THE ECOLOGICAL RISK OF ACID MINE DRAINAGE IN A SALINISING LANDSCAPE

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ABSTRACT

Acid mine drainage (AMD) and increasing salinity of freshwater ecosystems pose serious threats to water quality in water-stressed South Africa. These threats are exacerbated by mining activities, mainly gold and coal from both active and abandoned mines that continue to release acidic water that is rich in toxic metals and high sulphate concentrations. Therefore, the overarching hypothesis for this study was that "the combination of AMD and sulphate salts confers high ecological risk to the aquatic biota". The study employed both laboratory and field investigations to test this hypothesis and provide appropriate tools to protect freshwater ecosystems from increasing anthropogenic impacts. Firstly, a laboratory investigation was carried out to develop risk-based water quality guidelines (WQGs) for sulphates and treated AMD (TAMD) using the species sensitivity distributions (SSDs) technique. Five South African freshwater species belonging to five different taxonomic groupings, including *Adenophlebia auriculata* (insect), *Burnupia stenochorias* (mollusc), *Caridina nilotica* (crustacea), *Pseudokirchneriella subcapitata* (algae) and *Oreochromis mossambicus* (fish) were exposed to varying concentrations of sodium sulphate (Na_2SO_4), magnesium sulphate ($MgSO_4$) and calcium sulphate ($CaSO_4$), as well as TAMD in separate ecotoxicological experiments, applying short-term (96 h) non-renewal and long-term (240 h) renewal exposure test methods. Secondly, a novel trait-based approach (TBA) was also used to predict the vulnerability of taxa to treated acid mine drainage (TAMD). The TBA used a combination of carefully selected traits of organisms that are mechanistically linked to TAMD for their potential vulnerability predictions. Leptoceridae (caddisflies) and Leptophlebiidae (mayflies) were selected taxa for evaluation of the trait-based vulnerability predictions to TAMD for laboratory toxicity exposures. This was followed by a field investigation to assess macroinvertebrates assemblage responses, abundance and richness to a TAMD-impacted stream using the South African Scoring System version 5 (SASS5) protocol. Outcomes form the above three sources were combined in a multi-criteria analysis (MCA) to develop an appropriate water quality management strategy in a form of a trait-based decision-making support tool. Results of the risk-based WQGs revealed that Na_2SO_4 was the most toxic of the tested salts. A concentration of 0.020 g/L Na_2SO_4, 0.055 g/L $CaSO_4$, and 0.108 g/L $MgSO_4$ or a combined sulphate salts limit of 0.067 g/L were derived as long-term WQGs to protect over 95% of the population species in a natural environment considered as relatively pristine. This means that the generic 0.25 g/L sulphate compliance limit for South African freshwater systems is under-protective.

Burnupia stenochorias was the most sensitive to AMD after long-term exposures, and it was adjudged as a good indicator of AMD along with *P. subcapitata*. Long-term scenario-specific WQG for AMD for the protection of over 95% of the population species was derived as 0.014%. Results of the TBA revealed that the relative abundance and diversity of taxa at a site that received direct impact from TAMD generally corresponded to trait-based predictions. The site that received direct TAMD was largely dominated by members of the Corixidae and Naucoridae families. However, Leptoceridae was more vulnerable to TAMD than Leptophlebidae contrary to predictions. Its assemblage did not match the predictions although Leptophlebiidae corresponded to predictions in terms of its assemblage and diversity. As water quality improved further downstream of the TAMD source, macroinvertebrates assemblage and diversity also improved as predicted. However, it is important to note that other equally important traits that were not studied might influence the response of organisms during toxicity test exposures. The MCA findings suggest that the trait-based decision-making support tool is a useful management strategy for the predicting vulnerability of taxa aquatic stressors including AMD and increasing salinity. Overall, the outcome of this study suggests that AMD poses an ecological risk to aquatic biota, but this becomes riskier in the presence of excess sulphate salts. Albeit, the WQGs for sulphate salts and AMD as well as the developed trait-based decision support tool all contribute novel sound scientific knowledge basis for managing the AMD and increasing salinity in freshwater ecosystems. The study recommends incorporating different life stages of indigenous species tested to determine if their sensitivity to AMD and sulphate would correspond to current findings because early life stages could be more sensitive to aquatic stressors than juveniles or adults. This is important for the derivation of strong and relevant WQGs. The TBA requires further refinement for its incorporation in ecotoxicology on a wide scale.

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GLOSSARY

ANOVA	Analysis of variance
CI	Confidence interval
DSS	Decision support system
EC	Electrical conductivity, a common surrogate for salinity
EC_X	Effect concentration
EPT	Ephemeroptera, Plecoptera, Trichoptera, often regarded as salinity sensitive orders
g/L	Grams per litre
GLM	Generalised linear model
h	Hour(s)
LC_X	Concentration lethal to x% of a population
LDx	Dilution lethal to x% of a population
mS/cm	Millisiemens per metre, a unit of electrical conductivity
n	Sample size, for example, number of species, number of replicates
PC	Protective concentration
PC_P	The fraction of species expected to be protected at a given concentration
SD	Standard deviation
SE	Standard error of the means
SSD	Species sensitivity distribution, cumulative distributions of species' responses to a given toxicant
TBA	Trait-based approach
μL	Microlitre
WQG	Water quality guideline
RFT	Range finding test – used if the toxicity of the substance is not known to find range of concentrations to be used.

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"Your value does not decrease based on someone's inability to see your worth." Unknown

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"if you can just get started, God will do the rest" Rev. William Marrion Branham.

DEDICATION

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“The horse is prepared against the day of battle: but safety is of the LORD.” Proverbs 21:31

CHAPTER 1: GENERAL INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

Water is life; without it, there would be no life. The past and current forms of life on earth have been sustained by the availability of water, which still holds the key to future survival of all living organisms. The fundamental necessity of water is recognised in organisations such as the Global Water Partnership (GWP), which views water as a crucial resource in attaining socio-economic developments (GWP 2009). Water also drives energy production and ensures that people's health and food security through agricultural production are sustained (GWP 2009). Thus, water is an essential resource for maintaining human well-being and healthy ecosystems (Douglas 2015). Human survival and continuing social-economic developments depend on the supply of appropriate water quality and quantity. This means that a relationship exists between humans and ecosystems, as people strive to improve their well-being. Ecosystem services provide nature's assets to people, supporting their social and economic well-being (Palmer et al. 2002). Ecosystem services are beneficial to both humans and other living organisms in a number of ways (MEA 2005a). Water, for instance, contributes to ecological functions through provisioning of habitat for aquatic life, including fish and insect larvae. It also provides ecosystem services that include freshwater supply, regulatory functions such as dilution and water purification, and fulfilling cultural necessities (e.g. water for traditional, aesthetic and spiritual purposes). The benefits derived from ecosystem services are available to humans, regardless of where they live or where the services are generated. For example, people who live in urban areas enjoy river ecosystem service benefits such as fishing for food or growing trees along the river banks, which can be used to build houses or as firewood for cooking. It is therefore important to ensure sustainable management of aquatic ecosystems so they can continue to provide these important essential services (MEA 2005a). This is particularly true in urban areas where human activities may pollute distant ecosystems through effluent discharges caused by industrial and domestic or mining activities (Douglas 2015). Polluting water bodies through human activities has the potential of putting further socio-economic development at risk (Panagopoulos et al. 2015). Alterations to freshwater ecosystems need to be managed in such a way that there is a balance between short and long-term goals through a better understanding of causes and instream responses to such alterations.

In South Africa, one of the major sources of pollution causing deterioration in freshwater ecosystem functionality is effluent from old, abandoned mines. Coal and gold mining activities largely release acidic effluents commonly known as acid mine drainage (AMD) that are rich in metals and sulphates, which exacerbate freshwater salinity (Oelofse et al. 2002; Hancox and Götz 2014). The released effluents and increased instream salinity or their combined ecological risks require urgent scientific interventions to protect freshwater ecosystems.

The rest of this chapter is devoted to a detailed literature review, rationale, aim and objectives of the study.

1.2 Water quality threats

People have a right to access water of good quality to meet basic needs and improve their well-being (Un-Water 2014). However, the future availability and security of freshwater resources are still areas of concern, particularly in dryland and semi-arid environments (Un-Water 2014; Lopez and Sarni 2015). Water resources are increasingly under pressure from population growth, industrialisation and the influence of climate change on, for example, rainfall patterns (Fekete 2013; Li et al. 2015). Poor water quality adversely impacts economically disadvantaged communities, with global estimates of one billion people suffering from water-related diseases sourced from contaminated wells, springs, rivers and lakes (United Nations 2015). The production of wastewater through agricultural return flows (Slaughter 2005), mining (Sigg 2014), and industrial activities, as well as domestic wastewater treatment works (WWTWs) is the main driver of water quality degradation in freshwater ecosystems (Nielsen et al. 2003; Vargas-Amelin and Pindado 2014). Increasing toxins, nutrients, metals and salts are common water quality problems that arise from anthropogenic activities (MEA 2005a). In dry and semi-arid regions of the world, e.g. parts of Australia, North America, India, China, Peru and South Africa, low and variable rainfall patterns exacerbate these water quality problems through water transport and dilution (UNEP 2011; Bagatin et al. 2014). Although several water quality threats exist, increasing instream salinity and AMD pollution is among the major water quality issues threatening the sustainability of freshwater ecosystems, particularly in mining areas of South Africa (DWS 2011). If not properly managed, these could further escalate the already stressed water situation in South Africa.

1.3 Freshwater salinisation

Freshwater salinity refers to an increase in the total concentration of dissolved ions in freshwater (Cañedo-Argüelles et al. 2013b). It is an urgent ecological issue because of its associated problems to aquatic life. Depending on its origin, freshwater salinisation can be classified as either primary or secondary. Primary freshwater salinisation is of natural sources e.g. weathering, salt-water influxes from the seas or groundwater, while secondary salinisation is human-induced e.g. through agricultural return flows, wastewaters from domestic or industrial sources and mining activities (MEA 2005c). Natural processes are usually slower and tend to take longer compared with human-induced salinisation. Agricultural return flows and irrigation have been recognised as the commonest human-induced causes of freshwater salinisation (Nielsen et al. 2003). This is evident in many agricultural farmlands where stored salts are mobilised from groundwater or soil to the surface due to land-use disturbances such as land clearing or irrigation. Since salts do not degrade, flushed salts from the soil may be washed down to receiving freshwater ecosystems, thereby increasing instream salt concentrations (Marshall and Bailey 2004). Although salts do not degrade naturally, their instream concentrations can be diluted and their potential effects reduced. However, in semi-arid regions where rainfall is minimal and evaporation often higher than rainfall, the implication is that risk of adverse effects of salinity on aquatic organisms is higher in arid and semi-arid regions than regions with plenteous rainfall (MEA 2005a). It is therefore important that salinity is properly managed in semi-arid regions such as South Africa.

Freshwater ecosystems in semi-arid regions often experience high salinity concentrations because as a large volume of water is lost through evapotranspiration, the salt remains and accumulates in the aquatic medium (MEA 2005a: 2005b). Poorly and unevenly distributed low rainfall is not usually adequate to dilute or wash away the salts to oceans, which impacts on water quality (Kingwell and John 2007). Arid regions with no single river runoffs such as the Arabian Peninsula and Libya are at greatest threat from salinisation due to the accumulation of salts (Kundzewicz 2007). Furthermore, arid and semi-arid regions experience low aquifer recharges due to water scarcity (MEA 2005b). Water in aquifers frequently evaporates as the water table approaches the soil surface, a process that leaves the salts behind (Wanke et al. 2014). The Food and Agriculture Organisation (FAO) noted that aquifers are usually used up at rates that exceed their recharge rates, even in areas where groundwater is recharged (FAO 2000).

The salts left behind can negatively influence water quality and the aquatic biota downstream when runoff occurs because salts are toxic to aquatic organisms at higher concentrations (Kefford et al. 2002). This is a common threat to South African freshwater ecosystems because the country is located in a semi-arid region of the Earth.

In recent years, South Africa has shown many of the arid and semi-arid regional patterns in deteriorating water quality. The country receives relatively low annual rainfall of less than 450 mm, which is usually unreliable and unevenly distributed (Slaughter 2005). Increasing human population and the quest to improve well-being exerts a demand for food, which also leads to rapid agricultural developments and increased effluent discharges from sewage works (Holland et al. 2011). Irrigated agriculture flushes out salts from the soil, which find their way into aquatic ecosystems (Palmer et al. 2004a). High rates of evapotranspiration from agricultural activities, coupled with geological disturbances, including large-scale land clearing, infrastructural development and mining, intensify freshwater salinity by increasing the ionic concentrations through physical and chemical weathering of rocks (Muschal 2006; McCarthy and Pretorius 2009). Several studies have investigated impacts of salinisation on water quality in South African freshwater ecosystems (Goetsch and Palmer 1997; Kefford et al. 2004a; Slaughter 2005; Clercq et al. 2010). These impacts are worsened by substantial natural variations of chemical water constituents (Dallas and Day 2004).

1.3.1 Effects of freshwater salinisation on aquatic life

Although some organisms can tolerate saline environments, increased influx of salinity threatens sensitive life stages of most aquatic organisms. Salinity impacts may include oxygen depletion in aquatic ecosystems (McCarthy 2011), loss of sensitive species (Palmer et al. 2013); and disruption of ecological relationships such as mating, food sources and habits of organisms (Miranda et al. 2010). Excessive or insufficient contents of a certain component of salts may also disrupt the ecological balance of organisms at individual or community levels, which can affect their growth and reproduction (Nielsen et al. 2003; Miranda et al. 2010). Despite these concerns, studies that demonstrate salinity as a mining-related ecological problem are limited (Nieto et al. 2007; Cañedo-Argüelles et al. 2013b). In South Africa, salinity impacts on aquatic ecosystems are exacerbated by mining activities through the generation of AMD (Simate and Ndlovu 2014). The combined effects of salinity and AMD are likely to cause serious ecological impact in freshwater ecosystems.

It is therefore crucial that management measures aimed at protecting freshwater resources from salinisation in areas exposed to AMD consider the combined effects of both salinity and AMD, with a view of developing integrated measures for managing them. Investigating ecological risks of AMD in freshwater ecosystems would contribute to sound scientific knowledge for improved integrated environmental water quality (EWQ) management.

1.3.2 Acid mine drainage

Concerns about water quality management owing to the release of mining effluents into freshwater ecosystems have received substantial attention in the past (Barnes and Romberger 1968; Koryak et al. 1972; Whitehead et al. 1995). However, as human's demand for metals and minerals for socio-economic development continue to escalate, global mining activities also continue to cause water quality problems through discharges of mining effluents (Wessman et al. 2014). Mining discharges such as AMD pose serious ecological risks to the aquatic environment (Jooste and Thirion 1999; Sun et al. 2014; Martín-Crespo et al. 2015). Although, coal and gold mines are mainly responsible for the production of most AMD because they are often associated with pyrite, a sulphide mineral (Skousen et al. 1998; Name and Sheridan 2014), other minerals such as copper, silver, sulphur, lead and uranium have all been linked to AMD (Gray 1998). The chemistry of AMD shows a characteristic acidic water laden with ferric iron (Fe^{3+}), sulphate (SO_4^{2-}) and other metals, including manganese (Mn^{2+}) and aluminium (Al^{3+}) (Simate and Ndlovu 2014; Masukume et al. 2014). The production of AMD is mainly a function of geology, occurring naturally when the geological stratum containing pyrite and sulphide bearing minerals is exposed to water and oxygen in the presence of bacteria (Sima et al. 2011). There are four commonly accepted chemical reactions that represent the chemistry of pyrite weathering in the formation of AMD (Skousen et al. 1998; Boukhalfa and Chaguer 2012; Simate and Ndlovu 2014). These are summarised in the following equations:

Equation 1: $$2FeS_2 + 7O_2 + 2H_2O \leftrightarrow 2Fe^{2+} + 4SO_4^{2-} + 4H^+$$

In Equation 1, iron sulphide (pyrite) (FeS_2) is oxidised to release ferrous iron (Fe^{2+}), sulphate (SO_4^{2-}) and acid.

Equation 2: $$4Fe^{2+} + O_2 + 4H^+ \leftrightarrow 4Fe^{3+} + 2H_2O$$

In the presence of oxygen, the ferrous iron is oxidised to form ferric iron (Fe^{3+}).

Equation 3: $Fe^{3+} + 3H_2O \leftrightarrow Fe(OH)_3 + 3H^+$

Furthermore, ferric iron can either be hydrolysed to form ferric hydroxide, $Fe(OH)_3$, and H^+ acidity, or

Equation 4: $FeS_2 + 14Fe^{3+} + 8H_2O \leftrightarrow 15Fe^{2+} + 2SO_4^{2-} + 16H^+$

directly attacks pyrite (FeS_2) and act as a catalyst in generating greater amounts of ferrous iron, SO_4^{2-} and acid.

The above chemical reactions clearly indicate why surface waters contaminated by AMD are characterised by low pH, and elevated concentrations of iron and SO_4^{2-} (Boukhalfa and Chaguer 2012). The low pH of water facilitates solubility of metals as it moves down waterways (Gerhardt et al. 2004). Bacteria facilitate the formation of AMD through the oxidation of iron (Equation 2) to release sulphuric acid. Microbial activity is, however, a major part in the formation of AMD in comparison with mining influence. However, not all AMD water is characterised by low pH (Skousen et al. 1998).

1.3.2.1 Neutralisation of acid mine drainage

Neutralisation of AMD can refer to a reaction where a base or alkaline material reacts with an acid quantitatively with each other. It is a process that is dependent on significant levels of calcium and magnesium ions from dolomite dissolution (Madzivire et al. 2011). Carbonates can neutralise AMD by reducing hydrogen ions to release water and carbon dioxide through the dolomite dissolution. Calcium and magnesium ions remain after the reaction, making the water saline. This explains why some studies have focussed on the application of carbonates and other lime for AMD neutralisation (Barnes and Romberger 1968; Potgieter-Vermaak et al. 2006; Madzivire et al. 2011). The addition of lime for neutralisation purposes increases salinity concentrations, thereby potentially increasing its associated ecological risks in freshwater ecosystems – i.e. combined effects of increased salinity and AMD. Similarly, the uncertainty around natural neutralisation of AMD is questionable, especially when the surrounding rock contains higher proportions of pyrite than dolomite rocks because the effluent would still be highly acidic. It is common, however, that bacteria of the genus *Thiobacillus* hinder the natural neutralisation of AMD by accelerating its generation (Skousen et al. 1998; Simate and Ndlovu 2014).

This process is also influenced by other factors such as geological formations or enzymatic reactions. As a result, many of the ecological problems of AMD are reflected in freshwater ecosystems as effects on water quality.

1.3.2.2 Effects of acid mine drainage in freshwater ecosystems

Aquatic ecosystems that are unimpacted by AMD usually support many species with a moderate abundance of individuals, whereas impacted streams are dominated by fewer species and often have low to moderate numbers of only a few individuals (Jennings et al. 2008).

A study of the distribution of fish affected by AMD in Pennsylvania streams in the United States of America found that fish were severely impacted at pH 4.5 - 5.5 (Jennings et al. 2008). Ten species were tolerant to a pH of 5.5; 38 species survived in pH range between 5.6 and 6.4; while 68 species were found at pH levels greater than 6.4. In addition, there was a complete loss of fish in about 90% of streams with waters of pH 4.5 and total acidity of 0.015 g/L. DeNicola and Stapleton (2002) found that caddisflies were extremely impacted by AMD at lower sites in receiving streams at circumneutral pH values. Their study also noted that the mayfly *Isonychia* sp. survived, and suggested that its swimming trait characteristic could have played a role in escape from the AMD or it was the taxon most likely to be present due to drift during the study. Besides affecting the distribution of organisms, their study also reported clogging of gill surfaces, reduction in vision and disruption in feeding as other AMD effects on aquatic fauna. Acid mine drainage has also contributed to a deterioration in water quality in parts of Australia (Park et al. 2014; Parbhakar-Fox et al. 2014). Many of the affected rivers near Jumna in Queensland received metal-rich tailings associated with sulphide oxidation and formation of acidic water (Lottermoser and Ashley 2011). In North America and parts of Canada, river salinisation has increased due to AMD, with reported SO_4^{2-} toxicity to aquatic macroinvertebrates (Meays and Nordin 2013). AMD has also affected water quality and sediments of Essouk River located in the Sahara Desert in Algeria by increasing iron and SO_4^{2-} concentrations (Boukhalfa and Chaguer 2012). The problem with AMD impacts on water quality is that aquatic organisms in affected ecosystems must deal with acidic conditions, high ferric ion (Fe^{3+}) precipitation, low oxygen levels and an increase in toxic metals, including aluminium (Al), zinc (Zn) and manganese (Mn), which are associated with coal mining activities (Skousen et al. 1998; Davies et al. 2011; Simate and Ndlovu 2014). Acid-sensitive species, therefore, might be more prone to AMD impacts.

Considered as less sensitive species and best indicators of AMD, chironomids and some algae species dominate streams receiving acidic water from mining activities (Gerhardt et al. 2004). Micro-algae are primary producers in the food web and their presence should potentially result in the abundance of other organisms in the ecosystem. However, ecosystems receiving AMD usually have impaired ecological processes such as decomposition and are less productive because basal resources become inaccessible for species (Hogsden and Harding 2012). These ecosystems are also characterised by slow microbial processing of organic matter, the absence of many grazers or shredders and fish are replaced by invertebrates as top predators. Consequently, prey-predator relationships and breeding patterns become disrupted, especially for sensitive species.

Streams that are located in dryland or water-scarce regions receiving low rainfall to allow sufficient dilution of the acidic water might have a higher risk of AMD contamination. This would particularly be of great ecological concern for many South African freshwater ecosystems due to increased coal and gold mining activities. It is, thus, important to evaluate the toxicity of AMD using indigenous in South Africa to make appropriate water quality management decisions.

1.3.2.3 Acid mine drainage in South Africa and its influence on freshwater salinisation, pH and metals

An estimated 93% of electricity supply in South Africa is coal generated (Dabrowski et al. 2008; Chamier et al. 2015). The majority of coal mines are located in Mpumalanga and KwaZulu-Natal Provinces, while a few are also found in Limpopo, the Vaal and Gauteng regions (Sracek et al. 2004). Coal mining activities have been recognised as one of the major polluters of freshwater ecosystems in South Africa through AMD generation and discharge (Potgieter-Vermaak et al. 2006; Hancox and Götz 2014). Coal mines continuously release AMD to the environment, which pose threats to water quality due to low pH and high concentrations of SO_4^{2-} (Deloitte 2013; Oberholster et al. 2013). This is despite the country's existing water laws that are considered one of the best in the world (Griffin and Palmer 2015). The online water quality monitoring system (WQMS) hosted by the Department of Water and Sanitation (DWS) indicates that data for South African freshwater ecosystems receiving AMD have circumneutral pH levels (6-8), although acidic conditions are also common due to the geological formation of the rocks surrounding the AMD production (https://www.dwaf.gov.za/iwqs/wms/data/WMS_pri_map.asp).

Specific areas of South Africa with poor water quality data include the upper reaches of the Olifants, Vaal and Crocodile Rivers, which indicate low pH (as low as 3.2), high SO_4^{2-} (> 2 g/L) and elevated Al, Fe and Mn concentrations (Dabrowski et al. 2013). These levels exceed acceptable standard limits for SO_4^{2-} (0.08 g/L – 0.25 mg/L) and pH (6.5-8.4) in South African freshwater ecosystems (DWA 2011). In particular, the Olifants catchment is a major ecological concern where available data indicate that 43% of SO_4^{2-} and 57% of pH values exceed regulatory standards for South Africa (DWAF 1996; DWA 2011). The Vaal River has had unacceptable levels of SO_4^{2-} (44%) and pH (78%) as well due to gold and coal mine activities in the catchment (DWA 2011). These statistics demonstrate the urgent need for studies that address AMD and salinity interlinked, as their combined effects in freshwater ecosystems can be devastating.

Although pH may influence the bioavailability of ions in water, SO_4^{2-} is hardly altered due to its conservative ionic nature (Gray 1996; Olías et al. 2004). In addition, SO_4^{2-} is a very mobile anion in water (Olías et al. 2004) and are predominant in many AMD impacted freshwater ecosystems in South Africa, which is an urgent ecological issue (Slaughter 2015). Sulphates are useful indicators of AMD (Gray 1996). They can characterise the type of salinity in receiving streams such as reported for the Olifants River due to predominant coal mining activities in its upper catchment (Scherman et al. 2003). Since AMD is largely associated with high salinity (sulphates) and metals, it is critical to evaluate the combined effects of AMD, pH and sulphates to provide a complete picture of the range of possible ecological risks in aquatic ecosystems. The knowledge gained would contribute to the development of appropriate management approaches of AMD and salts.

The toxicity of SO_4^{2-} to aquatic life is well-documented (Goetsch and Palmer 1997; Oberholster et al. 2010b; Meays and Nordin 2013). For example, the toxicity of Na_2SO_4 reduces with increasing water hardness (Soucek and Kennedy 2005; Soucek 2007). This is due to the fact that increasing water hardness (more Ca and/or Mg) lessens sodium (Na) toxicity (Cañedo-Argüelles et al. 2013), perhaps through cation competition. However, Mg can be toxic at concentrations approaching natural background levels, but toxicity depends on the deficiency of Ca in water (van Dam et al. 2010). Thus, the ratio of major ions such as Mg and Ca result in physicochemical conditions that can promote the uptake and toxicity of various metals and other ions in water (van Dam et al. 2010). Conversely, more presence of Ca, as occurs in lime salts used for treating AMD in South Africa (Madzivire et al. 2011; Potgieter 2015 pers comm), potentially poses ecological threats to freshwater systems where dilution is insufficient.

The underlying idea of lime application to AMD for treatment is generally to raise the pH to circumneutral while reducing Fe, Al and SO_4^{2-} concentrations. While the application of lime from calcium carbonate and coal fly ash is capable of increasing the pH of the treated acid mine drainage (TAMD) to near-neutral (6 or higher) and reduce subsequent fraction of elements from the solution, not all sulphates and metals are removed from the AMD (Madzivire et al. 2011; Truter et al. 2014). This means that AMD and sulphate are not the only ecological concerns but metals as well need to be considered when addressing the problems.

AMD dissolves and increases the bioavailability of metals and in-stream salinity to the extent that even essential metals can become toxic because of high levels in water (DWAF 2008). The pH of the AMD influences the bioavailability of metals and their toxicity to aquatic organisms (Nieto et al. 2007). For example, iron (Fe) and zinc (Zn) are considered essential because they are beneficial for enzyme functions and optimal growth of aquatic life at low concentrations, whereas higher concentrations may exert toxic effects (Sigg 2014). Non-essential metals such as lead (Pb), mercury (Hg), cadmium (Cd) and silver (Ag) demonstrate toxic effects even in very low concentrations (Liu et al. 2014; Maanan et al. 2015). It is therefore important to understand the toxicity of metals in AMD as a water quality issue for appropriate management of freshwater resources due to their potential risks in the aquatic environment. Metals in the aquatic environment may determine the potential vulnerability of organisms to toxicants. For example, copper (Cu) can bind to an active site of an organism known as a biotic ligand such as gills (Rijstenbil and Gerringa 2002). The effect of the binding process can render it to become unavailable for the biological functions of a particular organism. The binding process may render Cu to become toxic to the organism when in excess and might also render other free ions induce toxicity (Di Toro et al. 2001). This implies that the more the essential metals can bind onto gills, the more the non-essential metals would induce toxicity for the organism. The degree of metal toxicity to organisms depends on how the metals compete at the ligand (Santore et al. 2001; Shuhaimi-Othman et al. 2013). From this perspective, it seems necessary to investigate the potential dangers of AMD in aquatic ecosystems beyond sulphates because the metals can greatly influence the vulnerability of organisms due to their binding effect on biotic ligands.

1.3.3 Current state of knowledge for mining-related salinisation

Globally, AMD-related literature is largely centred on either the metals released during AMD generation (Whitehead et al. 1995; Heviánková et al. 2014; Falayi and Ntuli 2014) or the treatment methods that deal with the acidic conditions (Wei et al. 2015; Lefticariu et al. 2015). However, AMD related salinisation, especially concerning SO_4^{2-}, is a less well-researched area and requires some more attention. The maximum compliance limit for SO_4^{2-} is 0.25 g/L for freshwater ecosystems in South Africa, but this limit is lower than concentrations usually reported in some streams impacted by AMD (DWA 2011; McCarthy 2011), which could mean that AMD is probably contributing to increasing salt levels beyond acceptable compliance limits. Limited studies have attempted to remove SO_4^{2-} in AMD with little or no success because the remaining concentrations after removal still exceed the 0.25 g/L limit (for example, Madzivire et al. 2011; Sánchez-Andrea et al. 2014).

Reverse osmosis appears to be a promising technique to treat AMD but it is costly (Name and Sheridan 2014; Nyale et al. 2014). It is therefore important to conduct studies that increase our scientific understanding about the combined ecological risks of AMD and sulphate in freshwater ecosystems for the development of appropriate water quality guidelines (WQGs). This would entail the investigation of AMD and sulphate, including its liming effects as potential toxicants due to increase in salinity. It also remains to be known about the ecological risk posed by AMD and sulphate in freshwater ecosystems in relation to different ecological categories (Warne 2004). The ecological consequences of the combined risks of AMD and sulphate, if not appropriately investigated, could undermine any management attempts. Thus, understanding the associated ecological risks posed by the combined effects of instream sulphate concentrations and AMD is an important step towards the development of appropriate and sustainable water quality management tools for South African water resources.

1.3.4 Environmental water quality management in South Africa

In South Africa, the management of environmental water quality (EWQ) involves the concurrent use of water chemistry, biomonitoring and aquatic ecotoxicology, usually referred to as the triad approach (Palmer et al. 2004b). Physical and chemical properties of freshwater ecosystems and the impact of anthropogenic activities on these properties are monitored, while the responses of instream biological organisms are managed using the biomonitoring approach.

The effects of specific toxicants on aquatic life are studied using aquatic ecotoxicology. The combined use of these three approaches provides a robust and integrated picture of EWQ, offering holistic management perspective. In the present study, all three approaches are employed to provide a holistic view of the ecological risk associated with AMD and its interactions with sulphate salts.

1.3.4.1 Water physicochemistry

Changes to water physicochemistry by natural processes and by human activities can adversely affect ecosystem structure and function (Odume 2014). It, therefore, remains important to measure, analyse and monitor water physicochemical variables in a water quality study. For example, dissolved oxygen, pH, electrical conductivity, temperature, nitrates and phosphates can be measured to assess water quality, which helps decision makers to protect or identify water quality anomalies (Palmer et al. 2004b).

For a very long time in South Africa, water chemical testing was the main and only approach used to manage water quality. Therefore, a large water chemistry dataset is available for different freshwater ecosystems in the country's resources (Hohls et al. 2002). This approach is relatively easy to understand as users can simply compare a sample parameter to a guideline of interest. However, one disadvantage of water chemical testing is that it only represents the time at which samples are collected, missing events in between, which may be critical to ecosystem health (Odume 2014). The biomonitoring approach makes it possible to monitor ecosystem health in order to detect ecological impairments, which physicochemistry would normally be unable to do so.

1.3.4.2 Biomonitoring

Biomonitoring measures both taxonomic and functional diversity, which are important components of the aquatic ecosystem (Odume 2014). Biomonitoring relies on the sound understanding that resident biota, i.e. plants, algae, animals and microorganisms provide an indication of ecosystem health. For example, organisms can be identified as suitable bioindicators because their presence/absence, abundance, diversity and behaviour might reflect the environmental conditions. In South Africa, biomonitoring approach is a relatively recent approach and therefore data are sparse.

However, it is important to understand that biomonitoring and physicochemistry approaches alone do not explain other important intrinsic effects or responses of organisms to toxicants such as mortality rate or percentage mortality with respect to time. Ecotoxicology provides a time-integrated indication of the consequences of exposure (Mensah 2012).

1.3.4.3 Ecotoxicology

Ecotoxicology is the study of the effects of chemical solutions and mixtures on living organisms (Palmer et al. 2004c). The ecotoxicological approach is important in EWQ management because it assesses direct responses of aquatic life to contaminants. Either short-term (acute) or long-term (chronic) toxicity tests can be used to evaluate effects of toxicants on organisms. Short-term effects occur rapidly within minutes (usually up to four days) of exposure of an organism to a chemical and is usually measured as the concentration that resulted in 50% mortality of the exposed organisms (LC50) (Mensah 2012). Long-term effects (weeks, months or years) occur after exposing organisms to lower concentrations of toxicants for an extended period.

For this study, short-term tests refer to toxicity exposures that did not exceed 96 hours, while long-term tests refer to any exposure duration that lasted beyond 96 hours. Ecotoxicology has not been firmly established in water quality monitoring and water use licenses in South Africa, making it less common than physicochemistry and biomonitoring approaches. Full integration of aquatic ecotoxicology with physicochemistry and biomonitoring approaches in water quality studies is crucial for sustainable water resources management in South Africa.

1.3.5 EWQ in the context of integrated water resources management in South Africa

The National Water Act (NWA) (Act No. 36 of 1998) recognises water resources as part of the integrated water cycle. It promotes the protection, management and sustainable use of water resources to meet societal and environmental requirements in terms of quantity and quality. Knowledge of water quality threats and implementation of appropriate interventions is necessary for the NWA to play its role effectively. In terms of achieving water quality, integration means understanding how the chemical, biological, radiological and physical characteristics of water (the water quality) link to the responses of living organisms and ecosystem processes (the environment) (Palmer et al. 2004d).

For AMD and salinity, this integration means understanding how the abiotic aquatic ecosystem components such as water quality provide appropriate conditions for aquatic life when freshwater ecosystems are polluted or become saline. In terms of water sustainability in a water-scarce South Africa, there is the need to balance water resource protection and use. The impacts of AMD can be environmentally damaging, even after mines close down (Deloitte 2013). It is therefore important to ensure that immediate and long-term measures are established as part of IWRM of AMD and salinity. The measures can prevent, control and remediate the effects of pollution by pollutants such as AMD and salts in freshwater ecosystems. In South Africa, Resource Directed Measures (RDMs) and Source Directed Controls (SDCs) ensure that pollution is controlled by employing appropriate measures and controls, respectively.

Water resource protection is implemented through RDMs. The measures include the development of quantifiable and descriptive goals for ecosystem conditions and water user requirements, which form the resource quality objectives (RQOs) (DWA 2013). It is therefore important for water resource managers to understand the need to balance the use and protection of resources. Source Directed Controls set abstraction and discharge licence conditions, financial and economic measures, and other regulatory processes for controlling water use (DWA 2013).

This strategy ensures that the potential impacts on the quality of all aspects of the resource, including water quality and aquatic life, are considered when granting a licence to a user to ensure that water resources are protected. In this case, water managers can then define the appropriate licence conditions to control water use. This is also in line with the concept of EWQ management aimed at providing methods for monitoring progress towards the achievement of resource quality objectives (Palmer et al. 2004d). This study contributes to the attainment of both RDMs and SDCs through the data generated, which can be translated into policy through the development of relevant WQGs as part of integrated water quality management of AMD and salinity.

Integrated water quality management requires a balance between water resource protection and use. Over-protection is expensive for users and under-protection is expensive for the environment (Palmer et al. 2004d). It is therefore important to conduct studies aimed at protecting freshwater ecosystems at an appropriate level. A good understanding of the EWQ approach is useful for water resource protection and water quality management, as well as for water users who deal with integrated water resource management, point and non-point source pollutions, licensing waste disposal and setting RQOs (Palmer et al. 2004d).

The EWQ approach requires the combined use of water physicochemistry, biomonitoring and ecotoxicology to assess ecosystem health and to make appropriate management decisions. However, the attainment of scientifically sound water management decisions will remain a challenge if ecotoxicology cannot be fully adopted in water monitoring studies. One important aspect of ecotoxicology is its contribution towards the development of WQGs, commonly aimed at the protection of resources from adverse ecological effects. WQGs are important because they evaluate the degree to which a toxicant might adversely affect aquatic life, and are designed to assist in the interpretation of water quality. The derivation and setting of WQGs is key to managing pollutant effects in aquatic ecosystems (Warne 1998). The process of deriving WQGs forms part of ecological risk assessment (ERA) studies.

1.3.6 Ecological risk assessment approaches

In ecological risk assessment (ERA) studies, hazard and exposure assessments are two approaches commonly practised. Hazard refers to the intrinsic properties of a chemical and its potential to do harm while risk is the combination of hazard and exposure (Heath et al. 2008). Different types of risk assessments exist, including human, chemical, ecological and environmental, although no frameworks incorporate all due to lack of common assessment terminologies (Mensah 2012). Ecotoxicologists strive to eliminate the risk by changing either or both hazard and exposure. Hazard-based and risk-based approaches have a common element in that identification of the hazard is the first step in both cases. In risk-based approaches, the process is followed by exposure assessment and the integration of exposure with hazard characterisation in the final risk characterisation step, to provide an overall risk assessment, from which to conclude on safety (Barlow et al. 2015).

The knowledge to understanding how to manage water quality from AMD and increasing sulphates or their combination in freshwater ecosystems is important in the context of risk assessments. Questions that may be asked are whether AMD or sulphate properties are enough to restrict their release into freshwater ecosystems or first assess the exposure to determine if there is a risk that can be limited by managing the exposure. Thus, risk-based approaches help to identify measures that could reduce the risk in critical scenarios where decisions have to be made.

For example, risk-based approaches may be useful if two alternatives exist, one that can achieve the desired result in low concentrations, but is more hazardous, and the other that is less hazardous but must be used in much higher concentrations. The ERA evaluates the likelihood that adverse ecological effects may occur or are occurring because of exposure to one or more stressors (Claassen et al. 2001). It assesses the threshold or limit of a stressor or toxicant, below which an ecosystem will not suffer unacceptable damage (Del Signore et al. 2016). The acceptable damage would depend on management goals. This assessment involves the combination of all information from the toxicity tests (ecological effects), the exposure information, assumptions, and uncertainties in a way that helps the risk assessor understand the relationship between the ecological effects and the stressors and helps support decision-making (USEPA 1998). Key basic concepts of ERA include exposure, risk and analysis of effects of a stressor in a habitat. These are done through problem formulation, characterising potential or existing exposure to stressors and their effects, and risk characterisation (USEPA 1998) (Figure 1.1)

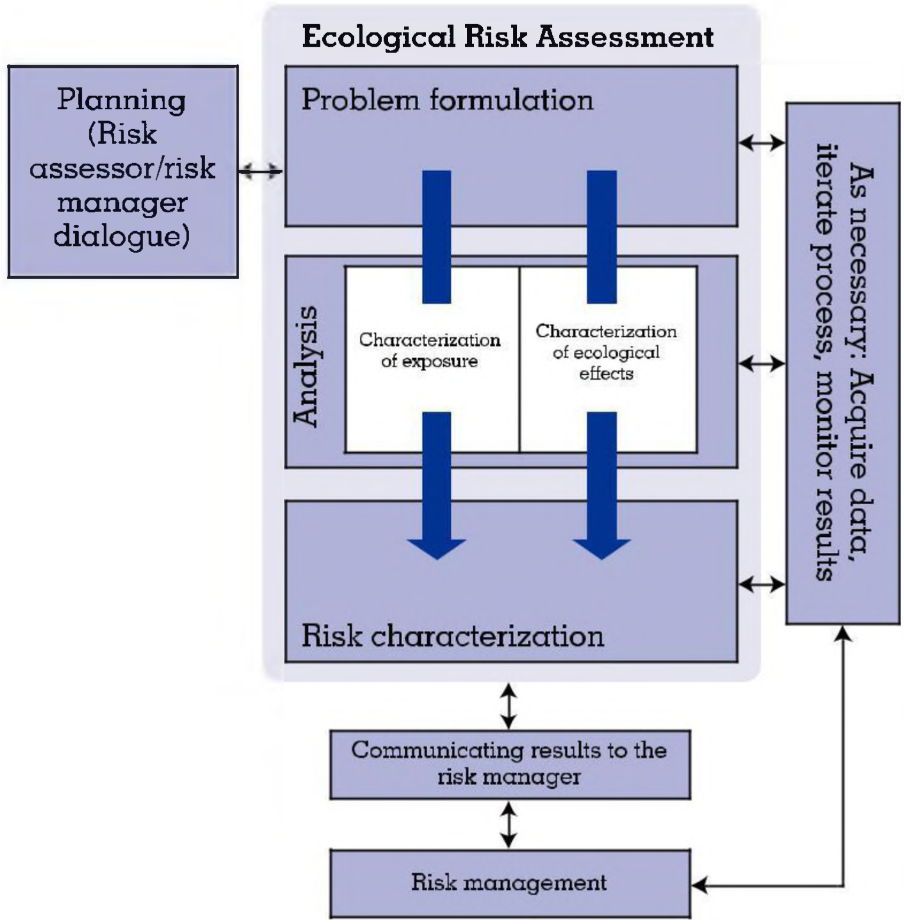

Figure 1.1: Ecological risk assessment processes (from https//goo.gl/images/MjXJZR)

After the risk has been assessed, results are communicated to the risk manager for appropriate decisions to remedy the problem. However, it is important that the endpoints for ERA should be of ecological relevance. Risk-based ecotoxicological studies can thus contribute to the prediction power of toxicants in aquatic ecosystems for better management practices, but thresholds can be difficult to assess if only data available are for a few species. For this reason, traditional approaches to risk assessment are based on observations of effects of individual chemicals on the survival, growth, and reproduction of a limited number of test species. Results from the observations are extrapolated using probability models related to ecologically relevant endpoints such as populations, communities and ecosystems.

Among many approaches to ERA, the assessment factor (AF) is often applied to extrapolate results from few species to ecosystem levels. This method assesses the most sensitive species to address the uncertainty in the extrapolation of available data (Del Signore et al. 2016). Given the growing extent and complexity of human impact on ecological systems, simplistic approaches that historically have dominated in ERA are insufficient (Galic and Forbes 2014). New ERA approaches which incorporate complexity in ways that can inform environmental management more effectively such as the Mechanistic Effect Models for Ecological Risk Assessment of Chemicals (CREAM) (Galic and Forbes 2014) are still being developed. The CREAM model integrates data for species habitat requirements, intra- and interspecific interactions and density dependence, heterogeneous exposure to chemical mixtures and multiple stressors, and long-term sub-lethal effects on populations. However, not many ecotoxicologists are trained modellers nor modellers are trained as ecotoxicologists to easily adopt this approach. Moreover, modelling biological systems is often challenging because of their infinite and complex nature. Another commonly used approach for extrapolating toxicity thresholds that are considered protective of ecosystem structure and functioning is the species sensitivity distributions (SSDs) (Del Signore et al. 2016). In this study, risk-based WQGs were developed using the SSD approach to help better manage increasing freshwater salinisation and AMD in South Africa by informing water managers with appropriate decisions about the potential risks of pollutants.

1.3.6.1 Species sensitivity distributions and water quality guidelines: A South African perspective

The value of risk-based approaches is becoming increasingly recognised in South Africa, but much still needs to be done. Some authors have suggested a review of the 1996 WQGs due to their generic nature of guideline values that did not consider local conditions and were majorly hazard-based (Warne et al. 2004; Heath et al. 2008). Certainly, the 1996 WQGs have limited water quality variables for limited water uses (DWAF 1996). For example, the guideline for sulphate is 0.25 g/L for all water uses, while 0.08 g/L is the ideal limit and 0.165 g/L is considered acceptable (DWAF 1996; DWA 2011). In addition, the toxicity data used for deriving the guidelines were mostly international that did not consider indigenous species responses in local conditions, which have been questioned (Slaughter et al. 2008a). The species sensitivity distribution (SSD) is a risk-based ERA approach widely used for the development of WQGs globally (ECETOC 2014). SSDs are the most preferred method for deriving WQGs (ANZECC/ARMCANZ 2000; CCME 2007). SSDs are useful in the protection of a defined fraction of species from toxicant exposures, which is dependent on management goals for the resource. Thus, SSDs can be used to estimate the potentially affected fraction of species at risk at a given exposure concentration. In this study, SSDs were used to estimate different protective concentrations as WQGs for sulphates as well as dilution levels for AMD for treated AMD. The findings are used to design appropriate freshwater monitoring programs that are aligned with the protection of water resources with respect to sulphate salts and AMD.

Traditionally, WQGs have been derived for individual chemicals rather than mixtures. One problem with this practice is that chemicals do not necessarily occur individually but as a mixture in the aquatic ecosystem. The composition of mixtures can change from time to time, even at site-specific level, and aquatic organisms have to cope with such changes while maintaining their ecological functions. Whole effluent toxicity (WET) testing becomes useful in ecological perspectives because the sporadic presence of effluents such as AMD can be assessed (Carbonell et al. 2010). WET testing mainly aims to protect the environment and not a particular species (Chapman 2000). However, if the most sensitive species to a particular toxicant can be protected then other less sensitive species are protected concomitantly. It is, thus, important to conduct WET testing for AMD for the development of scenario-specific WQGs that can protect local biota to contain ecological problems. WQGs are therefore used for setting up limits and standards for safe discharges of wastewater into receiving water bodies. WQGs can either be targeted to address short-term or long-term AMD and sulphate pollution.

Short-term WQGs protect species from robust or pulse releases of pollutants in an ecosystem. These may be a result of, but not limited to, unregulated application and discharges, spills or accidental events. The pollutants can be washed off into streams during heavy rains, wind or when instructions for use of the chemicals are not followed. They also protect species during unfortunate and disastrous events, but not for an indefinite period (Mensah 2012). However, long-term WQGs are important for the protection of species and life stages from mortality during chronic and severe exposures to pollutants. In this study, short-term WQGs were derived from 96 hours toxicity data while long-term WQGs used 240 hours toxicity data.

1.3.7 The potential use of traits to predict vulnerability of organisms to toxicants

Although WQGs are useful to protect aquatic life from pollutants, different taxa respond differently to pollution in aquatic ecosystems. Their adaptation to changing instream conditions due to pollution is a function of traits that the organism possesses (Townsend and Hildrew 1994; Hamid and Rawi 2014). The sensitivity of organisms to AMD and increasing saline conditions in their habitat can be attributed to a combination of traits such as morphology, life history, physiology or ecological preferences (Baird et al. 2008). Traits that impart resistance or resilience to toxicants are conserved in a population (Culp et al. 2011). If the potential vulnerability of organisms to toxicants can be predicted based on their traits, then a trait-based approach (TBA) has a potential to reduce costs of conducting ecotoxicological tests as the potential effects of toxicants could be predicted through the knowledge of existing species and trait combination of such species in a given environment. Therefore, while the development of WQGs is important in determining safe dilution levels and protective concentrations of toxicants, trait-based scientific knowledge has the potential to increase human's understanding as to why some organisms are relatively less, moderate or more sensitive to toxicants compared to others, thereby setting predictions as a precautionary approach to managing pollution effects. The ability to use trait combinations to predict the vulnerability of organisms to toxicants can increase the explanatory power of ecotoxicological findings. Trait knowledge can also improve biomonitoring approach by linking taxonomic data to identified traits. Trait-based findings would be useful to support a management decision support system (DSS) process of a resource. A DSS is a useful tool in EWQ management decision processes where there are often inadequate WQGs or no experienced ecotoxicologists to make decisions that would protect the ecosystems from AMD and increasing sulphates in freshwater ecosystems.

1.3.8 Rationale

Scientific knowledge gaps exist in understanding the ecological risks of AMD and sulphate in freshwater ecosystems. In South Africa, coal and gold mining activities continue to increase for various socio-economic reasons. With AMD linked to mining activities, deterioration of water quality is also increasing, potentially threatening ecosystem health, including aquatic organisms that play different ecological functions. The influx of treated and untreated AMD in freshwater ecosystems exacerbates freshwater salinisation. Moreover, AMD is associated with increased metal concentration and variable pH, rendering it a complex mining effluent, which organisms have to deal with in their habitats. Therefore, understanding the toxicity of AMD in the presence of increasing salinity is important for its management and protection of the freshwater resources. It is thus important to derive risk-based WQGs for appropriate management of AMD and sulphate salts.

1.3.8.1 Aim and specific objectives of the study

The following was the research question for this study: "What is the ecological risk of acid mine drainage in a salinising landscape?" Its hypothesis was that acid mine drainage does not pose any ecological risks to freshwater ecosystems in a salinising landscape. The overall aim of this study was to evaluate the potential ecological risk of AMD and sulphate salts in freshwater ecosystems to develop appropriate water quality management strategies. To achieve this aim, the following specific objectives were set:

- To investigate and compare the ecological risks associated with different sulphate salts: $MgSO_4$, Na_2SO_4, and $CaSO_4$ and develop appropriate water quality guidelines using a risk-based approach.
- To evaluate the toxicity of acid mine drainage (AMD) with a view of developing scenario-specific water quality guidelines using a risk-based approach.
- To develop a novel trait-based approach for predicting the vulnerability of selected taxa to treated acid mine drainage (TAMD).
- To develop water quality guidelines for the management of sulphate salts and AMD in South Africa
- To use the multi-criteria analysis for the development of an integrated management strategy for AMD and increasing salinity in South Africa

1.3.9 Thesis structure

This thesis consists of six chapters. For ease of reference, each chapter builds on the preceding one, although each chapter represents a major section of the study.

Chapter One

This chapter introduces the reader to water as a valuable global resource. It outlines the study background from global to South African perspective in terms of water quality threats, including AMD and sulphate in freshwater ecosystems. The chapter emphasises the need to protect South African freshwater resources from AMD and sulphate pollution or their combination as their effects could be devastating. The reader is then introduced to the potential roles of sulphates in AMD and the risk they pose to aquatic life. This is followed by a review of existing integrated water quality management approaches practised in South Africa. The reader is introduced to methods commonly used to derive WQGs globally and reviews the methods used in South Africa. The chapter argues that the 1996 South African WQGs are not adequate to protect all water uses in local conditions and that there is a need for revision using risk-based approaches rather than hazard-based approaches. The chapter concludes by introducing the reader to the trait-based approach (TBA) as a novel methodological framework that can be used to predict the vulnerability of organisms to stressors in aquatic environments in a decision support system (DSS). The general aims and specific objectives of the study are also outlined.

Chapter Two

This chapter provides insights into the ecotoxicity of sulphate salts to freshwater taxa. Using risk-based, species sensitivity distributions (SSD) approach, sulphate salts guidelines were derived for Na_2SO_4, $MgSO_4$, $CaSO_4$ and their combination. These salts were used as models of mining salinisation in South African ecosystems. These salts were tested on five indigenous freshwater species in South Africa under local conditions. The species include the mayfly *Adenophlebia auriculata* (Ephemeroptera: Leptophlebiidae), the limpet *Burnupia stenochorias* (Pulmonata, Ancylidae), freshwater shrimp (Atyidae: *Caridina nilotica*), tilapia *(Oreochromis mossambicus)* and green-algae *Pseudokirchneriella subcapitata.*

Details for the choice of organisms are given in the chapter. The WQGs derived are discussed in the context of protecting the tested species in South African freshwater ecosystems.

Chapter Three

Chapter three evaluates the toxicity of AMD on common freshwater taxa in South Africa. The chapter derived scenario-specific WQGs for AMD both in the short-term and long-term, using a risk-SSDs. The chapter concludes by introducing the reader to the application of the protective dilutions of South African freshwater ecosystems.

Chapter Four

This chapter provides insight into the ecotoxicity of (lime) treated acid mine drainage (TAMD) to freshwater taxa. Trait combinations were used as an approach to determine the vulnerability of organisms to TAMD. Different organisms were classified into groups as less, moderate or more vulnerable to TAMD based on literature review. Leptophlebiidae and Leptoceridae were chosen based on trait characteristics that they possess and the set combinations. This was followed by laboratory toxicity exposures to investigate the responses of the organisms to TAMD. Finally, a field validation exercise was undertaken to validate the laboratory findings. The reader is introduced to a novel trait-based prediction tool for use in a decision support system for management of stressors such as TAMD.

Chapter Five

This chapter reviews some of the major WQGs globally, to which South Africa partly corresponds to in terms of methods. This chapter discusses the application of the results in chapters two, three and four in the context of water quality management in South Africa. The chapter applies the derived WQGs in different ecosystem categories based on different protection levels generated through the SSD approach. Long-term and short-term WQGs for sulphates are also developed for the protection of species from AMD in different ecological categories are discussed in detail.

The chapter concludes by discussing the TBA findings and development of a decision support system as a possible water resource management tool.

Chapter Six

This chapter presents concluding summaries of the study in relation to the overall aim, specific objectives, research questions and hypotheses in the different chapters. It also outlines recommendations for future studies and how to implement the findings and conclusions emanating from this study.

CHAPTER 2: THE DERIVATION OF WATER QUALITY GUIDELINES FOR SULPHATE USING RISK-BASED APPROACH

The following manuscripts have been published from this chapter:

1. ***Vellemu EC**, Mensah PK, Griffin NJ, Odume ON (2017) Sensitivity of the mayfly Adenophlebia auriculata (Ephemeroptera: Leptophlebiidae) to $MgSO_4$ and Na_2SO_4. Phys Chem Earth 100:81–85. doi: 10.1016/j.pce.2017.06.009*

2. ***Vellemu EC**, Mensah PK, Griffin NJ, et al (2017) Using a Risk-based Approach for Derivation of Water Quality Guidelines for Sulphate. Mine Water Environ 0:1–8. doi: 10.1007/s10230-017-0480-2*

2.1 INTRODUCTION

Increasing freshwater salinity presents ecological concerns in freshwater ecosystems globally (Muschal 2006; Schäfer et al. 2011; Cañedo-Argüelles et al. 2013a). In particular, effluent releases from mining activities such as acid mine drainage (AMD) facilitate the availability of metals, including iron, aluminium, and manganese in freshwater that can combine with sulphate ions to form salt compounds, leading to high salinity levels (Gerhardt et al. 2004; Simate and Ndlovu 2014). Therefore, it is important to investigate the ecological risks of sulphates to indigenous freshwater species under local conditions prior to understanding the ecological risk of AMD or its combination with sulphate in freshwater ecosystems.

The evaluation of sulphate risks in freshwater ecosystems impacted by AMD is challenging due to data scarcity, especially with respect to indigenous South African species. The availability of toxicity data, particularly on long-term, is important for the development of relevant water quality guidelines (WQGs). The majority of sulphate toxicity data in the literature are acute or short-term conducted with aquatic invertebrates (Meays and Nordin 2013). This implies that long-term effects of sulphate on a wide range of freshwater taxa are required to inform appropriate management decisions for the protection of freshwater resources. Moreover, the uncertainties around the toxicity of different sulphate salts to freshwater organisms in South African context require consideration when assessing the ecological risks.

For example, although sodium sulphate (Na_2SO_4) dominates South African freshwater ecosystems impacted by mining activities (Goetsch and Palmer 1997), salts with Mg ions such as magnesium sulphate ($MgSO_4$) are presumed to be more toxic than Na_2SO_4 and calcium sulphate ($CaSO_4$) (Jooste and Rossouw 2002; Palmer et al. 2005). Thus, the ecological assessment of sulphate in freshwater ecosystems experiencing AMD pollution that continue to threaten water quality in South Africa becomes important. In this chapter, short-term (96 hours) and long-term (240 hours) risk-based water quality guidelines (WQGs) for sulphate were derived using the SSD approach, for the protection of species in freshwater ecosystems.

2.2 Materials and methods

This section outlines materials and methods employed in this study to the derivation of WQGs for sulphate.

2.2.1 Test solutions and water quality

Reference anhydrous sodium sulphate (Na_2SO_4), magnesium sulphate ($MgSO_4$) and calcium sulphate ($CaSO_4$) were purchased from Merck (Pty) LTD scientific laboratory store. Test solutions were prepared by dissolving weighed salts in dechlorinated tap water. These salts were used as models of mining salinisation in South African freshwater ecosystems. The following concentrations were used in each exposure tests: 0 (control), 0.25, 0.5, 1, 2, 4, 8, 16, and 32 g/L. These concentrations were determined after a range-finding test and from a local toxicity database (Palmer et al. 2004c). The higher concentrations were also used to model the sensitivity of the tested species to sulphates. Water quality parameters including dissolved oxygen (DO), pH and electrical conductivity (EC) were monitored at the beginning of each experiment and after water change at 96 hours to maintain water quality. The DO was measured using a Cyberscan® 1500 meter, pH by a Cyberscan® 5000 while the Cyberscan® 200 meter was used to measure the EC. These instruments were manufactured by Eutech Instruments, Singapore.

2.2.2 Test species

The toxicity of $MgSO_4$, Na_2SO_4 and $CaSO_4$ was assessed on indigenous freshwater species found in South African ecosystems (Table 2.1). These species are commonly used in toxicity tests. It is a requirement that, at least, five species from different taxonomic groupings are used to develop high-reliability WQGs in South Africa (Warne et al. 2004). For this reason, five freshwater organisms belonging to five different taxonomic groupings were used for this study. The mayfly nymphs *Adenophlebia auriculata* (Ephemeroptera: Leptophlebiidae) were chosen as insect representatives. They are indigenous and widespread in South African ecosystems. The limpet *Burnupia stenochorias* (Pulmonata: Ancylidae) is water quality indicator species that requires protection and is also a common indigenous species in South Africa (Palmer and Davies-Coleman 2004). Limpets with a shell length of 3 mm or less were used. The freshwater shrimp *Caridina nilotica* (Decapoda: Atyidae) was chosen because it is widespread in Southern Africa (Slaughter et al. 2008; Mensah et al. 2011). Shrimps were obtained from a laboratory culture maintained at the Institute for Water Research, Rhodes University in Grahamstown. Aquatic vertebrates were represented by the Mozambique tilapia, *Oreochromis mossambicus*. It is an indigenous and widespread species in Southern Africa and a valuable global species for good protein source (Murthy et al. 2011) with no known sulphate toxicity effects yet. *Pseudokirchneriella subcapitata* represented the algae group (OECD 1984). It is an indigenous and common species in freshwater ecosystems in South Africa. These species play various ecological roles in the food chain such that any disturbance due to mining activities can have devastating effects. Sampling sites were near-natural or pristine to avoid pre-exposure to pollutants. Appropriate care and handling were undertaken to avoid stressing organisms in the field and during transportation to the laboratory.

Table 2.1: Summary of test species used in the study

Common name	Family	Species	Age group	Site collected
Microalgae	Selenastraceae	*Pseudokirchneriella subcapitata*	-	Commercially obtained, MicroBioTests Inc., Nazareth, Belgium
Crustacean	Atyidae	*Caridina nilotica*	Juveniles (>7< 20 days post-hatch)	Laboratory-reared, Rhodes University, Grahamstown
Fish	Cichlidae	*Oreochromis mossambicus*	5 days old fingerlings	Rivendell hatchery, Eastern Cape, South Africa
Insect	Leptophlebiidae	*Adenophlebia auriculata*	Nymphs	Palmiet River, Eastern Cape, South Africa
Mollusc	Ancylidae	*Burnupia stenochorias*	Mixed	Kat River, Eastern Cape, South Africa

2.2.3 Experimental procedure

All experiments were conducted in a temperature-controlled laboratory (20 ± 2°C) after acclimation of organisms for 48 hours. For *C. nilotica*, *A. auriculata* and *O. mossambicus*, 10 individuals were randomly allocated into 600 mL glass beakers containing 300 mL of the test solution. Fifteen individuals of *B. stenochorias* were allocated in test beakers. Test organisms were exposed to increasing concentrations of $MgSO_4$, Na_2SO_4 and $CaSO_4$ in eight treatments excluding the control. Each treatment was replicated three times. Test organisms were not fed during the first 96 hours of exposure, after which TetraMin flakes (Tetra Company, Germany) were added daily including in the control groups. Only one life history stage was used for exposure tests.

The test endpoint was mortality. This was assessed by prodding the organism using a glass stirrer. Acceptable control mortality was less than 10% (DWAF 2000). Organisms were recorded as dead when they failed to respond to the stimulus. Organisms were counted daily and dead animals were removed from the test vessels. Except for green algae test, long-term renewal (240 hours) tests were conducted for all species. Test solutions were changed every 96 hours to maintain experimental water quality.

Micro-algal tests followed the Organisation for Economic Co-operation and Development (OECD) 201 guidelines (OECD 1984). The algal culture and stock solutions for the preparation of growth media were commercially obtained (MicroBioTests Inc., Nazareth, Belgium). The Toxkit contained healthy demobilised algae cells that were kept under 5°C prior to exposure, after which they were mobilised to prepare six concentrations of each salt experiment including the control groups. Test solutions were incubated at 23 ± 2°C for 96 hours (h) in 25 mL incubation vials containing 5 mL of algal growth medium. The vials were shaken thrice daily under constant illumination using fluorescent light during the incubation process. All toxicity tests were run in three replicates with algal cell count measured as optical density (OD). The readings were taken on a spectrophotometer at 670 nM (nanometre) wavelength. Culture samples were transferred into 5 cm well plates as a provision due to lack of a spectrophotometer fitting the 10 cm cell plates. The algal cell count readings were taken at 0, 24, 48, 72 and 96 hours. No feed was administered to algae during the entire exposure tests.

2.2.4 Data analyses

Lethal concentrations (LC) of each salt that killed 10% and 50% of the tested species were estimated using a regression curve in statistical software package 'DRC' within the R programming environment (Ritz and Streibig 2012). LC10 values were used for the derivation of long-term WQGs and LC50 values were used for short-term WQGs. The effective concentration (EC10 and EC50) values for algae were calculated using a linear regression analysis of transformed chemical concentration as natural logarithm data against percentage inhibition. Tests with algae conducted over 96 hours were considered both short-term and long-term as recommended by Hagen and Douglas (2014).

2.2.5 Construction of species sensitivity distributions (SSD)

The SSD Generator software was used to construct the SSDs (USEPA 2009). This software estimates protective concentrations (PC) with 95% upper and lower confidence limits (CL) (Mensah et al. 2013; Hagen and Douglas 2014). The log-probit (linearised lognormal) model was used to fit the data for concentrations at which different species exhibit a standard response to a stressor. An SSD was constructed as a combination of the three salts to model species responses to sulphate. Thereafter, three more SSDs were generated to rank the proportion of affected species to individual salts. This procedure was repeated both for short-term and long-term toxicity data.

2.3 Results

2.3.1 Short-term exposure results

The calculated algal effective concentrations (EC_X) values and associated goodness of fit for the models are presented in Table 2.2. The distributions of toxicities were characterised by both the 10th and the 50th percentile intercepts of the distributions. These were determined using a regression equation for the transformed data. Short-term and long-term EC_X values were supported strongly by the data models (R^2), which formed part of the species sensitivity distribution (SSD) construction.

Table 2.2: Regression coefficients (a, b) and intercepts for algae toxicity data distributions

		EC_x=ax+b		Correlation for the 10th and 50th percentile intercepts			
Salt	Exposure (hours)	a	b	R^2	10%	50%	Units
$MgSO_4$	96	-51.937	51.938	0.936	-	91.411	g/L
Na_2SO_4	96	-72.255	210.74	0.912	-	3.802	g/L
$CaSO_4$	96	-67.352	63.935	0.948	-	68.391	g/L
$MgSO_4$	240	-47.507	76.078	0.968	5.702	-	g/L
Na_2SO_4	240	-3.786	110	0.944	1.002	-	g/L
$CaSO_4$	240	-66.554	66.472	0.912	2.183	-	g/L

Note: x is calculated EC_X value.

Table 2.3 presents short-term toxicity results for Na_2SO_4, $MgSO_4$ and $CaSO_4$ on the tested species. $CaSO_4$ was more toxic to *C. nilotica* than the rest of the tested species. However, it was the least toxic salt to *A. auriculata*, *B. stenochorias* and *O. mossambicus*.

Table 2.3: Estimated 96 h lethal/effective concentration (LC/ECx) values for species responses to salts

Common name	**Family**	**Species**	**Taxonomic group**	**Salt**	**96 h EC/LC50 (g/L)**	**95% CL (g/L)**
Shrimp	Atyidae	*Caridina nilotica*	Crustacean	$MgSO_4$	10.665	7.788-13.542
				Na_2SO_4	2.052	1.587-2.5161
				$CaSO_4$	0.357	14.664
Mayfly	Leptophlebiidae	*Adenophlebia auriculata*	Insect	$MgSO_4$	4.139	2.529-5.749
				Na_2SO_4	6.439	3.594-9.283
				$CaSO_4$	306.863	1444.696
Limpet	Ancylidae	*Burnupia stenochorias*	Mollusc	$MgSO_4$	1.703	1.460-1.947
				Na_2SO_4	1.491	1.267-1.715
				$CaSO_4$	6.901	4.487-9.317
Mozambique tilapia	Cichlidae	*Oreochromis mossambicus*	Fish	$MgSO_4$	22.609	22.335-22.884
				Na_2SO_4	4.385	3.697-5.074
				$CaSO_4$	6463.01	6442.525-6483.5
Algae	Selenastraceae	*Pseudokirchneriella subcapitata*	Green algae	$MgSO_4$	91.411	-
				Na_2SO_4	3.802	-
				$CaSO_4$	68.391	-

Note: Individual CL values denote the upper limits. Lower values were negative and were thus considered not biologically meaningful. The readings for algae represent EC values.

2.3.1.1 SSD curve for combined sulphates

First, the three inorganic salts were modelled to evaluate their combined toxicity to the tested species after 96 hours of exposure. Species demonstrated varied responses to the combined sulphate after 96 hours of exposure. The species *C. nilotica* was the most sensitive of the tested species (Figure 2.1). *O mossambicus* was the least sensitive species to the three inorganic salts combined while *A. auriculata* were moderately sensitive. The model fit the data strongly ($R^2 = 0.922$) at a slope of 1.336.

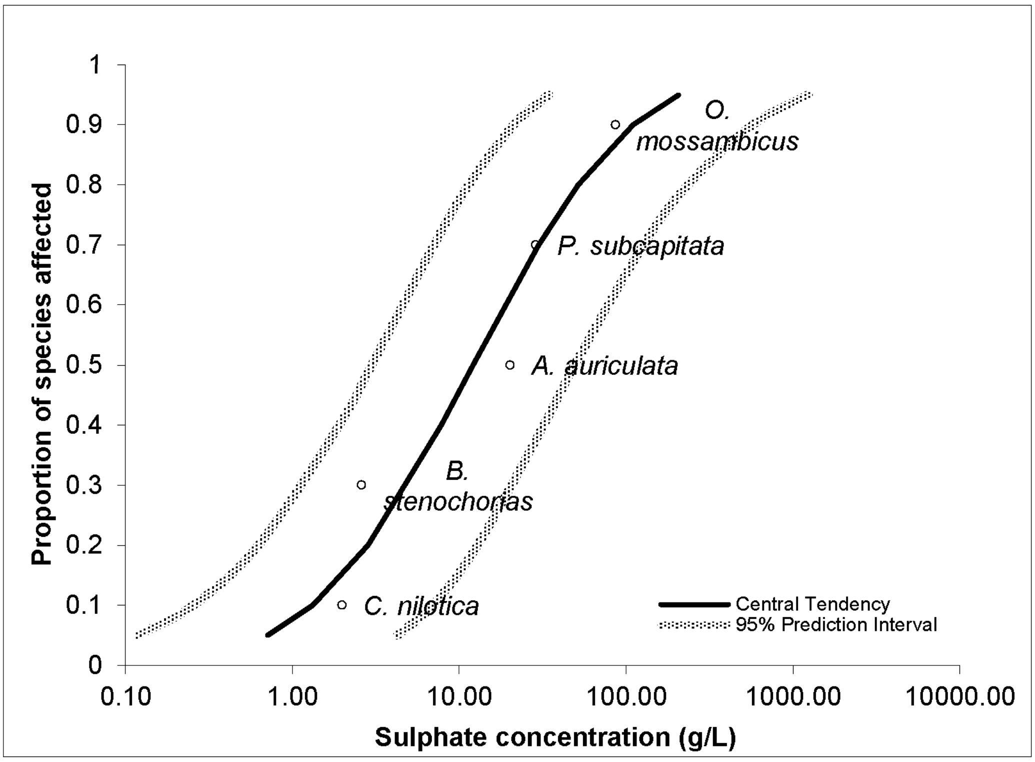

Figure 2.1: Species sensitivity distribution (SSD) showing the sensitivity of the test species *B. stenochorias, A. auriculata, C. nilotica, P. subcapitata* and *O. mossambicus* to the three inorganic salts combined based on 96 hour LC/EC50 toxicity data. The upper and lower 95% confidence intervals are also shown.

2.3.1.2 SSD curves for specific salts

The following SSD graphs represent short-term responses of the tested species to individual inorganic sulphate salts. *B. stenochorias* was the most sensitive species to Na_2SO_4 after 96 hours of exposure while the species *A. auriculata* was the least sensitive to this salt (Figure 2.2). The results revealed that *P. subcapitata* was moderately sensitive to Na_2SO_4. The model fit the data strongly ($R^2 = 0.963$) at a slope of 3.743.

Although *B. stenochorias* was most sensitive to $MgSO_4$, the species *P. subcapitata* was least sensitive while *C. nilotica* showed moderate sensitivity after 96 hours of exposure. However, *A. auriculata* was more sensitive to $MgSO_4$ than *O. mossambicus* (Figure 2.3). The model fit the data strongly ($R^2 = 0.993$) at a slope of 1.462. The salt $CaSO_4$ was most toxic to *C. nilotica* of the tested species while *O. mossambicus* was the least sensitive. *B. stenochorias* was more sensitive to $CaSO_4$ than *P. subcapitata* and *A. auriculata* after short-term exposures (Figure 2.4). The model fit the data strongly ($R^2 = 0.998$) at a slope of 0.605.

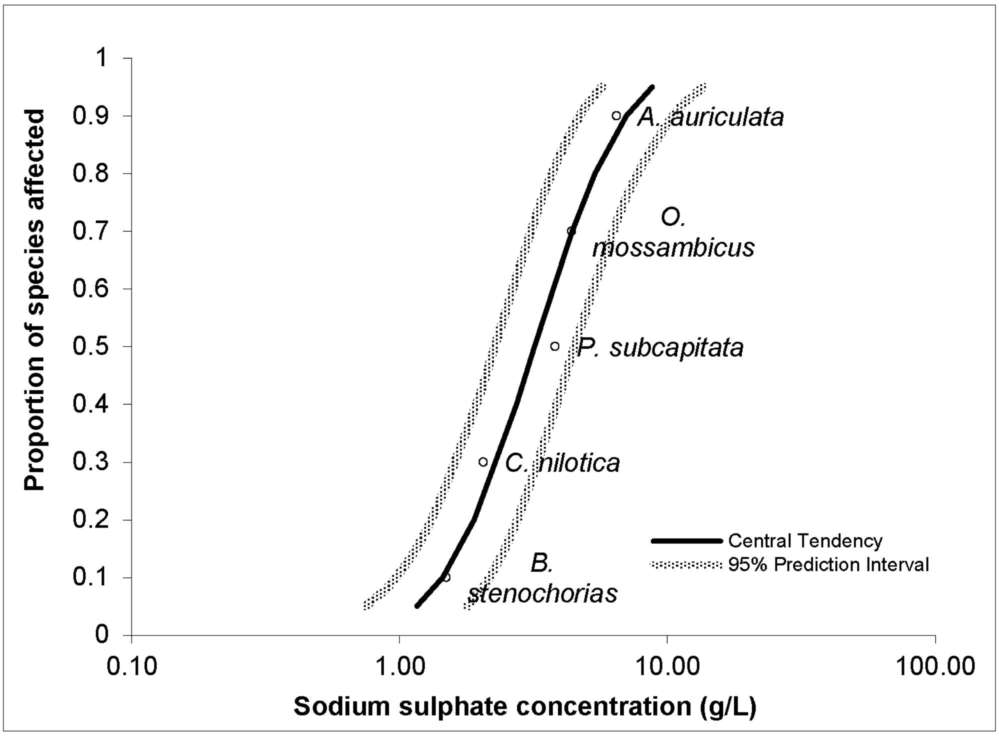

Figure 2.2: Species sensitivity distribution (SSD) showing the sensitivity of the test species *B. stenochorias, A. auriculata, C. nilotica, P. subcapitata* and *O. mossambicus* to sodium sulphate based on 96 hour LC/EC50 toxicity data. The upper and lower 95% confidence intervals are also shown.

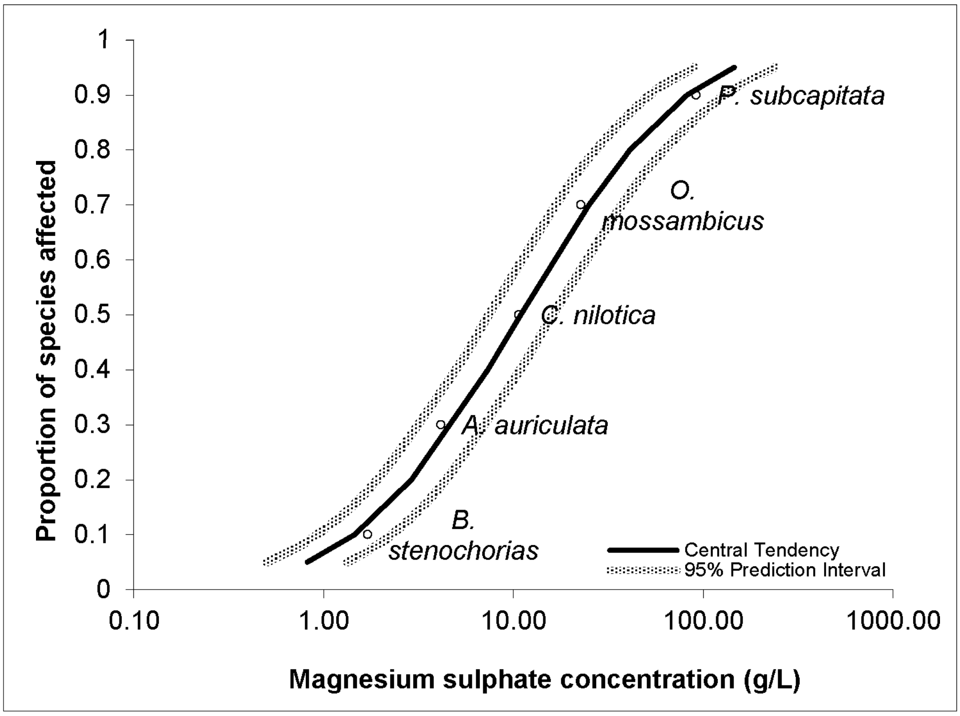

Figure 2.3: Species sensitivity distribution (SSD) showing the sensitivity of the test species *B. stenochorias, A. auriculata, C. nilotica, P. subcapitata* and *O. mossambicus* to magnesium sulphate based on 96 hour LC/EC50 toxicity data. The upper and lower 95% confidence intervals are also shown.

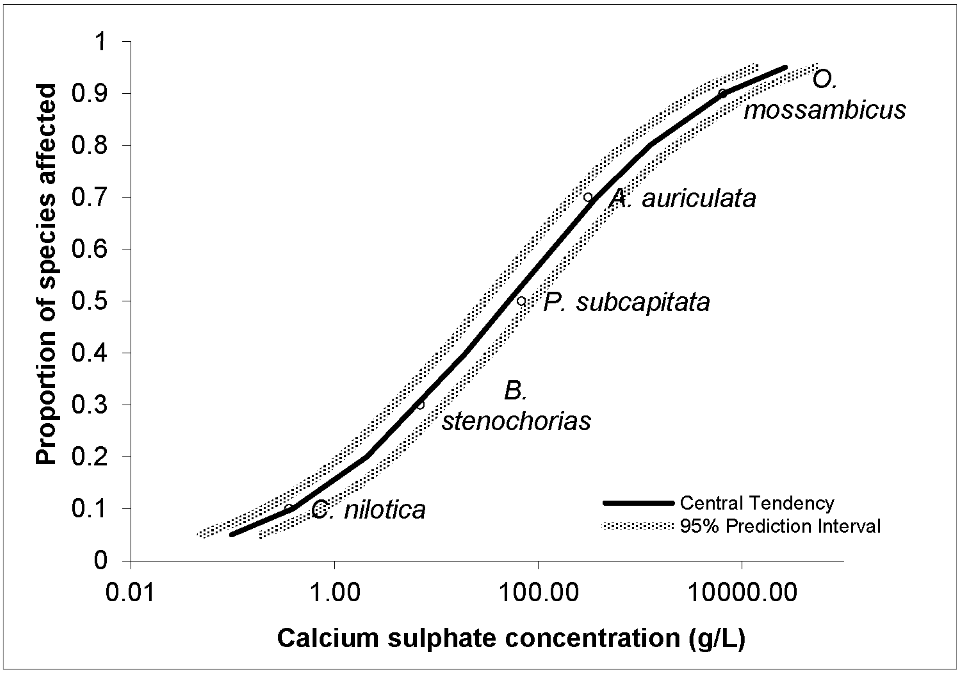

Figure 2.4: Species sensitivity distribution (SSD) showing the sensitivity of the test species *B. stenochorias, A. auriculata, C. nilotica, P. subcapitata* and *O. mossambicus* to calcium sulphate based on 96 hour LC/EC50 toxicity data. The upper and lower 95% confidence intervals are also shown.

2.3.2 Long-term exposure tests

Long-term toxicity data are recommended in the development of WQGs. The three inorganic salts had varied toxicity effects on the tested species (Table 2.4). Based on 240-hour exposure tests, Na_2SO_4 was most toxic to the species *A. auriculata*, *C. nilotica*, and *P. subcapitata* while the Mozambique tilapia, *O. mossambicus*, was generally the least sensitive species to Na_2SO_4. *B. stenochorias* and *O. mossambicus* were most sensitive to $CaSO_4$. It was observed that a gradient layer of the solution regularly formed in test beakers containing $CaSO_4$ during toxicity exposures. This necessitated frequent shaking of test beakers to allow mixing of the solutions, although nothing was done overnight until morning for the entire duration of the tests.

Table 2.4: Lethal and effective concentrations with 95% confidence limits for species exposed to sulphate salts

Common name	**Family**	**Species**	**Taxonomic group**	**Salt**	**240 h LC10 (g/L)**	**95% CL (g/L)**
Shrimp	Atyidae	*Caridina nilotica*	Crustacean	$MgSO_4$	1.321	0.766-1.876
				Na_2SO_4	0.339	0.217-0.462
				$CaSO_4$	7.251	3.678-10.825
Mayfly	Leptophlebiidae	*Adenophlebia auriculata*	Insect	$MgSO_4$	0.357	0.775
				Na_2SO_4	0.027	0.086
				$CaSO_4$	0.471	0.274-0.669
Limpet	Ancylidae	*Burnupia stenochorias*	Mollusc	$MgSO_4$	0.335	0.061-0.608
				Na_2SO_4	0.334	0.273-0.394
				$CaSO_4$	0.084	0.242
Mozambique tilapia	Cichlidae	*Oreochromis mossambicus*	Fish	$MgSO_4$	6.369	13.21
				Na_2SO_4	1.893	1.220-2.565
				$CaSO_4$	1.167	0.660-1.674
Algae	Selenastraceae	*Pseudokirchneriella subcapitata*	Green algae	$MgSO_4$	5.702	-
				Na_2SO_4	1.002	-
				$CaSO_4$	2.183	-

Individual CL values denote the upper limits. Lower values were negative and were thus considered not biologically meaningful. The readings for algae are EC values.

2.3.2.1 SSD curve for combined sulphates

Similar to short-term exposures, SSD predictions showed that species had a varied sensitivity to the salts. The three inorganic salts were combined to statistically model species responses to sulphate during long-term exposures (Figure 2.5). The species *A. auriculata* was the most sensitive of the tested species to the combined sulphate after 240 hours of exposure. However, *O. mossambicus* were the most and least sensitive species to sulphate respectively after 240 hours of exposure. *P. subcapitata* was relatively less sensitive to individual sulphate salts and the combined SSD (Figures 2.6 – 2.8). The species *B. stenochorias* was generally more sensitive to increasing sulphate salinity.

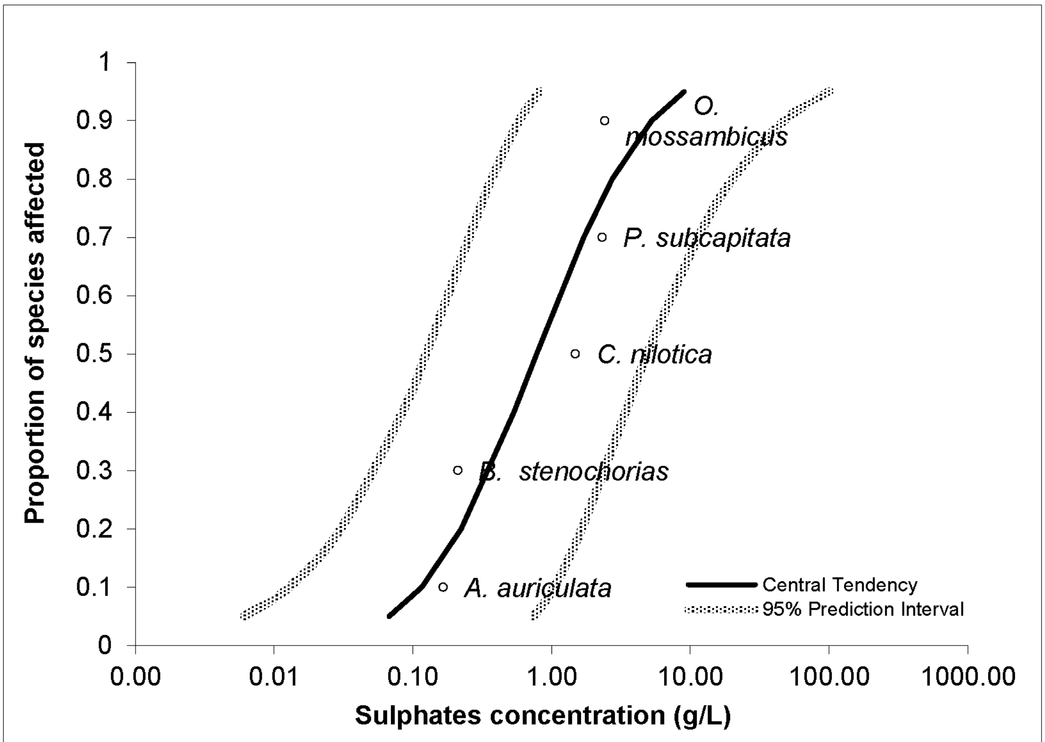

Figure 2.5: Species sensitivity distribution (SSD) showing the sensitivity of the test species *B. stenochorias, A. auriculata, C. nilotica, P. subcapitata* and *O. mossambicus* to the three inorganic salts combined based on 240 hour LC/EC10 toxicity data. The upper and lower 95% confidence intervals are also shown.

2.3.2.2 SSD curves for specific salts

With reference to specific salts, the SSD curves showed that the tested species were more sensitive to Na_2SO_4 than $MgSO_4$ and $CaSO_4$, suggesting that Na_2SO_4 was relatively more toxic (Figure 2.6). The species were also more sensitive to Na_2SO_4 than the modelled combined sulphates. The results further showed corresponding sensitivity pattern of species between the modelled combined sulphates and Na_2SO_4, as there were no changes in the rank responses. Mortalities began to occur at low concentrations of Na_2SO_4 compared to $MgSO_4$ and $CaSO_4$ (Figure 2.7 and 2.8).

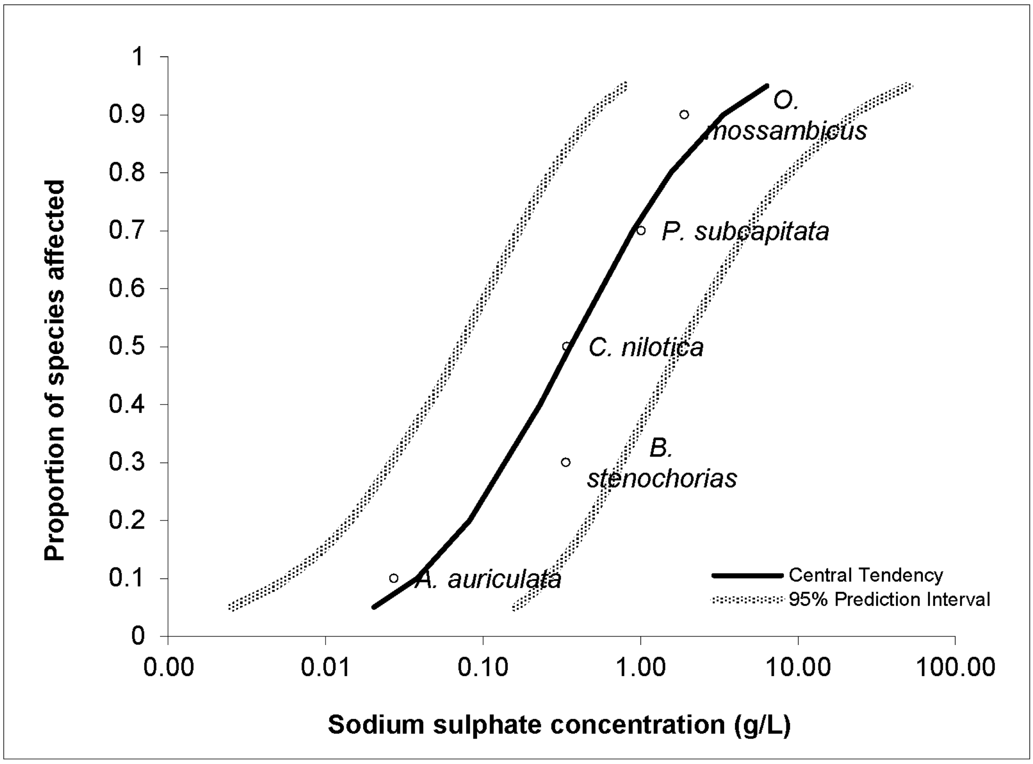

Figure 2.6: Species sensitivity distribution (SSD) showing the sensitivity of the test species *B. stenochorias, A. auriculata, C. nilotica, P. subcapitata and O. mossambicus* to $MgSO_4$ based on 240 hour LC/EC10 toxicity data. The upper and lower 95% confidence intervals are also shown

The species *B. stenochorias* was the most sensitive to $MgSO_4$ while *O. mossambicus* was the least sensitive to the salt. *B. stenochorias* was also the most sensitive to $CaSO_4$. Surprisingly, the crustacean was relatively less sensitive to $MgSO_4$ than *B. stenochorias*.

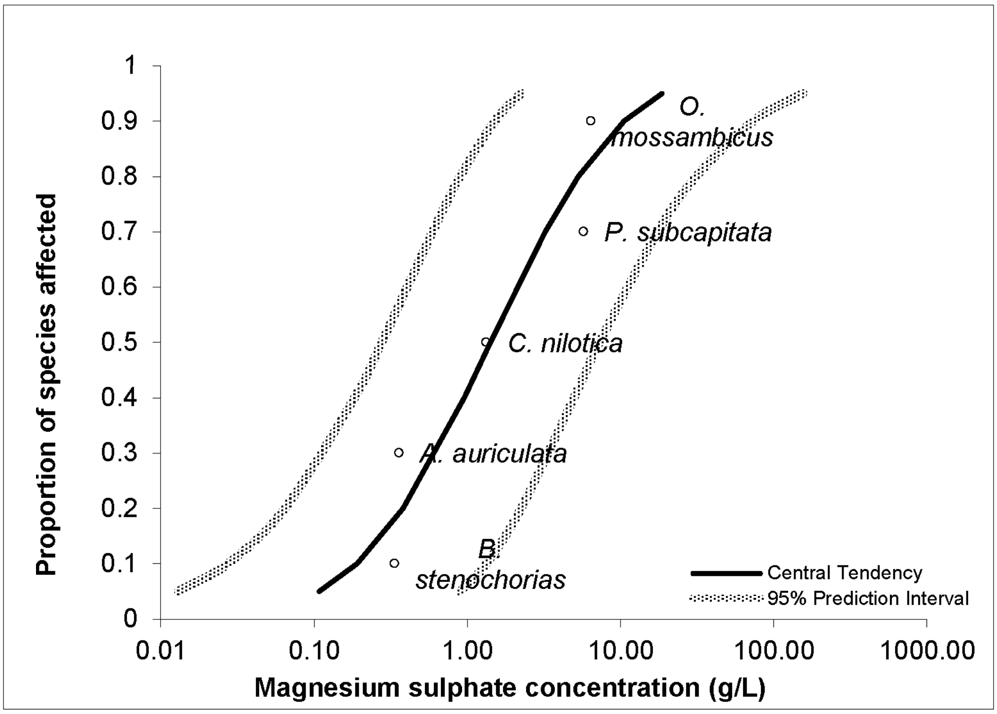

Figure 2.7: Species sensitivity distribution (SSD) showing the sensitivity of the test species *B. stenochorias, A. auriculata, C. nilotica, P. subcapitata* and *O. mossambicus* to Na_2SO_4 based on 240 hour LC/EC10 toxicity data. The upper and lower 95% confidence intervals are also shown.

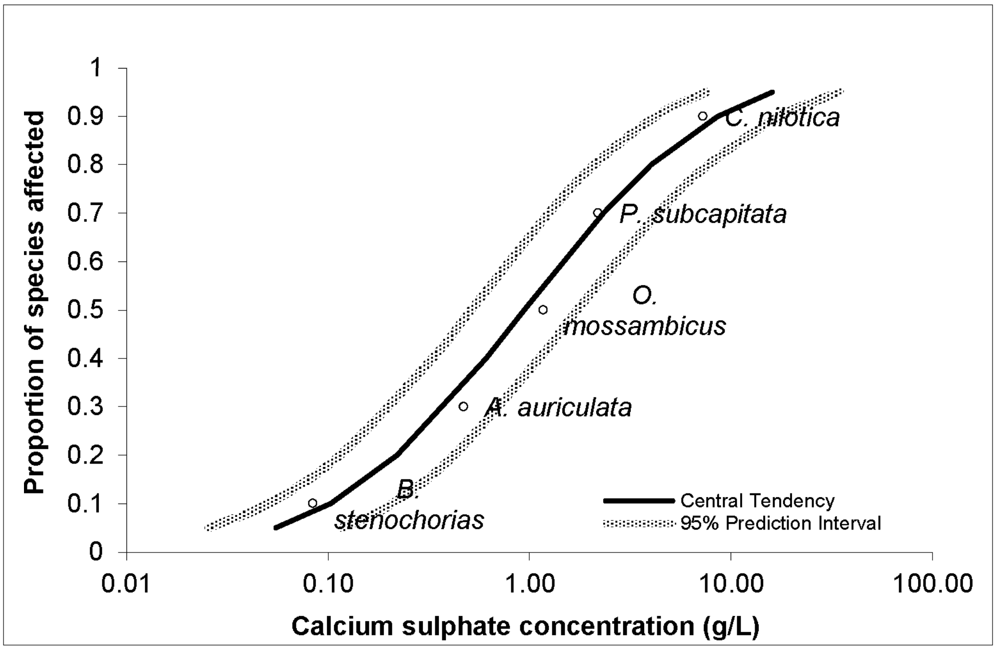

Figure 2.8: Species sensitivity distribution (SSD) showing the sensitivity of the test species *B. stenochorias, A. auriculata, C. nilotica, P. subcapitata* and *O. mossambicus* to $CaSO_4$ based on 240 hour LC/EC10 toxicity data. The upper and lower 95% confidence intervals are also shown.

2.3.3 Derivation of water quality guidelines for sulphate

Tables 2.5 and 2.6 present risk-based short-term and long-term WQGs for the sulphate salts as estimated from the SSD approach. These WQGs, if not exceeded, can protect at least 95% of the tested species in an ecosystem that is considered relatively pristine or natural.

2.3.3.1 Short-term sulphate WQGs

The results indicated $CaSO_4$ and Na_2SO_4 as the most and least protective salts for the tested species. The modelled combination of the three inorganic sulphates WQG was 0.71 g/L. However, short-term WQG for $CaSO_4$ was overprotective than the three salts combined.

Table 2.5: 95% protective concentrations for sulphate salts as were experimentally derived from the five tested species after short-term exposure

Salt	Central tendency (g/L)	Lower predicted interval (g/L)	Upper predicted interval (g/L)
$MgSO_4$	0.819	0.502	1.337
Na_2SO_4	1.158	0.750	1.790
$CaSO_4$	0.097	0.049	0.193
Modelled sulphate (combined)	0.710	0.118	4.259

2.3.3.2 Long-term sulphate WQGs

The results indicated that Na_2SO_4 had the lowest protective concentration (0.020 g/L). The WQG value for $MgSO_4$ was the highest of the three salts at 0.108 g/L. The model goodness of fit (R^2) for $MgSO_4$ was 0.871 with confidence boundaries ranging between 0.013 g/L and 0.899 g/L. However, the slope for Na_2SO_4 was steeper (1.318) compared to 1.471 for $MgSO_4$. The regression models (R^2 = 0.98, 0.90 and 0.87) for $CaSO_4$, Na_2SO_4 and $MgSO_4$ fit the data strongly respectively (Appendix 1D).

Table 2.6: 95% protective concentrations for sulphate salts as were experimentally derived from the five tested species after long-term exposure

Salt	Central tendency (g/L)	Lower predicted interval (g/L)	Upper predicted interval (g/L)
$MgSO_4$	0.108	0.013	0.899
Na_2SO_4	0.020	0.003	0.162
$CaSO_4$	0.055	0.026	0.118
Modelled sulphate (combined)	0.067	0.006	0.745

2.4 Discussion

The South African Ecological Reserve requires that any proposed WQGs for toxicants in waterways classified as category A (i.e. 'excellent') should protect 95% or more of species (Warne et al. 2004). Protecting the majority of species in an ecosystem is the goal for most managers (Kefford et al. 2006). However, currently, in South Africa, there are no risk-based derived WQGs for sulphates that are directed at mining discharges, particularly AMD. Furthermore, no short-term and long-term specific guidelines exist with regards to sulphate risks to freshwater species in South Africa. This is worrisome considering that South Africa is a semi-arid and water-stressed country that continues to receive sulphates through AMD from coal and gold mining activities. Thus, the risk-based WQGs derived in this study are important for the management of sulphates both in the short-term and long-term exposure scenarios for the protection of freshwater ecosystems. Although long-term WQGs are preferred, short-term WQGs are still essential for South Africa to address the urgent deterioration of freshwater water quality, particularly in mining regions.

2.4.1 Ecological risks of sulphate to the tested species

2.4.1.1 Short-term salinity toxicity

This study has shown that the tested species were sensitive to sulphates after short-term exposures. Significant species sensitivity differences to the salts were observed between the different species. Modelling the combination of the salts was necessary to give an overall picture of the effects of sulphates to the tested species because sulphates do not easily change their form in nature systems (Gray 1998). During short-term exposures, the modelled sulphate toxicity effects to the tested species demonstrated that *C. nilotica* was the most affected species. This result could indicate the potential for sulphates to affect the shell of shrimps adversely, which would impede other biological functions such as growth. This result suggests that juvenile shrimps are highly sensitive to sulphate after short-term exposures than the nymphs of the mayfly used in this study. Similarly, *B. stenochorias* was more affected by sulphate compared to *A. auriculata*, which was moderately tolerant than both *P. subcapitata* and *O. mossambicus* respectively.

Both Na_2SO_4 and $MgSO_4$ were more toxic to *B. stenochorias* compared to $CaSO_4$ after 96 hours of exposure. However, Na_2SO_4 was more toxic to *B. stenochorias* than $MgSO_4$. This may be due to ionic composition differences between the salts.

The salt Na_2SO_4 is heavier (142.04 g/mol) than $MgSO_4$ (120.366 g/mol) and $MgSO_4$ (136.14 g/mol) in terms of molar mass. In addition, Na_2SO_4 has two Na ions per mole compared to one mole of Mg and Ca for $MgSO_4$ and $CaSO_4$ respectively.

2.4.1.2 Long-term salinity toxicity

The ionic effect of salts on the tested species during short-term exposures might not vary substantially with respect to long-term exposures. The ionic composition of salts, either percent of salt or type of salt, in water can influence the sensitivity of species, although effects over different time periods is an area that needs more research (Zalizniak et al. 2007). Pure sodium chloride (NaCl) is more toxic than sea water, which is 85% NaCl (Cañedo-Argüelles et al. 2013). Water hardness (more Ca and/or Mg) is also known to lessen sodium (Na) toxicity (Cañedo-Argüelles et al. 2013), perhaps through cation competition. The ionic ratio differences within each salt in the solution might have yielded significant responses for the tested species.

A difference in enzyme activation within the species may also affect species responses to salinity. Kefford et al. (2006) reported that enzymes induce the sensitivity of species to increasing salinity, resulting in different responses for different species. The tested species in this study started to become more sensitive to Na_2SO_4 at very low concentrations. This corresponds with a study that found insects and molluscs to be more sensitive to low concentrations of salinity compared to crustaceans such as *C. nilotica* (Kefford et al. 2006). For example, the Na_2SO_4 LC value (0.339 g/L) for *C. nilotica* was about five-fold more toxic compared to 1.80 g/L reported by Palmer et al. (2004d) for the same species but was less toxic for *A. auriculata* (0.027 g/L). Contrary to current results, another study suggested that insects are generally better regulators of Na than crustaceans such as shrimps especially if they have a haemoglobin buffer system (Gerhardt et al. 2004). However, current results revealed that *A. auriculata* did not regulate Na better compared to *C. nilotica* to support the suggestion based on this study.

The mollusc *B. stenochorias* was consistently one of the most sensitive species to sulphate. This may be due to the molluscs being relatively sessile in nature and unable to easily escape from toxicants exposure. Its high sensitivity to sulphates patterns its responses to other toxicants including pesticides and metals (Gerhardt and Palmer 1998; Mensah et al. 2013). The sensitivity of molluscs to calcium salts is unclear. In this study, *B. stenochorias* was most sensitive in test waters rich in Ca.

This is similar to a study that found the mollusc species *Physa acuta* sensitive to alkaline conditions or water with higher Ca concentrations (Cañedo-Argüelles et al. 2013). However, other studies have shown that saline waters with low Ca are more toxic to aquatic macroinvertebrates (Zalizniak et al. 2007).

The LC values for *B. stenochorias* (0.084 g/L) and *A. auriculata* (0.471 g/L) for $CaSO_4$ were particularly low compared to the other tested species in this study. This may be related to the test design, influenced by the formation of some salt in beakers, particularly for $CaSO_4$. A microscopic view of dead organisms collected from the bottom in test beakers had blocked gills, which is a potential occurrence in streams that receive lime-treated AMD with minimal dilution for sufficient mixing of the effluent. This finding potentially suggests that salts might not only affect organisms through internal mechanisms but externally as well.

2.4.2 Water quality guidelines for sulphate

This study has developed risk-based WQGs for sulphate to protect over 95% of the tested species in South African freshwater ecosystems. If not exceeded, a WQG of 0.097 g/L would protect over 95% of the tested species during short-term exposures. Similarly, a WQG of 0.020 g/L would protect over 95% of the tested species if not exceeded. These guidelines imply that the ecological risk of sulphates to the tested species is higher in the presence of the $CaSO_4$ and Na_2SO_4 during short-term and long-term exposures. Species that are found in streams receiving AMD can exhibit diverse sensitivity patterns due to changing water quality variables and as such might not match SSD findings. However, when AMD streams host endangered species, the SSD approach provides alternative management options for the risk over general uncertainty factors (ECETOC 2014).

SSDs have been criticised for their biases in species selection, relevance to ecological functions with no assessment of measurement error or uncertainties in input data and their lack of an accepted method of updating results including trigger or threshold concentrations, among others (Fox 2011; Kefford et al. 2006; Xu et al. 2015). To add to the ecological relevance of results from this study, indigenous species from known communities were used for exposures to derive risk-based WQGs that reflect local conditions. These species belonged to different taxonomic groups. However, SSD validations have proved to conform with field realism, although slight variability could occur in terms of species percentage protected (Kefford et al. 2006; Xu et al. 2015).

The present study has demonstrated that the five tested freshwater species exhibited sensitivity differences to increasing sulphate salinity after both 96 hours and 240 hours of exposure. The current study has also demonstrated a consistent sensitivity response pattern for the species to Na_2SO_4 and $MgSO_4$ exposures, with Na_2SO_4 as the most salt the tested species were most sensitive to and $MgSO_4$ the salt to which they were least sensitive in the long-term. These findings suggest that protecting *C. nilotica* from $CaSO_4$ potentially protects over 95% of the tested species. Similarly, protecting *A. auriculata* from Na_2SO_4 exposure would potentially protect at least 95% of the tested species in an ecosystem.

The sulphate WQGs were significantly lower than the existing maximum compliance guideline for South Africa in freshwater ecosystems. This implies that the 0.25 g/L guideline may be insufficient to protect species in the long-term. It also undermines the toxicity of $CaSO_4$ during short-term exposures, except for $MgSO_4$. With the exception of $MgSO_4$, setting individual salt guidelines at 0.25 g/L would still wipe out over 95% of the tested species in the long-term based on the current results. If salinity sensitive species are lost through mortality, it can disrupt the entire food chain in an ecosystem, including ecological roles. Given the evidence in this study that WQGs are too low, South Africa might want to require strong compliance limit for sulphates such as that for Canada of 0.1 g/L (Meays and Nordin 2013). Thus, risk-based WQGs for sulphate from the current study is an essential contribution to the management of freshwater resources in South Africa.

2.5 Conclusion

The objective of this chapter was to derive short-term (96 hours) and long-term (240 hours) risk-based WQGs for sulphate, a major salt component in AMD, using SSD approach for the protection of at least 95% of species in a natural ecosystem with little or no anthropogenic disturbances. The study concludes that:

- The tested species were sensitive to increasing sulphate salinity at varying levels both during short-term and long-term. Correspondingly, WQGs for sulphate salts have been derived as 0.097 g/L for short-term and 0.020 g/L for long-term.
- The tested species were generally most sensitive to $CaSO_4$, followed by$MgSO_4$ and then Na_2SO_4 after short-term exposures respectively.
- The tested species were generally most sensitive to Na_2SO_4, followed by $CaSO_4$ and then $MgSO_4$ after long-term exposures respectively.
- The current sulphate compliance limit of 0.25 g/L for freshwater ecosystems in South Africa underestimates the toxicity of Na_2SO_4 and $CaSO_4$ for the tested species, and therefore a compliance limit of 0.020 g/L is recommended based on the finding of this study.

The data provided in this study are important ecotoxicological information that contributes to better understanding of ecological risk associated with sulphates occasioned by the release of AMD in freshwater ecosystems. They guide the review and development of further risk-based WQGs for sulphate in regions where AMD continues to threaten water quality, particularly in arid and semi-arid areas.

CHAPTER 3: A RISK-BASED APPROACH TO DERIVING SCENARIO-SPECIFIC WATER QUALITY GUIDELINES FOR COAL-ACID MINE DRAINAGE: THE CASE OF THE BOESMANSPRUIT, MPUMALANGA, SOUTH AFRICA

The following manuscript has been accepted in the African Journal of Aquatic Sciences:

1. *"**EC Vellemu**, PK Mensah, NJ Griffin and ON Odume (2017) The derivation of water quality guidelines for acid mine drainage using a risk-based approach". African Journal of Aquatic Sciences.*

3.1 Introduction

Chapter two of this thesis investigated the ecological risks of sulphate salts and derived water quality guidelines (WQGs) using a risk-based approach. This chapter focuses on assessing AMD as a whole effluent in order to evaluate the combined effects of its constituents. Investigating AMD effects on indigenous aquatic species in South Africa as a whole effluent is a promising integrative and holistic approach because it provides insights into the combined toxicity of all its chemical constituents in a natural environment. It also provides some degree of environmental realism. However, whole effluents can vary in volume, compositions and concentrations of constituents considerably.

An understanding of AMD toxicity as a whole effluent in aquatic ecosystems can provide robust and important insight into the management of mine water. Although individual contaminants such as salts or metals have specific impacts on aquatic organisms, their effects may be aggravated in receiving streams when combined, as in AMD. Whole effluent toxicity (WET) testing can provide important synergistic effects information for receiving streams. Relating the effects to the potential toxicological impacts of the AMD from mines is often a challenge as different mines produce different effluents due to various processes, including geological formations of the mine (Falkenberg and Styan 2015). Despite the challenges of relating effects to exposures, whole effluent tests are used to assess the sporadic presence of concentrations of contaminants (Carbonell et al. 2010). They help to identify, characterise and eliminate toxic effects of effluents in ecosystems (Chapman 2000). Although WET testing does not reliably predict effects in receiving ecosystems, they are an important first step in risk assessment to identifying hazards in the environment (Chapman 2000).

WET testing also provides important endpoints, especially when more than one test organism is used in the exposure tests (Falkenberg and Styan 2015). Therefore, investigating AMD as WET can help understand the management of AMD and associated impacts on freshwater ecosystems.

The potential impacts of AMD in freshwater ecosystems need to be considered in water management approaches to protect the local environment, which hosts diverse aquatic organisms. WET studies are generally limited in scientific studies (Davies-Coleman 2001; Zokufa et al. 2001; Falkenberg and Styan 2015), partly due to their complexity in composition and variation. However, scenario-specific WQGs for AMD can be formulated to manage and protect local biota. Scenario-specific WQGs can be useful because they can be applied widely in areas with similar environmental conditions (e.g. geological formations and soils). Heath (2008) noted that the same industrial process using different industrial raw materials might require or tolerate different water qualities, suggesting the need for scenario-specific WQGs. Therefore, the aim of this chapter was to evaluate the toxicity of AMD with a view to developing short-term and long-term risk-based scenario-specific WQGs. Although it is preferred to protect over 95% of species in a natural ecosystem (Warne et al. 2004), mining activities are key drivers of the economy in South Africa (Manders et al. 2009), and some level of pollution would still occur to impede the achievement of 95% protection goal. It is thus appropriate to derive WQGs for AMD based on specific-scenarios occurring at a receiving water resource.

3.2 Materials and methods

3.2.1 Study site and collection of mine water

Acid mine drainage samples were collected from an abandoned coal mine located near Carolina in Mpumalanga Province, South Africa (Latitude: 26.173706° S; Longitude: 30.112451° E) (Figure 3.1). The AMD effluent samples were collected at the end of an underground pipe just before it discharged into a holding dam on-site (Figure 3.2). The dam was built to hold decanting AMD, as well as to allow sedimentation and evaporation in efforts to minimise environmental contamination in the receiving stream. Mechanisms to treat the AMD are in place by the application of lime (lime bags placed in the middle of the dam) mainly to raise the pH, although continuous seepage and spills were observed during sampling. The area had not received any rainfall for over a month at the time of sampling. The AMD spill flows into the Boesmanspruit, which flows into the Nooitgedacht Dam in Carolina. Samples were collected in pre-acid washed bottles, kept in a cooler-box that contained ice cubes. Collected AMD samples were transported to the laboratory at Rhodes University for the WET tests. The pH of the AMD at the time of sampling was 3.01. A full scan elemental analysis of the 100% AMD, 50% AMD and the diluent (dechlorinated tap water) was run using both the Inductively Coupled Plasma Mass Spectrometry (ICP-MS) and Gas Chromatography-Mass Spectrometry (GC-MS) methods at InnoVenton analytical laboratory in Port Elizabeth, South Africa.

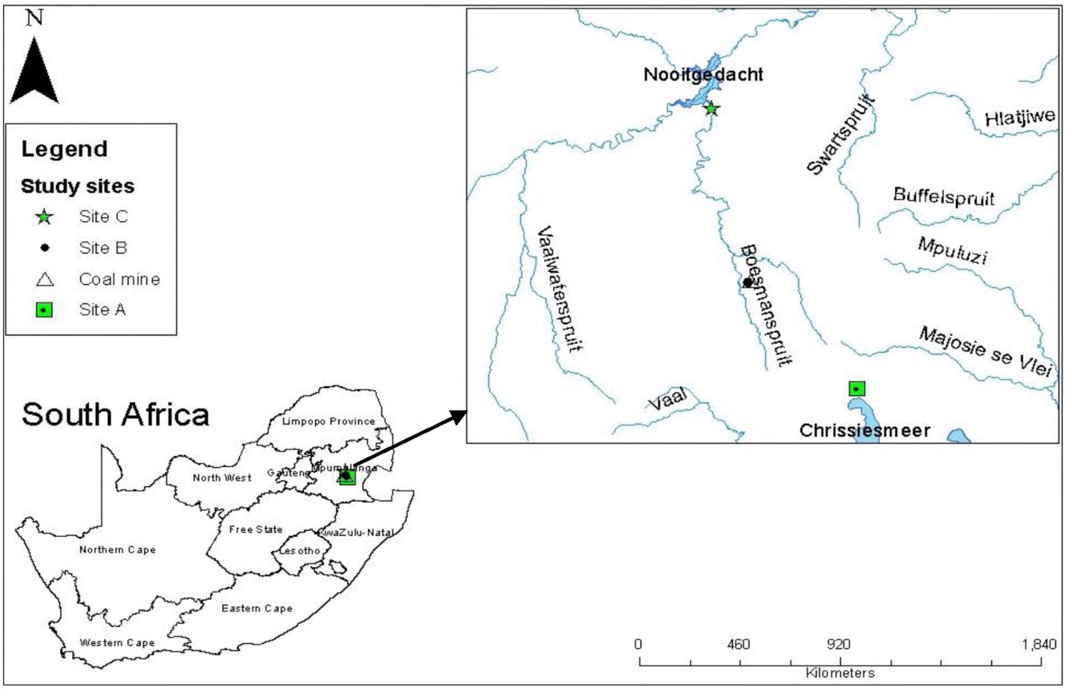

Figure 3.1 Map showing the location of the coal mine (near Site B).

Figure 3.2: Coalmine-holding dam where AMD samples were collected in the Boesmanspruit catchment in Mpumalanga.

3.2.2 Test dilutions for the exposure

In the laboratory, AMD samples were diluted using dechlorinated tap water for test solutions. A range finding test was performed to determine appropriate dilutions, which resulted in the following definitive dilutions; 0%, 0.048%, 0.098%, 0.195%, 0.39%, 0.78%, 1.56%, 3.12% and 6.25% (0% dilution was the control, i.e. dechlorinated tap water without AMD) following a protocol in Prasad et al. (2012). Each dilution was replicated three times. The pH for the 100% undiluted AMD (initial concentration) was 3.01, 1500 mg/L for sulphates and 4.07 mS/m for electrical conductivity (EC). Water quality parameters, including dissolved oxygen (DO), pH and EC were monitored throughout the exposure tests.

3.2.3 Test species

The toxicity of AMD was assessed on indigenous freshwater species belonging to five different taxonomic groups in South African ecosystems (Table 3.1).

Table 3.1: Summary of species used for acid mine drainage exposure tests and their origin

Common name	Family	Species	Age group	Site collected
Microalgae	Selenastraceae	*Pseudokirchneriella subcapitata*	-	Commercially obtained, MicroBioTests Inc., Nazareth, Belgium
Crustacean	Atyidae	*Caridina nilotica*	Juveniles (>7< 20 days post-hatch)	Laboratory-reared, Rhodes University, Grahamstown
Fish	Cichlidae	*Oreochromis mossambicus*	5 days old fingerlings	Rivendell hatchery, Eastern Cape, South Africa
Insect	Leptophlebiidae	*Adenophlebia auriculata*	nymphs	Palmiet River, Eastern Cape, South Africa
Mollusc	Ancylidae	*Burnupia stenochorias*	mixed	Kat River, Eastern Cape, South Africa

3.2.4 Experimental procedure

The experimental procedure discussed in Chapter Two was followed for AMD experiments. All experiments were conducted in a temperature-controlled laboratory (20 °C ± 2) after acclimation of the organisms. About 1000 mL of the AMD sample was transferred into a beaker for dilution.

Dechlorinated tap water was added into the AMD by a factor of two for dilution until all test solutions and replicates were prepared. Ten individuals of *C. nilotica*, *A. auriculata* and *O. mossambicus* were randomly allocated into 600 mL glass beakers containing 350 mL of the test solution during exposure. Fifteen individuals of *B. stenochorias* were allocated in test beakers, as these were highly abundant. Organisms were not fed during the first 96 hours of exposure, after which TetraMin flakes (Tetra Company, Germany) were added daily including in the control groups for each experiment. The 0% dilution was the control group (i.e. dechlorinated tap water without AMD).

Only one life history stage was used for each tested species with mortality being the test endpoint. This was assessed by prodding the organism using a glass stirrer. Acceptable control mortality was less than 10% (DWAF 2000). Organisms that did not respond to the stimulus were presumed dead. Organisms were counted daily and dead animals were removed from the test vessels. Except for green algae test, long-term renewal (240 hours) tests were conducted for all species. Fresh test solutions were prepared and changed every 96 hours to maintain the water quality.

Micro-algal tests followed the Organisation for Economic Co-operation and Development (OECD) 201 guidelines (OECD 1984). The algal culture and stock solutions for the preparation of growth media were commercially obtained (MicroBioTests Inc., Nazareth, Belgium). The Toxkit had healthy demobilised algae cells that were kept under 5 °C prior to exposure, after which they were mobilised to prepare six concentrations for each experiment including the control groups. Test solutions were incubated at 23 ± 2 °C for 96 hours in 25 mL incubation vials containing 5 mL of algal growth medium. The vials were shaken once daily under constant illumination using fluorescent light during the incubation process. The pH ranged between 7.8 and 8.5. Toxicity tests were run in three replicates and algal cell count measured as optical density. Culture samples were transferred into 5 cm well plates as a provision due to lack of a spectrophotometer fitting the 10 cm cell plates. Algal cell counts were read on a spectrophotometer at 670 nM (nanometre) wavelength at 0, 24, 48, 72 and 96 hours. No feed was administered to algae during the entire exposure tests.

3.2.5 Estimation of lethal and effective dilutions

Lethal dilutions (LDs) of the AMD that killed 10% and 50% of the tested species were estimated using a regression curve in statistical software package 'DRC' within the R programming environment (Ritz and Streibig 2012).

LD10 values were used for the derivation of long-term scenario-specific WQGs and LD50 values were used for short-term scenario-specific WQGs. The effective dilution (ED10 and ED50) values for algae were calculated using a linear regression analysis of transformed chemical concentration as natural logarithm data against percentage inhibition. Tests with algae conducted over 96 hours were considered both short-term and long-term as recommended by some authors (Hagen and Douglas 2014).

3.2.6 Generation of species sensitivity distribution curves

The LD and ED values were used to generate Species Sensitivity Distribution (SSD) curves for short-term and long-term using the SSD Generator (USEPA 2009). This software estimates protective dilutions (*PDs*) for the tested species with 95% upper and lower confidence limits (CL) (Mensah et al. 2013; Hagen and Douglas 2014). The log-probit (linearised lognormal) model was used to fit the data for dilutions at which different species exhibited a standard response to AMD. The *PDs* are important because they serve as WQGs.

3.3 Results

3.3.1 Summary of metal concentrations in acid mine drainage and diluent

At a pH 3.01, a full scan of the AMD for metals before dilution, 50% dilution and diluent (dechlorinated tap water) by ICP-MS and GC-MS methods indicated that the study site contained ores of pyrite (Table 3.2). It was evident from the results that dechlorinated tap water did not contain metals that could have interfered with the AMD during exposure tests. High concentrations of manganese, lithium, sulphur and nickel were found in the 100% AMD compared to the 50% diluted AMD. Generally, the 50% diluted AMD had about half the concentrations of metals recorded in the 100% AMD.

Table 3.2: Summary of metal concentrations that were present in 100% and 50% diluted AMD, as well as the diluent

Metal	**100% AMD**	**50% AMD**	**Dechlorinated tap water (diluent)**
Iron as Fe mg/l	96.4	65.9	ND (not detected)
Chromium as Cr µg/l	4.1	ND	ND
Copper as Cu µg/l	10.2	5.3	ND
Nickel as Ni µg/l	697.3	430.1	ND
Zinc as Zn µg/l	1000	665.4	ND
Cobalt as Co µg/l	1100	655.6	ND
Lead as Pb µg/l	5.8	1.5	ND
Manganese as Mn µg/l	23 300	14600	ND
Sodium as Na mg/l	1.2	0.6843	0.051
Magnesium as Mg mg/l	30.8	19.4	ND
Sulphur as S mg/l	101	61.0	ND
Potassium as K µg/l	1.5	818.8	ND
Strontium as Sr µg/l	386.5	227.1	ND
Iodide as I µg/l	4.8	2.6	0.5
Lithium as Li µg/l	718.8	479.2	ND
Beryllium as Be µg/l	6.7	2.2	ND
Calcium as Ca mg/l	9.2	5.1	ND
Aluminium as Al mg/l	7.4	4.7	ND
Barium as Ba µg/l	4.9	0.9	ND

3.3.2 Short-term toxicity tests

A layer of gradient regularly formed in test beakers during toxicity exposures. This was also observed in the holding dam, while the streams were regularly mixed due to flow. This necessitated frequent shaking of test beakers to allow mixing of the test solutions, although nothing was done overnight until morning for the entire duration of the tests. The estimated lethal dilutions (LDs) for short-term 96 hour exposures are presented in Table 3.3. The AMD had different toxicity effects on the tested species. The minimum and maximum LDs of AMD that resulted in the mortality of 50% species were 0.157% and 7.204%, respectively.

Table 3.3: Estimated short-term lethal/effective dilutions (%) of acid mine drainage for the tested species

Common name	Family	Species	96 h ED/LD50 (%)	95% CL (%)
Shrimp	Atyidae	*C. nilotica*	2.783	1.698-3.869
Mayfly	Leptophlebiidae	*A. auriculata*	1.978	1.668-2.288
Limpet	Ancylidae	*B. Stenochorias*	0.792	0.492-1.092
Mozambique tilapia	Cichlidae	*O. mossambicus*	5.112	0.174-7.204
Green-algae	Selenastraceae	*P. subcapitata*	0.157	-

3.3.2.1 SSD curves for short-term AMD exposure

The results in Table 3.3 were used for the construction of short-term species sensitivity distribution (SSD) curves (Figure 3.3). The species *P. subcapitata* was the most sensitive while *O. mossambicus* was the least sensitive to AMD. The species *A. auriculata* was moderately sensitive to AMD but most sensitive compared to *C. nilotica*. Based on the proposed 95% protocol for species protective levels in South African freshwater ecosystems (Warne et al. 2004), the 96 hour median LD/ED AMD exposures was 0.122% for a natural ecosystem. The lowest *PD* boundary for this category was 0.030% and the highest was 0.489%. Other *PDs* required protecting the tested species in different ecological categories have been documented in Appendix 2A.

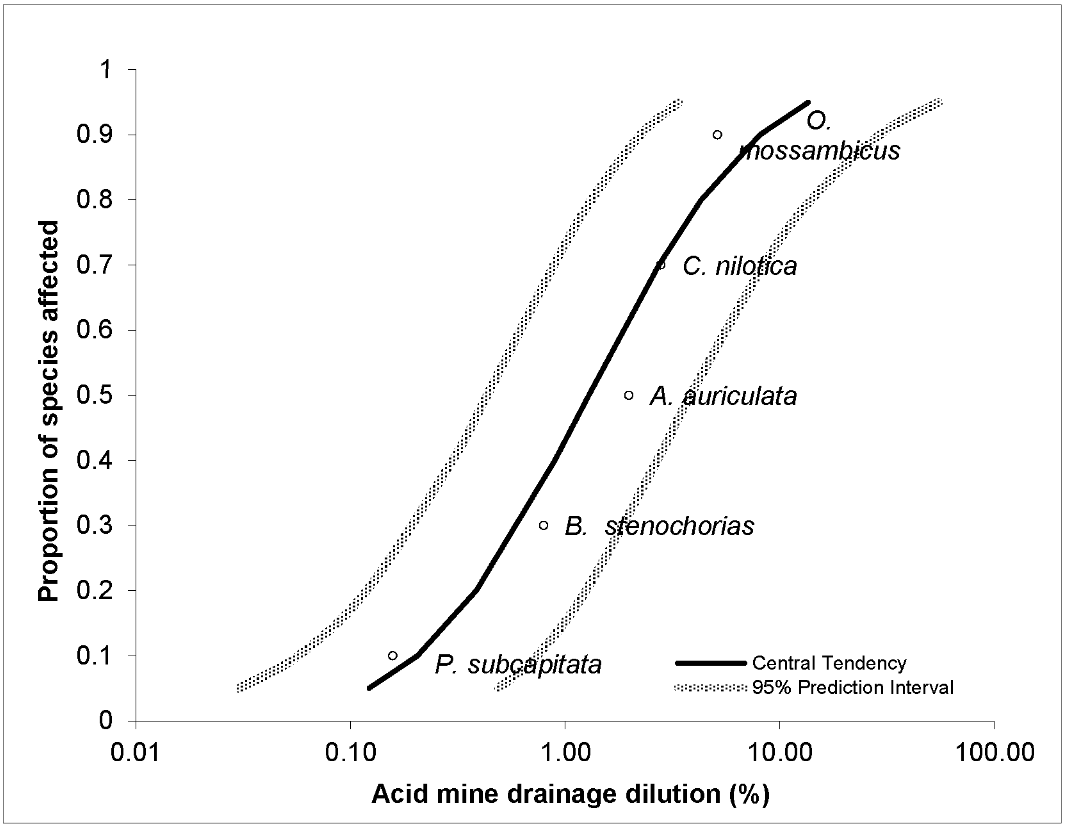

Figure 3.3: 96 hour LD/ED50 species sensitivity distribution (SSD) showing the sensitivity of the tested species *B. stenochorias, A. auriculata, C. nilotica, P. subcapitata and O. mossambicus* to acid mine drainage. The upper and lower 95% confidence intervals are also shown

3.3.3 Long-term toxicity tests

Similar to the short-term results, the tested species had a varied sensitivity to AMD (Table 3.4). The minimum and maximum lethal dilutions of AMD that resulted in the mortality of 10% species were 0.001% and 0.269% respectively. The method used to estimate ED values for *P. subcapitata* did not produce any confidence limits.

Table 3.4: Estimated long-term lethal/effective dilutions (%) of acid mine drainage for the tested species

Common name	Family name	Species	240 h ED/LCD10 (%)	95% CL (%)
Shrimp	Atyidae	*C. nilotica*	0.053	0.080-0.269
Mayfly	Leptophlebiidae	*A. auriculata*	0.032	0.084 (upper CL)
Limpet	Ancylidae	*B. Stenochorias*	0.020	0.001-0.038
Mozambique tilapia	Cichlidae	*O. mossambicus*	0.027	0.007-0.046
Green-algae	Selenastraceae	*P. subcapitata*	0.084	-

3.3.3.1 SSD curves for long-term AMD exposure

The results in Table 3.4 were used for the construction of long-term SSD curves to rank the sensitivity of the tested species to AMD (Figure 3.4). The species *B. stenochorias* was the most sensitive to AMD after 240 hours of exposure. Interestingly, the species *P. subcapitata* was the least sensitive to AMD after 240 hours of exposure to AMD. However, the species *A. auriculata* was still moderately sensitive to AMD compared to short-term exposures. Mozambique tilapia, *O. mossambicus,* was more sensitive to AMD after 240 hours of exposure compared to 96 hours of exposure. The estimated 240 hours LD/ED10 dilution of AMD that would protect over 95% of the tested species from AMD exposure was 0.014% for a natural ecosystem (Warne et al. 2004). The lowest *PD* boundary for this category was 0.010% and the highest was 0.021%.

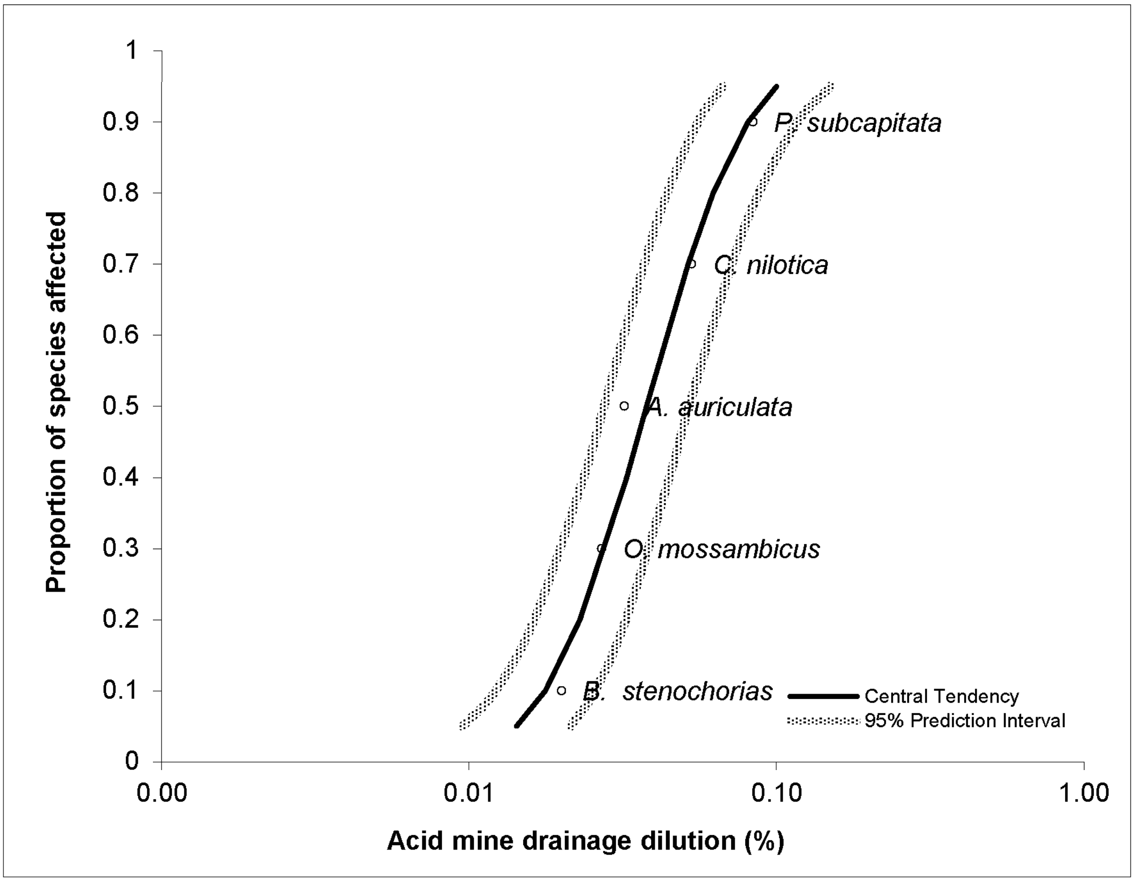

Figure 3.4: 240 hour LD/ED10 species sensitivity distribution (SSD) showing the sensitivity of the tested species *B. stenochorias, A. auriculata, C nilotica, P. subcapitata* and *O. mossambicus* to acid mine drainage. The upper and lower 95% confidence intervals are also shown

3.3.4 Derivation of water quality guidelines for AMD

Tables 3.5 and 3.6 present risk-based derived short-term and long-term WQGs for AMD as estimated from the SSD approach. These WQGs, if not exceeded, would protect at least 95%, 90% and 80% of the tested species in an ecosystem.

3.3.4.1 Short-term WQGs for AMD

Table 3.5 and Table 3.6 present WQGs for AMD for the protection of ecosystems using the tested species at different levels. These guidelines are scenario-specific but can be applied widely if the scenarios are similar to those of the study area. The derived guidelines were 0.122%, 0.205% and 0.384% for the protection of 95%, 90% and 80% of the tested species. The model goodness of fit (R^2) for short-term WQG for AMD was 0.932 at a slope of 1.606 with confidence boundaries ranging between 0.030% and 1.274%.

Table 3.5: WQGs for AMD as were experimentally derived from the five tested species after short-term exposure

Species protection level	Central tendency (%)	Lower predicted interval (%)	Upper predicted interval (%)
95%	0.122	0.030	0.489
90%	0.205	0.057	0.738
80%	0.384	0.118	1.247

3.3.4.2 Long-term WQGs for AMD

The results indicated that 0.014% as a WQG for AMD to protect over 95% of the tested species. Similarly, 0.018% and 0.023% were derived guidelines to protect 90% and 80% of the tested species from AMD. The model goodness of fit (R^2) was 0.966 at a slope of 3.890 with confidence boundaries ranging between 0.010% and 1.274 0.032%.

Table 3.6: WQGs for AMD as were experimentally derived from the five tested species after long-term exposure

Species protection level	Central tendency (%)	Lower predicted interval (%)	Upper predicted interval (%)
95%	0.014	0.010	0.021
90%	0.018	0.012	0.026
80%	0.023	0.016	0.032

3.4 Discussion

This study investigated the toxicity of AMD with a view to developing scenario-specific WQGs. The findings indicated that AMD was toxic to the five tested freshwater species. Several studies have related the toxicity of AMD to associated impacts in freshwater ecosystems (DeNicola and Stapleton 2002; Jennings et al. 2008; Boukhalfa and Chaguer 2012; Park et al. 2014; Parbhakar-Fox et al. 2014). The toxicity of AMD as a whole effluent on a wide range of aquatic species remains relatively unexplored, partly due to the difficulty of working with whole effluents (Chapman 2000). The micro-algae, *P. subcapitata*, were the most sensitive species to AMD after short-term exposures. Micro-algae are primary producers in the food web and their absence can determine the abundance of other organisms in an ecosystem. The presence of micro-algae in AMD affected ecosystems can vary substantially. The high sensitivity of algae during short-term exposures could be due to the presence of metal hydroxides in the AMD. Metal hydroxide deposits can suppress algal presence by direct toxicity or by smothering (Hogsden and Harding 2012). Metal deposits such as Al and Fe are more harmful to algae than others, although the mechanism for their toxicity difference is not clear (Hogsden and Harding 2012). Orlekowsky et al. (2013) study reported that algal growth had a negative correlation with metals such as strontium (Sr), lithium (Li), copper (Cu) and sodium (Na). However, algal higher abundance was positively correlated with the presence of Al and Mn. Although their study did not report the time duration of the investigation, they acknowledged that algal growth varies from species to species. In this study, high concentrations of these metals in the AMD could have potentially exacerbated the toxicity of the AMD to *P. subcapitata* in the long-term.

A 0.157% dilution of the AMD was effective to reduce 50% algal growth after 96 hours of exposure in this study. The result supported an earlier study, which reported an almost 50% algal biomass reduction at sites with high Fe hydroxide deposition than at sites where it was absent (Hogsden and Harding 2012). In this study, the acidic water also reduced algae diversity. In addition, the higher sensitivity of *P. subcapitata* to AMD could be attributed to changes in water quality and nutrients depletion to concentrations incapable of sustaining their growth (FAO 2014). This might imply that the cell density decreased rapidly at the end of 96 hours of exposure.

Algae can tolerate AMD pollution. For example, the presence of some algal species is known as a good indicator of the presence of AMD (Gerhardt et al. 2004). High biomass has been linked to the dominance of large-celled filamentous green algae instead of diatoms (Hogsden and Harding 2012).

Other tolerant filamentous green algae, such as *Ulothrix*, can proliferate when metals remain dissolved in highly acidic waters with sufficient light that increases their growth in nature (Hogsden and Harding 2012).

Compared to the other tested species, *P. subcapitata* was the least sensitive species to AMD beyond 96 hours of exposure. The result should be interpreted with caution because algae tests lasted for only 96 hours, but the data represented both short-term and long-term (Hagen and Douglas 2014). This result would affirm the possibility of algae to reproduce and survive in habitats that are rich in metals (Oberholster et al. 2010a). Enzymes produced by algae might also begin to work against the AMD mechanism of toxicity as a defence mechanism. Kefford et al. (2006) reported that enzymes induce the sensitivity of species to increasing salinity, resulting in different responses for different species. This would be particularly true for AMD rich in sulphate.

The moderate sensitivity of *A. auriculata* to AMD in this study suggests its feeding trait characteristic. Predatory insects such as *A. auriculata* dominate streams affected by AMD (Hogsden and Harding 2012). Generally, insects are better regulators of sodium and chloride ions than crustaceans in acidic waters due to haemoglobin buffer effect in their system (Gerhardt et al. 2004). Thus, *A. auriculata* might have been able to regulate ions, allowing it some degree of AMD tolerance during exposures. However, this result does not support that the insect taxon is a good indicator of water quality despite their dominance in freshwater ecosystems (Arimoro and Muller 2010; Kefford et al. 2016). Based on the results of this study, *P. subcapitata* and *B. stenochorias* would be good indicator species of AMD in the short-term and long-term respectively.

This study showed that *O. mossambicus* was the least sensitive species to AMD after 96 hours of exposure. In a related study, fish have demonstrated to avoid AMD within 96 hours of exposure. Moreira-Santos et al. (2008) studied the avoidance of small fish in a linear contamination gradient along test chambers and concluded that the species *Danio rerio* evaded AMD within 96 hours of exposure based on their uniform distribution in the test system, which was not limited by the design. The authors contended that contaminants could enter into aquatic ecosystems in non-linear or steep pathways that might create a chemical gradient, thereby making it difficult for organisms to escape the chemical. The effect of an AMD gradient on the reduction of diversity of organisms is also reported for bacterial communities at low pH (Hogsden and Harding 2012). Since exposure tests were conducted in a static system design in this study, the AMD gradient that could support avoidance mechanism was that observed during the interval of solution mixing in test beakers.

Test solutions were shaken three times a day to allow adequate mixing. However, an AMD layer always formed at the bottom of the test solutions overnight, which *O. mossambicus* could have avoided during the 96 hours of exposure.

The continued exposure of *O. mossambicus* to AMD beyond 96 hours resulted in more sensitivity effects. Generally, fish accumulate metals on gill surfaces that impairs ion regulation when exposed to acidic waters for longer durations (Hogsden and Harding 2012). The sensitivity of *O. mossambicus* could be attributed to the increased sulphates in AMD as discussed in Chapter Two of this thesis. Increased salinity has been reported to affect the osmotic balance in fish species such as *Poecilia reticulata* and *D. rerio* by negatively disrupting oxygen uptake (Holland et al. 2011). Further, *O. mossambicus* might have spent its energy by avoiding contact with the bottom layer of the AMD due to prolonged exposure in static systems. This result suggests that, apart from ecological or biological factors, appropriate mixing, time interval and compositions of the AMD are important factors that contributed to the sensitivity of *O. mossambicus.*

The species *C. nilotica* was more sensitive to AMD than *O. mossambicus* and *P. subcapitata* after 96 hours and 240 hours of exposure respectively. Although the *C. nilotica* could have also avoided the AMD gradient, studies have attributed pH changes in AMD as a factor that influences the sensitivity of organisms in acidic aquatic ecosystems. Temporary seasonal, physical and chemical changes in streams are usually key toxicity trigger variables (Gerhardt et al. 2004). In the natural environment, these changes exacerbate the breakdown of metals in AMD, which induce toxicity aquatic organisms. For example, Mohti et al. (2012) reported increased mortalities for the freshwater prawn *Macrobrachium lachesteri* (Palaemonidae) in the presence of metal hydroxides after 96 hours of exposure; similar to the algal biomass reduction as reported by Hogsden and Harding (2012). In a related study, the crustacean *Ceriodaphnia dubia* was sensitive to AMD at pH 3, with rapid mortalities at pH 2.5 (Lopes et al. 1999).

The sensitivity of crustaceans in AMD affected ecosystems can also vary for different species. Long-term exposure of the freshwater shrimp, *Atyaephyra desmaresti,* to AMD resulted in high mortalities with increasing pH from 4.0 to 5.2 (De Bisthoven et al. 2006). Thus, the species *A. desmaresti* could be acid-tolerant. The current results for *C. nilotica* do not confirm that crustaceans are acid-sensitive (Gerhardt et al. 2004) when compared with the other tested species. Although the sensitivity of different crustaceans varies, the results suggest the importance of enhancing water quality monitoring in regions where AMD continues to threaten freshwater ecosystems.

With the exception of *P. subcapitata* during short-term exposures, the species *B. stenochorias* was most sensitive to AMD. Its sensitivity greatly increased after 240 hours of exposure more than the other tested species. Higher calcium in water promotes shell building in molluscs (Fent and Gies 1996).

However, their calcified exoskeleton sheds off in acidic conditions and usually leads to mortality (Wurts and Masser 2013). This would explain the high sensitivity of the species *B. stenochorias* to AMD since the test solutions had very low concentrations of calcium (9.2 mg/L) and magnesium (30.8 mg/L) (Table 3.2). Magnesium can be toxic at concentrations approaching natural background levels, but its toxicity depends on the deficiency of calcium concentrations in water (van Dam et al. 2010). Thus, the ratio of magnesium and calcium might have influenced the responses of *B. stenochorias* to AMD despite their low concentrations. Low concentrations of major ions such as magnesium and calcium result in physicochemical conditions that can promote the uptake and toxicity of various metals and other ions (van Dam et al. 2010). In this study, the concentrations of both magnesium (Mg) and calcium (Ca), which determine the hardness of water were relatively in compliance with South African limits in freshwater ecosystems (DWA 2011).

3.4.1 The toxicity of metals and salts in AMD

The toxicity of metals in AMD to freshwater organisms is well documented. Aluminium (Al) and ferric iron cause toxicity in streams affected by AMD (Hill 1997; Last 2001). In particular, the availability of biotic ligands in a body of an organism increases the toxicity of Al (Hill 1997). It is, therefore, possible that the concentrations of Al and Fe in AMD used in this study could have influenced the toxicity of the tested species due to their attachments on the ligand. In addition, sulphates (SO_4^{2-}) and magnesium play important biological roles in protein synthesis, enzyme activation, energy transfer and cellular homeostasis, but greatly contribute to electrical conductivity (EC) (van Dam et al. 2010). High salinity concentrations are toxic to aquatic organisms and influence their abundance and diversity in freshwater ecosystems (Kefford et al. 2002; Cañedo-Argüelles et al. 2014).

3.4.2 Scenario-specific WQGs for AMD

AMD can vary spatially or at regionally close sites with similar geology although its constituents such as sulphates may remain relatively unchanged in chemical formation regardless of pH changes (Olías et al. 2004). This study has derived risk-based WQGs for AMD for the protection of the tested species at 95%, 90% and 80% dilution levels in riverine ecosystems. The application of these guidelines is dependent on the management goals.

For example, if the goal is to ensure that majority of the species should be protected from AMD exposure, the choice of the 95% protection level would be ideal. However, it might be difficult to protect over 95% of species in an ecosystem receiving AMD in a country such as South Africa that relies on mining as an economic driver. For this reason, the resources in question, lower protective level such as 90% or 80% were considered appropriate. These WQGs are scenario-specific for AMD generated at a coal mine in the Boesmanspruit catchment. They are important for management decisions aimed at protecting the Boesmanspruit, which receives the AMD directly, and other catchments that might be experiencing similar AMD conditions.

WQGs provide important information regarding the mixing and discharging of AMD. They can also be useful in the appropriate plan of treatment designs for AMD. For example, understanding the dilution levels would help engineers to design treatment plants that would accommodate operate at required scientific merits. In particular, instead of constructing holding dams to treat AMD by application of lime, it is possible to protect species from AMD if appropriate mixing or dilutions are followed if water is adequate. However, in water-scarce regions like South Africa, the mixing can be done at the source other than the resource itself. The treatment designs may include gravitational or pressure, movement of the discharge or outlets and their sizes that can provide the required mixing proportions to save time and costs. In addition, WQGs can be used to model projections of mixing rates of treatment liquid used and most impacted sites in locations that receive effluents to assess potential impacts both in the short-term and long-term (Falkenberg and Styan 2015). Therefore, the WQGs generated in this study, if not exceeded, would protect the tested species in the Boesmanspruit during short-term and long-term exposures respectively.

3.5 Conclusion

This study has demonstrated that AMD presents ecological risks to aquatic organisms both during short-term and long-term exposures. The degree of risk varies across different species. The study generally found *P. subcapitata* as the most sensitive species to AMD after 96 hours of exposure while the species *B. stenochorias* was the most sensitive after 240 hours of exposure. For this study, *P. subcapitata* and *B. stenochorias* are good indicators of AMD. While these findings are scenario-specific, they may be adopted and applied widely for management of similar AMD from coalmines with similar geologies.

Although AMD pollutes freshwater ecosystems, adequate dilution appears to be an effective approach to managing it from an ecotoxicological risk perspective but it is not the ultimate solution, as mortalities still existed due to potential accumulation of salts and other toxic metals which can increasing become toxic over time. In addition, the findings demonstrate that AMD is toxic to aquatic organisms.

The present study further revealed that sulphates are toxic to the tested species; the findings demonstrate added effects of AMD in freshwater ecosystems. However, it is still a problem to evaluate whether synergistic and antagonistic effects occur during AMD exposure. In natural systems where the diluent might contain various minerals that interfere with AMD constituents, it is appropriate to find management measures that would protect biodiversity. Thus, approaches that can help water users to make effective rapid management decisions to protect the species are useful. One approach would be to predict the vulnerability of organisms to pollutants based on their trait characteristics in a decision support system.

CHAPTER 4: A TRAIT-BASED APPROACH TO BIOMONITORING AND TOXICITY TESTING OF TREATED ACID MINE DRAINAGE

4.1 Introduction

In this Chapter, the study employed a trait-based approach (TBA) to predict the vulnerability of aquatic organisms to treated acid mine drainage (TAMD). The TBA is based on the link between traits and the environment, which offers a great opportunity to predict the potential sensitivity or vulnerability of organisms to the stressors of interest. To this far, the present study has shown that sulphates and acid mine drainage (AMD) adversely affect indigenous and common freshwater organisms in South Africa. Water quality guidelines (WQGs) were derived using a risk-based approach for the management of sulphates and AMD both in the short-term and long-term exposure durations. However, it is important to treat AMD before it reaches instream to minimise its ecological risks. AMD treatment approaches might include liming, designing of bioreactors, metallurgical slags and the use of microorganisms such as bacteria or algae (Madzivire et al. 2011; Rojas et al. 2014; Simate and Ndlovu 2014; Masindi et al. 2016; Tozsin 2016). These methods are aimed at neutralising acidic water by raising the water pH to alkaline condition while removing metals and sulphates, which are toxic to aquatic life (Xingyu et al. 2013; Kefeni et al. 2015). Among these methods, liming AMD is one of the commonest treatment approach in South Africa because it is simple and cost-effective (Madzivire et al. 2011).

The liming process involves the addition of alkaline salts to raise the pH of the AMD to circumneutral before reuse or discharge into a receiving resource (Tozsin 2016). Common salts used to liming AMD include calcium carbonate ($CaCO_3$), calcium oxide (CaO), coal fly ash or cryptocrystalline magnesite (Madzivire et al. 2011; Masindi et al. 2016). In particular, coal fly ash contains high concentrations of calcium sulphate due to inherent CaO, which fixes sulphur dioxide during the combustion process (Jia et al. 2016). The addition of lime increases the salinity content of the AMD after the treatment process, an additional stressor to aquatic organisms (Marshall and Bailey 2004). Therefore, it is important to evaluate the potential toxicity of treated acid mine drainage (TAMD) to ensure that relevant freshwater monitoring programs are designed. Further, the potential effects of TAMD in aquatic ecosystems located in arid and semi-arid regions can be greater compared to regions that experience sufficient rainfall due to dilution differences (Bagatin et al. 2014).

Therefore, although WQGs were derived in Chapter Two and Three of this thesis for the protection of species from freshwater pollution induced by sulphates, AMD or their combination, alternative approaches that can predict the potential vulnerability of organisms or species to pollutants prior to pollution occurrence are essential for appropriate management and protection of aquatic resources. Vulnerability has been defined as a function of susceptibility to exposure, direct sensitivity and recovery capability (Ippolito et al. 2012).

The risk-based approach as undertaken in Chapters Two and Three of this thesis, although useful for the derivation of WQGs, only considered the trophic position in selecting the organisms for toxicity testing. This is the traditional and common approach in the field of ecotoxicology. However, in this study, a novel trait-based approach (TBA) was used to select organisms for their vulnerability assessment in relation to TAMD. The TBA can be used in ecotoxicological studies to predict the potential vulnerability of organisms to pollutants. In the TBA approach, the selection of organisms is considered in light of many traits that are deemed mechanistically linked to the stressor of interest such as sulphates, AMD or TAMD. Traits are described as biological and behavioural characteristics of an organism, generally measured at the individual level (Violle et al. 2007). The TBA approach offers a great opportunity to predict the potential vulnerability of organisms to the stressors of interest. The TBA approach might thus enable the concomitant prediction of the potential vulnerability of organisms to toxicants in ecotoxicological studies (Liess and Beketov 2011).

4.1.1 The trait-based approach and its potential application in ecotoxicology

In the habitat templet theory (HTT) described by Townsend and Hildrew (1994), a river system was used as a templet to hypothesise that there are historic and phylogenetic constraints on the match between organisms and environment. Their study derived predictions about traits of species, including size, body form, reproduction tactics, mobility and potential for regeneration that were likely to occur in particular regions under specific environmental context. Since aquatic species can respond differently to pollution (Hamid and Rawi 2014), the HTT argues that hypothesis can be tested by comparing traits predicted for particular habitats whose spatial and temporal heterogeneity have been quantified with those actually observed. Successful predictions would entail, for example, the development of appropriate predictive freshwater monitoring programs.

It is also possible to apply the HTT concept in ecotoxicology if traits of species can be linked to exposure mechanisms to predict species vulnerability to TAMD. The sensitivity of organisms to stressors can be attributed to their biological traits such as morphology, life history, physiology or feeding ecology, which can assist in predicting the effects of the particular stressor (Baird et al. 2008). If successful predictions can be made, the TBA would contribute important and critical scientific information about species responses to stressors beyond the risk-based approach using the species sensitivity distributions (SSDs). Other possible contributions of the TBA include time saving and low costs since no laboratory experiments would be required, provided prediction has been made and sufficiently validated and all possible uncertainty accounted for. Generally, the SSD approach requires large numbers of organisms/species, which is often tedious. It also emphasises on one trait, food and trophic position, over other potentially very important traits that may be mechanistically linked to a stressor. However, the TBA can form the basis of predicting the potential effect of stressors on species by carefully selecting several traits linked to a stressor. The TBA also expands on the notion of trophic position to include and consider other kinds of traits. Therefore, in this chapter, a novel TBA for predicting the vulnerability of selected macroinvertebrate taxa to TAMD was developed and tested both in the laboratory and in the field.

4.2 Materials and methods

4.2.1 Selection of traits for predicting vulnerability of organisms to TAMD

It is important to identify appropriate traits that are mechanistically linked to the stressor of interest for different organisms prior to toxicity predictions. For treated acid mine drainage (TAMD), inherent toxic metals and sulphates can still cause problems to organisms through different modes. For example, results in Chapter Three indicated that *Pseudokirchneriella subcapitata* and *Burnupia stenochorias* could be better regulators of ions such as sodium and chloride compared to *Caridina nilotica*. Similarly, the calcified exoskeleton in *B. stenochorias* sheds off in low acidic conditions and resulted in mortality (Wurts and Masser 2013). Moreover, magnesium and calcium deficiencies can result in physiological conditions that promote the uptake and toxicity of various metals and other ions in aquatic organisms (van Dam et al. 2010). Through osmosis, metals and sulphate in TAMD can enter the bodies of organisms via permeable membranes. These examples demonstrate that the mechanisms of stressor toxicity in organisms vary, and in many cases, not clear (Hogsden and Harding 2012). However, the possession of certain traits provides protection for organisms possessing them. Trait selection, therefore, must be guided by a mechanistic understanding of the link between the mode of action of TAMD and the biological entity. For this study, body size, respiration, feeding behaviour and locomotion were selected as traits that are mechanistically linked to the vulnerability of organisms to TAMD. It is important to note that these traits are by no means exhaustive, but were selected to test the potential application of the TBA in ecotoxicology. In addition, these selected traits are those that ecotoxicological studies have wittingly engaged in terms of exposure to chemical substances over the years.

4.2.1.1 Selected traits

Body size

Sulphates and toxic metals in TAMD can be absorbed by organisms in aquatic ecosystems. The internal distribution of toxicants in organisms is a function of the partitioning either through passive diffusion or active biological transport (Rubach et al. 2011). Some organisms lack barriers that separate the site of action of a toxicant from the rest of the body. This implies that such organisms might be unable to metabolise the toxicants, potentially becoming vulnerable. However, absorbed toxicants are potentially mediated by the degree and extent of exposure.

This is directly related to body size, which defines the capacity of toxicant concentration the organism can hold. For example, organisms with large bodies do not easily lose heat because they can retain or decrease metabolic rates per unit mass compared to organisms with smaller bodies (Sharma and McNeill 2009). In addition to the heat loss concept, the smaller the organism's body size, the greater the surface area to volume ratio. This implies that when smaller organisms are exposed to TAMD, the organisms might absorb more toxicants and become more vulnerable than larger organisms. Thus, body size through surface area to volume ratio is an important trait that has an influence on the degree and extent of vulnerability of an organism to toxicants; hence, it was selected as part of suits of traits for this study. Selection of body sizes as a trait also considered the flexibility of organism exposures in test media in the laboratory. Based on literature and visual observation, body sizes of organisms less than 10 mm and more than 20 mm were considered small and large, respectively.

Gill

The mechanism through which TAMD can be transported from the external environment to a site where it elicits toxic effects in an organism is also important. For example, presence and absence of gills for respiration and ion exchange can determine the vulnerability of an organism to TAMD during exposures. Since gills act as a site of action (biotic ligand) for toxic metals (Di Toro et al. 2001; Smith et al. 2015), gill-bearing taxa would be more vulnerable than those without gills. However, the degree of vulnerability would be higher in small body-sized organisms with gills, which have large surface area to volume ratio than large body-sized organisms because their metal absorption would increase beyond the threshold of the rate of action of their gills. Thus, non-gill bearing taxa are hypothesised to be less vulnerable to TAMD exposure than gill-bearing taxa.

The number of gill pairs can also have an influence on the absorption and metals exchange rate in organisms. When metals bind on the gills for regular ion exchange process, free available ions such as calcium and hydrogen compete for binding on gills and mitigate toxicity within the organism. The toxicity effect depends on the strength and concentration of binding on the gills (Santore et al. 2001). For example, the presence of more calcium lessens sodium toxicity (Cañedo-Argüelles et al. 2013), perhaps through cation competition on the gills. However, the toxicity of magnesium depends on the deficiency of calcium concentrations in a solution (van Dam et al. 2010).

Since TAMD is inherently rich with toxic metals, gill-bearing taxa with many gill pairs (for example, > 3) are predicted to be potentially more vulnerable to TAMD than non-gill bearing taxa or those that have fewer gill pairs (< 3 or none) for two reasons. First, more gill pairs imply that there are increased sites of action and binding effect for metals to induce or increase toxicity. Second, small body size taxa with many gill pairs would be potentially vulnerable to TAMD due to the surface area to volume ratio. The categorisation of gill pairs was derived based on the concept of the biotic ligand.

Further, gill-bearing taxa are potentially more vulnerable to TAMD than non-gill bearing taxa because gills are naturally exposed. The lack of opercula, which conceal gills in some taxa, might render the organisms vulnerable to toxic metals and sulphate in TAMD. However, taxa with opercula can easily evade toxicant exposure in short durations (Rubach et al. 2011). During laboratory exposures, organisms with opercula might be more affected by TAMD during long-term exposures when their energy is spent. Thus, presence and absence of gills in taxa become an important aspect of consideration with respect to TAMD exposure.

Predation

Food choice plays an important mediating role between exposure of a toxicant and the organism. Predator-prey interactions are potential characteristics to predict vulnerability of taxa to TAMD. Small-bodied organisms which are top predators usually select their type of prey carefully to maximise their net rate of energy gain in relation to the energetic costs (Rodríguez-Lozano et al. 2016). In this trade-off, prey size is the primary determinant of predator choice, as it reflects well the costs of handling time and benefits of foraging such as prey energy content. The predator may prefer a particular size range because prey size is a trade-off between costs and benefits of foraging. This implies that the predator spends more energy in search for its prey, and is thus more vulnerable to ingesting toxic metals from TAMD. Prey drift tendency in the water column may also increase vulnerability to predation, and subsequent TAMD exposure. High tolerant and predaceous macroinvertebrate orders such as Coleoptera and Odonata usually dominate streams receiving AMD effluent (Gerhardt et al. 2004). As such, toxic metals can bio-accumulate in the food chain. Predators can be exposed to higher concentrations of the metals, even with limited exposure as they search and select their prey. Thus, food type presents an important route of contamination in an organism when ingested. If food is the main exposure route, the daily food intake determines the extent of exposure to a toxicant (Rubach et al. 2011). Predators are therefore vulnerable to TAMD compared to their prey because many traits may be correlated. Predation may also increase at low prey densities in ecosystems and increase the vulnerability of predatory taxa to TAMD.

Predatory taxa may spend considerable time and energy if prey density is low and scarce. However, in the laboratory exposures predation is not an issue because predators are not mixed with their prey. In the field, predation becomes an important trait.

Locomotion

The degree of mobility of organisms may be a determining factor for vulnerability to TAMD in receiving ecosystems. Mobility is linked to food choice trait since organisms have to move to locate food. Crawlers, burrowers and sessile taxa may be vulnerable to predation, as well as TAMD as they are unable to escape or migrate to less polluted sites/patches. Similarly, fast swimming (active) taxa may easily avoid pollution by migrating to less polluted river patches compared to sessile taxa (Moreira-Santos et al. 2008). It is important to note that locomotion only becomes an important trait in the field but not in the laboratory since organisms are relatively confined and can hardly escape from toxicants. The selected traits were combined to define *a priori* vulnerability classes, which then informed taxa selection for toxicity testing and formulation of hypothesis.

4.2.2 Taxa selection based on vulnerability classes

Body size, respiration, predation and locomotion were selected traits for this study. Other trait attributes within these traits such as water or air-breathing that would make taxa vulnerable to TAMD were also considered. Thereafter, three classes of vulnerability were established as follows: i) less vulnerable, ii) moderately vulnerable, and iii) more vulnerable. Each of these classes was defined based on the vulnerable trait attributes possessed by the organism by means of a scoring process (Table 4.1). First, taxon belonging to less vulnerable class was considered to have large body sizes, less (< 3) or no gill pairs, lung breathers and non-predatory. Second, a taxon belonging to vulnerable class was considered to have small body sizes, (> 3) gill pairs, water breathers and predatory lifestyle. Lastly, taxon belonging to moderately vulnerable class was considered to have overlapping trait attributes for less and vulnerable classes such as less than three-gill pairs, small body size, vice-versa or their combination. Taxa were identified and allocated to each of the described vulnerability classes (Table 4.1). One taxon was identified from each vulnerability class if it possesses more than one vulnerable trait attribute within each trait for TAMD toxicity testing based on the scoring. The selection of taxa for toxicity testing also considered the availability of organisms at the time of this study.

Based on these descriptions, Leptoceridae and Leptophlebiidae were predicted to be less and more vulnerable respectively, and their toxicity testing was undertaken to test this prediction (Table 4.2). The vulnerability of these families to TAMD was also compared between short-term and long-term exposures during toxicity testing. It is important to note that in classifying a taxon into a vulnerability class, a potential source of uncertainty and error is that the taxon has been classified at the family level, without considering potential functional trait diversity and plasticity within such a family. Instead, once a family was indicated as possessing a trait attribute based on the literature and visual observation, the taxon was assumed to possess the trait attributes across its entire component species.

Table 4.1: Scoring sheet that guided preliminary trait selection before toxicity testing

Name	**Trait**	**Body size (mm)**			**Gills**				**Predation**				**Locomotion**			
	Trait attributes	**Small (≤10)**	**Medium (>10<20)**	**Large (>20)**	**Gills (≤3)**	**Gills (>3)**	**Water breather**	**Air breather**	**Filter**	**shredder**	**Grazer**	**Predator**	**Active**	**Passive**	**Sluggish**	**Sessile**
Beetle (Coleoptera)	Dytiscidae	1	0	0		1		1				1	1	0	1	0
Beetle (Coleoptera)	Gyrinidae	1	0	0		1		1				1	1	0	1	0
Beetle (Coleoptera)	Elmidae	1	0	0		1		1	0	1	1	0	0	1	0	1
Cased Caddisfly	Leptoceridae			1				1		1	1		1	0	1	0
Cased Caddisfly	Petrothrincidae		1			1				1	0		0	1	0	1
Caseless Caddisfly	Hydropsyche sp		0	1		1			1	1			1		1	
Caseless Caddisfly	Amphisyche sp					1										
Caseless Caddisfly	Philopotamidae				0	0										
Crustacean	Potamonautidae (crabs)			1								1				
Crustacean	Atyidae			1		1			0	0	1	0				
Diptera	Chironomidae			1		1										
Hemiptera (bugs)	Naucoridae											1				
Lepidoptera	Pyralidae		1			1					1					
Mayfly	Leptophlebiidae		1		0	1	1		1	0	0	1				
Mayfly	Baetidae	1				1	1			1			1		1	
Mayfly	Caenidae						1						1		1	
Mayfly	Prosopistomatida e	1					1						1		1	
Mayfly	Teloganodidae		1		1		1						1		1	
Mayfly	Heptageniidae		1			1	1			1			1		1	
Stonefly	Stoneflies					1	1		0	0	0	1	1		1	
Odonata (dragonfly)	Aeshnidae		1											1		1
Odonata (dragonfly)	Gomphidae			1	1	0						1		1		1
Odonata (dragonfly)	Dragonflies			1		1			0	0	1	1		1		1
Plecoptera	Perlidae		1									1				
Turbellaria	Planaria	1								0	1	1		1		1

Note: A score of "1" indicates that a particular organism or taxon possess the corresponding trait in that category. A score of "0" indicates that the concerned organism does not meet the assigned category or does not possess that particular trait. A blank cell means data paucity during literature search.

Table 4.2: Taxa selection based on trait combinations

Macroinvertebrates		**Predicted vulnerability**		
Order	**Family/species**	**Less vulnerable taxa**	**Moderately vulnerable taxa**	**More vulnerable taxa**
Coleoptera	Dytiscidae	Leptoceridae (Hydropsyche)	Leptophlebiidae	Gyrinidae
Coleoptera	Gyrinidae	Hydropsyche sp1	Baetidae	Elmidae
Coleoptera	Elmidae	Potamonautidae (crabs)	Teloganodidae	Petrothrincidae
Trichoptera	Leptoceridae	Chironomidae	Stoneflies	Pyralidae
Trichoptera	Petrothrincidae	Atyidae	Dytiscidae	Baetidae
Trichoptera	Hydropsyche sp	Gomphidae	Gyrinidae	Prosopistomatidae
Trichoptera	Amphisyche scottae	Dragonflies (Odonata)	Elmidae	Leptophlebiidae
Trichoptera	Philopotamidae	Pleidae	Naucoridae	Teloganodidae
Decapoda	Potamonautidae	Planariidae	Nepidae	Heptageniidae
Atyidae	Caridina	Dytiscidae	Corixidae	Aeshnidae
Diptera	Chironomidae	Naucoridae	Dragonflies	Gyrinidae
Hemiptera	Naucoridae		Planaria	Teloganodidae
Lepidoptera	Pyralidae		Elmidae	Gomphidae
Ephemeroptera	Leptophlebiidae		Pyralidae	Leptophlebiidae
Ephemeroptera	Baetidae		Leptoceridae	Baetidae
Ephemeroptera	Caenidae		Hydropsyche sp	Heptageniidae
Ephemeroptera	Prosopistomatidae			Stoneflies
Ephemeroptera	Teloganodidae			Dragonflies
Ephemeroptera	Heptageniidae			Pyralidae
Ephemeroptera	Baetidae			Chironomidae
Odonata	Aeshnidae			Atyidae
Odonata	Gomphidae			*Amphisyche*

Macroinvertebrates		Predicted vulnerability		
Order	**Family/species**	**Less vulnerable taxa**	**Moderately vulnerable taxa**	**More vulnerable taxa**
				Dytiscidae
Odonata	Libellulidae			
Plecoptera	Perlidae			
Turbellaria	Planariidae			

4.3 Laboratory evaluation of the trait-based approach to ecotoxicological study

This section focusses on the toxicity of treated acid mine drainage (TAMD) to selected macroinvertebrates based on the developed trait-based approach discussed in previous sections. Leptoceridae (Trichoptera) and Leptophlebiidae (Ephemeroptera) were sampled from Palmiet River in Grahamstown using South African Scoring System (SASS) version 5. Leptoceridae was selected as less vulnerable taxon while Leptophlebiidae was chosen as a taxon that was more vulnerable to TAMD based on the trait-combinations described. No sampling for predicted potentially moderately vulnerable taxon was conducted. The body sizes of sampled macroinvertebrates used for toxicity testing ranged between 5 mm and 30 mm. AMD samples were collected from a coal mine Boesmanspruit catchment in Mpumalanga according to the methods described in Section 3.2.1 of Chapter Three.

4.3.1 Liming of acid mine drainage in preparation for test solutions

Reference anhydrous calcium oxide (CaO) for liming AMD was purchased from Spellbound Laboratory in Port Elizabeth, South Africa. About 1000 mL of the AMD was poured into a mixing beaker in a laboratory controlled environment. The pH of the AMD before liming/treatment was 3.47, which had increased by 0.46 fold in comparison to the sampling period after transportation and storage. The gradual addition of CaO into the mixing beaker raised the pH to an alkaline level. Approximately 2 g of CaO was used to raise the pH to 7.25. During the liming process, contents were shaken frequently for thorough mixing but settled and formed a layer after a few minutes.

Dechlorinated tap water, which was analysed for full metal elements as described in Chapter Three, was used both as a diluent and a medium for the control group. Seven dilutions (treatments) including the control (0% AMD) were prepared for the exposure tests. The dilutions were 0%, 3.125%, 6.25%, 12.5%, 25%, 50%. The 100% solution was TAMD before serial dilutions were prepared. Its pH was 7.25 to ensure it was alkaline. The values of dissolved oxygen (DO), pH and electrical conductivity (EC) were monitored in test solutions throughout exposure tests using Cyberscan® 1500, Cyberscan® 5000 and Cyberscan® 200 metres respectively manufactured by Eutech Instruments, Singapore. Test solution temperatures (^{O}C) were also monitored.

4.3.2 Toxicity testing of taxa to treated acid mine drainage

Two separate experiments, one for Leptophlebidae and another for Leptoceridae were performed under similar test conditions in a temperature-controlled laboratory (20°C ± 1) in static design systems. Ten individual Leptophlebiids and Leptocerids were randomly allocated into 600 mL glass beakers (treatment) containing 300 mL of the TAMD solution including the control. Each treatment was replicated three times. The organisms were not fed during the first 96 hours of exposure, after which about 0.20 grams of TetraMin flakes (Tetra Company, Germany) were added daily. Small stones were provided in test beakers for the animals to hold and limit their energy expenditure, particularly for the mayflies which were mostly sampled from stone biotope. The toxicity test endpoint was mortality, which was assessed by prodding the organism using a glass stirrer. Organisms were recorded as dead if they did not respond to the stimulus. Acceptable control mortality was set at 10% (DWAF 2000). Organisms were monitored each day for routine quality assessment that included count and removal of those that had died. Test solutions were changed after every 96 hours for a test duration of 240 hours.

4.3.2.1 Data analyses based on toxicity exposures for selected taxa

Water quality variables of test solutions were analysed using a general omnibus test to assess main effects and their interactions. Mortality data were analysed using a two-parameter log-logistic regression in R software package "DRC" (Ritz et al. 2015). In this method, dilutions at which both 10% and 50% of the test organisms died (LD10 and LD50) were estimated using the model function,

Y_{ijk} <- drm (μ/w ~ $\alpha_{i,}$ β_j, Z_n

Where,

Y_{ijzk} is the model's observation /unit given the i^{th} level of mortality, the j^{th} level of total mortality and the z^{th} level of dilution or concentration

μ/w is the mortality fraction of organisms

α_i is the dilution levels (dilutions; i=0%, 3.125, 6.25%, 12.5%, 25%, 50% and 100%)

β_j is the organisms being tested (macroinvertebrate families; j=Leptophlebiidae, Leptoceridae)

Z_k is the time or duration of exposure for the experiment (time; 96 hours or 240 hours)

The LD values determined after 96 hours were considered short-term lethal endpoints while those determined after 240 hours were considered long-term lethal endpoints. To determine the mortality differences between the two tests, curves for each exposure duration were compared (comparison test for curves) using the "comped" function in R software package "DRC" (Ritz et al. 2015).

4.3.3 Results

4.3.3.1 Water quality results for toxicity tests

Treated acid mine drainage (TAMD) had an adverse effect on water quality in test solutions. The results for EC, pH and DO are presented in Tables 4.3 and 4.4. Test solution pH became acidic with increasing dilutions. DO and EC levels improved with increased dilutions of the AMD (i.e. in lower treatments). Statistical tests only revealed significant DO concentrations in test solutions. Liming AMD had a significant impact on the tested organisms. In addition, there were significant interaction effects ($p < 0.05$) between DO and EC values across the different treatments.

Table 4.3: Summary of measured water quality parameters for Leptoceridae experiment

	Treatments (TAMD dilution) in %							
Variables	0	3.125	6.25	12.5	25	50	100	p-value
EC (mS/m)	0.67	0.78	0.98	2.78	3.41	3.98	5.59	0.887932
pH	7.73	3.81	3.95	4.12	4.37	5.37	7.25	0.347862
Mean DO (mg/L)	7.54	6.83	6.69	6.33	5.38	5.23	5.17	0.000185

Table 4.4: Summary of measured average water quality parameters for Leptophlebiidae experiment

	Treatments (TAMD dilution) in %							
Variables	0	3.125	6.25	12.5	25	50	100	p-value
EC (mS/m)	0.66	0.98	1.86	2.7	3.98	5.43	6.03	0.887932
pH	8.11	3.95	4.2	4.29	5.37	6.85	7.25	0.347862
Mean DO (mg/L)	9.52	8.96	8.42	7.58	6.37	5.66	5.26	0.000185

4.3.3.2 Toxicity results for 96 hours and 240 hours exposure tests

Toxicity test results after 96 hours and 240 hours of exposure are presented in Table 4.5. The log-logistic regression model revealed significant macroinvertebrate mortalities ($p < 0.05$) in both experiments between 96 hours and 240 hours of exposure. There was no consistent mortality trend in the different treatments. Many of the mortalities for Leptoceridae occurred within 96 hours of exposure.

The dilution of TAMD that killed 50% of the organisms after 96 hours of exposure was about twice higher for Leptophlebiidae compared to Leptoceridae. This implies that TAMD was relatively less toxic to Leptophlebiidae in the short-term. However, TAMD was particularly more toxic to Leptophlebiidae after 240 hours of exposure. This pattern was similar for Leptoceridae. These results indicate that both families were sensitive to TAMD after long-term exposures, suggesting that exposure duration had an influence on the toxicity of each taxon.

The maximum TAMD treatments that had a mortality effect on 50% of the organisms in the short-term were 11.195% and 5.98% for Leptophlebiidae and Leptoceridae respectively. The long-term maximum treatments that killed 10% of the organisms were 2.146% and 0.54% for Leptophlebiidae and Leptoceridae respectively.

Table 4.5: Estimated lethal percent dilutions (LDx) for Leptophlebiidae and Leptoceridae

Family	Exposure time (hours)	LD10 (%)	95% confidence limit	LD50 (%)	95% confidence limit (%)
Leptophlebiidae	96	-	-	7.919	4.643-11.195
Leptoceridae	96	-	-	3.989	2.00-5.980
Leptophlebiidae	240	1.80	1.014-2.146	-	-
Leptoceridae	240	0.109	0.540	-	-

4.3.3.3 Treatment-response relationships between Leptophlebiidae and Leptoceridae

The sensitivity responses of Leptophlebiidae and Leptoceridae to TAMD after 96 hours are shown in Figure 4.1. Leptoceridae was more affected by TAMD compared to Leptophlebiidae. A high number of mortalities occurred in the 100% TAMD treatments after 96 hours of exposure for both taxa. The treatments that had 50% TAMD affected over 80% of the two tested macroinvertebrate families. Only less than 10% of each family was affected by TAMD after 96 hours of exposure, indicating that short-term exposure to TAMD had minimal effect on mortalities of both taxa.

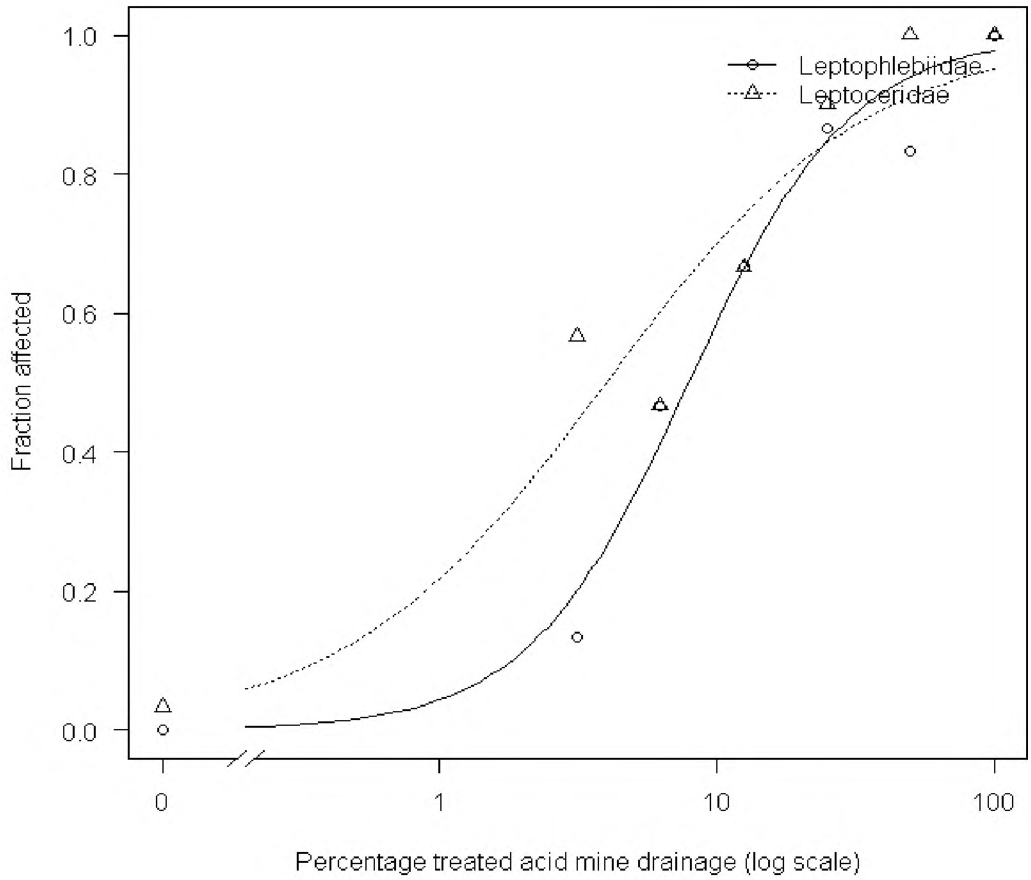

Figure 4.1: Short-term (96 hours) responses of Leptophlebiidae and Leptoceridae to the toxic effect of treated acid mine drainage.

Similar to the short-term trends, Leptoceridae was more sensitive to TAMD compared to Leptophlebiidae after 240 hours of exposure (Figure 4.2). Many mortalities occurred at pH range between 7.25 – 7.46. Over 60% of the tested organisms for both families were affected by TAMD treatments exceeding 12.5% dilution. Mortalities in the control beakers were within the acceptable range of not more than 10%.

A comparison of the curves after long-term exposures revealed that TAMD was 14.5 fold more toxic to Leptoceridae than Leptophlebiidae.

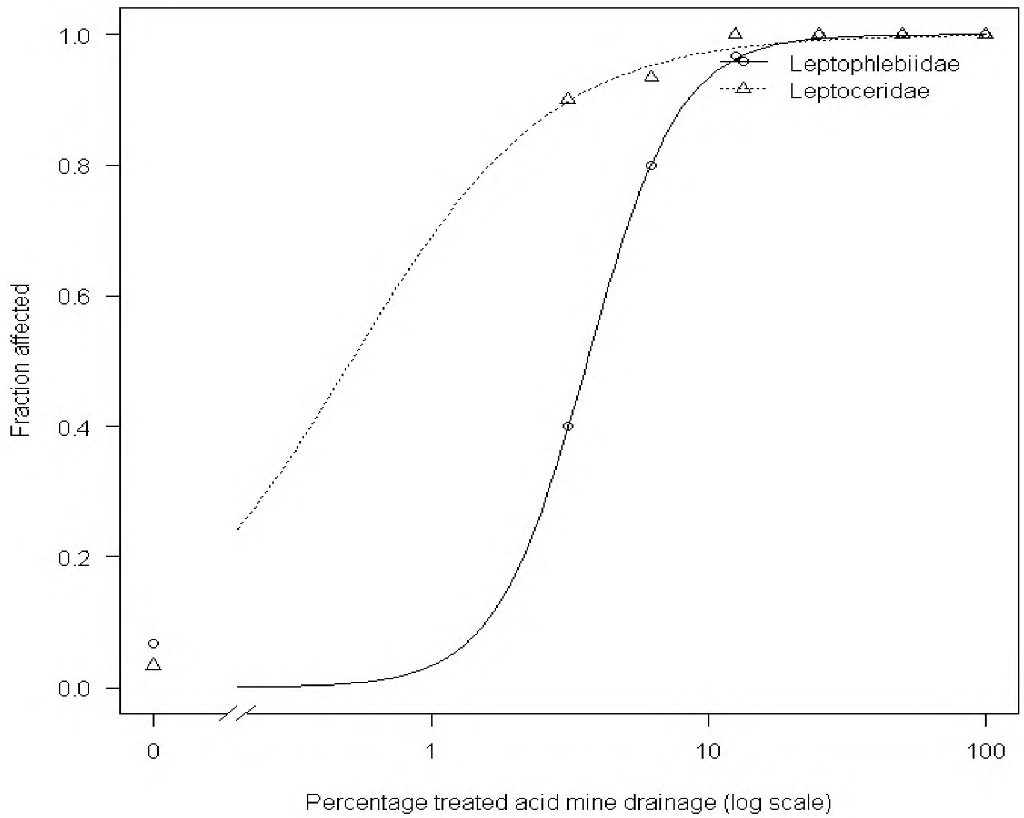

Figure 4.2: Long-term (240 hours) responses of Leptophlebiidae and Leptoceridae to the toxic effect of treated acid mine drainage.

4.3.4 Discussion

4.3.4.1 Laboratory toxicity findings

This study predicted Leptoceridae and Leptophlebiidae as less and vulnerable to TAMD after 96 hours and 240 hours of exposure, respectively. However, findings have demonstrated that Leptoceridae was more vulnerable to TAMD than Leptophlebiidae after 96 hours of exposure. Similarly, Leptoceridae was also more vulnerable to TAMD than Leptophlebiidae after 240 hours of exposure. However, Leptophlebiidae was more vulnerable to TAMD in the long-term compared to short-term exposures. Leptoceridae was also more vulnerable to TAMD after long-term exposure than short-term exposures. These results suggest the need to compare vulnerability of taxa to toxicants between exposure durations as time is an important factor for the development of WQGs. Since TAMD was 1.99 fold more toxic to Leptoceridae than Leptophlebiidae after short-term exposures, it implies that taxa, which were predicted as more vulnerable, did not support the hypothesis. In the long-term, TAMD was 14.5 fold more toxic to Leptoceridae than Leptophlebiidae. The majority of the traits used for the predictions in this study are field relevant and not experimentally confirmed. For example, locomotion becomes a relevant trait in the natural environment where organisms can easily avoid toxicants as opposed to laboratory experiments. Food choice or predatory lifestyle of organisms might not be easily accommodated by the organism's body parts in test beakers, which are confined. As such, trait-based prediction results might not have confirmed toxicity findings. However, it is essential to conduct field observations to relate the trait-based predictions for conformity.

Nonetheless, current laboratory findings are important with respect to exposure duration, which is key to deriving relevant WQGs (Warne 1998), as results supported the vulnerability prediction for Leptophlebiidae. Overall, TAMD was toxic to both Leptoceridae and Leptophlebiidae after 96 hours and 240 hours of exposure. Their sensitivity pattern to TAMD did not change much regardless of exposure duration. Over 50% of mortalities for Leptoceridae occurred within 12 hours of exposure. The relative inability of Leptoceridae to escape from exposure due to the static design of the experiments could have contributed to its vulnerability to TAMD. This suggests that locomotion as a trait would be better tested in the field than in the laboratory when evaluating the predictions. Other traits, including the presence and type of integumentary structure that were not considered in this study could also influence responses of organisms to TAMD.

4.3.4.2 Effects of water chemistry variables on mortalities of organisms

Although mortalities could be attributed to the liming effect, interaction of water quality variables such as dissolved oxygen (DO) and electrical conductivity (EC) might have influenced the sensitivity of the tested families. DO levels in both experiments exceeded the lower limit (4 mg/L) threshold for toxicity experiments (USEPA 2002). However, changes in DO could have been below the tolerance threshold of the organisms as reflected in mortality changes in higher treatments compared to the control. The formation of a layer in test beakers due to the static design nature of the experiment or density differences of the AMD and diluent might have also depleted oxygen levels. DO has been linked to increased gill movements in the mayfly Heptageniidae (Campbell 1990). Since Leptophlebiidae contains more than three-gill pairs, there might have been increased binging effect on the gills by metals from TAMD to influence appropriate regulation. Other mayflies have demonstrated the ability to change their locations in water due to the regulation of oxygen in the gills. For example, Wiley & Kohler (1980) reported that Heptageniidae occupied the bottom of stones except at very low DO levels (4 mg/L) but moved to the surface of the water at night in the absence of light. The authors concluded that most Ephemeropterans change positions when physiological means of regulating oxygen consumption (gill ventilation) are insufficient to meet respiratory needs. This might suggest that many gill pairs on Leptophlebiidae might have greatly affected its respiratory rates as the oxygen levels decreased in test beakers. The oxygen levels at the bottom of the test beakers might have been compromised as the lime contents after shaking or mixing settled immediately. This, in turn, might have affected proper ion exchange in the gills by blockage during exposure after 96 hours of exposure.

Generally, pH is an important variable that influences the sensitivity of organisms to toxicants. In this study, test solutions became more acidic in lower treatments such as the 3.13% and 6.25% compared to higher treatments, although more mortalities occurred in higher than in lower treatments. The current prediction findings based on toxicity results are thus scenario-specific pertaining to the AMD contents similar to that which occurs in the study area. Calcium ions binding effect on the gills possibly rendered the ambient solution acidic because they ameliorate the toxicity of other ions including magnesium and sodium by acting as a buffer (van Dam et al. 2010). Increased EC also influenced the sensitivity of the tested families to TAMD because high salinity concentrations are toxic to organisms (Kefford et al. 2002). Thus, water chemistry can greatly influence the toxicity of TAMD. The current trait-based findings, therefore, suggest the need for potential linkage between predictions and water quality variables.

4.3.4.3 Comparing the vulnerability of the tested taxa to TAMD with respect to predicted vulnerabilities

The 96 hour LD50 value for Leptoceridae was lower than the LD50 for Leptophlebiidae. Liming AMD by 12.5% was lethal to approximately 80% of the tested families after 96 hours of exposure. It is clear that both Leptoceridae and Leptophlebiidae were vulnerable to TAMD although Leptophlebiidae was predicted to be more vulnerable compared with Leptoceridae.

The ionic composition of TAMD might have induced toxicity. It remains to be investigated whether such an influence occurs antagonistically or as a combined effect of the ions after 96 hours and 240 hours of exposure. A complex and synergistic relationship that exists between water quality variables and the presence of macroinvertebrate communities in AMD impacted waters has been suggested (Last 2001). Both families were more vulnerable to TAMD within short-term and long-term exposures despite earlier predictions that Leptoceridae would be less vulnerable. These findings suggest a 50% success rate of the vulnerability predictions and provide a basis for further refinement with other traits or their combinations.

The laboratory results suggest that the application of lime to treat AMD may not be the best management approach because inherent sulphates in AMD do not easily break down for mitigating the toxicity of TAMD. Other management options need to be explored including the use of microorganisms such as bacteria and algae to minimise ecological effects. Further refinement of the current findings using more traits and their combinations are necessary to improve the approach. The toxicity data generated herewith are useful for the derivation of scenario-specific WQGs for TAMD for the protection of freshwater resources in South Africa.

4.3.5 Conclusion

The laboratory-based results revealed that TAMD was lethal to both Leptophlebiidae and Leptoceridae, although the latter was more vulnerable to TAMD than the former, and did not support the predictions. It is important to consider the duration of exposure in trait-based predictions as vulnerability variations might occur. These findings show that trait-based studies have the potential to increase prediction of taxa vulnerability to aquatic stressors. Further combinations of traits are needed to enhance the predictions.

Since the toxicity test results did not fully support the trait-based predictions because Leptoceridae was more vulnerable to TAMD than Leptophlebidae, a biomonitoring field-based study was undertaken to evaluate the potential vulnerabilities of taxa to their pattern of distribution. The biomonitoring exercise also assisted to validate laboratory findings.

4.4 Field evaluation of the trait-based approach to ecotoxicological study

The purpose of this subsection was to collect macroinvertebrates from a TAMD impacted river to elucidate the pattern of macroinvertebrate distribution in relation to trait-based predicted vulnerability of taxa (Table 4.2). Macroinvertebrate samples were collected from three sites in the Boesmanspruit River following the South African Scoring System version 5 protocol (SASS5) (Dickens and Graham 2002). Macroinvertebrate samples were collected once in September 2016 from stones, vegetation, gravel, sand and mud. SASS5 is a well-established protocol for the sampling of macroinvertebrates in South Africa (Dickens and Graham 2002; Ollis et al. 2006). SASS requires that macroinvertebrates are collected from three distinct biotope groups: vegetation (marginal and aquatic), sediments (gravel, sand and mud (GSM)) and stones (stone-in-and-out-of-current). Macroinvertebrates were sorted and identified to family level in the laboratory at Rhodes University, Grahamstown.

4.4.1 Description of macroinvertebrates sampling sites

Generally, the sites were selected to form a gradient of TAMD impact. Site A received no observable TAMD impact, Site B with high and Site C being minimally impacted by TAMD. Macroinvertebrates were collected from three sites: Site A, B and C (Figure 4.3). Site A is situated in the upper reaches of the Boesmanspruit River in Chrissiesmeer, above the point where the TAMD is being released into the river. This site has no TAMD influence and appeared to have poor habitat diversity due to trampling by livestock in the area. Site B is situated in a small tributary to the Boesmanspruit River, which receives TAMD from the Septhethe AMD holding dam. Macroinvertebrates at this site were collected about 500 m downstream of the TAMD discharge point. Site B is therefore considered highly impacted by TAMD. Site B also experienced significant agricultural impacts from crop and animal farming with little or no flow of water in some sections of the river. Site C is situated about 15 km further downstream of the TAMD discharge point. The site is situated close to the Nooitgedacht Dam in Carolina Town. Site C was mainly characterised by stone biotope with relatively clear fast flowing water. All sites had a diverse range of macroinvertebrate biotopes except Site B, which had limited macroinvertebrate taxa in vegetation.

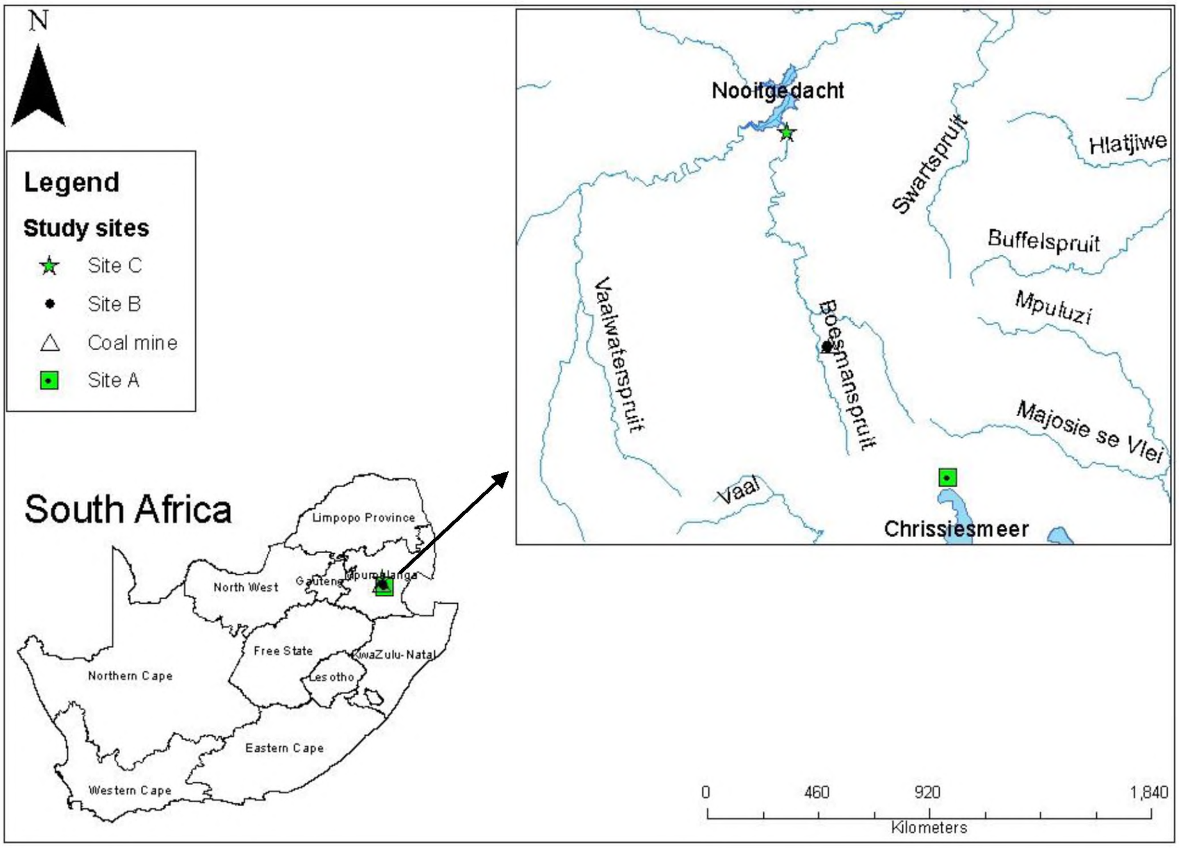

Figure 4.3: Study map showing macroinvertebrate sampling sites in Boesmanspruit in Mpumalanga Province in South Africa. The holding dam is located near Site B.

4.4.2 Physicochemical sampling

Water quality parameters including dissolved oxygen (DO), pH and electrical conductivity (EC) were monitored at each site during sampling. The DO was measured using a Cyberscan® 1500 meter, pH by a Cyberscan® 5000 while the Cyberscan® 200 meter was used to measure the EC. These instruments were manufactured by Eutech Instruments, Singapore.

4.4.2.1 Data analyses

The indicator species analysis was used to evaluate the association of macroinvertebrate families with sites as well as the significance of the association to the sites (De cáceres and Legendre 2009). The hypothesis was formulated that taxa predicted as less vulnerable to TAMD would be dominant at the impacted sites, particularly Site B. Similarly, taxa predicted as moderately vulnerable and vulnerable to TAMD were predicted to be less dominant at the impacted sites, particularly at Site B. The Shannon diversity index and Non-metric multidimensional scaling were computed to compare the diversity of taxa across sites, and the clustering of the assemblages, respectively. The Jaccard distance metric test was used to assess the similarity of family overlaps between sites, as well as the significance of treating AMD by liming as a management option. The lowest observed probability values of each family were analysed and statistically allocated to sites where the families would be abundant.

4.4.3 Results

4.4.3.1 Presence of macroinvertebrates in study sites

Table 4.6 shows the relative abundance of sampled macroinvertebrates from Sites A, B and C. Relatively high abundance of macroinvertebrates were sampled from Sites A and C. Site B that received high TAMD impact had less abundance and diversity of taxa. None of the predicted taxa (Leptoceridae and Leptophlebiidae) was sampled from Site B. Leptocerids were present at Site A and C only while Leptophlebiids were sampled at Site C. However, other mayfly families including Baetidae were present at Site A. Corixidae and Naucoridae were dominant families at Site B. Planariidae was the dominant family at Site A (46.7%) followed by Corixidae (27.2%). These two families were predicted as less and moderately vulnerable to TAMD.

Taxa were allocated into sites where there were likely in abundance. For example, since most Ephemeroptera families are relatively pollution sensitive, these families were clustered as taxa associated with Site A and C. Similarly, Coleoptera families were associated with Site B. This comparison allowed matching the trait-based predictions with prior pollution knowledge of the taxon in question.

The results in Table 4.6 indicated overlapping macroinvertebrate presence at Site A and C against predictions. Thus, it is clear that although a taxon was predicted as less, vulnerable or more vulnerable to TAMD, it still occurred at sites with less or more impact than its prediction. For example, Leptophlebiidae was predicted as more vulnerable to TAMD than Leptoceridae but it was abundant (36.2%) at Site C with moderate impact. However, Leptoceridae was present at both Site A (3%) and C (9.4%).

Table 4.6: Relative abundance of sampled taxa with the trait-based predicted vulnerability to TAMD

Order	Family	Site A (no impact)		Site B (high impact)		Site C (moderate impact)		Predicted vulnerability (Less vulnerable =1; moderately vulnerable = 2 and vulnerable = 3)
		Abundance	% abundance	Abundance	% abundance	Abundance	% abundance	
Ephemeroptera	Baetidae 1sp	75	4.4	0	0	0	0.0	3
	Baetidae 2sp	65	3.8	0	0	34	4.6	3
	Leptophlebiidae	0	0.0	0	0	268	36.2	3
	Heptageniidae	1	0.1	0	0	0	0.0	2
	Caenidae	24	1.4	0	0	7	0.9	2
Odonata	Aeshnidae	5	0.3	0	0	0	0.0	2
	Synlestidae	5	0.3	0	0	0	0.0	2
	Gomphidae	2	0.1	0	0	0	0.0	2
	Proitoneuridae	1	0.1	0	0	0	0.0	2
	Chlorocyphidae	0	0.0	0	0	5	0.7	2
	Lestidae	1	0.1	0	0	0	0.0	2
Trichoptera	Hydropsychidae 1sp (Leptoceridae)	52	3.0	0	0	70	9.4	1
	Hydropsychidae 2sp	0	0.0	0	0	0	0.0	1
	Hydropsychidae >2sp	10	0.6	0	0	0	0.0	1
	Ecnomidae	126	7.4	0	0	124	16.7	2
Coleoptera	Elmidae	12	0.7	0	0	1	0.1	1
	Dytiscidae	6	0.4	44	88	10	1.3	1
Diptera	Ceratopogonidae	7	0.4	0	0	0	0.0	1

Order	Family	Site A (no impact)		Site B (high impact)		Site C (moderate impact)		Predicted vulnerability (Less vulnerable =1; moderately vulnerable = 2 and vulnerable = 3)
		Abundance	% abundance	Abundance	% abundance	Abundance	% abundance	
	Chironomidae	19	1.2	0	0	87	11.7	2
	Muscidae	2	0.1	0	0	12	1.6	2
	Simuliidae	1	0.1	0	0	0	0.0	2
	Syphidae	1	0.1	0	0	0	0.0	2
	Ephydridae	0	0.0	0	0	1	0.1	2
	Culicidae	0	0.0	0	0	11	1.5	2
	Tabanidae	2	0.1	0	0	0	0.0	2
	Athericidae	0	0.0	0	0	2	0.3	2
	Tipulidae	0	0.0	0	0	1	0.1	2
	Dixidae	0	0.0	0	0	0	0.0	2
	Psychodidae	5	0.3	0	0	2	0.3	2
Hemiptera	Corixidae	465	27.2	2	4	23	3.1	2
	Naucoridae	0	0.0	3	6	1	0.1	1
	Nepidea	0	0.0	0	0	1	0.1	1
	Pleidae	0	0.0	1	2	0	0.0	2
Gastropoda	Physidae	19	1.1	0	0	9	1.2	1
	Lymnaeidae	2	0.1	0	0	3	0.4	1
Turbellaria	Planariidae	799	46.7	0	0	28	3.8	1
Pelecypoda	Corbiculidae	4	0.2	0	0	41	5.5	1
Total abundance		**1711**	**100**	**50**	**100**	**741**	**100**	

4.4.3.2 Physicochemistry and macroinvertebrate assemblage structure in the Boesmanspruit catchment

The physicochemical results indicated that EC was generally good across the sampling sites with respect to current South African standards (DWA 2011) (Table 4.7). The lowest DO and pH values were recorded at Site B, compared to Sites A and C. However, Site B had higher EC value than Site A and C. These values were still within acceptable standards (50 mS/m) in South Africa (DWA 2011). Overall, Site B, downstream of the discharge point of the TAMD was highly acidic, compared to Sites A and C that were relatively alkaline. The majority of sampled taxa were abundant at Sites A and C at pH ranging between 8.02 and 8.54. Neither Leptophlebiidae nor Leptoceridae was present at Site B with pH less than 4 and EC levels higher than 0.46. Corixidae, Naucoridae and Pleidae were the only families that survived the mean DO levels of 6.35 mg/L at pH 3.77. These families supported the predictions of vulnerability to TAMD.

Table 4.7: Average concentrations of water chemistry variables measured at the sampling sites in the Boesmanspruit catchment during macroinvertebrates collecting exercise (September 2016).

Site	pH	DO (mg/L)	EC (mS/m)
Site A (no mining impact)	8.02	7.75	0.46
Site B (highly impacted)	3.77	6.35	2.07
Site C (moderate impact)	8.54	11.29	0.35

4.4.3.3 Macroinvertebrate assemblage distribution and richness in the Boesmanspruit catchment

Macroinvertebrate families were analysed for diversity and richness. Thirty-five (35) families were sampled from rivers in the Boesmanspruit catchment (Table 4.8). There were 25 families at Site A, 4 at Site B and 21 were collected from Site C. Leptophlebiids were more abundant at Site C than at A and B. Leptocerids were more abundant at Site C than A. Families in the order Diptera were more diverse at Sites C and A, respectively than at Site B, which had none. Site A, with no mining impacts, was characterised by increased taxon richness (25) than any site including sensitive orders such as Ephemeroptera and Trichoptera but was also dominated by Turbellaria and Hemiptera. Diptera and Odonata families were also present at Site A. Site B (highly impacted) was only dominated by Coleoptera, particularly Dytiscidae that was predicted as less vulnerable, and Hemiptera with a loss of sensitive orders.

No other macroinvertebrate families were recorded at Site B. Site C (moderately impacted) was characterised by increased taxon richness (21) compared to Site B (4) but was lower than that for Site A (25). Sensitive taxa including Ephemeroptera and Trichoptera with some tolerant groups such as Diptera and Hemiptera were more dominant at Site C. The most abundant family for Diptera was Chironomidae. This site had other tolerant groups such as Pelecypoda and Turbellaria. Pelecypoda was only present at Site C.

Table 4.8: Total macroinvertebrate community measures per site. Richness represents families per order**.**

<table>
<tr><th>Order</th><th>Family</th><th>Abundance and diversity of families per order</th><th>Site A (no impact)</th><th>Site B (high impact)</th><th>Site C (moderate impact)</th></tr>
<tr><td rowspan="2">Ephemeroptera</td><td rowspan="2">Baetidae (1sp + 2sp)
Leptophlebiidae
Heptageniidae
Caenidae</td><td>Abundance</td><td>165</td><td>0</td><td>309</td></tr>
<tr><td>Richness</td><td>4</td><td>0</td><td>3</td></tr>
<tr><td rowspan="2">Odonata</td><td rowspan="2">Aeshnidae
Synlestidae
Gomphidae
Proitoneuridae
Chlorocyphidae
Lestidae</td><td>Abundance</td><td>13</td><td>0</td><td>5</td></tr>
<tr><td>Richness</td><td>5</td><td>0</td><td>1</td></tr>
<tr><td rowspan="2">Trichoptera</td><td rowspan="2">Hydropsychidae 1sp (Leptoceridae)
Hydropsychidae 2sp
Hydropsychidae >2sp
Ecnomidae</td><td>Abundance</td><td>188</td><td>0</td><td>194</td></tr>
<tr><td>Richness</td><td>3</td><td>0</td><td>1</td></tr>
<tr><td rowspan="2">Coleoptera</td><td rowspan="2">Elmidae
Dytiscidae</td><td>Abundance</td><td>18</td><td>44</td><td>11</td></tr>
<tr><td>Richness</td><td>2</td><td>1</td><td>2</td></tr>
<tr><td rowspan="2">Diptera</td><td rowspan="2">Ceratopogonidae
Chironomidae
Muscidae
Simuliidae
Syphidae
Ephydridae
Culicidae
Tabanidae
Athericidae
Tipulidae
Dixidae
Psychodidae</td><td>Abundance</td><td>38</td><td>0</td><td>116</td></tr>
<tr><td>Richness</td><td>6</td><td>0</td><td>7</td></tr>
</table>

Order	Family	Abundance and diversity of families per order	Site A (no impact)	Site B (high impact)	Site C (moderate impact)
Hemiptera	Corixidae Naucoridae Nepidea Pleidae	Abundance	465	6	25
		Richness	1	3	3
Gastropoda	Physidae Lymnaeidae	Abundance	21	0	12
		Richness	2	0	2
Turbellaria	Planariidae	Abundance	799	0	28
		Richness	1	0	1
Pelecypoda	Corbiculidae	Abundance	4	0	41
		Richness	1	0	1
Total taxa		**Abundance**	**1711**	**50**	**741**
		Richness	**25**	**4**	**21**

4.4.3.4 Macroinvertebrate families and sites association – evaluating the trait-based vulnerability prediction

To evaluate whether the predicted vulnerability of taxa to TAMD corresponded with field observations, an indicator species permutation test was performed following De cáceres and Legendre (2009) protocol. Results revealed that of the 35 families recorded, 14 were exclusively associated with Site A; and 1 with Site B and 11 with Site C. Nine families were associated with more than one site, with 8 families being associated with Sites A and C, and one with Sites B and C (Table 4.9). Of those families that were associated with Site A, Elmidae, Baetidae and Heptageniidae were predicted as taxa that were vulnerable to TAMD. Of the families associated with Site B, none was predicted as less vulnerable to TAMD. However, Leptoceridae and Leptophlebiidae were associated with Site A and Site C respectively somewhat supporting the predictions.

Table 4.9: Permutation test results showing the association of all families with sites (Association function: IndVal.g; alpha =1.

Site A #families. 14	statistic	p.value
Elmidae	0.961	0.23
Baetidae.1sp	0.707	1
Heptageniidae	0.707	1
Aeshnidae	0.707	1
Synlestidae	0.707	1
Gomphidae	0.707	1
Proitoneuridae	0.707	1
Lestidae	0.707	1
Leptoceridae	0.707	1
Hydropsychidae. 2sp	0.707	1
Ceratopogonidae	0.707	1
Simuliidae	0.707	1
Syphidae	0.707	1
Tabanidae	0.707	1
Site B #families. 1		
Pleidae	0.707	1
Site C #families. 11		
Leptophlebiidae	1	0.199
Hydropsychidae.2sp	1	0.199
Corbiculidae	0.955	0.199
Muscidae	0.926	0.199
Chironomidae	0.902	0.377
Chlorocyphidae	0.707	1
Ephydridae	0.707	1
Culicidae	0.707	1
Athericidae	0.707	1
Tipulidae	0.707	1
Nepidea	0.707	1
Site A+C #families.		
Ecnomidae	1	0.197
Physidae	1	0.197
Planariidae	1	0.197
Corixidae	0.998	0.197
Lymnaeidae	0.866	0.592
Beatidae.2sp	0.707	1
Caenidae	0.707	1
Psychodidae	0.707	1
Site B+C #families.		
Naucoridae	0.707	1

The Shannon diversity indices (Site A= 1.6, Site B = 0.4, Site C = 2.0) per site are illustrated in Figure 4.4. It is clear that Site B, which received direct TAMD, had the lowest diversity of macroinvertebrate families. Site C located in the lower reaches of the Boesmanspruit had the most diversity of macroinvertebrate families. The distribution of families was assessed for evenness across the three sites (Site A = 1.5, Site B = 0.4 and Site C = 1.9) and results revealed that Site C had higher evenness followed by Site A and then Site B (Figure 4.5). The Jaccard distance (dendrogram) revealed that some families were present both at Sites A and C (Figure 4.6).

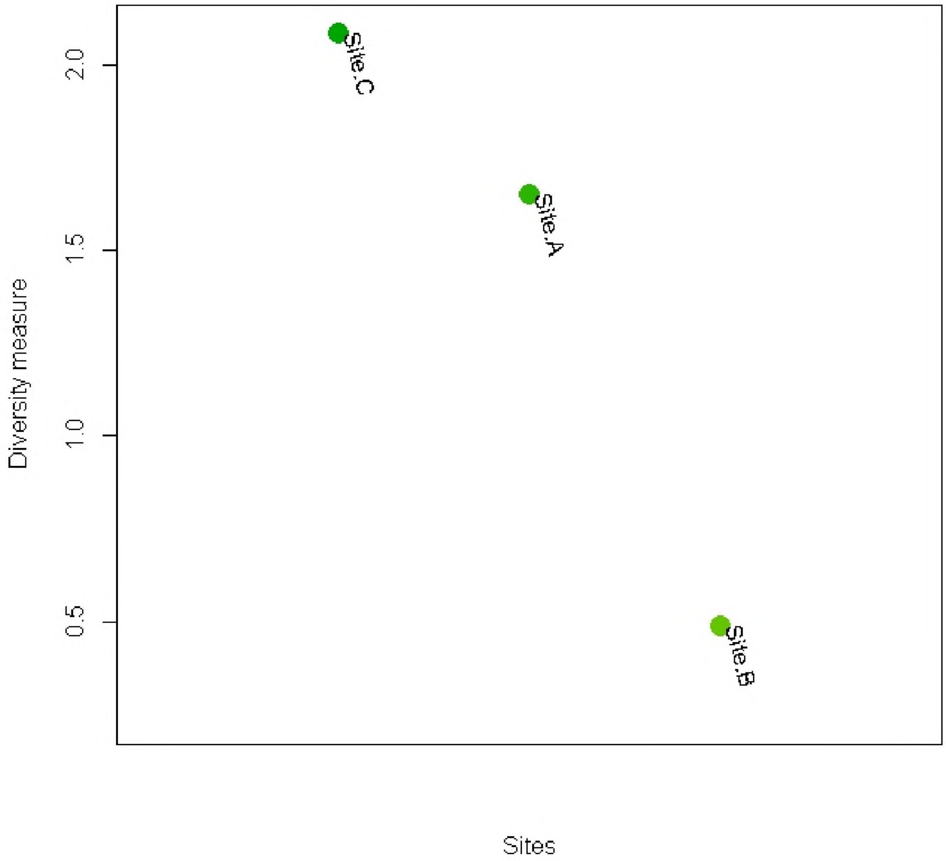

Figure 4.4: Shannon diversity indices for macroinvertebrate families collected from the three sites in the Boesmanspruit in Mpumalanga Province, South Africa

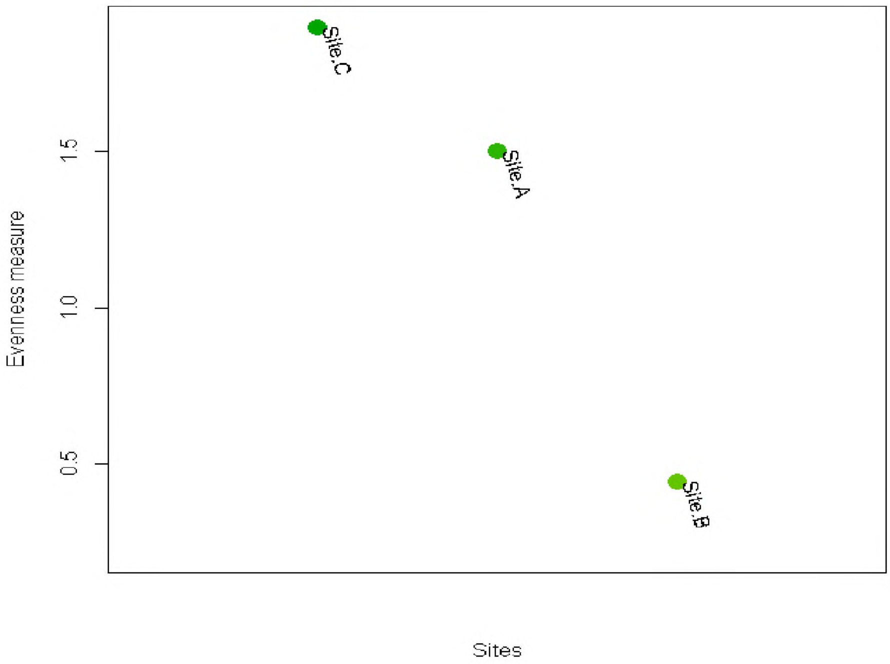

Figure 4.5: Evenness for the macroinvertebrate families collected at the three sampling sites in the Boesmanspruit in Mpumalanga Province, South Africa

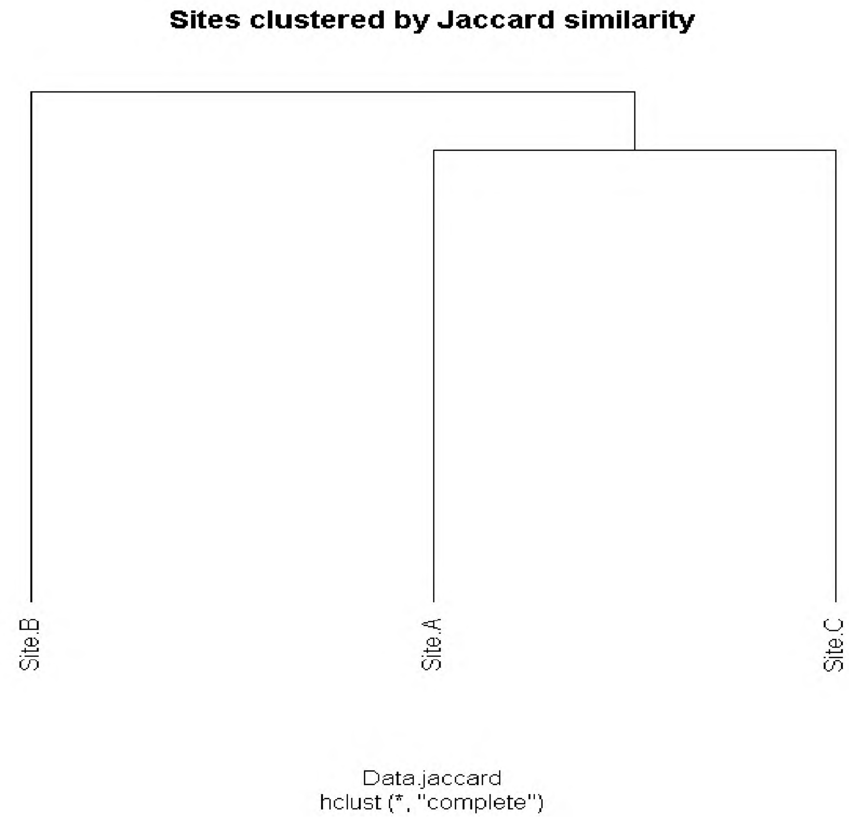

Figure 4.6: Dendrogram depicting clustered sites for similarity of macroinvertebrate family assemblages for the three sites in the Boesmanspruit, Mpumalanga, South Africa

4.4.3.5 Relationship between macroinvertebrate assemblage and liming AMD as a management option

A relationship between the Shannon diversity index values and the management intensity in the Boesmanspruit showed a downward trend, where the application of lime to treat AMD decreased macroinvertebrate diversity (Figure 4.7). However, a linear regression test revealed that, while the trend was negative, it was not significant (p = 1699; R^2 = 0.93). Thus, the addition of the lime to AMD as a treatment option is not important because it still reduces macroinvertebrate assemblages. In this case, management intensity summarised the management system, where high values mean more addition of lime to AMD for treatment and a reduction of macroinvertebrate assemblages.

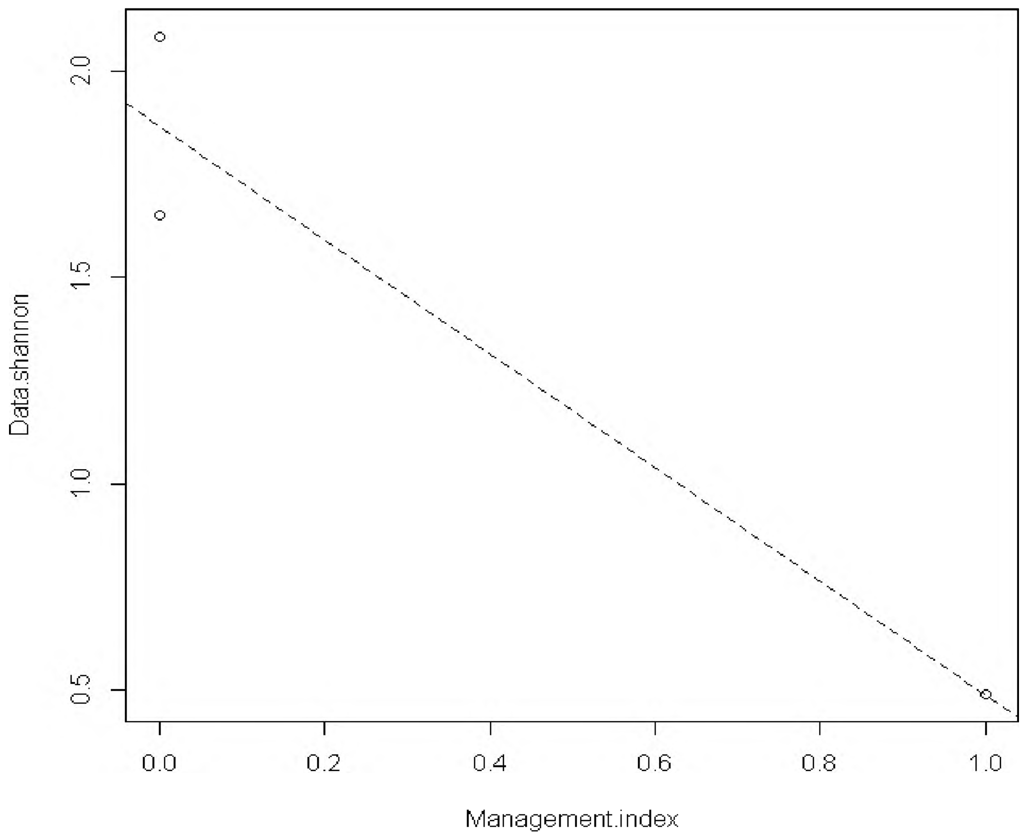

Figure 4.7: Relationship between the Shannon diversity index values and liming AMD as management option

4.4.4 Discussion

4.4.4.1 Macroinvertebrate biomonitoring findings

This section discusses whether taxa collected from the study sites corresponded with the trait-based predictions. Leptoceridae was predicted as a less vulnerable taxon than Leptophlebiidae but findings revealed that Leptoceridae was more vulnerable to TAMD than Leptophlebiidae. Despite this finding, Leptophlebiidae was vulnerable to TAMD after 240 hours of exposure in comparison to 96 hours of exposure. Therefore, it becomes important to define vulnerability predictions of taxa to toxicants based on the duration of exposures when comparing with other taxon responses. In terms of their presence in the natural environment, Leptoceridae was not sampled at Site B but was present at Site C, which had improved water quality. This did not support the prediction that Leptoceridae was less vulnerable to TAMD. However, as noted in the results, the absence of a taxon at a particular habitat does depend on both biotic and abiotic factors. Similarly, the predictions did not entirely match laboratory responses due to probably design nature of the experiments for all traits to be functional in test beakers.

Leptophlebiidae was present at Site C. No Leptoceridae and Leptophlebiidae were present at Site B with impaired water quality in comparison with Sites A and C. Site A had moderate water quality despite high EC in relation to Site C that could be attributed to agricultural impacts or high evaporation rates, as the site is a pan. Sites A and C, where many macroinvertebrates were sampled, had DO levels above 7.50 mg/L. However, the mayfly Heptageniidae is known to survive in water with very low DO (but not acidic) of 1 mg/L (Wiley and Kohler 1980). Based on current results, the Jaccard similarity revealed that the presence of taxa at Sites A and C were similar with some families present at both sites. This was also evident from the relative abundance distribution of individuals across the families at Sites A and C compared to A. This could be a result of improved water quality.

Water quality deteriorated at Site B but improved downstream at Site C. At Site B, water quality might have been affected negatively because of spillovers of the AMD from the holding dam or seepage and percolation. The pH at Site B conformed to laboratory findings, as both were acidic despite the AMD treatment. In addition, as the TAMD reached Site B from the holding dam, water quality slightly deteriorated again except for DO that appeared to improve, probably due to moderate water flow in the stream. This trend was not consistent as water quality at Site C located at the downstream of the catchment greatly improved with lower salinity levels with respect to Site A and B. The improved water quality downstream could be due to dilution of the effluent, probably from small tributary streams.

Based on macroinvertebrate diversity metric, the addition of CaO (lime) to treat AMD as a management option likely resulted to a decrease of macroinvertebrate diversity and abundance of taxa. Site B, which received direct TAMD, had relatively impaired water quality associated with very low taxa abundance. As water quality improved downstream of the Boesmanspruit at Site C, macroinvertebrate diversity and abundance also increased in relation to Site B. It was apparent that taxa abundance and richness decreased with a decline in water quality in the study area. Only two tolerant families were sampled at Site B. Other field studies have reported similar declines of macroinvertebrate assemblages due to AMD in particular (Last 2001; Gerhardt et al. 2004; Herbst 2013). Although data for TAMD toxicity studies are scarce, it appears that the effects of TAMD in aquatic ecosystems depend on variables that are likely to act synergistically (Gerhardt et al. 2004).

The absence of predicted vulnerable taxa such as Ephemeroptera, Plecoptera and Trichoptera and other less predicted vulnerable taxa at Site B was similar to changes in community structure observed in areas where AMD or metals are a problem. Hill (1997) reported a decreased correlation of species richness at low pH while the diversity of micro-algae decreased significantly in streams that received direct AMD (Hogsden and Harding 2012). Similarly, Chapter 3 of this thesis found that Leptophlebiidae was moderately tolerant to AMD although Hogsden & Harding (2012) noted that other mayfly families and passive filter feeder macroinvertebrates were dominant in similar ecosystems. For TAMD therefore, moderately and vulnerable taxa were eliminated at sites that received direct TAMD with pH values greater than 4. This is not always the case because other mayflies such as the Siphlonuridae tolerate sites impacted by AMD (Rosemond et al. 1992) or the Ephemerellidae that tolerates pH levels between 4.9 and 5.5 (Last 2001). Leptophlebiidae and Baetidae were present at Sites A and C, which had none or moderate mining impacts with pH levels above 8. These families can also dominate sites with pH levels below 4.9 (Last 2001). However, sites associated with moderate mining disturbances such as Site C have previously been reported to have abundant macroinvertebrates diversity (Gerhardt et al. 2004).

Although predictions were made for most Trichoptera families in this study as more vulnerable to TAMD based on traits possessed, Redell et al. (2009) demonstrated that variations do occur whereby Phryganeidae abundant at a site contaminated by AMD with a low pH of 2.58. The order Trichoptera was more abundant at Site C where waters were cooler and fast flowing with no notable mining impacts at the time of sampling. The lack of water flow and observed animal impacts at Site A might have contributed to the low abundance of Trichoptera compared to Site C where waters were clear and oxygenated due to the flow.

The dominance of Dytiscidae and Naucoridae at Site B was similar to a study that found a very high presence of these orders within a pH range of 4 and 6 (Gerhardt et al. 2004). Temporary and seasonal physical and chemical factors might affect macroinvertebrate presence in such streams. Further, tolerance of organisms in polluted streams can be attributed to their physiological and ecological characteristics (Gerhardt et al. 2004). For example, insects are better regulators of sodium and chloride ions than crustaceans while most families in the order Hemiptera and Coleoptera are fugitive, opportunistic and predacious (Gerhardt et al. 2004). Based on the trait-based predictions, these families were less vulnerable to TAMD as predicted.

Most organisms thrive better in unperturbed ecosystems that are well aerated. In this study, the scarcity of other families at Site B may not be entirely related to TAMD toxicity. Last (2001) suggested that the abundance measures may not be a reliable indicator of AMD impacts as sensitive organisms can be replaced by tolerant taxa due to other factors such as seasonal changes or breeding patterns. For example, at Site A there was abundant Turbellaria compared to Ephemeroptera, Trichoptera and Plecoptera. The absence of Leptoceridae and other Trichoptera families at Site B could thus reflect their relative inability to escape toxicants in nature (Wells 2004), although this is unlikely for this study. Site B was heavily impacted by not only TAMD but also other crop and animal farming impacts and the site had little or no flow of water. However, it is surprising that chironomids were absent at Site B despite the trait-based predictions.

Taxon richness was greatly responsive to TAMD impact, with Site B being greatly impacted compared with Sites A and C, which had relatively improved water quality. This is consistent with other studies that have documented low species richness due to, for example, low pH (Rosemond et al. 1992) and saline freshwater ecosystems (Cañedo-Argüelles et al. 2012). Water chemistry often limits and control species richness in ecosystems (Stockdale et al. 2014), as evident in the current study. In particular, Van Damme et al. (2010) suggested pH as the best predictor of macroinvertebrate community richness after observing a gradual decline of macroinvertebrate numbers in streams that received AMD at pH levels lower than 3. This current study, which has demonstrated that TAMD was more lethal to macroinvertebrate families in higher treatments with circumneutral pH than in lower treatments; pH may not be the best predictor of macroinvertebrate community richness in this case for Sites A and C but not at Site B.

Trait-based ecotoxicity studies are still in infant stages to support results of this study. However, other environmental factors might influence traits of relative importance for macroinvertebrates. For example, EC was inversely proportional to the abundance of macroinvertebrate families and richness across the three sites. Some authors have reported that aluminium and ferric iron in AMD might contribute to the toxicity of organisms in AMD impacted streams (Hill 1997; Last 2001). An increase in EC might have influenced the sensitivity and abundance of Leptoceridae as evidenced in a study that reported a higher sensitivity of shredders to increasing salinity (Cañedo-Argüelles et al. 2014).

TAMD contains high concentrations of sulphate and calcium ions and total dissolved solids (TDS) (Soucek and Kennedy 2005). Other ions potentially present at high concentrations in TAMD are magnesium and chlorides; therefore, the interacting effects of these various ions should be considered in assessing the toxicity of TAMD and macroinvertebrates community studies. It can thus be suggested that TAMD is a complex effluent mixture with a number of synergistic relationships with water quality variables that influence the survival of macroinvertebrates and determine their abundances in impacted streams. Still, differences that conform with the hypothesis that large body-sized organisms which possess less than three or no gill pairs and are prey for other organisms were less vulnerable to TAMD have been documented in other Trichoptera family. Phryganeidae was abundant on a site contaminated by AMD with pH as low as 2.58 (Redell et al. 2009). Although Leptoceridae was vulnerable to TAMD than predicted, Phryganeidae has been demonstrated to be less vulnerable. One of the reasons for the differences in vulnerability to toxicants between Leptoceridae and Phryganeidae could be different trait characteristics possessed by the organisms or species.

This study has therefore shown that water characterised by low pH and DO levels can easily eliminate sensitive taxa in a community. In such waters, metals might not be of primary importance compared to sites where pH is high. The abundance of Dytiscidae and Naucoridae in TAMD impacted site could imply that these families possess other traits that enable them to survive (by either swimming on the water surface or breathing using atmospheric oxygen) in mining-impacted ecosystems. In addition, toxicity exposure test systems can easily stress organisms (Liess and Beketov 2011). Traits related to competition and predation can also increase the vulnerability of organisms to toxicants (Liess and Beketov 2011). This might be linked to stress that could stem from the organisms being in a confined system, thereby affecting the survival organisms.

The findings of this study would contribute to the rapid and improved management decisions for the protection of aquatic resources. This would particularly be crucial when traits information can easily inform decision makers through a decision support system (DSS) about the options to take prior to an environmental project or during exposure. Trait-based studies can also explain ecotoxicological data for improved understanding of community structure of a population or organism responses and recovery from disturbances.

Although seemingly impressive for their potential contribution to science, trait-based data are largely sparse in ecotoxicology. Thus, it is difficult to provide links between traits and the affected processes in organisms for sound management strategies of freshwater ecosystems as a precautionary principle (Rubach et al. 2011). In particular, the TBA has not been applied in ecotoxicology in South Africa or most part of the world for that matter because it is new in this field. Species possess a wide range of traits that might play synergistic or antagonistic roles in response to stressors, making predictions a challenge in ecotoxicology. In addition, predictions of species vulnerability can be challenging as they are a function of the mode of action of a particular stressor (Ippolito et al. 2012). Despite challenges of using traits in ecotoxicology, they offer promising critical contributions to water quality management because of their approach to understanding in-depth knowledge about species or organisms with respect to stressor exposure and responses beyond mortality endpoints as employed by the SSD approach.

4.4.5 Conclusion

TAMD affected macroinvertebrates diversity, relative abundance and richness of macroinvertebrates. Taxon relative abundance and richness also decreased with a decline in water quality. Corixidae and Dytiscidae were the only dominant families in a highly impacted site and could be considered as best indicators of AMD and TAMD pollution. Corixidae and Dytiscidae were also predicted as less vulnerable to TAMD. However, based on predictions, Leptoceridae can be regarded as the best indicator for TAMD. The findings of this study suggest that the abundance and richness of macroinvertebrate families are dependent on water quality. Low pH and DO levels can eliminate sensitive taxa in a community easily. In such ecosystems, metals are not likely of primary concern compared to sites where pH is high. Therefore, environmental variables and the complexity of the TAMD might play synergistic roles in the determination of vulnerability of organisms. These should be carefully considered when making predictions based on trait combinations. Although Leptophlebiidae did not entirely satisfy the predictions, trait-based studies demonstrate the potential to predict the vulnerability of organisms to aquatic stressors in general. It is important to note that other traits of organisms play roles to influence the presence and abundance of a taxon in a habitat that might vary from predictions. Similarly, although predictions might not match laboratory test results, it is important to note that the trait-based approach is not exhaustive of traits during selection. Other equally important traits might influence the response of organisms in test chambers or their presence and absence in a natural environment. The outcomes of this study are important to form part of an integrated water resources management including the development of a decision support system.

CHAPTER 5: MANAGEMENT OPTIONS FOR ACID MINE DRAINAGE AND SALINITY

5.1 Introduction

Chapter one of this thesis introduced water as a valuable natural resource that provides important ecosystem services for social-economic development. Increases in AMD and high salinity were indicated as aquatic stressors of concerns in South Africa and the need to manage them was articulated in the chapter. In the chapters that followed, ecological risks of AMD in a salinising landscape were investigated using both laboratory and field studies in order to achieve the overall aim and objectives of this study. Using inorganic sulphate salts as models of mining salinisation in South Africa, the study demonstrated the role of sulphate salts in AMD as it occurs in mining-impacted freshwater ecosystems in South Africa. The study used a risk-based approach, the species sensitivity distribution (SSD), to derive water quality guidelines (WQGs) for sulphate and AMD for the protection of freshwater ecosystems in South Africa (Chapters two and three). In Chapter four, a novel trait-based approach developed for the prediction of the potential vulnerability of organisms to treated acid mine drainage (TAMD) using both laboratory and field-based studies. The present chapter is aimed at proposing an environmental water quality management framework that integrates the findings of all these previous chapters for the management of AMD and salinity in freshwater ecosystems. In this regard, current global practices with regard to managing AMD and salts are reviewed and a framework developed based on the findings of the present study and global best practices.

5.1.1 A review of water quality guidelines for acid mine drainage and salinity

Acid mine drainage and increasing salinity are considered as water quality stressors of urgent global concern (Cañedo-Argüelles et al. 2013a). As a consequence, countries in Europe and North America have developed water quality guidelines (WQGs) that can inform appropriate environmental water quality management framework for managing AMD and salinity. WQGs can act as a science-only-based benchmark or a science-and policy-based benchmark to ensure the protection of aquatic resources (CCME 2007). A brief review of WQGs and water quality management strategies in relation to salinity and/or AMD in selected countries is therefore presented.

5.1.1.1 Australia and New Zealand

Several factors influence the development of WQGs, including specific management goals, levels of protection and factors at the site that modify a toxicant or determine its bioavailability. It is important that the application of a WQG should acknowledge that ecosystem types vary at different conditions, which can influence effects or presence, transport and degradation of toxicants. For example, Australia and New Zealand have three established ecosystem classes .i.e. Class 1, 2 and 3 based on their condition and levels of protection (ANZECC/ARMCANZ 2000). Class 1 is regarded as an ecosystem with "high conservation ecological value" requiring 99% level of protection of biodiversity, particularly where there are limited scientific data. The Australian guidelines also require that a Direct Toxicity Assessment (DTA) be conducted for any effluent discharges such as AMD. Class 2 ecosystems are described as those moderately disturbed, requiring about 95% protection of biodiversity with a DTA process for effluent discharges. Finally, ecosystems considered as "highly disturbed" i.e. Class 3 follow the same 95% level of biodiversity protection. The only difference between Classes 2 and 3 is that for Class 3, the guideline stipulates the need for a DTA process as an alternative approach for setting site-specific guidelines. The choice of the level of protection is still subject to management goals. For instance, where the management goal is to ensure there are no or minimal changes in biodiversity for ecosystem Class 2, the 99% protection level may thus be preferred – making the system flexible in practice and strict in principle. These guidelines are necessary for the protection of diverse biodiversity from increasing salinity and various effluents.

Salinity management appears to be a challenge in Australia due to its saline landscape. The trigger value (TV) for sulphate salts in Australia and New Zealand is 0.40 g/L, which was majorly derived from short-term acute toxicity data (from tests $\leq$ 96 hours duration) (ANZECC/ARMCANZ 2000). Due to its saline landscape, it appears that more salinity studies are conducted in Australia than elsewhere, to ensure management and protection of its biodiversity. Key management strategies range from the design of diagnostic tools that define timeframes for salinity development, the biophysical features of the landscape within which salinity develops and the riskiness of current and alternative management systems and matching with a complementary community process structured for knowledge building and intervention (Grundy et al. 2007).

Ecosystems that are generally considered to be at risk from increasing salinity are mapped on a database with diagnostic information. These maps form part of salinity knowledge dissemination materials for local communities and they also guide investment in salinity prevention. For example, monitoring programs based on the presence of species that are salinity sensitive provide direct evidence of impacts. Salinity sensitivity scores (SSS) (based on field distributions of macroinvertebrates), generalised salinity scores (GSS) (based on both field distributions of macroinvertebrates and laboratory toxicity results) and salinity indices (for identifying localities of potential salinity impact on biodiversity at the catchment scale) are tools that help Australia to assess water quality impacts from increasing salinity concentrations (Kefford et al. 1999). It is such information that is mapped to show areas where biodiversity is being impacted so that that management actions could be triggered, following the "precautionary principle".

Australia and New Zealand adopted the "Precautionary Principle" as a water quality management strategy to allow policy decisions to be made when scientific data are inadequate. The principle states that if there are threats of serious or irreversible environmental damage, lack of full scientific certainty should not be used as a reason for postponing measures to prevent environmental degradation (Warne 1998). This implies that these countries are more proactive to the management of freshwater ecosystems with regard to salinity.

5.1.1.2 The United States of America

The general sulphate guideline for the United States of America (USA) is 0.25 g/L, which is the same for South African (DNR 2009). However, many states have individual guidelines depending on water users where limits can range as high as 1.5 g/L for Ohio to no limit at all for other States such as Michigan and Kentucky. In the USA, sulphate is commonly regarded as a natural salt component that does not carry the risk of a true toxic substance. Similar to Australia/New Zealand, sulphate guidelines in the USA are usually derived based on short-term toxicity data with some authors suggesting that long-term exposures would not result in reduced survival of species compared to short-term (DNR 2009). However, the lowa Department of Natural Resources highly recommends that sulphate guidelines be developed based on site-specific criteria rather than relying on a national-wide numerical standard. To properly manage sulphates in freshwater resources, the Department suggests the consideration of water ambient chemical characteristics such as hardness and chlorides, which influence the toxicity of sulphates (DNR 2009).

Although it is thought that an explicit consideration and analysis of hardness and chloride could influence protection level for ecosystems, it seems the approach is still not being implemented in the USA.

In terms of management of salinity and mining effluents, mining companies in the USA are actively involved in informing the public on the safe discharges of aquatic toxicants (DNR 2009). This often includes the mixing of effluents with freshwater to reduce the salinity levels. However, the USA guidelines prohibit mixing of effluents in streams that have a zero flow for a minimum of seven consecutive days to protect aquatic life from discharges occurring at drought. Similar to the Australia/New Zealand DTA approach, key management approaches require that whole effluent toxicity (WET) tests be conducted, although no clearly defined ecosystem classes are stipulated (DNR 2009). This includes short-term (acute) WET tests for 100% of the effluent flow, alongside chemical analyses of ions of toxicological importance.

For AMD discharge, the USEPA (2000) guidelines recognise effluent toxicity findings as part of an integral tool in the assessment of water quality. Companies require permits to discharge effluents to ensure the protection of aquatic life (DNR 2009). Challenges still exist as current effluent and sulphate standards appear to have a huge impact on the coal mining industry, which calls for a review of the standards for economic reasons. According to the lowa Department of Natural Resources, for coal mines to meet the existing water quality standards would mean shutting down of majority of the active mines and almost all reclamation projects (DNR 2009). The development of site-specific sulphate guidelines is advantageous because technologies for the removal of dissolved sulphate salts are prohibitively expensive (DNR 2009). Reverse osmosis, which helps to evaporate the salts, is relatively good management approach but expensive and cannot be applied on a large-scale such as a river. In some cases, deep well injection of high salt content waters has been used, but the technique is increasingly difficult to implement due to groundwater protection regulations. As a result, it would appear that preventing the salts from dissolving in wastewaters is the best management approach. For the USA, it is suggested that this should be done at the mines as part of best management practices. Unlike the USA, Canada appears to have strict water quality regulations.

5.1.1.3 Canada

In Canada, where the general limit for sulphate is 0.1 g/L and applicable to all water users, provincial jurisdictions may aim for greater or lesser protection levels depending on circumstances (CCME 2007; Meays and Nordin 2013). However, the Canadian Council of Ministers of the Environment (CCME) Water Quality Task Group selects substances for WQG development after stakeholders' consultations. The CCME guidelines are set for both short-term and long-term exposures protection. Short-term WQGs protect most species against lethality during intermittent and transient events as opposed to long-term exposure guidelines, which are meant to protect against all negative effects during indefinite exposures.

Similar to ANZECC/ARMCANZ (2000), CCME guidelines are derived using species sensitivity distributions (SSDs) approach. However, the alternative method mostly used when data are inadequate to perform the SSD estimations in Canada is the Assessment Factor (AF) (Warne et al. 2004). Thus, the SSD derived guidelines are referred to as "Type A" guidelines while "Type B" guidelines refer to those that are derived using the AF method.

For effluents such as AMD, CCME guidelines are not designed to deal with mixtures but rather single-substances, although they recommend WET tests and the development of site-specific WQGs (CCME 2007). Contrary to the USA management approach, the CCME, just like Australian/New Zealand guidelines, do not simultaneously factor in chloride and hardness in evaluating sulphate protection levels in WQGs.

5.1.1.4 South Africa

As noted in Chapter One of this thesis, AMD and increasing salinity are widely recognised as threats to freshwater ecosystems in South Africa (DWA 2011). In particular, the instream water quality compliance regulations indicate that 30% of the national rivers monitoring sites in South Africa have unacceptable high levels of salinity (DWA 2011). Similar to Australian/New Zealand and Canada WQGs, South African guidelines do not simultaneously consider the interaction of chlorides and hardness when estimating protective levels for sulphates. Currently, South African WQGs for organic toxicants follow the Australia/New Zealand and Hong Kong guidelines (Warne et al. 2004), with few modifications due to practical constraints such as finance and administrative overload.

The modifications resulted, for example, in reducing the 99% protection level as practised by Australia/New Zealand to 95% protection level for South Africa, which is relatively less strict by comparison. A 4% difference of protection level may result in a loss of sensitive keystone species in an ecosystem. The adoption of the Australia/New Zealand guidelines implies that any developed South African WQGs have the potential inherent weaknesses and strengths of the ANZECC/ARMCANZ (2000).

Although the South African WQGs development process follows the Australian/New Zealand guidelines, in practice, differences exist in terms of experimental approach to assessing the biological effects of toxicants. Few species of many individuals with an acclimation of no less than 24 hours are used during toxicity tests in South Africa (DWAF 2000; Slaughter et al. 2008a; Mensah et al. 2012). The organisms are acclimated prior to exposures, which generally run for 96 hours (short-term) or 240 hours (long-term) (Mensah 2012). The Australian approach normally uses many species of few individuals with no acclimation for 72 hours (example, Kefford et al. 2003). Although every approach has its advantages, the statistical power of the results such as confidence limits can be compromised adversely. In addition, short-term (acute) toxicity tests have been criticised, often due to unrealistic laboratory conditions without consideration of sub-lethal effects or interaction between species (Kefford et al. 2004b). Thus, there was a need for South Africa to derive its WQGs for both short-term and long-term using indigenous species under prevailing local conditions to minimise uncertainties. Although setting South African WQGs on the basis of international toxicity data has been long questioned (Zokufa et al. 2001; Slaughter et al. 2008b), few studies have adequately addressed this concern to initiate a review of existing WQGs with a view to revising them or setting new WQGs based on indigenous species.

In South Africa, water quality management, through WQGs, is placed within the broader context of resource-directed measures (RDMs) and source-directed controls (SDC), which are two complementary strategies for ensuring the use and protection of water resources. The results of the current study can contribute to SDC measures such as setting of water license conditions for AMD mining facilities and other water users discharging effluents containing salts. The proposed WQGs based on the results of this study are therefore discussed below and their implications in the broader context of RDM and SDC highlighted.

5.2 Proposed South African water quality guidelines for acid mine drainage and salinity

In South Africa, the Tool for Ecological Aquatic Chemical Habitat Assessment (TEACHA) was developed to determine the inorganic salt concentrations from the available salt ions found in solutions (Jooste and Rossouw 2002). TEACHA was based on short-term acute toxicity test data (Holland et al. 2011a). To date, inconsistencies with electrical conductivity and biotic response data have been reported with TEACHA as it either overestimates or underestimates some salt boundaries in the ecological reserve, e.g. for $MgSO_4$ (Holland et al. 2011a; Palmer et al. 2013). Although TEACHA is still being used in the reserve determination process, its wide application has been problematic because of inconsistent results as well as the mathematics rigour required for its application. For these reasons, stakeholders have advocated for better WQGs applicable for the ecological Reserve and general water resource management in South Africa. The proposed guidelines from the study contribute to this effect.

5.2.1 Levels of protection

Five major levels of protection that represent different ecological categories i.e. Categories A – E are recommended in South Africa in terms of the toxicity of substances (Warne et al. 2004). Ecological category A is considered as excellent or natural and is proposed to protect over 95% of species. The second category (B) is considered good and has a protection level of over 90% of species and categories C-D, described as good-fair targets for the protection of 80% of species. The last category is considered poor (E) and is proposed to protect less than 80% of species. In this study, less than 80% protection level represented a poor ecological category (Table 5.1). Based on these ecological categories and outcomes of this study, appropriate WQGs for sulphate salts are proposed in Tables 5.2 - 5.3.

Table 5.1: Ecological categories and their proposed levels of protection of species recommended for South Africa (Warne et al 2004).

<table>
<tr><th colspan="2">Alternate class description for ecological integrity</th><th>Level of protection</th></tr>
<tr><td>Excellent (natural)</td><td>A</td><td>PC>95</td></tr>
<tr><td rowspan="3">Good</td><td>B</td><td rowspan="2">PC>90</td></tr>
<tr><td rowspan="3">C</td></tr>
<tr><td rowspan="3">PC>80</td></tr>
<tr><td rowspan="2">Fair</td></tr>
<tr><td>D</td></tr>
<tr><td>Poor</td><td>E</td><td>PC<80</td></tr>
</table>

5.2.1.1 Short-term water quality guidelines for sulphates

The short-term WQGs for sulphates for the tested species as calculated from the species sensitivity distributions (SSDs) are shown in Table 5.2. The current 0.25 g/L sulphate compliance limit for South Africa would protect the species almost in all ecological categories. However, this value would under-protect over 90% of species when exposed to $CaSO_4$ in a natural category.

Table 5.2: Short-term WQG values at different levels of ecosystem protection using the risk-based SSD approach derived in the present study.

<table>
<tr><th colspan="2">Ecological category description</th><th>Protection level</th><th>Guideline values for Na_2SO_4 in g/L with 95% lower and upper CI in parenthesis</th><th>Guideline values for $MgSO_4$ in g/L with 95% lower and upper CI in parenthesis</th><th>Guideline values for $CaSO_4$ in g/L with 95% lower and upper CI in parenthesis</th><th>Guideline values for combined sulphates in g/L with 95% lower and upper CI in parenthesis</th></tr>
<tr><td>Excellent (natural)</td><td>A</td><td>PC>95</td><td>1.158 (0.750-1.79)</td><td>0.819 (0.502-.337)</td><td>0.097 (0.049-0.193)</td><td>0.710 (0.118-4.259)</td></tr>
<tr><td rowspan="2">Good</td><td>B</td><td rowspan="2">PC>90</td><td rowspan="3">1.448 (0.969-2.164)</td><td rowspan="3">1.452 (0.924-2.282)</td><td rowspan="3">0.387 (0.205-0.731)</td><td rowspan="3">1.328 (0.255-6.905)</td></tr>
<tr><td rowspan="2">C</td></tr>
<tr><td rowspan="2">Fair</td><td rowspan="2">PC>80</td></tr>
<tr><td>D</td><td>1.899 (1.312-2.746)</td><td>2.902 (1.913 - 4.404)</td><td>2.061 (1.146-3.707)</td><td>2.833 (0.624-12.857)</td></tr>
<tr><td>Poor</td><td>E</td><td>PC<80</td><td>4.40 (3.09-6.264)</td><td>24.938 (16.726-37.182)</td><td>372.615 (212.342-653.859)</td><td>29.823 (7.032-126.47)</td></tr>
</table>

CI = confidence interval

5.2.1.2 Long-term water quality guidelines for sulphates

As stated already in the thesis, long-term WQGs are recommended for the protection of aquatic ecosystems over short-term WQGs. The South African 0.25 g/L sulphate compliance guideline clearly undermines the protection of an ecosystem with an excellent class description (Table 5.3). As discussed in Chapter two, most species in that ecological class would be wiped out after long-term exposures to increasing sulphate concentrations. Similarly, except for $MgSO_4$, over 80% of species (Chapter two) would still be wiped out in an ecological class considered poor or good. These guidelines demonstrate the need for further studies on a wide range of other species in order to inform the review of the current sulphate compliance limit for South Africa. In comparison with the CCME guideline of 0.1 g/L, it appears to protect most of the tested species in the different ecological classes in South Africa.

Table 5.3: Long-term WQG values at different levels of ecosystem protection using the risk-based SSD approach.

<table>
<tr><th colspan="2">Ecological class description</th><th>Protection level</th><th>Guideline values for Na_2SO_4 in g/L with 95% lower and upper CI in parenthesis</th><th>Guideline values for $MgSO_4$ in g/L with 95% lower and upper CI in parenthesis</th><th>Guideline values for $CaSO_4$ in g/L with 95% lower and upper CI in parenthesis</th><th>Guideline values for combined sulphates in g/L with 95% lower and upper CI in parenthesis</th></tr>
<tr><td>Excellent (natural)</td><td>A</td><td>PC>95</td><td>0.02 (0.003-0.162)</td><td>0.108 (0.013-0.899)</td><td>0.055 (0.026-0.118)</td><td>0.067 (0.006-0.745)</td></tr>
<tr><td rowspan="3">Good</td><td>B</td><td rowspan="2">PC>90</td><td rowspan="4">0.038 (0.006-0.257)</td><td rowspan="4">0.191(0.027-1.332)</td><td rowspan="4">0.103 (0.051-0.209)</td><td rowspan="4">0.116 (0.013-1.042)</td></tr>
<tr><td rowspan="3">C</td></tr>
<tr><td rowspan="3">PC>80</td></tr>
<tr><td rowspan="2">Fair</td></tr>
<tr><td>D</td><td>0.082 (0.014-0.472)</td><td>0.379 (0.064-2.243)</td><td>0.220 (0.115-0.422)</td><td>0.223 (0.030-1.645)</td></tr>
<tr><td>Poor</td><td>E</td><td>PC<80</td><td>0.892 (0.168-4.742)</td><td>3.222 (0.593-17.515)</td><td>2.320 (1.242-4.334)</td><td>1.704 (0.255 -11.401)</td></tr>
</table>

CI = confidence interval

5.3 Risk-based scenario-specific WQGs for acid mine drainage occurring in the Boesmanspruit catchment in Mpumalanga

The following are proposed short-term and long-term WQGs for AMD as it occurs in the Boesmanspruit catchment in Mpumalanga Province (Tables 5.4 and 5.5). A guideline of 0.122% is capable of protecting over 95% of species in a natural ecosystem (Table 5.4). Similarly, depending on management goals and the resource in question, some ecosystems might require protecting over or less than 80% of species. In such scenarios, WQGs of 0.384% and 2.724% is suitable to protect species during short-term exposures. In the long-term, 0.018% and 0.052% dilution of AMD are proposed guidelines to protect over 90% and 80% of species in good and poor ecological classes (Table 5.5). These proposed WQGs are important for the improvement of water quality in receiving streams. They can also aid in the development of wastewater treatment designs. Based on these guidelines, even if the resource is considered polluted and classified as a poor ecosystem class, it is still desirable to protect over 80% of the species in that particular ecosystem. Therefore, although AMD might vary in different regions, these guidelines are necessary as a precautionary management approach. These WQGs support Warne et al. (2004) who suggested the need to use South African derived background data for scenario-specific guidelines.

5.3.1 Short-term water quality guidelines for acid mine drainage

Table 5.4: Short-term protective dilutions for acid mine drainage at different protection levels of species

<table>
<tr><th colspan="2">Ecological class description</th><th>Protection level</th><th>Guideline values as % dilution with 95% lower and upper CI in parenthesis (%)</th></tr>
<tr><td>Excellent (natural)</td><td>A</td><td>PD>95</td><td>0.122 (0.030-0.489)</td></tr>
<tr><td rowspan="3">Good</td><td>B</td><td rowspan="2">PD>90</td><td rowspan="4">0.205 (0.057-0.738)</td></tr>
<tr><td rowspan="3">C</td></tr>
<tr><td rowspan="3">PD>80</td></tr>
<tr><td rowspan="2">Fair</td></tr>
<tr><td>D</td><td>0.384 (0.118-1.247)</td></tr>
<tr><td>Poor</td><td>E</td><td>PD<80</td><td>2.724 (0.885-8.390)</td></tr>
</table>

CI = confidence interval

5.3.2 Long-term water quality guidelines

Table 5.5: Long-term protective dilutions for acid mine drainage at different protection levels of species

<table>
<tr><th colspan="2">Ecological class description</th><th>Protection level</th><th>Guideline values with 95% lower and upper CI in parenthesis (%)</th></tr>
<tr><td>Excellent (natural)</td><td>A</td><td>PD>95</td><td>0.014 (0.010- 0.021)</td></tr>
<tr><td rowspan="3">Good</td><td>B</td><td rowspan="2">PD>90</td><td rowspan="4">0.018 (0.012- 0.026)</td></tr>
<tr><td rowspan="3">C</td></tr>
<tr><td rowspan="3">PD>80</td></tr>
<tr><td rowspan="2">Fair</td></tr>
<tr><td>D</td><td>0.023 (0.016- 0.032)</td></tr>
<tr><td>Poor</td><td>E</td><td>PD<80</td><td>0.052 (0.037- 0.072)</td></tr>
</table>

CI = confidence interval

Although management goals greatly determine which protection level would be taken for the resource, the successful implementation of any management choice relies on the integration of available options. Mensah (2012) noted that it is important to consider risks and benefits of alternative management actions along with preferences of stakeholders, available technology, economic costs, regulatory requirements, legal considerations and other factors in the decision-making process prior to the implementation of a particular level of protection. Another important consideration is that the WQGs for AMD are scenario-specific. However, these guidelines may be important indicative dilutions derived from the basis of the precautionary principle for other mines discharging similar AMD. In addition, the guidelines presented so far do not consider trait-based ecotoxicity information, which, if considered in the management decision process, would contribute important knowledge in terms of decision making for appropriate management of AMD and sulphates at any particular level of protection.

5.3.3 Integration of management approaches as part of a holistic decision-making process

The outcomes of the species sensitivity distributions (SSDs) approach include protective concentrations (dilutions for AMD), confidence boundaries, and model goodness of fit (R^2) and slope of the distribution curve. The SSD approach also produces different levels of protective concentrations as WQGs. However, one important outcome of the SSD approach is to rank the most and least sensitive species to the toxicant being investigated. Management decisions for the resource are based on such rankings as they are considered guidelines. They merely point estimates generated from a regression analyses with many assumptions that do not necessarily sufficiently consider the complexity of ecological and biological attributes such as traits of organisms.

Certainly, protective concentrations are aimed at addressing the ecological risk of the tested species to the toxicant. The risk is mainly designated to the most sensitive species because if that particular species is protected, the technical assumption is that protective concentrations would protect other species of similar taxonomic grouping in an ecosystem if not exceeded. This approach is adopted because it is practically impossible to test all species present in a particular ecosystem to generate a "precise or accurate" protective concentration. This is a limitation of the SSD approach, which has made many studies to include toxicity data from other studies or databases to estimate protective concentrations for particular toxicants (Del Signore et al. 2016).

In principle, the SSD approach is largely based on the organisms' feeding traits or trophic level position. The trait-based approach described in Chapter Four of this thesis chose organisms based on their potential vulnerability to toxicants using a range of traits that are mechanistically linked to the stressor. The prediction findings revealed that Leptoceridae was more vulnerable to TAMD than Leptophlebiidae, although both families were vulnerable to TAMD between 96 hours and 240 hours of exposure. The vulnerabilities of taxa to TAMD were validated using both laboratory and field investigations. For example, Baetidae were abundant at Site C besides being predicted to be more vulnerable to TAMD. Pleidae and Dytiscidae were less vulnerable to TAMD based on predictions. However, no single Leptophlebiid was collected at Site B, which received direct TAMD. This finding corresponded with the SSD findings where Leptophlebiidae was also more sensitive to increasing sulphate concentrations in the long-term (Chapter Two) of the tested species. Leptophlebiidae was particularly sensitive to sulphate salts used in the current study. It is thus ecologically important to predict the vulnerability of taxa to stressors beyond laboratory tests as part of suits of management approaches to EWQ management.

Moreover, as noted in Chapter two, laboratory experiments largely demand technical expertise to execute; they are time-consuming and constrained by the availability of resources and organisms. When WQGs cannot be implemented due to lack of expertise, ecological problems may escalate and become complicated to manage and protect organisms. The ability of stakeholders to decide on what to do about the ecological problems in a precautionary manner regardless of the approach used would help to make swift management decisions for the resources in question.

5.4 Trait-based decision-making tool for management of AMD, TAMD and salinity

The trait-based predictions can help management of the resource because it enables a priori prediction. This is so because the field based results supported a range of predictions made. Thus, the vulnerability levels and taxa occurred in terms of their relative abundance generally matched. This finding can thus support decision making with regard to ecosystem protection. This would be particularly useful for management of both short-term and long-term exposure events, which might pollute freshwater ecosystems. The application of a trait-based support tool for management of a resource would save time and accelerate problem-solving based on its predictions of the vulnerability of a taxon to toxicants in question. Since no trait-based toxicity data are represented in current methods of developing WQGs, this study proposes a decision support system (DSS) framework (Figure 5.1) for the potential integration of the trait-based approach into managing AMD and salinity as a rapid decision support tool.

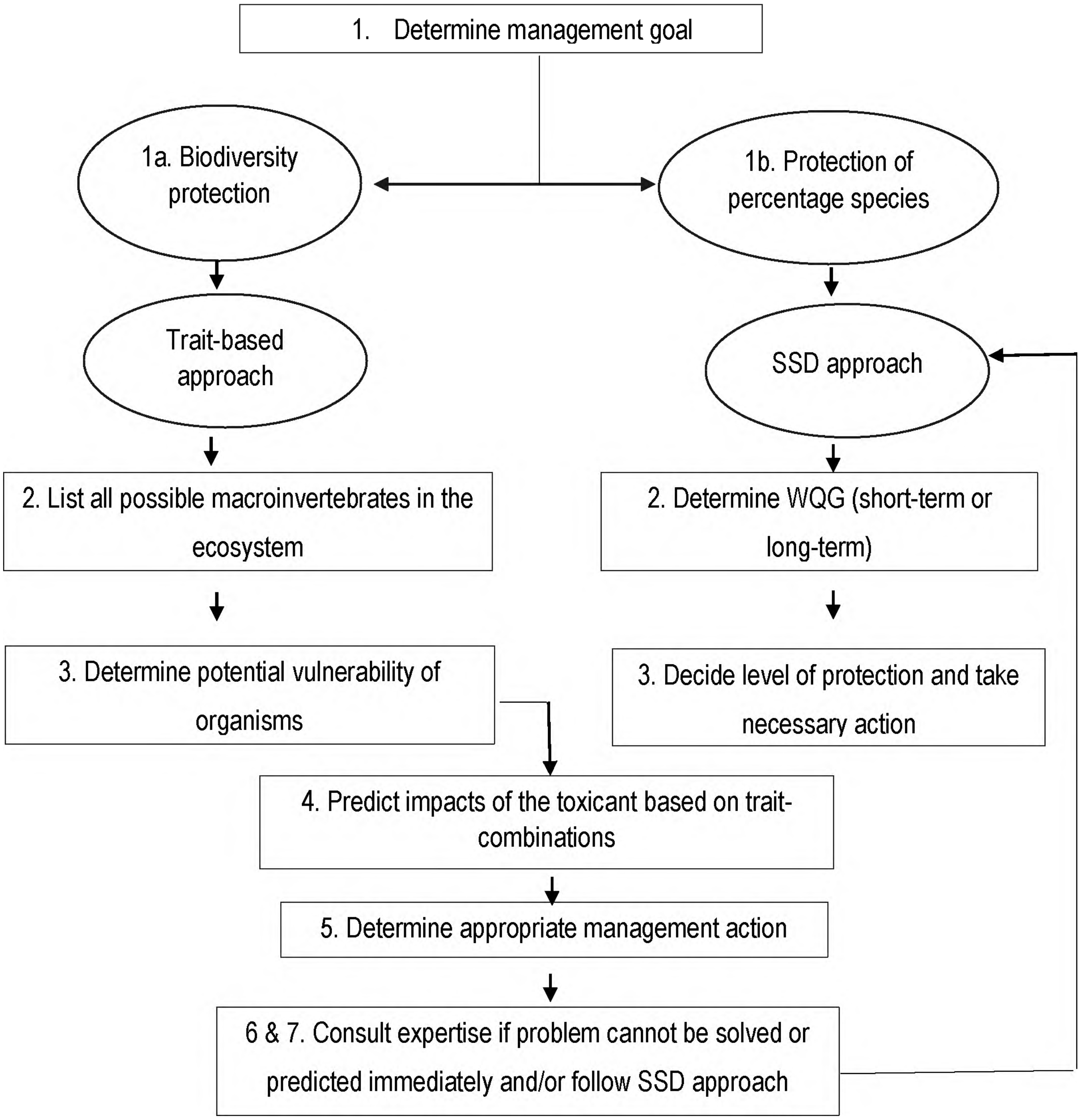

Figure 5.1: Trait-based versus species sensitivity distribution decision support tool for management of acid mine drainage and salinity

1. Determine the choice of WQGs as either short-term or long-term based on the goals.
2. List all possible macroinvertebrate families in the ecosystem
3. Determine the potential vulnerability of organisms in particular ecosystem.
4. Decide level of species protection according to management goals if using the SSD approach
5. Assess and predict potential impacts based on trait-combinations
6. Take appropriate management action to proceed with the proposed project or consult.
7. The user should consult experts if problem continues

For example, if there is continued water pollution in a particular freshwater resource through the release of AMD, the decision of managers would entail diluting AMD using CaO at the proposed concentrations to protect different levels of species. This would be dependent on the value of the resource and the ecological category of the ecosystem. In such scenarios, it would be useful to apply the SSD approach. In the event that there are no known WQGs for AMD or TAMD, the decision would depend on the trait-based predictions for the protection of the ecosystem. The predictions would inform which species would be most likely affected by the toxicant based on vulnerability results of this study. Such a decision can facilitate quick decision to halt the operation (if already running) or inform the next available steps for the protection of the resource if project is yet to be initiated.

5.5 Conclusions

This chapter has demonstrated that management knowledge gaps in the protection of aquatic life from increasing sulphate concentrations and AMD in freshwater ecosystems exist despite several WQGs in the literature. WQGs are commendable but cannot protect all biodiversity in an ecosystem and inconsistencies exist. This necessitates the need for more scenario-specific guidelines based on the toxicant and region where the site is located. The chapter, therefore, argued that it is possible to protect freshwater ecosystems with an understanding of trait-based prediction. The application of the trait-based predictive tool can be useful for different users including non-specialists to predict beyond normal toxicological approaches in a precautionary manner. It is recommended that more trait-based toxicity studies be conducted to aid in decision-making processes for rapid predictions of organisms' vulnerability to toxicants. The proposed WQGs for AMD in this study are scenario-specific for a coal mine in the Boesmanspruit catchment but can also act as a precautionary tool for similar mines elsewhere. Similarly, the sulphate WQGs reported here are useful in many areas affected by AMD or salinity in general. In particular, the data used to generate the WQGs in this study were from indigenous species found in freshwater ecosystems across South Africa. Finally, it is recommended that approaches to managing AMD and salinity in South Africa should be reconsidered to reflect derived guidelines in view of the current findings.

CHAPTER 6: GENERAL CONCLUSION AND RECOMMENDATIONS

6.1 Introduction

The overall aim of this study was to understand the ecological risk of acid mine drainage in a salinising landscape and series of laboratory and field experiments were conducted to achieve this. The following are summary conclusions with reference to the aim and specific objectives of this study.

6.1.1 Summary conclusions

In chapter one of this thesis, a literature review of threats posed by acid mine drainage (AMD) and sulphates to freshwater ecosystems in South Africa was conducted. The review identified key knowledge gaps in the context of ecological risks and combinations of AMD and sulphates in freshwater ecosystems. This led to the development of short-term and long-term risk-based WQGs for sulphate salts and AMD in chapters two and three. The following are general conclusions based on the specific objectives of this study.

Specific objective 1: To investigate and compare the ecological risks associated with different sulphate salts: $MgSO_4$, Na_2SO_4, and $CaSO_4$ and develop appropriate water quality guidelines using a risk-based approach (Chapter 2)

The above objective was formulated based on the notion that sulphates are a major salt component present in AMD and an ecological threat in many coal and gold mining regions globally. However, there remains a paucity of data on sulphate toxicity on a wide range of indigenous freshwater taxa in South Africa to derive relevant WQGs. This necessitated the derivation of risk-based WQGs using the sensitivity distribution (SSD) approach. Three inorganic sulphate salts were used as models of mining salinisation in South Africa. Findings revealed that a concentration of 1.158 g/L for Na_2SO_4, 0.097 g/L for $CaSO_4$, or 1.158, 0.819 g/L $MgSO_4$, or a combined salts limit of 0.71 g/L as WQGs to protect over 95% of the species after 96 hours of exposure. In addition, $CaSO_4$ was the most toxic salt to the species during short-term exposures.

The findings further revealed that Na_2SO_4 was the most toxic to the tested salts after 240 hours of exposure; and a concentration of 0.020 g/L Na_2SO_4, 0.055 g/L $CaSO_4$, or 0.108 g/L $MgSO_4$, or a combined salts limit of 0.067 g/L would be protective of over 95% species after 240 hours of exposure. Since long-term WQGs are recommended, these findings indicate that the 0.25 g/L compliance limit for South Africa is inadequate. South Africa might need a stronger regulation such as 0.10 g/L similar to that of Canada to protect its freshwater resources. Other WQGs for these salts were also derived to protect the species both in the short-term and long-term.

Specific objective 2: To evaluate the toxicity of acid mine drainage (AMD) with a view of developing scenario-specific water quality guidelines using a risk-based approach (Chapter 3)

Findings from this study revealed that AMD was toxic to the five tested freshwater species (*Adenophlebia auriculata, Burnupia stenochorias, Caridina nilotica, Pseudokirchneriella subcapitata* and *Oreochromis mossambicus*) after 96 hours and 240 hours of exposure. The species showed varied sensitivity to AMD both after short-term and long-term exposures. Generally, *B. stenochorias* was the most sensitive species to AMD during both exposure durations. *P. subcapitata* was most sensitive to AMD during short-term exposure. *O. mossambicus* tolerated AMD within short-term exposures but was more sensitive after long-term exposure than the rest of the species. Short-term and long-term scenario-specific WQGs based on AMD generated at an abandoned coal mine in the Boesmanspruit catchment in Mpumalanga Province were derived using a risk-based approach. The short-term guidelines of 0.122% and 2.724% would be protective of over 95% and about 80% of species in ecosystems considered natural and poor respectively based on Warne et al. (2004) categories. Long-term risk-based WQGs for AMD for the protection of over 95% and about 80% of species in natural and poor ecosystems are 0.014% and 0.052% respectively. These derived WQGs can act as precautionary guidelines for other AMD prior to the development of relevant and specific guidelines (if needed) to protect the ecosystem in question.

Specific objective 3: To develop a novel trait-based approach for predicting the vulnerability of taxa to TAMD (Chapter 4)

In Chapter four, the potential of using traits of organisms to predict their vulnerability to treated acid mine drainage (TAMD) was demonstrated. Laboratory test results were used to validate field based macroinvertebrate assemblages for correspondence. Findings revealed that both Leptoceridae and Leptophlebiidae were not present at a site that received direct TAMD in the study area although the majority of taxa relatively matched predictions in terms of assemblages.

Taxa relative abundance and diversity at sites that received impact from TAMD corresponded to trait-based predictions. As water quality improved further downstream from TAMD source to a moderately impacted site, taxa relative abundance and diversity also improved based on predictions. The findings also revealed that Leptoceridae was more vulnerable to TAMD than Leptophlebiidae contrary to predictions. However, it is important to note that other traits play roles in laboratory tests, and as such, predictions in the laboratory could not have been the same in the field study for all taxa. These findings add important novelty to the field of ecotoxicology for evaluation of the effects of toxicants in addition to the derived risk-based WQGs. These findings enabled the development of a novel trait-based approach for predicting the vulnerability of organisms to TAMD. This tool can be used as a rapid decision support tool for management of sulphates and AMD.

Specific objective 4: To develop water quality guidelines for the management of sulphate salts and AMD in South Africa (Chapter 5a)

Chapter five assessed management options for AMD and salinity using well-known global WQGs in relation to South Africa prior to the development of risk-based guidelines using common and indigenous species. WQGs for three inorganic sulphate salts and their combination were derived. Unlike in Chapters two and three, WQGs derived in Chapter five were derived for the protection of species at different levels with a view of different management goals. For example, the generated short-term 95% WQGs for sulphate salts were 1.158 g/L (0.750 - 1.79) Na_2SO_4, 0.819 g/L (0.502 - 1.337) $MgSO_4$, 0.097 g/L (0.049 - 0.193) $CaSO_4$ and 0.710 g/L (0.118 - 4.259) sulphate combination for the protection of over 95% of species in a natural ecosystem. The derived long-term 95% WQGs were 0.02 g/L (0.003 - 0.162) Na_2SO_4, 0.108 g/L (0.013 - 0.899) $MgSO_4$, 0.055 g/L (0.026 - 0.118) $CaSO_4$ and 0.067 g/L (0.006 - 0.745) sulphate combination for the protection of over 95% of species in an ecosystem. Different protective concentrations were also derived for the protection of species in various ecosystem categories. These guidelines serve as trigger values for different management strategies. Similarly, risk-based WQGs for AMD were also derived in Chapter five for the protection of species in different ecosystem classes.

Specific objective 5: To combine the multi-criteria analysis for the development of an integrated management strategy for AMD and increasing salinity in South Africa (Chapter 5b)

A trait-based management tool that predicts the vulnerability of taxa to aquatic stressors such as salinity and AMD and TAMD was developed. The tool integrates two sections to assist decision makers on which approach to be followed to solve ecological problems. The first approach is the trait-based protocol in simple steps that can be followed by inexperienced ecotoxicologists or non-experts to assess potential impacts of a proposed project in aquatic ecosystems. The user simply decides on the size of biodiversity of organisms to be protected in the resource. When the resource is considered natural then it is likely that there might be vulnerable organisms to the toxicant in question. This informs management of the need to strengthen the protection decisions as opposed to an ecosystem that is relatively fair or poor. However, the decisions will also be dependent on management goals and the value of the particular project's socio-economic or political factors. The second approach incorporates the SSD approach to assist decision makers in the setting of guidelines for management of salinity, AMD or TAMD. This would be useful for setting protective levels or dilutions when the decision is to protect some percentage number of species in an ecosystem category. These two approaches integrate in the sense that they guide both experienced and inexperienced ecotoxicologists about the management of the resource from aquatic stressors in once combined protocol. Although the risk-based approach is generally preferred over the hazard approach to deriving WQGs, it is quite data demanding where its strength would lie in using indigenous species tested in local environmental conditions. Similarly, there are other important traits that organisms possess which can influence their vulnerability to various aquatic stressors. Further use of the trait-based approach would refine the trait-based management tool developed in Chapter five.

6.2 Application of the study findings in the context of AMD and sulphate management

The 1996 South African WQGs are largely generic derived mostly from international short-term toxicity data. This study derived risk-based WQGs using indigenous species employing both short-term and long-term toxicity tests under local South African conditions for the protection of freshwater ecosystems from salinity and AMD. Since no sulphate salt guidelines existed in South Africa prior to this study, the findings could contribute to the refinement and improvement of the 1996 WQGs.

The trait-based predictive management tool could also be applied in different scenarios and on a wide scale of resources since it is practically impossible to exhaustively develop WQGs for each toxicant found in the environment. Further refinement of the predictive tool could have greater importance in data scarce regions for decision making about ecological problems. Since scenario-specific WQGs for AMD in South Africa are lacking despite the escalating environmental problems, the derived risk-based WQGs for AMD could contribute to management strategies during both short-term exposure and long-term exposures events. Although the guidelines are scenario-specific for AMD generated at a coal mine in Mpumalanga Province, they are important indicative guidelines for similar AMD that might be generated at other mines for precautionary management measures until site-specific WQGs are developed if deemed necessary.

6.3 Recommendations

Based on the findings of this study, the following recommendations are made for management of AMD and salinity as well as for future studies:

6.3.1 Management recommendations

- For managing decanting AMD, there is need to construct holding dams or to build structures for treating AMD at appropriate concentrations prior to release. Since water is a scarce commodity in South Africa, treated AMD can be reused, for example in irrigation. This will lessen its negative impact on the freshwater resources.
- A policy could be formulated to require any proposed mining project that potentially would generate AMD to possibly include the trait-based decision support tool in the environmental impact assessment phase prior to the actual mining activities

6.3.2 Recommendations for future studies

- The trait-based approach requires further refinement for its increased adoption in ecotoxicology. The refinement should include the development of a trait-based vulnerability index for indigenous taxa for the management of different aquatic stressors, including the ones reported in this study. The index can also guide investment priority and critical areas deemed most vulnerable to the stressors under consideration.

- The need to incorporate different life stages of the tested species to determine if their sensitivity to AMD and sulphate would correspond to current findings. This is important for the derivation of strong and relevant WQGs.
- Integration of trait-based toxicity data with SSD findings to develop a lucid decision support tool should be explored further as this would enhance the power of the WQGs.
- There is the need to revise current South African WQGs for sulphates using locally derived toxicity data. In particular, the 0.25 g/L does not seem to protect the majority of freshwater species tested in this study, especially in the long-term exposure tests.

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APPENDICES

Appendix 1A: Protocol for Acid-Wash of Glassware

Procedure

1. Take off any stick-on labels, parafilm, etc.

2. Rinse once with tap water.

3. Wash thoroughly with 5 % Extran (phosphate free soap).

4. Rinse with tap water.

5. Soak in 10 % hydrochloric acid (HCl) for 10 minutes.

6. Rinse well with tap water.

7. Rinse three times with boiling water (slightly cooled).

8. Rinse three times with deionised water.

9. Leave to drip dry on paper towelling next to the sink.

Precautions

1. Use containers provided for acid wash only.

2. Wear protective gloves, laboratory coat and safety goggles when acid washing glassware.

Appendix 1B: Step-by-step calculation of ECx values using Microsoft Excel 2013

1. List concentrations in one column (A).

2. List corresponding mortality in next column (B).

3. Change concentrations to log base in next column (C) using Excel as follows: **=log(concentration value)**.

4. Change mortality to percent mortality in next column (D) using Excel as follows: **=(subjects respond/total number of subjects)*100**.

5. Select columns C and D.

6. Go to **insert**, select **scatter**, and click on **with only markers** (a scatter graph appears).

7. Click on any of the points to select them.

8. Right-click, and click on **Add Trendline.**

9. Select the boxes **Display Equation and Chart** and **Display R-squared value on Chart**, and close the display box.

10. The equation, in the form $y = ax + b$, and R-squared value are displayed on the chart.

11. Make $\boldsymbol{x}$ the subject of the formula, and substitute $\boldsymbol{y}$ with 50, 20 or 10 if you are estimating EC50, 20 or EC10, respectively ($\boldsymbol{x}$ is the calculated EC value) in Excel as follows:

=(y-b)/a.

12. Change the value of $\boldsymbol{x}$ (which is in log form) back to a non-log form as in Excel follows **=power (10,*x*)**.

Appendix 1C: Short-term protective concentrations for different salts based on short-term 96-hour LC/EC50 values

Table C.1: Model parameters **(a)** and statistics **(b)** for species sensitivity distribution (SSD) curve of indigenous and common South African freshwater aquatic species based on short-term 96-hour LC/EC50 values for sodium sulphate using the Species Sensitivity Distribution Generator

a.

PARAMETERS	
Slope	3.743
Intercept	3.116
R^2	0.963
GrandMean	0.503
SumSQ	1.530
CSSQ	0.264
MSE	0.047
Tcrit	2.353
N	5
df	3

b.

Proportion	**Probit**	**Log Central Tendency**	**SSQ**	**Log Upper PI**	**Log Lower PI**	**Central Tendency**	**Upper PI**	**Lower PI**
0.05	3.355	0.064	0.006	0.253	-0.125	1.158	1.790	0.750
0.1	3.718	0.161	0.005	0.335	-0.013	1.448	2.164	0.969
0.2	4.158	0.278	0.005	0.439	0.118	1.899	2.746	1.312
0.4	4.747	0.436	0.004	0.586	0.286	2.726	3.851	1.930
0.5	5.000	0.503	0.004	0.652	0.354	3.186	4.490	2.261
0.7	5.524	0.643	0.004	0.797	0.490	4.400	6.264	3.090
0.8	5.842	0.728	0.005	0.889	0.568	5.348	7.736	3.697
0.9	6.282	0.846	0.005	1.020	0.671	7.010	10.472	4.692
0.95	6.645	0.943	0.006	1.132	0.754	8.766	13.544	5.673

Table C.2: Model parameters **(a)** and statistics **(b)** for species sensitivity distribution (SSD) curve of indigenous and common South African freshwater aquatic species based on short-term 96-hour LC/EC50 values for magnesium sulphate using the Species Sensitivity Distribution Generator

a.

PARAMETERS	
Slope	1.462
Intercept	3.482
R^2	0.993
GrandMean	1.038
SumSQ	7.170
CSSQ	1.780
MSE	0.009
Tcrit	2.353
N	5
df	3

b.

Proportion	**Probit**	**Log Central Tendency**	**SSQ**	**Log Upper PI**	**Log Lower PI**	**Central Tendency**	**Upper PI**	**Lower PI**
0.05	3.355	-0.086	0.008	0.126	-0.299	0.819	1.337	0.502
0.1	3.718	0.162	0.007	0.358	-0.035	1.452	2.282	0.924
0.2	4.158	0.463	0.006	0.644	0.282	2.902	4.404	1.913
0.4	4.747	0.865	0.005	1.035	0.695	7.329	10.831	4.959
0.5	5.000	1.038	0.005	1.207	0.870	10.921	16.097	7.410
0.7	5.524	1.397	0.005	1.570	1.223	24.938	37.182	16.726
0.8	5.842	1.614	0.006	1.795	1.433	41.094	62.352	27.084
0.9	6.282	1.915	0.007	2.111	1.718	82.150	129.136	52.259
0.95	6.645	2.163	0.008	2.376	1.950	145.558	237.480	89.217

Table C.3: Model parameters **(a)** and statistics **(b)** for species sensitivity distribution (SSD) curve of indigenous and common South African freshwater aquatic species based on short-term 96-hour LC/EC50 values for calcium sulphate using the Species Sensitivity Distribution Generator

a.

PARAMETERS	
Slope	0.605
Intercept	3.968
R^2	0.998
GrandMean	1.705
SumSQ	24.975
CSSQ	10.444
MSE	0.003
Tcrit	2.353
N	5
df	3

b.

Proportion	Probit	Log Central Tendency	SSQ	Log Upper PI	Log Lower PI	Central Tendency	Upper PI	Lower PI
0.05	3.355	-1.013	0.016	-0.714	-1.312	0.097	0.193	0.049
0.1	3.718	-0.413	0.014	-0.136	-0.689	0.387	0.731	0.205
0.2	4.158	0.314	0.012	0.569	0.059	2.061	3.707	1.146
0.4	4.747	1.286	0.010	1.525	1.047	19.328	33.501	11.151
0.5	5.000	1.705	0.010	1.942	1.468	50.675	87.501	29.347
0.7	5.524	2.571	0.011	2.815	2.327	372.615	653.859	212.342
0.8	5.842	3.095	0.012	3.350	2.841	1245.674	2240.123	692.686
0.9	6.282	3.822	0.014	4.099	3.546	6642.050	12552.256	3514.653
0.95	6.645	4.423	0.016	4.722	4.124	26459.823	52680.975	13289.850

Table C.4: Model parameters **(a)** and statistics **(b)** for species sensitivity distribution (SSD) curve of indigenous and common South African freshwater aquatic species based on short-term 96-hour LC/EC50 values for combined sulphate salts using the Species Sensitivity Distribution Generator

a.

PARAMETERS	
Slope	1.336
Intercept	3.554
R^2	0.922
GrandMean	1.082
SumSQ	7.836
CSSQ	1.981
MSE	0.099
Tcrit	2.353
N	5
df	3

b.

Proportion	**Probit**	**Log Central Tendency**	**SSQ**	**Log Upper PI**	**Log Lower PI**	**Central Tendency**	**Upper PI**	**Lower PI**
0.05	3.355	-0.149	0.109	0.629	-0.927	0.710	4.259	0.118
0.1	3.718	0.123	0.093	0.839	-0.593	1.328	6.905	0.255
0.2	4.158	0.452	0.078	1.109	-0.205	2.833	12.857	0.624
0.4	4.747	0.893	0.068	1.505	0.280	7.808	32.001	1.905
0.5	5.000	1.082	0.067	1.690	0.474	12.081	48.998	2.979
0.7	5.524	1.475	0.071	2.102	0.847	29.823	126.473	7.032
0.8	5.842	1.712	0.078	2.369	1.055	51.516	233.767	11.353
0.9	6.282	2.041	0.093	2.757	1.325	109.940	571.833	21.137
0.95	6.645	2.313	0.109	3.091	1.535	205.604	1233.476	34.271

Appendix 1D: Long-term protective concentrations for different salts based on long-term 240-hour LC/EC10 values

Table D.1: Model parameters **(a)** and statistics **(b)** for species sensitivity distribution (SSD) curve of indigenous and common South African freshwater aquatic species based on long-term 240-hour LC/EC10 values for sodium sulphate using the Species Sensitivity Distribution Generator

a.

PARAMETERS	
Slope	1.318
Intercept	5.590
R^2	0.899
GrandMean	-0.447
SumSQ	2.985
CSSQ	1.984
MSE	0.129
Tcrit	2.353
N	5
df	3

b.

Proportion	**Probit**	**Log Central Tendency**	**SSQ**	**Log Upper PI**	**Log Lower PI**	**Central Tendency**	**Upper PI**	**Lower PI**
0.05	3.355	-1.695	0.147	-0.792	-2.599	0.020	0.162	0.003
0.1	3.718	-1.420	0.124	-0.589	-2.250	0.038	0.257	0.006
0.2	4.158	-1.086	0.104	-0.326	-1.846	0.082	0.472	0.014
0.4	4.747	-0.640	0.090	0.068	-1.347	0.229	1.170	0.045
0.5	5.000	-0.447	0.089	0.255	-1.150	0.357	1.799	0.071
0.7	5.524	-0.049	0.095	0.676	-0.775	0.892	4.742	0.168
0.8	5.842	0.191	0.104	0.951	-0.569	1.553	8.942	0.270
0.9	6.282	0.525	0.124	1.355	-0.305	3.349	22.658	0.495
0.95	6.645	0.801	0.147	1.704	-0.103	6.318	50.581	0.789

Table D.2: Model parameters **(a)** and statistics **(b)** for species sensitivity distribution (SSD) curve of indigenous and common South African freshwater aquatic species based on long-term 240-hour LC/EC10 values for magnesium sulphate using the Species Sensitivity Distribution Generator

a.

PARAMETERS	
Slope	1.471
Intercept	4.777
R^2	0.871
GrandMean	0.151
SumSQ	1.660
CSSQ	1.545
MSE	0.165
Tcrit	2.353
N	5
df	3

b.

Proportion	**Probit**	**Log Central Tendency**	**SSQ**	**Log Upper PI**	**Log Lower PI**	**Central Tendency**	**Upper PI**	**Lower PI**
0.05	3.355	-0.967	0.153	-0.046	-1.888	0.108	0.899	0.013
0.1	3.718	-0.720	0.129	0.125	-1.565	0.191	1.332	0.027
0.2	4.158	-0.421	0.108	0.351	-1.192	0.379	2.243	0.064
0.4	4.747	-0.021	0.093	0.696	-0.738	0.953	4.968	0.183
0.5	5.000	0.151	0.091	0.863	-0.560	1.417	7.291	0.276
0.7	5.524	0.508	0.098	1.243	-0.227	3.222	17.515	0.593
0.8	5.842	0.724	0.108	1.495	-0.048	5.294	31.291	0.896
0.9	6.282	1.023	0.129	1.868	0.178	10.543	73.711	1.508
0.95	6.645	1.270	0.153	2.191	0.349	18.622	155.099	2.236

Table D.3: Model parameters **(a)** and statistics **(b)** for species sensitivity distribution (SSD) curve of indigenous and common South African freshwater aquatic species based on long-term 240-hour LC/EC10 values for calcium sulphate using the Species Sensitivity Distribution Generator

a.

PARAMETERS	
Slope	1.335
Intercept	5.036
R^2	0.985
GrandMean	-0.027
SumSQ	2.124
CSSQ	2.120
MSE	0.019
Tcrit	2.353
N	5
df	3

b.

Proportion	**Probit**	**Log Central Tendency**	**SSQ**	**Log Upper PI**	**Log Lower PI**	**Central Tendency**	**Upper PI**	**Lower PI**
0.05	3.355	-1.259	0.020	-0.926	-1.592	0.055	0.118	0.026
0.1	3.718	-0.987	0.017	-0.680	-1.295	0.103	0.209	0.051
0.2	4.158	-0.658	0.014	-0.374	-0.941	0.220	0.422	0.115
0.4	4.747	-0.217	0.013	0.048	-0.482	0.607	1.118	0.329
0.5	5.000	-0.027	0.013	0.236	-0.291	0.939	1.723	0.512
0.7	5.524	0.366	0.013	0.637	0.094	2.320	4.334	1.242
0.8	5.842	0.603	0.014	0.886	0.320	4.010	7.699	2.089
0.9	6.282	0.933	0.017	1.240	0.625	8.564	17.383	4.219
0.95	6.645	1.205	0.020	1.538	0.872	16.025	34.487	7.446

Table D.4: Model parameters **(a)** and statistics **(b)** for species sensitivity distribution (SSD) curve of indigenous and common South African freshwater aquatic species based on long-term 240-hour LC/EC10 values for combined sulphate salts using the Species Sensitivity Generator

a.

PARAMETERS	
Slope	1.546
Intercept	5.166
R^2	0.821
GrandMean	-0.108
SumSQ	1.376
CSSQ	1.318
MSE	0.228
Tcrit	2.353
N	5
df	3

b.

Proportion	**Probit**	**Log Central Tendency**	**SSQ**	**Log Upper PI**	**Log Lower PI**	**Central Tendency**	**Upper PI**	**Lower PI**
0.05	3.355	-1.172	0.197	-0.128	-2.216	0.067	0.745	0.006
0.1	3.718	-0.937	0.165	0.018	-1.891	0.116	1.042	0.013
0.2	4.158	-0.652	0.136	0.216	-1.521	0.223	1.645	0.030
0.4	4.747	-0.272	0.117	0.532	-1.075	0.535	3.405	0.084
0.5	5.000	-0.108	0.115	0.689	-0.905	0.780	4.889	0.125
0.7	5.524	0.232	0.123	1.057	-0.594	1.704	11.401	0.255
0.8	5.842	0.437	0.136	1.305	-0.432	2.734	20.191	0.370
0.9	6.282	0.721	0.165	1.676	-0.233	5.264	47.406	0.585
0.95	6.645	0.956	0.197	2.000	-0.088	9.044	100.058	0.817

Appendix 2A: Short-term protective dilutions for acid mine drainage occurring at a coal mine in the Boesmanspruit catchment

Table B.1: Model parameters **(a)** and statistics **(b)** for species sensitivity distribution (SSD) curve of the tested indigenous and common South African freshwater aquatic species based on short-term (96 hours) LD/ED50 values for acid mine drainage using the Species Sensitivity Generator

a.

PARAMETERS	
Slope	1.606
Intercept	4.825
R^2	0.932
GrandMean	0.109
SumSQ	1.444
CSSQ	1.385
MSE	0.087
Tcrit	2.353
N	5
df	3

b.

Proportion	**Probit**	**Log Central Tendency**	**SSQ**	**Log Upper PI**	**Log Lower PI**	**Central Tendency**	**Upper PI**	**Lower PI**
0.05	3.355	-0.915	0.066	-0.310	-1.520	0.122	0.489	0.030
0.1	3.718	-0.689	0.056	-0.132	-1.246	0.205	0.738	0.057
0.2	4.158	-0.415	0.047	0.096	-0.926	0.384	1.247	0.118
0.4	4.747	-0.049	0.041	0.428	-0.526	0.893	2.680	0.298
0.5	5.000	0.109	0.040	0.582	-0.365	1.285	3.822	0.432
0.7	5.524	0.435	0.043	0.924	-0.053	2.724	8.390	0.885
0.8	5.842	0.633	0.047	1.144	0.122	4.293	13.929	1.323
0.9	6.282	0.907	0.056	1.464	0.350	8.066	29.077	2.237
0.95	6.645	1.133	0.066	1.738	0.528	13.578	54.651	3.373

Appendix 2B: Long-term protective dilutions for acid mine drainage occurring at a coal mine in the Boesmanspruit catchment.

Table B.2: Model parameters **(a)** and statistics **(b)** for species sensitivity distribution (SSD) curve of the tested indigenous and common South African freshwater aquatic species based on long-term (240 hours) LD/ED10 values for acid mine drainage using the Species Sensitivity Generator

a.

PARAMETERS	
Slope	3.890
Intercept	10.534
R^2	0.966
GrandMean	-1.423
SumSQ	10.366
CSSQ	0.245
MSE	0.043
Tcrit	2.353
N	5
df	3

b.

Proportion	**Probit**	**Log Central Tendency**	**SSQ**	**Log Upper PI**	**Log Lower PI**	**Central Tendency**	**Upper PI**	**Lower PI**
0.05	3.355	-1.846	0.006	-1.670	-2.021	0.014	0.021	0.010
0.1	3.718	-1.752	0.005	-1.591	-1.914	0.018	0.026	0.012
0.2	4.158	-1.639	0.004	-1.490	-1.788	0.023	0.032	0.016
0.4	4.747	-1.488	0.003	-1.349	-1.627	0.033	0.045	0.024
0.5	5.000	-1.423	0.003	-1.285	-1.561	0.038	0.052	0.027
0.7	5.524	-1.288	0.004	-1.146	-1.430	0.052	0.072	0.037
0.8	5.842	-1.206	0.004	-1.058	-1.355	0.062	0.088	0.044
0.9	6.282	-1.093	0.005	-0.932	-1.255	0.081	0.117	0.056
0.95	6.645	-1.000	0.006	-0.825	-1.175	0.100	0.150	0.067

Appendix 2C: Water quality parameter readings during exposures

Organism	Toxicant	Time	% Dilution	DO	pH	EC
Mayfly	AMD	96	Control	7.03	6.46	0.15
Mayfly	AMD	96	Control	7.03	6.46	0.15
Mayfly	AMD	96	Control	7.03	6.46	0.15
Mayfly	AMD	96	0.048	6.98	6.32	0.168
Mayfly	AMD	96	0.048	6.98	6.32	0.168
Mayfly	AMD	96	0.048	6.98	6.32	0.168
Mayfly	AMD	96	0.078	7.04	6.24	0.2
Mayfly	AMD	96	0.078	7.04	6.24	0.2
Mayfly	AMD	96	0.078	7.04	6.24	0.2
Mayfly	AMD	96	0.097	6.45	6.21	0.21
Mayfly	AMD	96	0.097	6.45	6.21	0.21
Mayfly	AMD	96	0.097	6.45	6.21	0.21
Mayfly	AMD	96	0.195	6.64	6.2	0.23
Mayfly	AMD	96	0.195	6.64	6.2	0.23
Mayfly	AMD	96	0.195	6.64	6.2	0.23
Mayfly	AMD	96	0.39	6.15	6.19	0.25
Mayfly	AMD	96	0.39	6.15	6.19	0.25
Mayfly	AMD	96	0.39	6.15	6.19	0.25
Mayfly	AMD	96	1.56	5.84	6.05	0.26
Mayfly	AMD	96	1.56	5.84	6.05	0.26
Mayfly	AMD	96	1.56	5.84	6.05	0.26
Mayfly	AMD	96	3.125	5.68	5.96	0.34
Mayfly	AMD	96	3.125	5.68	5.96	0.34
Mayfly	AMD	96	3.125	5.68	5.96	0.34

Organism	Toxicant	Time	% Dilution	DO	pH	EC
Mayfly	AMD	96	6.25	5.54	5.51	0.49
Mayfly	AMD	96	6.25	5.54	5.51	0.49
Mayfly	AMD	96	6.25	5.54	5.51	0.49
Mayfly	AMD	240	Control	7.03	6.46	0.15
Mayfly	AMD	240	Control	7.03	6.46	0.15
Mayfly	AMD	240	Control	7.03	6.46	0.15
Mayfly	AMD	240	0.048	6.98	6.32	0.168
Mayfly	AMD	240	0.048	6.98	6.32	0.168
Mayfly	AMD	240	0.048	6.98	6.32	0.168
Mayfly	AMD	240	0.078	7.04	6.24	0.2
Mayfly	AMD	240	0.078	7.04	6.24	0.2
Mayfly	AMD	240	0.078	7.04	6.24	0.2
Mayfly	AMD	240	0.097	6.45	6.21	0.21
Mayfly	AMD	240	0.097	6.45	6.21	0.21
Mayfly	AMD	240	0.097	6.45	6.21	0.21
Mayfly	AMD	240	0.195	6.64	6.2	0.23
Mayfly	AMD	240	0.195	6.64	6.2	0.23
Mayfly	AMD	240	0.195	6.64	6.2	0.23
Mayfly	AMD	240	0.39	6.15	6.19	0.25
Mayfly	AMD	240	0.39	6.15	6.19	0.25
Mayfly	AMD	240	0.39	6.15	6.19	0.25
Mayfly	AMD	240	1.56	5.84	6.05	0.26
Mayfly	AMD	240	1.56	5.84	6.05	0.26
Mayfly	AMD	240	1.56	5.84	6.05	0.26

Organism	Toxicant	Time	% Dilution	DO	pH	EC
Mayfly	AMD	240	3.125	5.68	5.96	0.34
Mayfly	AMD	240	3.125	5.68	5.96	0.34
Mayfly	AMD	240	3.125	5.68	5.96	0.34
Mayfly	AMD	240	6.25	5.54	5.51	0.49
Mayfly	AMD	240	6.25	5.54	5.51	0.49
Mayfly	AMD	240	6.25	5.54	5.51	0.49
Shrimp	AMD	96	Control	6.78	8.22	0.272
Shrimp	AMD	96	Control	6.78	8.22	0.272
Shrimp	AMD	96	Control	6.78	8.22	0.272
Shrimp	AMD	96	0.048	6.68	6.09	0.281
Shrimp	AMD	96	0.048	6.68	6.09	0.281
Shrimp	AMD	96	0.048	6.68	6.09	0.281
Shrimp	AMD	96	0.078	6.69	6.04	0.303
Shrimp	AMD	96	0.078	6.69	6.04	0.303
Shrimp	AMD	96	0.078	6.69	6.04	0.303
Shrimp	AMD	96	0.097	6.82	5.81	0.314
Shrimp	AMD	96	0.097	6.82	5.81	0.314
Shrimp	AMD	96	0.097	6.82	5.81	0.314
Shrimp	AMD	96	0.195	6.93	5.73	0.318
Shrimp	AMD	96	0.195	6.93	5.73	0.318
Shrimp	AMD	96	0.195	6.93	5.73	0.318
Shrimp	AMD	96	0.39	6.89	4.98	0.324
Shrimp	AMD	96	0.39	6.89	4.98	0.324
Shrimp	AMD	96	0.39	6.89	4.98	0.324

Organism	Toxicant	Time	% Dilution	DO	pH	EC
Shrimp	AMD	96	1.56	6.88	4.88	0.405
Shrimp	AMD	96	1.56	6.88	4.88	0.405
Shrimp	AMD	96	1.56	6.88	4.88	0.405
Shrimp	AMD	96	3.125	7	4.53	0.538
Shrimp	AMD	96	3.125	7	4.53	0.538
Shrimp	AMD	96	3.125	7	4.53	0.538
Shrimp	AMD	96	6.25	6.85	4	0.752
Shrimp	AMD	96	6.25	6.85	4	0.752
Shrimp	AMD	96	6.25	6.85	4	0.752
Shrimp	AMD	240	Control	6.78	8.22	0.272
Shrimp	AMD	240	Control	6.78	8.22	0.272
Shrimp	AMD	240	Control	6.78	8.22	0.272
Shrimp	AMD	240	0.048	6.68	6.09	0.281
Shrimp	AMD	240	0.048	6.68	6.09	0.281
Shrimp	AMD	240	0.048	6.68	6.09	0.281
Shrimp	AMD	240	0.078	6.69	6.04	0.303
Shrimp	AMD	240	0.078	6.69	6.04	0.303
Shrimp	AMD	240	0.078	6.69	6.04	0.303
Shrimp	AMD	240	0.097	6.82	5.81	0.314
Shrimp	AMD	240	0.097	6.82	5.81	0.314
Shrimp	AMD	240	0.097	6.82	5.81	0.314
Shrimp	AMD	240	0.195	6.93	5.73	0.318
Shrimp	AMD	240	0.195	6.93	5.73	0.318
Shrimp	AMD	240	0.195	6.93	5.73	0.318

Organism	Toxicant	Time	% Dilution	DO	pH	EC
Shrimp	AMD	240	0.39	6.89	4.98	0.324
Shrimp	AMD	240	0.39	6.89	4.98	0.324
Shrimp	AMD	240	0.39	6.89	4.98	0.324
Shrimp	AMD	240	1.56	6.88	4.88	0.405
Shrimp	AMD	240	1.56	6.88	4.88	0.405
Shrimp	AMD	240	1.56	6.88	4.88	0.405
Shrimp	AMD	240	3.125	7	4.53	0.538
Shrimp	AMD	240	3.125	7	4.53	0.538
Shrimp	AMD	240	3.125	7	4.53	0.538
Shrimp	AMD	240	6.25	6.85	4	0.752
Shrimp	AMD	240	6.25	6.85	4	0.752
Shrimp	AMD	240	6.25	6.85	4	0.752
Limpet	AMD	96	Control	6.51	8.16	0.18
Limpet	AMD	96	Control	6.51	8.16	0.18
Limpet	AMD	96	Control	6.51	8.16	0.18
Limpet	AMD	96	0.048	6.39	6.48	0.27
Limpet	AMD	96	0.048	6.39	6.48	0.27
Limpet	AMD	96	0.048	6.39	6.48	0.27
Limpet	AMD	96	0.078	6.46	6.46	0.28
Limpet	AMD	96	0.078	6.46	6.46	0.28
Limpet	AMD	96	0.078	6.46	6.46	0.28
Limpet	AMD	96	0.097	6.38	6.42	0.34
Limpet	AMD	96	0.097	6.38	6.42	0.34
Limpet	AMD	96	0.097	6.38	6.42	0.34

Organism	**Toxicant**	**Time**	**% Dilution**	**DO**	**pH**	**EC**
Limpet	AMD	96	0.195	6.39	6.27	0.37
Limpet	AMD	96	0.195	6.39	6.27	0.37
Limpet	AMD	96	0.195	6.39	6.27	0.37
Limpet	AMD	96	0.39	6.83	5.76	0.46
Limpet	AMD	96	0.39	6.83	5.76	0.46
Limpet	AMD	96	0.39	6.83	5.76	0.46
Limpet	AMD	96	1.56	6.83	4.92	0.352
Limpet	AMD	96	1.56	6.83	4.92	0.352
Limpet	AMD	96	1.56	6.83	4.92	0.352
Limpet	AMD	96	3.125	6.87	4.37	0.483
Limpet	AMD	96	3.125	6.87	4.37	0.483
Limpet	AMD	96	3.125	6.87	4.37	0.483
Limpet	AMD	96	6.25	6.79	5.02	0.821
Limpet	AMD	96	6.25	6.79	5.02	0.821
Limpet	AMD	96	6.25	6.79	5.02	0.821
Limpet	AMD	240	Control	6.51	8.16	0.18
Limpet	AMD	240	Control	6.51	8.16	0.18
Limpet	AMD	240	Control	6.51	8.16	0.18
Limpet	AMD	240	0.048	6.39	6.48	0.27
Limpet	AMD	240	0.048	6.39	6.48	0.27
Limpet	AMD	240	0.048	6.39	6.48	0.27
Limpet	AMD	240	0.078	6.46	6.46	0.28
Limpet	AMD	240	0.078	6.46	6.46	0.28
Limpet	AMD	240	0.078	6.46	6.46	0.28

Organism	Toxicant	Time	% Dilution	DO	pH	EC
Limpet	AMD	240	0.097	6.38	6.42	0.34
Limpet	AMD	240	0.097	6.38	6.42	0.34
Limpet	AMD	240	0.097	6.38	6.42	0.34
Limpet	AMD	240	0.195	6.39	6.27	0.37
Limpet	AMD	240	0.195	6.39	6.27	0.37
Limpet	AMD	240	0.195	6.39	6.27	0.37
Limpet	AMD	240	0.39	6.83	5.76	0.46
Limpet	AMD	240	0.39	6.83	5.76	0.46
Limpet	AMD	240	0.39	6.83	5.76	0.46
Limpet	AMD	240	1.56	6.83	4.92	0.352
Limpet	AMD	240	1.56	6.83	4.92	0.352
Limpet	AMD	240	1.56	6.83	4.92	0.352
Limpet	AMD	240	3.125	6.87	4.37	0.483
Limpet	AMD	240	3.125	6.87	4.37	0.483
Limpet	AMD	240	3.125	6.87	4.37	0.483
Limpet	AMD	96	6.25	6.79	5.02	0.821
Limpet	AMD	96	6.25	6.79	5.02	0.821
Limpet	AMD	96	6.25	6.79	5.02	0.821
Fish	AMD	96	Control	6.33	8.6	0.065
Fish	AMD	96	Control	6.33	8.6	0.065
Fish	AMD	96	Control	6.33	8.6	0.065
Fish	AMD	96	0.048	6.01	8.61	0.122
Fish	AMD	96	0.048	6.01	8.61	0.122
Fish	AMD	96	0.048	6.01	8.61	0.122

Organism	Toxicant	Time	% Dilution	DO	pH	EC
Fish	AMD	96	0.078	5.97	8.6	0.191
Fish	AMD	96	0.078	5.97	8.6	0.191
Fish	AMD	96	0.078	5.97	8.6	0.191
Fish	AMD	96	0.097	5.95	8.55	0.341
Fish	AMD	96	0.097	5.95	8.55	0.341
Fish	AMD	96	0.097	5.95	8.55	0.341
Fish	AMD	96	0.195	5.89	8.46	0.392
Fish	AMD	96	0.195	5.89	8.46	0.392
Fish	AMD	96	0.195	5.89	8.46	0.392
Fish	AMD	96	0.39	5.85	8.28	0.536
Fish	AMD	96	0.39	5.85	8.28	0.536
Fish	AMD	96	0.39	5.85	8.28	0.536
Fish	AMD	96	1.56	5.8	7.83	1.568
Fish	AMD	96	1.56	5.8	7.83	1.568
Fish	AMD	96	1.56	5.8	7.83	1.568
Fish	AMD	96	3.125	5.81	7.69	1.79
Fish	AMD	96	3.125	5.81	7.69	1.79
Fish	AMD	96	3.125	5.81	7.69	1.79
Fish	AMD	96	6.25	5.78	7.51	2.56
Fish	AMD	96	6.25	5.78	7.51	2.56
Fish	AMD	96	6.25	5.78	7.51	2.56
Fish	AMD	240	Control	6.33	8.6	0.065
Fish	AMD	240	Control	6.33	8.6	0.065
Fish	AMD	240	Control	6.33	8.6	0.065

Organism	Toxicant	Time	% Dilution	DO	pH	EC
Fish	AMD	240	0.048	6.01	8.61	0.122
Fish	AMD	240	0.048	6.01	8.61	0.122
Fish	AMD	240	0.048	6.01	8.61	0.122
Fish	AMD	240	0.078	5.97	8.6	0.191
Fish	AMD	240	0.078	5.97	8.6	0.191
Fish	AMD	240	0.078	5.97	8.6	0.191
Fish	AMD	240	0.097	5.95	8.55	0.341
Fish	AMD	240	0.097	5.95	8.55	0.341
Fish	AMD	240	0.097	5.95	8.55	0.341
Fish	AMD	240	0.195	5.89	8.46	0.392
Fish	AMD	240	0.195	5.89	8.46	0.392
Fish	AMD	240	0.195	5.89	8.46	0.392
Fish	AMD	240	0.39	5.85	8.28	0.536
Fish	AMD	240	0.39	5.85	8.28	0.536
Fish	AMD	240	0.39	5.85	8.28	0.536
Fish	AMD	240	1.56	5.8	7.83	1.568
Fish	AMD	240	1.56	5.8	7.83	1.568
Fish	AMD	240	1.56	5.8	7.83	1.568
Fish	AMD	240	3.125	5.81	7.69	1.79
Fish	AMD	240	3.125	5.81	7.69	1.79
Fish	AMD	240	3.125	5.81	7.69	1.79
Fish	AMD	240	6.25	5.78	7.51	2.56
Fish	AMD	240	6.25	5.78	7.51	2.56
Fish	AMD	240	6.25	5.78	7.51	2.56

Appendix 3A: Ecotoxicological data analysis summary tables

a. **96 hours toxicity summary output**

```
model1<- drm(Mortality/Total~Lime,Organism,subset=Time=="96",weights=Total, fct=LL.2(),data=
mydata)
> summary(model1)

Model fitted: Log-logistic (ED50 as parameter) with lower limit at 0 and upper limit at 1 (2 parms)

Parameter estimates:

                   Estimate Std. Error  t-value p-value
b:Leptophlebiidae -1.49262    0.38358 -3.89127  0.0004
b:Leptoceridae    -0.92365    0.24603 -3.75426  0.0006
e:Leptophlebiidae  7.91887    1.30858  6.05149  0.0000
e:Leptoceridae     3.98858    1.26130  3.16229  0.0031

Residual standard error: 1.684936 (38 degrees of freedom)
```

b. **240 h toxicity summary output**

```
model2<- drm(Mortality/Total~Lime,Organism,subset=Time=="240",weights=Total, fct=LL.2(),data
=mydata)
> summary(model2)

Model fitted: Log-logistic (ED50 as parameter) with lower limit at 0 and upper limit at 1 (2 parms)

Parameter estimates:
                   Estimate Std. Error  t-value p-value
b:Leptophlebiidae -2.62030    0.39137 -6.69523  0.0000
b:Leptoceridae    -1.20560    0.78081 -1.54402  0.1309
e:Leptophlebiidae  3.65448    0.19915 18.35055  0.0000
e:Leptoceridae     0.51618    0.69430  0.74345  0.4618
```

Residual standard error: 0.7003131 (38 degrees of freedom)

c. Test for model lack of fit summary

---

1. modelFit(model1) – 96 hours

Lack-of-fit test

	Model Df	RSS	Df	F value	p value
ANOVA	28	0.1667			
DRC model	38	18.6367	10	310.3	0.0

2. modelFit(model2) – 240 hours

Lack-of-fit test

	Model Df	RSS	Df	F value	p value
ANOVA	28	0.1667			
DRC model	38	18.6367	10	310.3	0.0

d. Summary output for water quality parameters' analyses - Leptophlebiidae

---

```
model.1 <- (lm(Mortality/Total~Lime*DO*EC*pH, data=mydata))
Residuals:
    Min       1Q   Median       3Q      Max
-0.44560 -0.08482 -0.00211  0.09528  0.40742

Coefficients:
              Estimate Std. Error t value Pr(>|t|)
(Intercept)  -3.777171   4.504206  -0.839 0.404639
Lime          0.728969   0.290507   2.509 0.014485 *
DO            0.679323   0.908204   0.748 0.457049
EC           -0.339883   2.402830  -0.141 0.887932
pH            0.589360   0.623476   0.945 0.347862
Lime:DO      -0.179468   0.045376  -3.955 0.000185 ***
Lime:EC      -0.130995   0.124452  -1.053 0.296264
DO:EC         0.117520   0.286170   0.411 0.682609
Lime:pH       0.058957   0.048978   1.204 0.232863
DO:pH        -0.097922   0.112289  -0.872 0.386246
EC:pH        -0.137152   0.608960  -0.225 0.822481
Lime:DO:EC    0.034566   0.015953   2.167 0.033763 *
```

```
Lime:DO:pH     0.006782   0.006187   1.096 0.276833
Lime:EC:pH    -0.012052   0.017971  -0.671 0.504735
DO:EC:pH       0.006788   0.066464   0.102 0.918949
Lime:DO:EC:pH -0.001381   0.002156  -0.641 0.523934
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.177 on 68 degrees of freedom
Multiple R-squared:  0.8143,            Adjusted R-squared:  0.7734
F-statistic: 19.88 on 15 and 68 DF,  p-value: < 2.2e-16
```

e. Summary output for water quality parameters' analyses - Leptoceridae

```
model.1 <- (lm(Mortality/Total~Lime*DO*EC*pH, data=mydata))
Residuals:
     Min       1Q   Median       3Q      Max
-0.44560 -0.08482 -0.00211  0.09528  0.40742

Coefficients:
              Estimate Std. Error t value Pr(>|t|)
(Intercept)   -3.777171   4.504206  -0.839 0.404639
Lime           0.728969   0.290507   2.509 0.014485 *
DO             0.679323   0.908204   0.748 0.457049
EC            -0.339883   2.402830  -0.141 0.887932
pH             0.589360   0.623476   0.945 0.347862
Lime:DO       -0.179468   0.045376  -3.955 0.000185 ***
Lime:EC       -0.130995   0.124452  -1.053 0.296264
DO:EC          0.117520   0.286170   0.411 0.682609
Lime:pH        0.058957   0.048978   1.204 0.232863
DO:pH         -0.097922   0.112289  -0.872 0.386246
EC:pH         -0.137152   0.608960  -0.225 0.822481
Lime:DO:EC     0.034566   0.015953   2.167 0.033763 *
Lime:DO:pH     0.006782   0.006187   1.096 0.276833
Lime:EC:pH    -0.012052   0.017971  -0.671 0.504735
```

```
DO:EC:pH       0.006788   0.066464   0.102 0.918949
Lime:DO:EC:pH -0.001381   0.002156  -0.641 0.523934
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 0.177 on 68 degrees of freedom
Multiple R-squared:  0.8143,	Adjusted R-squared:  0.7734
F-statistic: 19.88 on 15 and 68 DF,  p-value: < 2.2e-16
```

Appendix 3B: Summary of Permutation test results to assess association of macroinvertebrates occurring in all sites (s.1=Site A, s.2=Site B, s.3=Site C).

indval$sign

	s.1	**s.2**	**s.3**	**index**	**stat**	**p.value**
Baetidae.1sp	1	0	0	1	0.7071068	1
Beatidae.2sp	1	0	1	5	0.7071068	1
Leptophlebiidae	0	0	1	3	1	0.199
Heptageniidae	1	0	0	1	0.7071068	1
Caenidae	1	0	1	5	0.7071068	1
Aeshnidae	1	0	0	1	0.7071068	1
Synlestidae	1	0	0	1	0.7071068	1
Gomphidae	1	0	0	1	0.7071068	1
Proitoneuridae	1	0	0	1	0.7071068	1
Chlorocyphidae	0	0	1	3	0.7071068	1
Lestidae	1	0	0	1	0.7071068	1
Hydropsychidae.1sp	1	0	0	1	0.7071068	1
Hydropsychidae.2sp	0	0	1	3	1	0.199
Hydropsychidae..2sp	1	0	0	1	0.7071068	1
Ecnomidae	1	0	1	5	1	0.197
Elmidae	1	0	0	1	0.9607689	0.23
Dytiscidae	**1**	**1**	**1**	7	**0.7071068**	**NA**
Ceratopogonidae	1	0	0	1	0.7071068	1
Chironomidae	0	0	1	3	0.9017118	0.377
Muscidae	0	0	1	3	0.9258201	0.199
Simuliidae	1	0	0	1	0.7071068	1
Syphidae	1	0	0	1	0.7071068	1
Ephydridae.shore.flies.	0	0	1	3	0.7071068	1
Culicidae	0	0	1	3	0.7071068	1
Tabanidae	1	0	0	1	0.7071068	1
Athericidae	0	0	1	3	0.7071068	1
Tipulidae	0	0	1	3	0.7071068	1
Psychodidae	1	0	1	5	0.7071068	1
Corixidae	1	0	1	5	0.9979571	0.197

Naucoridae	0	1	1	6	0.7071068	1
Nepidea	0	0	1	3	0.7071068	1
Pleidae	0	1	0	2	0.7071068	1
Physidae	1	0	1	5	1	0.197
Lymnaeidae	1	0	1	5	0.8660254	0.592
flatworms	1	0	1	5	1	0.197
Corbiculidae	0	0	1	3	0.9545214	0.199

Appendix 3C: Summary of Permutation test using the function signassoc to assess association of macroinvertebrates with sites of sampling where p-value was the lowest

Sidak = signassoc(mydata,cluster=groups, alternative = "two.sided",control = how(nperm=199))

> Sidak

	1	**2**	**3**	**best**	**psidak**
Baetidae.1sp	0.66	1	1	1	0.960696
Beatidae.2sp	0.64	0.84	1	1	0.953344
Leptophlebiidae	0.75	0.93	0.11	3	0.295031
Heptageniidae	0.64	1	1	1	0.953344
Caenidae	0.64	0.84	1	1	0.953344
Aeshnidae	0.66	1	1	1	0.960696
Synlestidae	0.66	1	1	1	0.960696
Gomphidae	0.64	1	1	1	0.953344
Proitoneuridae	0.66	1	1	1	0.960696
Chlorocyphidae	1	1	0.78	3	0.989352
Lestidae	0.66	1	1	1	0.960696
Hydropsychidae.1sp	0.64	1	1	1	0.953344
Hydropsychidae.2sp	0.75	0.93	0.11	3	0.295031
Hydropsychidae..2sp	0.66	1	1	1	0.960696
Ecnomidae	0.4	0.16	0.51	2	0.407296
Elmidae	0.12	0.4	0.83	1	0.318528
Dytiscidae	0.82	0.74	1	2	0.982424
Ceratopogonidae	0.64	1	1	1	0.953344
Chironomidae	1	0.48	0.29	3	0.642089

Muscidae	0.75	0.48	0.11	3	0.295031
Simuliidae	0.66	1	1	1	0.960696
Syphidae	0.66	1	1	1	0.960696
Ephydridae	1	1	0.78	3	0.989352
Tabanidae	0.64	1	1	1	0.953344
Athericidae	1	1	0.78	3	0.989352
Tipulidae	1	1	0.78	3	0.989352
Psychodidae	0.64	0.84	1	1	0.953344
Corixidae	0.12	0.16	0.83	1	0.318528
Naucoridae	0.82	0.74	1	2	0.982424
Nepidea	1	1	0.71	3	0.975611
Pleidae	1	0.66	1	2	0.960696
Physidae	0.44	0.16	0.89	2	0.407296
Lymnaeidae	1	0.47	0.42	3	0.804888
Planaridae	0.12	0.16	0.83	1	0.318528
Corbiculidae	0.75	0.47	0.11	3	0.295031