<i>Pheidole sp2.</i>	PRED
		<i>Anaplolepis sp.</i>	PRED
		<i>Plagiolepis sp.</i>	PRED
		<i>Tetramonium sp.</i>	PRED
		<i>Monomorium sp.</i>	PRED
		<i>Technomyrmex sp.</i>	PRED
		<i>Cerapachys sp.</i>	PRED
		<i>Crematogaster sp.</i>	PRED
		<i>Myrmicaria sp.</i>	PRED
		<i>Tetraponera sp.</i>	SO
Hemiptera	Coccidae		PPAR
Isoptera	Hodotermitidae	<i>Hodotermes sp.</i>	PPAR
Diptera	Brachycera		SO
	Nematocera		SO
	Muscidae		SO
	Cecidomyiidae		SO
Lepidoptera	Geometridae		PPAR
Collembola	Poduridae		MP
	Isotomidae		MP
	Entomobryidae		MP
	Sminthuridae		MP
Blattodea			SO