Validation of artificial mussels as indicators of platinum group element exposure in the Hex River System

M Labuschagne

orcid.org 0000-0002-6215-9223

Dissertation submitted in fulfilment of the requirements for the degree *Masters of Science in Environmental Sciences* at the North-West University

Supervisor:	Prof V Wepener
Co-supervisor:	Dr S Zimmermann

Graduation May 2019

24279862

Abstract

Platinum group elements (PGE) occur naturally in the environment in very low concentrations. However, these elements have increased over the last couple of decades, since they have been increasingly used in a number of applications. Mining activities in South Africa have been reported to contribute prominently to PGE emissions in the environment. The knowledge of (PGE) contamination in freshwater systems is important for the risk assessment for aquatic health and water resource management. From this study it could be seen that laboratory bioaccumulation studies alone are not sufficient since organisms in natural environments are affected by different environmental conditions that influence the bioavailability of metals to organisms. PGE concentrations in living organisms from field studies give valuable information on the metals that are associated with the mining activities and the bioavailable PGE fraction and the effective PGE concentrations inside of the organisms. The use of the artificial mussel (AM) have been successfully applied for a wide range of metals in marine, and more recently, fresh water systems, but never before used for determining PGE concentrations. The design of the AM was validated and optimised in the laboratory for uptake of PGEs. An optimum loading and elution protocol was developed for platinum (Pt). It was demonstrated that the Chelex® beads in the AMs accumulate Pt in a dose-response relationship and reflect the bioavailable environmental concentrations. The AMs were exposed to environmental PGEs alongside an established bioindicator species. This obtained information on the bioaccumulation and effects of the biota and how the bioaccumulation correlates with the measured uptake by the AMs when exposed for the same period. Both the AMs and the transplanted organisms were exposed in plastic baskets at each site. Metals that were analysed during this study include As, Cd, Co, Cr, Ni, Pb, Pd, Pt, V and Zn. When the AMs were exposed in the tailings dam and the two impoundments different PGE concentrations could be found. The AMs accumulated significantly higher Pd concentrations in Bospoort Dam during the second survey, while these concentrations were similar in the two impoundments during the first survey. For Pt it could be seen that these concentrations were similar in both impoundments during the second survey and Bospoort Dam contained slightly higher Pt concentrations during the first survey. The water concentrations reflect that there is a Pd concentration gradient along the Hex River, while the Pt concentrations were similar in the two impoundments, and the tailings dam contained significantly higher PGE concentrations. The accumulation patterns within in the AMs and the transplanted clams indicated different uptake patterns. Clams will take up metals from the water both in the dissolved form and as complexes bound to particulate matter. Thus the physical and chemical conditions at the different sites and during the different surveys influence the form of the metal available for uptake by the clams. On the other hand the AMs showed great accumulation patterns for all metals. The

AMs correlated well with the concentrations found in the water column, which indicated that Bospoort Dam had higher metal concentrations, while the transplanted clams indicated that Olifantsnek Dam had higher concentrations. It was found that the clams from the reference site were exposed to high metal concentrations at the source site prior to deployment. It is therefore highly likely that the responses following deployment in the study sites were masked by the pre-exposed conditions. Mechanisms against oxidative stress were observed with increased CAT activities (non-significant increases) when exposed to conditions in Olifantsnek Dam. These responses in turn caused a depletion in energy reserves and damage to lipid and protein content of the clams. Metallothionein induction (based on total metals) were higher in Bospoort Dam, while the Pt-MT were higher in the reference clams and to a degree in Olifantsnek Dam, which correlated with Pt concentrations found in the clam tissues. This study provided some insight into PGE concentrations that can be found in PGE mining areas in South Africa, in areas that are not impacted (Olifantsnek Dam) by these activities to areas that are heavily impacted (tailings dam).

Keywords:

PGE mining activities, artificial mussel, transplantation studies, bioaccumulation, biomarker response

Acknowledgements

Writing a dissertation gives you great excitement leading to many early mornings and fieldtrips, and ends with a few mini breakdowns and some tears. Without all of these stages this dissertation would not have been possible, especially all the people who assisted, contributed and supported me along the way. I want to extend my greatest appreciation to the following:

To my main supervisor, Prof Victor Wepener, without you none of this would have been possible. Thank you for all of the opportunities that you have given me over the last couple of years, especially the support and the wise words you always have.

To my co-supervisor, Sonja Zimmermann, in a really short time period you have taught me so many new things in a field I knew nothing about. You made me feel at home in a faraway country. Thank you for joining in on the excitement when we made our first AM and the endless guidance throughout this project. Thank you for the contribution that you have made to my dissertation.

The financial assistance of the National Research Foundation (NRF) towards this research is hereby acknowledged. Opinions expressed and conclusions arrived at, are those of the author and are not necessarily to be attributed to the NRF.

Prof. Bernd Sures, Milen Nachev and everyone at the Universitat Duisburg-Essen. Thank you for making Essen feel like a second home, for the help analysing my samples, the funding to make this possible and for making my first, second and third trip abroad so much fun and leaving your mark on my academic career.

I would also like to extend my appreciation to my colleagues and friends. Wynand, Ruan and Tarryn you are always there to give advice and help with everything, I am really thankful for having Post-docs like you in the WRG. Hannes Erasmus, you have been the best senior student on this project ever. Thank you for assisting on every fieldtrip, for the endless discussions on the results that we have gathered and making overseas trips SO much fun. Divan, Rian and Hannes thank you for “risking” your lives to collect my samples when it is almost impossible. Anja, thank you for always being there, from making the map for my dissertation to giving some of your formatting-OCD to me. Thank you to all of my friends and everyone at the NWU Water Research Group for all the support, advice and coffee breaks.

To my family, thank you for EVERYTHING. The endless support and motivation through every step of my academic career. Thank you for knowing that I will never stop studying even if I start getting old. Dylan, thank you for all of your support, love and motivation throughout the last couple of months.

Thank you God for giving me the opportunities and the strength do the things that I love.

Table of Contents

List of tables

List of figures

List of abbreviations

Ag-MT – Total metallothioneins

AM - Artificial mussel

As – Arsenic

CAT – Catalase

Cd – Cadmium

CEA – Cellular energy allocation

Co – Cobalt

Ea – Energy available

Ec – Energy consumed

MDA – Malondialdehyde

MT – Metallothioneins

Ni – Nickel

Pb – Lead

Pd – Palladium

PGE - Platinum group elements

Pt – Platinum

Pt-MT – Platinum bound metallothioneins

V – Vanadium

Zn – Zinc

Chapter 1: Introduction

1.1 Problem statement and potential impact

Platinum group elements (PGE) occur naturally in the environment in very low concentrations. These elements have increased over the last couple of decades since they have been used more and more in a number of applications; various chemical processes, the pharmaceutical industry and other production processes. One of the biggest contributors to PGEs in the environment is the use of automotive catalytic converters and mining activities. Mining activities in South Africa have been reported to greatly contribute to PGE emissions in the environment (Rauch and Peucker-Ehrenbrink, 2015).

The focus of this study is on providing insight into application of monitoring tools, the artificial mussel (AM), to determine the impact of mining thereby contributing to sustainable management and production. Mining and the raw material industries are of high economic significance in South Africa and during these processes a lot of raw materials are produced. During this process the wastes that are disposed are discharged into the rivers and surface waters, in this case the Hex River system. The raw material industries are the main suppliers of platinum group elements that include platinum (Pt) and palladium (Pd).

The bioaccumulation of PGEs in the aquatic environment has been demonstrated in laboratory investigations as well as in field studies from non-mining areas. Different aquatic organisms have different accumulation potentials for PGEs (Zimmermann *et alet al.*, 2015). Species react differently to metal stressors and although exposure to these stressors are normally external these organisms are also capable of regulating the elements internally. Thus multi-species studies are necessary to address the uptake and effects of chronic exposure to PGEs (Sures *et al.*, 2015).

It is important to understand the behaviour of PGEs in aquatic ecosystems to evaluate the potential impacts and risks of these mining activities. Accumulated pollutants usually have adverse effects on the organisms. There will be a deviation from the normal physiological homeostasis when these organisms are exposed to PGE levels that are not suitable for their survival. These changes are measurable and can be used for effect indication and biomarkers can be used to determine these changes (Sures *et al.*, 2015).

There is limited research on PGE emissions, their fate, distribution and methods on how to determine these levels and the possible risks. This study will contribute by gathering information on how these levels will affect aquatic organisms. The PGE concentrations in living organisms (e.g. bivalves) from field studies give valuable information on the bioavailability of

PGE and the effective concentrations within the organism which may cause adverse effects. During such studies both resident and transplanted indicator organisms are sacrificed to determine the uptake and effects and thus the study will attempt to develop and validate an artificial biomonitoring device to replace and / or improve these methods.

1.2 Aims and objectives

The knowledge of PGE contamination in freshwater systems is important for the risk assessment for aquatic animals and human health as well as water resource management. Laboratory bioaccumulation studies alone are not sufficient since organisms in natural conditions are influenced by all environmental conditions that in turn have an effect on the bioavailability of PGEs (Balcerzak, 2011). Therefore, monitoring studies analysing the pollutant concentrations in different environmental matrices are essential. Bioaccumulation studies of PGE concentrations in living organisms from field studies give valuable information on the bioavailable PGE fraction and the effective PGE concentrations inside of the organisms.

An alternative monitoring method to indicator organisms is the use of artificial monitoring devices to detect metals in freshwater environments (Claassens *et al.*, 2016). The use of artificial monitoring devices may be a useful tool to detect PGEs in freshwater systems. The artificial mussel (AM), a passive sampling device that accumulates and collects soluble metals from the environment, was originally developed by Wu *et al.* (2007). The artificial mussel has been used successfully for a wide range of metals in marine and more recently freshwater systems (Degger *et al.*, 2011, Claassens *et al.*, 2016, Degger *et al.*, 2016).

This sampling device is not affected by factors that can influence the bioaccumulation of metals, such as temperature, and can thus be used as a standardized monitoring method. This device is beneficial since it allow the monitoring of water quality without having to sacrifice organisms. Other benefits of a passive sampling device is that they allow the comparison between water bodies that are not able to support a specific bio-indicator species and the monitoring of the water quality without having to kill organisms (Kibria *et al.*, 2012). The results generated from studies using these devices can be used in comparative studies for different systems, since they are not limited to geographical borders or species distribution.

This study aims to develop and validate the artificial mussel (AM) as a monitoring device for PGE in freshwater systems.

To achieve this aim the following objectives were set:

- The development of an artificial mussel as a monitoring device for PGE (Pt and Pd) from freshwater systems
- Validate the design of the AM as a tool that is able to take up PGEs following exposure to Pt in the laboratory.
- Apply the AM in field studies in the aquatic ecosystems in a PGE mining region.
- Testing the AM in exposure studies alongside an established bioindicator species to obtain information on bioaccumulation and effects in the biota and how these correlate with the measured uptake by the AMs.

1.3 Hypotheses

The validation and application of the AM is a novel method to detect bioavailable PGE from freshwater ecosystems, therefore:

Hypothesis 1: When the artificial mussel is exposed to freshwater containing platinum (Pt) this metal will be accumulated by the AM device. It is posited that the Pt concentration accumulated by the AMs will correlate with the external Pt concentrations provided the binding capacity of the AM is not reached (Chapter 3).

Hypothesis 2: When the AM is exposed to environmental PGEs there will be a gradient of PGE exposure along the Hex River system, implying that there will be higher PGE levels closer to the mining areas (Chapter 4).

Hypothesis 3: During the concomitant exposure of transplanted organisms the uptake patterns within the AMs and transplanted organisms will be similar (Chapter 4).

Hypothesis 4: The biological responses in the form of biomarkers will reflect a dose-response relationship with increased PGE exposure (Chapter 5).

1.4 Potential impact

Metal production is an important source of metals into the environment, but the emission of PGE have received little attention (Rauch and Fatoki, 2015). There has been an increase in the attention towards mining and water issues, leading to more severe regulations governing water rights and responsibilities. The focus has been shifted to water management and potential environmental impacts associated with mining (Mudd, 2008). The mining industry has a close association with water resources, mining activities need substantive amounts of water but can have major impacts on surface and ground water resources (Mudd, 2008).

South Africa is the world's largest PGE producer, with 72% of Pt, 37% Pd and 80% of Rh production (Rauch and Fatoki, 2015). Variation in PGE concentrations can be influenced by

the type of activity in the direct vicinity of the aquatic environment, smelters are more important sources than mining shafts or ore processing (Rauch and Fatoki, 2015). A study done on the importance of automobile catalysts found that it is a minor source that contributes to the environmental concentration in South Africa. However elevated concentrations are associated with PGE mining and production activities. It is therefore necessary to determine emission rates and to assess environmental contamination (Rauch and Fatoki, 2015). There are many reasons for using water during mining processes (Prosser *et al*., 2011):

- Transport of ore and waste in slurries and suspension
- Separation of minerals through chemical processes
- Physical separation of material e.g. centrifugal separation
- Cooling systems around power generation
- Suppression of dust during mineral processing and around conveyors
- Washing of equipment
- Dewatering of mines

The AM, developed by Wu *et al.* (2007), is a passive sampling device that accumulates soluble metals (bioavailable fraction) and has been successfully used in marine systems. So far AMs have been successfully tested for a wide range of metals in marine and more recently freshwater systems (Leung *et al.*, 2008, Degger *et al.*, 2011, Kibria *et al.*, 2012, Claassens *et al.*, 2016, Degger *et al.*, 2016, Kibria *et al.*, 2016, Dahms-Verster *et al.*, 2018, Genç *et al.*, 2018, Ruiz-Fernández *et al.*, 2018). This tool has never been applied to monitor PGE, for which it could present a cost-effective and reliable assessment tool. The validation of an artificial device such as AMs in conjunction with traditional bioaccumulation indicator organisms will provide insight into PGE contamination in natural aquatic environments.

This study will provide valuable knowledge to identify potential risks associated with the exposure of aquatic environments to PGEs. Both aquatic ecosystems and human populations, situated in the vicinity of these PGE mines, are potentially exposed to high PGE levels. The results from this study will contribute towards a better understanding of the risks that are involved and it will provide a basis for a more sustainable management of aquatic ecosystems in South Africa. This will form a balance between the protection of aquatic ecosystems, human health and permitting to the social and economic needs of society.

References

Balcerzak, M. 2011. Methods for the determination of platinum group elements in environmental and biological materials: a review. *Analytical Chemistry,* 41**:**214-235.

Claassens, L., Dahms, S., Van Vuren, J. and Greenfield, R. 2016. Artificial mussels as indicators of metal pollution in freshwater systems: A field evaluation in the Koekemoer Spruit, South Africa. *Ecological Indicators,* 60**:**940-946.

Dahms-Verster, S., Baker, N. and Greenfield, R. 2018. A multivariate examination of 'artificial mussels' in conjunction with spot water tests in freshwater ecosystems. *Environmental monitoring and assessment,* 190**:**427.

Degger, N., Chiu, J. M. Y., Po, B. H. K., Tse, A. C. K., Zheng, G. J., Zhao, D.-M., Xu, D., Cheng, Y.-S., Wang, X.-H., Liu, W.-H., Lau, T. C. and Wu, R. S. S. 2016. Heavy metal contamination along the China coastline: A comprehensive study using Artificial Mussels and native mussels. *Environmental Management,* 180**:**238-246.

Degger, N., Wepener, V., Richardson, B. and Wu, R. 2011. Application of artificial mussels (AMs) under South African marine conditions: A validation study. *Marine Pollution Bulletin,* 63**:**108-118.

Genç, T. O., Po, B. H., Yılmaz, F., Lau, T.-C., Wu, R. S. and Chiu, J. M. 2018. Differences in metal profiles revealed by native mussels and artificial mussels in Sarıçay Stream, Turkey: implications for pollution monitoring. *Marine and Freshwater Research,* 69**:**1372-1378.

Kibria, G., Hossain, M. M., Mallick, D., Lau, T. and Wu, R. 2016. Monitoring of metal pollution in waterways across Bangladesh and ecological and public health implications of pollution. *Chemosphere,* 165**:**1-9.

Kibria, G., Lau, T. and Wu, R. 2012. Innovative 'Artificial Mussels' technology for assessing spatial and temporal distribution of metals in Goulburn–Murray catchments waterways, Victoria, Australia: Effects of climate variability (dry vs. wet years). *Environment international,* 50**:**38-46.

Leung, K. M., Furness, R. W., Svavarsson, J., Lau, T. and Wu, R. S. 2008. Field validation, in Scotland and Iceland, of the artificial mussel for monitoring trace metals in temperate seas. *Marine Pollution Bulletin,* 57**:**790-800.

Mudd, G. M. 2008. Sustainability reporting and water resources: a preliminary assessment of embodied water and sustainable mining. *Mine Water and the Environment,* 27**:**136.

Prosser, I., Wolf, L. and Littleboy, A. 2011. Water in mining and industry. *Water: Science and Solutions for Australia*:135-146.

Rauch, S. and Fatoki, O. S. 2015. Impact of Platinum Group Element Emissions from Mining and Production Activities. *In:* Zereini, F. and Wiseman, C. L. S. (eds.) *Platinum Metals in the Environment.* Heidelberg: Springer.

Rauch, S. and Peucker-Ehrenbrink, B. 2015. Sources of Platinum Group Elements in the Environment. *In:* Zereini, F. and Wiseman, C. L. S. (eds.) *Platinum Metals in the Environment.* Heidelberg: Springer.

Ruiz-Fernández, A. C., Wu, R. S., Lau, T.-C., Pérez-Bernal, L. H., Sánchez-Cabeza, J. A. and Chiu, J. M. 2018. A comparative study on metal contamination in Estero de Urias lagoon, Gulf of California, using oysters, mussels and artificial mussels: Implications on pollution monitoring and public health risk. *Environmental pollution,* 243**:**197-205.

Sures, B., Ruchter, N. and Zimmermann, S. 2015. Biological effects of PGE on aquatic organisms. *In:* Zereini, F. and Wiseman, C. L. S. (eds.) *Platinum metals in the environment.* Heidelberg: Springer.

Wu, R. S., Lau, T., Fung, W. K., Ko, P. and Leung, K. M. 2007. An ‘artificial mussel’ for monitoring heavy metals in marine environments. *Environmental pollution,* 145**:**104-110.

Zimmermann, S., Sures, B. and Ruchter, N. 2015. Laboratory Studies on the Uptake and Bioaccumulation of PGE by Aquatic Plants and Animals. *In:* Zereini, F. and Wiseman, C. L. S. (eds.) *Platinum Metals in the Environment.* Heidelberg: Springer.

Chapter 2: Literature Review

The aim of this chapter is to provide the context of the different aspects that are dealt with in this study. It therefore does not serve to provide a detailed review of the available literature but rather just a literature-based overview of the components of the study.

2.1 Platinum Group Elements

What are Platinum Group Elements?

The discovery of the platinum-bearing reef in Rustenburg in 1925 marked the beginning of the exploitation of platinum group elements from the world's largest deposits (Wittmann and Förstner, 1976). In the mineral sector, the Bushveld Complex is well-known for its overwhelming deposits of platinum group elements (Cawthorn, 2010). The Bushveld Complex is a large, layered geological formation that is found in the north west of South Africa (Cawthorn, 2010). Platinum group elements (PGE) are six rare metals, i.e. platinum (Pt), palladium (Pd), iridium (Ir), rhodium (Rh), ruthenium (Ru) and osmium (Os). Platinum group elements are noble metals and occur in the upper crust of the earth (Singer *et al.*, 2005) of which the most used are Pt, Pd and Rh. Over the last few decades the use of these metals in a variety of applications has increased tremendously (Zereini and Wiseman, 2015).

Increased mining and use of PGEs are influencing the release of PGE into the environment to such an extent that the anthropogenic PGE fluxes exceed the natural fluxes at the earth's surface (Rauch and Fatoki, 2015). The main point sources of PGEs into the environment include automotive catalytic converters, clinical wastewaters, jewellery industry, sewage plants and the chemical industry (Schindl and Leopold, 2015). These sources emit the PGEs into the water, soils and the atmosphere, from here they enter plants, animals and humans. To determine the extent to which these elements affect organisms, all environmental compartments should be studied (Zereini and Wiseman, 2015).

Sources of Platinum group elements

Platinum group elements occur naturally in the earth's crust, but due to anthropogenic activities the natural cycles and concentrations of these PGE are affected. Automobile catalysts are one of the major sources of PGE. These devices are placed in the exhaust system of vehicles to convert gaseous pollutants emitted from the engine into less hazardous forms (Rauch and Peucker-Ehrenbrink, 2015). The most potent PGE sources according to

Rauch and Peucker-Ehrenbrink (2015) are these catalysts because they use Pt, Pd and Rh as the main active components (Ravindra *et al.*, 2004, Singer *et al.*, 2005, Moldovan, 2007).

Studies in urban settings suggest that there are multiple sources that contribute to PGE fluxes in the environment. An important use of Pt is jewellery, where 33% of Pt is used for this purpose (Rauch and Peucker-Ehrenbrink, 2015). Jewellery is a very unlikely source of high Pt emissions in the environment, since it has a high recycling rate. Platinum is also used in drugs (Pt-based) for cancer treatment, in this case it can enter the environment through the excretion of the administered drugs. In addition, it has been found that patients that have undergone these types of treatments can excrete Pt for long periods of time, even as long as 8 years post treatment (Rauch and Peucker-Ehrenbrink, 2015). This indicates that Pt is not only released by the medical facility during the treatment, but it is also released outside of the facility. The Pt is then diluted in the wastewater system to much lower concentrations.

Even though these sources contribute negligibly to the overall PGE levels, if they are combined with other applications, it may still contribute to critical PGE releases in the environment. These other applications include; electrical applications, uses by the chemical industry, jewellery and dental applications (Rauch and Fatoki, 2015). Industrial activities are also associated with PGE emissions. Platinum group elements abundance ratios can be used to differentiate between the sources of PGE emissions (Rauch and Fatoki, 2015). Industrial and automobile emissions can be characterized by different PGE ratios, however the ratios for industrial sources depend on the type of activity that is taking place.

Applications that make use of PGE may contribute to elevated PGE loadings in waste and sewage streams, despite the active recycling of electronic components and automobile catalysts (Rauch and Fatoki, 2015). Sewage and waste are secondary PGE sources, this leads to PGE emissions during treatment, reuse and disposal (Rauch and Peucker-Ehrenbrink, 2015). Depending on the sewage network, these discharges can result in the release of PGE into the aquatic environment. Therefore, it can be said that anthropogenic PGE emissions stem from a range of anthropogenic activities. Many of these emissions are equally important sources of PGE, thus it is important to assess and identify all sources that contribute to PGE in the environment.

Platinum group elements from mining activities

Platinum group elements are mined primarily from deposits that usually consist of igneous minerals and associate to the other elements that are found in the igneous rocks (Rauch and Fatoki, 2015). Primary deposits account for most of the PGE productions. These deposits can be found in the Bushveld Igneous Complex in South Africa and the Norilsk/Talnakh complex in Russia (Rauch and Fatoki, 2015). It is estimated that 82% of the global PGE production in

2013 was produced by South Africa and Russia, the remaining 18% was produced by Canada, USA and Zimbabwe (Rauch and Fatoki, 2015).

Until recently mining has been concentrated in the shallower reef that forms part of the western limb of the Bushveld Igneous Complex (Rauch and Fatoki, 2013). South Africa is the world's largest PGE producer, Anglo American Platinum, Impala Platinum, Sibanye and Lonmin are some of the main suppliers of PGEs. Mining and PGE production activities are known to discharge PGE into the environment. Variations in PGE concentrations can be attributed to the type of activity in the vicinity of sampling points. It has been found that smelters are more important sources of PGEs than the underground shafts, or the ore processing plants (Rauch and Fatoki, 2015).

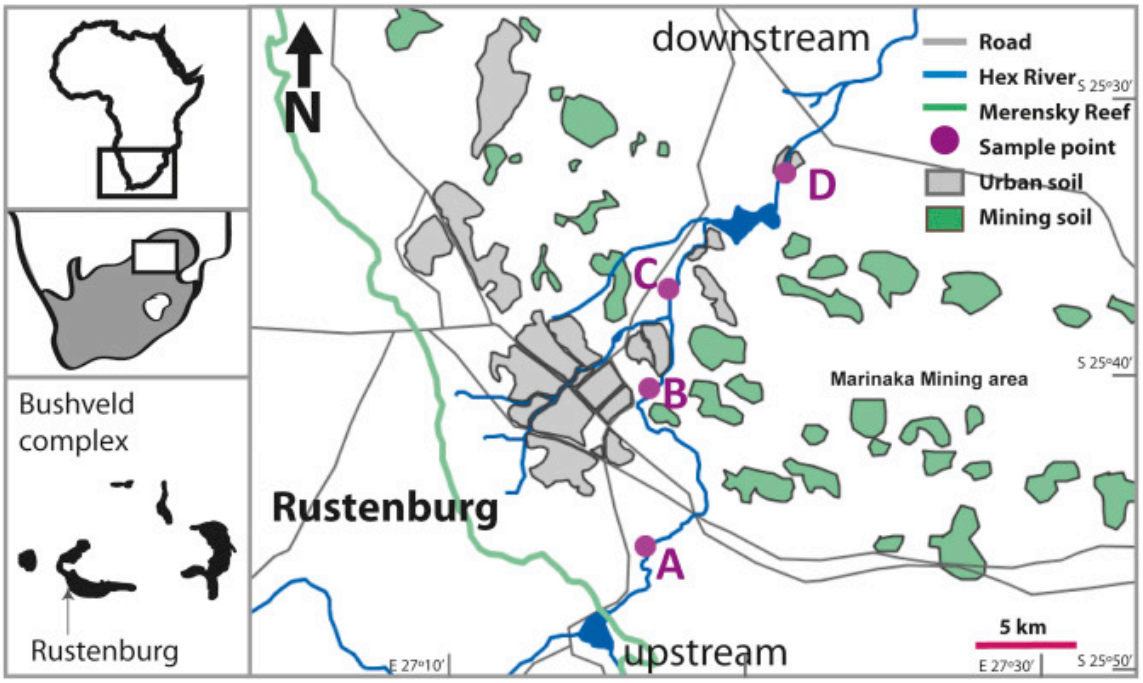

Figure 2-1: The Bushveld Igneous Complex in South Africa (Almécija *et al*., 2017).

It has not been proven that the elevated levels of PGE concentrations have an impact on the environment, this is due to the fact that there is limited data available on the chronic effects (Ruchter *et al.*, 2015). There is an absence of data regarding PGE in biota near PGE mining and production sites (Rauch and Fatoki, 2015). Many of the main PGE mining and production sites are located in the vicinity of human settlements (Rauch and Fatoki, 2015). Rustenburg is located within a few kilometres of the mines, smelters and refineries. This is a cause for concern for the local population that depends on the rivers and dams that are affected by these activities. Many of the inhabitants in this area are employed by the mining companies, thus they are exposed to a combination of environmental and occupational factors (Rauch and Fatoki, 2015).

Platinum group elements in aquatic ecosystems

It was thought that PGE are relatively inert, but it has been shown that these metals can undergo environmental transformation that can make them biologically available (Ek *et al.*, 2004). The biological availability of these metals can lead to their bioaccumulation in various environmental compartments (Balcerzak, 2011). Platinum is more soluble in the environment than Pd and Rh. The soluble fraction of Pt is less than 10%, where for Pd and Rh the soluble fraction is in the same order of magnitude as the particulate fraction (Ek *et al.*, 2004). Therefore the Pd and Rh emissions may have more severe effects on the environment than Pt (Ek *et al.*, 2004). Mining and ore transport can result in the PGEs being deposited in the soils surrounding the mines and roads. The deposited particles can then be washed into rivers and other water bodies during rain events, here the PGE accumulate in the sediments and in very low levels within the water (Ek *et al.*, 2004).

The low natural background of PGEs facilitate the detection of even small anthropogenic additions to the natural PGE cycles (Rauch and Peucker-Ehrenbrink, 2015). The natural environmental levels of PGEs do not exhibit a direct hazard to the living organisms within ecosystems. However, the intensive mining and associated activities in the Bushveld Complex may lead to an anthropogenic increase in PGE concentrations (Almécija *et al.*, 2017). According to Sures *et al.* (2015) free living organisms are threatened by environmental stressors such as PGE, which may alter their physiology. Organisms that are affected by these substances have the ability to accumulate the metals inside their bodies.

Uptake and bioaccumulation of Platinum group elements

When determining PGE toxicity there are different factors to keep in mind in order to ensure that accurate results are obtained and to prevent contamination. Liquid samples are commonly filtered to remove any unwanted particles in the water, the water samples are acidified in order to prevent the adsorption of PGE onto the vessel and to keep the PGE in solution (Schindl and Leopold, 2015). Polypropylene or polyethylene vessels are most practical for storage, but it is better to keep the storage period for as short as possible. There are many test parameters that can influence the uptake and accumulation of PGE by biota in laboratory exposure studies, as well as in the field. These parameters include test systems, PGE source, route of exposure, exposure concentration, exposure medium, test organisms, temperature and exposure period (Zimmermann *et al.*, 2015).

According to Zimmermann *et al.* (2015) most studies that were published made use of static tests or semi-static test. Static exposure tests entail that the test organisms are exposed to the same test solution for the duration of the test, the test substance is administered at the

beginning of the exposure period and not renewed. Semi-static exposure tests are a compromise between a flow-through and static system, with this system the organisms are periodically transferred to fresh solutions or a proportion of the test solution is removed and renewed. The benefit of using these kind of systems is that you are guaranteed that the PGE are within the system at the beginning of any exposure. The PGE concentrations can be affected in many ways before the exposure has even started. Many aquatic exposure studies have showed that PGE concentrations quantified within the system are far less than the concentrations applied (Zimmermann *et al.*, 2015).

The loss of PGE within such a system can be explained by adsorption processes and precipitation. The PGE can adsorb to the exposure container material, as well as to test organisms that are present in the system, such as the shells of mussels (Zimmermann *et al*. 2018). When using glass and other types of containers it was found that problems occurred in future exposure studies, after each exposure the containers could not be used again and this was not very cost effective. To overcome this problem plastic bags are being used. By adding the plastic bags with the PGE concentration before the commencement of the study, the unwanted loss of PGE by adsorption processes is eliminated. This also ensures that the bags can be discarded after the exposure and not the containers.

As mentioned, the PGE source can influence exposures in a considerable way, this can affect the routes of uptake and the biological availability. Soluble PGE are taken up much quicker than particle bound PGE. The size of the particle bound PGE and the binding strength can enhance the uptake of PGE via ingestion (Zimmermann *et al.*, 2015). Different organisms have different mechanisms for feeding thus, it can influence the bioaccumulation of PGE within an organism. These elements are usually applied by way of the exposure medium, if test organisms are fed the uptake of contaminated food is possible. Generally, the soluble PGE concentration is much higher in the water than in the test organisms, this should be kept in mind during exposure studies (Zimmermann *et al*., 2015).

In exposure studies that used multiple PGE it was found that there is no competition between Pt, Pd and Rh for the same uptake mechanisms (Zimmermann *et al.*, 2015). Ek *et al.* (2004) also mentioned that no interactions occur between different PGE when organisms are exposed to mixed solutions. Uptake may occur through ingestion of metals within the sediments, or via absorption from metals that are dissolved in the water. The elevated PGE concentration within the organisms are indicative of their bioavailability (Balcerzak, 2011). Aquatic ecosystems are complex, there is a mixture of elements available in the water and these concentrations are normally much lower than those used for laboratory studies (Zimmermann *et al.*, 2015).

According to Ruchter *et al.* (2015) up to now it has not been possible to do a risk assessment on PGEs in the environment, due to field studies being rare. However, there is some knowledge about PGEs from field studies that focus specifically on PGEs. It has been found that metals accumulate in the sediments, where these concentrations ranged between 3 – 14 ng/g Pt and 3 – 11 ng/g in 2002 (Ruchter *et al.*, 2015). Water concentrations for Pt ranged between 0.006 – 2.6 ng/L and Pd between 0.4 – 10.2 ng/L. A few different taxa have been analysed that comprise of fish, crustaceans and molluscs. Zebra mussels (*Dreissena polymorpha)* from Lake Mondsee in Austria have been analysed, where the mean PGE concentrations of 0.1 ng/g Pt and 1 ng/g Pd were found in the freeze dried soft tissue (Zimmermann *et al.*, 2002). *Corbicula* sp. were also analysed, it was found that Pt concentrations were about 1.3 ng/g (Haus *et al.*, 2007). These concentrations are two to three times lower than some of the other metals found within the organisms (Ruchter *et al.*, 2015).

Field studies have indicated that when the PGEs accumulate within the sediment, the biota get into contact with the metals in the sediment. There are a few studies that deal with the accumulation of PGE in biota in laboratory studies, this include plants, annelids, crustaceans, molluscs, fish and amphibians (Zimmermann *et al.*, 2015). A range of different concentrations (0 to 1000 µg/L) and exposure periods were used during these studies. It has been proven in several studies, that as the exposure concentration increase, an increase in metals can be found within the soft tissue of the organisms (Zimmermann *et al.*, 2005, Sures and Zimmermann, 2007, Zimmermann and Sures, 2018). It is important to relate the metal concentrations to the biomarker response of the organisms, i.e. metal sensitive organelles, heat-sensitive proteins or the biologically detoxified metal fraction (Zimmermann and Sures, 2018).

2.2 Bioaccumulation monitoring in metal exposure studies

Water quality deterioration has become a more common issue in South Africa. To solve these issues water monitoring has been taking place in various forms and with various methods that incorporate chemical and biological monitoring (Claassens *et al.*, 2016). Bioindicators are used to determine the status of an ecosystem. The use of the individual biota, or otherwise known as biological indicators (bio-indicators), in monitoring programs have increased drastically in the last few decades (Wu and Lau, 1996). For an ecosystem to be healthy, the individual biota in the ecosystem also need to be healthy (Ruchter *et al.*, 2015). During biomonitoring studies the accumulation of substances within an organism is measured to determine the environmental status.

Aquatic organisms are sensitive to fluctuations in metal concentrations, they reflect the ambient metal concentrations of the environment and are widely utilized for these type of

studies (Claassens *et al.*, 2016). During such assessments there are numerous factors that can influence the mechanisms in which organisms are affected and the degree to which it can accumulate these pollutants. When organisms are exposed to pollutants they have the ability to accumulate these substances and if they are exposed for a long enough period a biochemical response may be induced. Monitoring programs involving metal concentrations are required to detect the spatial and temporal changes that occur in all water types, this aids with protecting living resources and public health (Wu and Lau, 1996).

The concentration of metals found in the organisms are affected by many biological and physical factors. The metal release and uptake in these organisms are affected by temperature, life cycles, size, depth and reproduction. It has been found that different species have different accumulation strategies, and that the indicator species may have different natural distribution patterns (Wepener, 2008). Various monitoring programs make use of bivalves as transplanted organisms. These organisms are useful as bioindicators since they are distributed worldwide, they are universally abundant, they have a sedentary lifestyle and they have the ability to bioconcentrate pollutants (Greenfield *et al.*, 2014).

Bivalves and their sedentary nature can monitor temporal trends within the bioavailable fraction in the environment. Freshwater bivalves such as *Corbicula fluminalis africana* have the ability to take up metals via three pathways; the first is filtering soluble metals from discharge, feeding on particles within the water column or from the sediment via pedal feeding (Ruchter and Sures, 2015). *Corbicula* sp. are often used in biomonitoring studies for determining metal pollution, several studies have shown that tissue samples indicated clear changes in metal concentrations at different sites (Ruchter and Sures, 2015). Every now and then the population may not be able to survive in certain environmental conditions. Indicator species may not always be present in all ecosystems that need to be monitored, thus it prevents the comparison between different geographical areas. Sometimes the kinetics behind the uptake and depuration of these metals are not well understood (Wu and Lau, 1996).

Transplantation studies

To rule out some of these factors and to avoid too much variability, the use of active biomonitoring is beneficial (Wepener, 2008). This method is different from traditional biomonitoring methods, it does not assess the responses of the resident organisms but it makes use of bioindicators that are deployed for a predetermined period (Wepener, 2013). This method makes use of caged organisms, organisms are collected from an unpolluted population and afterwards they are translocated to the polluted site. The chemical and biological changes that occur in the translocated organisms can then be followed in space and time (Wepener, 2008, Wepener, 2013).

These organisms are placed in cages with mesh screens at different sampling sites. This allows for free-flowing water to move through the cage, especially if the bioindicators are filter feeders. To avoid a loss or theft, these cages are attached to floats or anchored at each of the sites. The cages with the translocated organisms can be deployed from four to six weeks, or for longer periods. This period allows enough time for the transplanted organisms to recover from any stress as a result of the translocation process and to react to the environmental conditions (Wepener, 2013). The exposure period to which the transplanted organisms are exposed to the ambient conditions depends on the specific organism that is used. For bivalves and fish the exposure period can be anywhere from four weeks to as long as eight weeks (Wepener, 2013).

There are many advantages and a few disadvantages that go hand in hand with using this method. First of all, the transplanted organisms are acclimated to changing environmental conditions, since they have been removed from the environment, relocated to the laboratory and then relocated again to the polluted site. This will limit the impact that the field exposure may have on the organisms (Wepener, 2008). Since these organisms are relocated to the study area the pollution history of the organism is known as well as the exposure period of the organism. Before the organisms are transplanted to the study site, control samples can be taken to determine the background metal levels.

By using this method it is easy to compare different sites with each other, since it can be guaranteed that the same organisms can be located at each of the exposure locations (Giarratano *et al.*, 2010). This can lead to investigations that can make use of the transplanted and indigenous organisms to determine the degree to which the organisms are adapted to pollutants within the system. When the indigenous species adapt to pollutants in the ecosystem it is difficult to use them as bioindicators and to compare different sites to one another. In most studies this method protects the indigenous species that occur naturally in the environment since they aren't harmed during the experiments.

These advantages support the use of active biomonitoring, but there are also a few disadvantages that can influence the results. One of the major drawbacks is food availability and the toxicity of the study site. A decrease in the quality or the quantity of the food resources can influence the organisms, to little food can cause the organisms to die and too much food can lessen the effect of the pollutants (Wepener, 2008). When using these caged organisms, the experiment can be influenced by theft, the loss of the cages and the transplanted organisms can make it difficult to interpret the results. When organisms are exposed to low levels of contamination over a long period of time the indigenous organisms can adapt genetically, thus when using the transplanted organisms evolutionary processes should be kept in mind (Wepener, 2008).

Prospective organisms for transplantation studies

Different organisms have different mechanisms when bioaccumulating pollutants as well as surviving in these circumstances. The choice of organism can thus influence the relevance, success and the interpretation of the results (Wepener, 2008). Therefore a list of criteria were compiled to recognise an organism as prospective bioindicator (Wepener, 2008, Wepener, 2013). First of all, the organisms should be abundant, this ensures that the ecosystem where the organisms are removed from isn't influenced in a negative way. A suitable organism should commonly be immobile to represent the specific study area. The organisms should be able to accumulate pollutants to levels that are present in the environment and it should be relatively tolerant to pollutants.

A few other characteristics can include that they should be easy to collect, identify, handle and they should be able to survive for a relatively long period. It is important to use organisms that is a reasonable size, this insures that there are enough tissue sample to be used for analyses. Along with this a sufficient number of organisms should be deployed at each site. When an organism meets the requirements of this list it can be used as a transplanted organism. Bioindicator organisms can be obtained from a variety of sources, such as the laboratory, a hatchery or even an unpolluted reference site within the same system. According to a study done by Greenfield *et al.* (2014) transplanted mussels are able to accumulate metal concentrations at much higher concentrations than the indigenous mussels. This is due to the fact that resident organisms can get adapted to exposure concentrations. Exposure periods are also very important since the transplanted mussels have the ability to start regulating metals.

Artificial mussels

To resolve some of these problems Wu *et al.* (2007) developed a passive sampling device that can be implemented as a tool in water bodies to provide time integrated results. This method is more beneficial, since it allows the comparison between water bodies that are not able to support a specific bio-indicator species, they also allow the monitoring of the water quality without having to kill organisms. Other advantages of using artificial monitoring devices include continuous monitoring and no power or energy is required to use it, it is simple to handle, deploy, recover and to analyse. These devices are not affected by biotic and abiotic factors, thus it can be deployed in marine and freshwater ecosystems (Hossain *et al.*, 2015).

Artificial mussels (AMs) are defined as passive sampling devices that accumulate soluble and bioavailable metals from the environment (Wu *et al.*, 2007). This device makes use of a polymer ligand which can absorb metals from the aquatic environment. The accumulation

patterns of the polymer ligand respond to fluctuations in the environmental metal concentrations. This sampling device is not affected by factors that can influence the bioaccumulation of metals, such as temperature, and can thus be used as a standardized monitoring method (Claassens *et al.*, 2016). The results generated from studies using these devices can be used in comparative studies for different systems, since they are not limited to geographical borders or species distribution (Claassens *et al.*, 2016).

Wu and Lau (1996) tested a series of different polymer ligands and found that Chelex®-100 resin beads work the best for the purpose of metal monitoring. The Chelex® beads have a few advantages that include; the uptake and release of metals is relative insensitive to changes in pH between 6 and 8. The use of a passive sampling device that makes use of Chelex® beads involves simple and predictable physical processes, while the biological processes within bioindicators are much more complex (Wu and Lau, 1996). The biggest advantage is the fact that these devices can be deployed without geographical or hydrographic limitations and allows direct comparison of results worldwide.

The AM consists of Perspex® tubing that contains a spacer and two semi-permeable polyacrylamide gel plugs on both sides of the spacer. The Chelex® beads are suspended in deionized water in between these two polyacrylamide gel plugs. The AM works on the principle that water enters through the polyacrylamide gel by means of diffusion, the bioavailable metals then bind to the Chelex® beads. Artificial mussels have successfully been used in various bioaccumulation studies as seen in Table 2-1, predominantly in marine environments but recently it has been used more frequently in the freshwater environment (Claassens *et al.*, 2016, Degger *et al.*, 2016, Kibria *et al.*, 2016a). It has also successfully been used in freshwater monitoring of gold mining areas of South Africa (Claassens *et al.*, 2016).

Table 2-1: Previous literature pertaining artificial mussels and where it has been implemented successfully.

	Study area	Metals measured	Reference
Marine	Marine environments (developed)	Cd, Cr, Cu, Pb and Zn	Wu *et al*. (2007)
	Scotland and Iceland	Cd, Cu, Cr, Pb and Zn	Leung *et al*. (2008)
	South African coastline	Cd, Cr, Cu, Pb and Zn	Degger *et al*. (2011)
	Portuguese Coast	Cd, Cr, Cu, Pb and Zn	Gonzalez-Rey *et al*. (2011)
	China coastline	Cd, Cr, Cu, Hg, Pb and Zn	Degger *et al*. (2016)

	Bay of Bengal, Bangladesh	Cd, Cu, Fe, Mn, Ni, Pb, U and Zn	Kibria *et al.* (2016a), Kibria *et al.* (2016b)
	Estero de Urias lagoon, Gulf of California	Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, U and Zn	Ruiz-Fernández *et al*. (2018)
	Sarıçay Stream, Turkey	Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, U and Zn	Genç *et al*. (2018)
Freshwater	Goulburn-Murray catchment, Victoria, Australia	Cd, Cu, Hg, Pb, and Zn	Kibria *et al*. (2012)
	Koekemoer Spruit, South Africa	As, Cd, Cr, Co, Cu, Pb, Mn, Ni, U, V and Zn	Claassens *et al*. (2016)
	Nyl River floodplain, South Africa	Al, Cd, Cr, Co, Cu, Fe, Mn, Ni, Pb and Zn	Dahms-Verster *et al*. (2018)

2.3 Biomarkers

According to De Coen and Janssen (1997) a variety of methods have been developed to evaluate the effects of pollutants on the abundance, production and persistence of populations, communities and ecosystems. In-stream toxicity assessments makes use of endpoints that measure biological responses. Biomarkers are defined as biochemical or physiological changes that occur in organisms that are exposed to contaminants, this represents the initial responses to environmental perturbations and contamination (de Lafontaine *et al.*, 2000). The biological responses of an organism to contaminants has become a useful tool for the evaluation of environmental quality and risk assessments (Wepener, 2008).

These biological responses are termed biomarkers and can be related to exposures to or the toxic effect of environmental contaminants (Wu *et al.*, 2005). The aim of biomarkers is to indicate that a certain organism has been exposed to a certain contaminant or that organisms are likely to suffer future impairments that are ecologically relevant (Forbes *et al.*, 2006). Biomarkers are used as complementary tools to link sublethal biochemical alterations and effects that may occur within the natural populations (Howcroft *et al.*, 2009). Biomarker responses are associated with growth impairment, reproductive output and metabolic functions (Howcroft *et al.*, 2009).

When organisms are exposed to a certain toxicant a response of biomarkers can be observed as biological of biochemical effects (Wepener *et al.*, 2005). Biomarkers are implemented to identify the exposure of organisms to certain chemicals, such as acetylcholinesterase (AChE) for organophosphates, metallothionein (MTs) for metals and Cytochrome P450 enzymes for organic pollutants (Wu *et al.*, 2005). Biomarkers can help with monitoring spatial and temporal changes in contamination levels, this can help with early detection of deteriorating environmental conditions.

Changes in the biological responses of organisms are important because it can help to interpret the extent and the significance of exposure. Information on the time that is required for a biological response to be induced is very important, this will determine the frequency and period necessary for field sampling or transplantation (Wu *et al.*, 2005). It is very important to ensure that samples for biomarker analyses are handled and analysed correctly. False positive results can be very expensive in the short term, but false negatives on the other hand may not pick up on adverse effects that can be reversed before they can cause major damage (Forbes *et al.*, 2006)

There are several measures that makes a biomarker useful and practical for biomonitoring. The results obtained from biomarkers should be able to exhibit a dose-response relationship to levels of environmental contaminants. Biomarkers ought to help with estimating contaminant levels within an organism over the period of monitoring and should exhibit clear endpoints. The specific biomarker that is used needs to be ecologically relevant, this will help in making predictions on the ecological risk in the environment (Wu *et al.*, 2005). Every so often biomarkers respond much faster in lower biological levels and may possibly provide better warning signs to changes in environmental conditions (Wu *et al*. 2005).

When organisms are exposed to contaminants there is a period before any biological response is observable. While the organisms are exposed to the contaminants, the contaminants binds to the minimum number of receptors that are available, in turn this brings about an observable response (Wu *et al.*, 2005). When the organisms are continuously exposed to the contaminants the number of receptors increase and the response reach its maximum level (Wu *et al.*, 2005). The maximum response is likely to show a quantitative relationship to the concentration of the contamination. However, it may occur that the induced response may decline when the organisms are exposed for a prolonged period.

A series of responses occur within an organism if they are exposed to contaminants. Primary responses are rapid and reversible at a cellular biochemical level. While the secondary responses are physiological changes that take more time to occur in organisms, and they can also be reversed over time. Tertiary responses at individual and population level are the least reversible having the longest-lasting effect (Wepener *et al.*, 2005, Wu *et al.*, 2005, Wepener, 2008). There are mechanisms within an organism that restore homeostasis, these mechanisms include detoxification or depuration.

Another factor that can influence the biological response is the contamination levels within the system, when these levels drop the dose-response will also decline (Wu *et al.*, 2005). This can be described as follows; when an organism is returned to clean water the number of receptors will decrease an therefore the biological response with it (Wu *et al.*, 2005). An article

by Wu *et al.* (2005) made three observations; the first is that there is a positive relationship between the induction and recovery of biochemical responses. The second observation is that different types of contaminants have different required times for the induction and adaptation of biological responses. The last and third observation is that these times and biological responses will be different in different organisms.

The advantage of using biomarkers

Simple measurements normally make use of the contaminants that accumulate within the organisms' tissue. Biomarkers offer more complete and relevant biological information on the potential impact that toxic pollutants may have on the health of the aquatic ecosystem (de Lafontaine *et al.*, 2000). There are many advantages that biomarkers have over the traditional chemical methods (Wu *et al.*, 2005). First of all, the environmental fate, bioavailability and interactions of the contaminants can be monitored. With biomarkers one can determine how environmental levels effect the organism and how the levels found correlate with the observed response, thus exposure and adverse effects can be related. The concentration of contaminants is generally high in the biological tissues, therefore making chemical analyses easier. According to Wu *et al.* (2005) the most important advantage is that the biological effects can be linked to environmental consequences. This makes it easier to address environmental concerns.

A suite of different biomarkers

A variety of biomarkers have been developed and implemented by various national and international monitoring programs (Wu *et al.*, 2005). Biomarkers can be grouped in different categories, since they can differ in significance and terminology. Some are biomarkers of exposure, others are of effect, stress, alteration or even susceptibility (de Lafontaine *et al.*, 2000). Biomarkers are usually characterised or labelled by the terms "exposure to" or "effect of", this is determined by the biochemical response that it provokes (de Lafontaine *et al.*, 2000). There is a link between exposure and biological effects, but this does not change the fact that these are different types of biomarkers.

It is important to indicate whether a biomarker is intended to provide an estimate of exposure or if it is intended to indicate relevant effects (Forbes *et al.*, 2006). Among the various ecotoxicological biomarkers, those based on responses at molecular and cellular levels represent the earliest signals of environmental disturbance and are commonly used for biomonitoring (Giarratano *et al.*, 2010). To evaluate the effects of pollutants on individuals it can be useful to measure several biomarkers at the same time in the same organism (Wepener *et al.*, 2005).

Measuring the same biomarker in different areas at the same time, gives information about the pollution status and provides a better comprehension of the mechanism of response of the organism to pollutants (Giarratano *et al.*, 2010). A wide variety of biomarkers has been tested for their response to toxic substances and their use as biomarkers of effect and exposure (Wepener *et al.*, 2005). If biomarker and multivariate analysis are used as monitoring tools it is important to find a set of biomarkers that complement one another (Wepener *et al.*, 2005).

In response to stressors in the environment, all living cells have defence mechanisms. These mechanisms include the induction of antioxidant enzymes, one of these is catalase. Catalase activity (CAT) is considered as the primary defence against oxidative damage, it has been used in several bivalve mollusc studies around the world (Giarratano *et al.*, 2010). It is an enzymatic intracellular antioxidant involved in defence systems against environmental oxidative pollutants (Giarratano *et al.*, 2010). This defence system protects the organisms' cells from the harmful effects of oxyradicals, such as H_2O_2.

Metallothioneins (MTs) are generally known for being a biomarker specific and sensitive to metal contamination (de Lafontaine *et al.*, 2000). Metallothioneins are low molecular weight, cysteine-rich, heat-resistant cytosolic proteins that binds to metals (Atli and Canli, 2008). Metallothioneins occur naturally in organisms, they have a significant role in the homeostasis of essential metals. These proteins are usually not saturated by a single element, but it contains several elements such as Cu, Zn, Cd, Ag or Hg (Amiard *et al.*, 2006). After organisms are exposed to these elements in the environment metallothioneins are synthesized to regulate the concentrations within the organism. Essential and non-essential metals that occur in the environment stimulate metallothionein synthesis (Atli and Canli, 2008). The MTs act as essential metal stores, however non-essential metals are able to displace the MT-associated essential metals (Amiard *et al.*, 2006). Therefore MTs are involved in the detoxification of both essential and non-essential metals. Metallothionein levels within an organisms vary depending on the element and the exposure concentration to that metal (Frank *et al.*, 2008).

In many different species the induction of MT synthesis by metal contamination has been proven. Therefore, metallothionein concentrations can be used in organisms as a biomarker of metal exposure. Some laboratory experiments have shown the induction of MT synthesis over a very short period, while long field exposures did not indicate any significant rise in MT concentrations (Amiard *et al.*, 2006). According to Amiard *et al.* (2006) it is easier to demonstrate the MT-metal exposure relationship in fish than in invertebrates, since invertebrate species make use of biomineralization processes that detoxify trace metals. Metallothioneins play an important role in the potential of species to adapt to conditions where they are exposed to high metal concentrations.

When organisms live in environments that are under stress there will be a cost of dealing with these conditions when regarding metabolic resources (Smolders *et al.*, 2004). Cellular Energy Allocation is an approach that integrates the amount of energy available to and the energy consumed by an organism (Gomes *et al.*, 2015). It is known that organisms use their energy reserves to survive in stressful conditions, this can influence other biological functions such as reproduction and growth (Gomes *et al.*, 2015). The amount of energy consumed is estimated by measuring the electron transport activity at the mitochondrial level. The electron transport system activity measures the oxygen consumption process and the cellular respiration rate (De Coen and Janssen, 2003, Novais *et al.*, 2013). On the other hand the energy available is assessed by measuring the total lipid, protein and sugar content of the organism (De Coen and Janssen, 1997).

The difference between the amount of energy consumed and the amount of energy used is equal to the energy budget of the organism (De Coen and Janssen, 2003). Effects at cellular levels will result to lower energy that is available for growth and the maintenance of the condition of the organism, this will ultimately result in the impairment of reproduction and the development of the organism (Smolders *et al.*, 2004). This endpoint is considered to be a sensitive biomarker to measure the energy status of an organism (Novais *et al.*, 2013).

Lipid peroxidation generates a variety of products, some of these products reacts with DNA and proteins which can result in toxic or mutagenic effects (Marnett, 1999). Malondialdehyde (MDA) is an intermediate product of lipid peroxidation and is degraded rapidly. DNA and protein damage is often irreversible and affects cell functionality (Del Rio *et al*., 2005). It is used as a non-enzymatic marker of oxidation of membrane phospholipids through lipid peroxidation (Giarratano *et al.*, 2010). Malondialdehyde levels in organisms can be related to the degradation of the environment and the water quality (Giarratano *et al.*, 2010). There are many more biomarkers that can be used but this study will focus on the four mentioned.

References

Almécija, C., Cobelo-García, A., Wepener, V. and Prego, R. 2017. Platinum group elements in stream sediments of mining zones: the Hex River (Bushveld Igneous Complex, South Africa). *African Earth Sciences,* 129**:**934-943.

Amiard, J.-C., Amiard-Triquet, C., Barka, S., Pellerin, J. and Rainbow, P. 2006. Metallothioneins in aquatic invertebrates: their role in metal detoxification and their use as biomarkers. *Aquatic toxicology,* 76**:**160-202.

Atli, G. and Canli, M. 2008. Responses of metallothionein and reduced glutathione in a freshwater fish *Oreochromis niloticus* following metal exposures. *Environmental toxicology and pharmacology,* 25**:**33-38.

Balcerzak, M. 2011. Methods for the determination of platinum group elements in environmental and biological materials: a review. *Analytical Chemistry,* 41**:**214-235.

Cawthorn, R. G. 2010. The platinum group element deposits of the Bushveld Complex in South Africa. *Platinum Metals Review,* 54**:**205-215.

Claassens, L., Dahms, S., Van Vuren, J. and Greenfield, R. 2016. Artificial mussels as indicators of metal pollution in freshwater systems: A field evaluation in the Koekemoer Spruit, South Africa. *Ecological Indicators,* 60**:**940-946.

Dahms-Verster, S., Baker, N. and Greenfield, R. 2018. A multivariate examination of 'artificial mussels' in conjunction with spot water tests in freshwater ecosystems. *Environmental monitoring and assessment,* 190**:**427.

De Coen, W. M. and Janssen, C. 1997. The use of biomarkers in *Daphnia magna* toxicity testing. IV. Cellular energy allocation: a new methodology to assess the energy budget of toxicant-stressed *Daphnia* populations. *Aquatic Ecosystem Stress and Recovery,* 6**:**43-55.

De Coen, W. M. and Janssen, C. R. 2003. The missing biomarker link: Relationships between effects on the cellular energy allocation biomarker of toxicant-stressed *Daphnia magna* and corresponding population characteristics. *Environmental toxicology and chemistry,* 22**:**1632-1641.

De Lafontaine, Y., Gagné, F., Blaise, C., Costan, G., Gagnon, P. and Chan, H. 2000. Biomarkers in zebra mussels (*Dreissena polymorpha*) for the assessment and monitoring of water quality of the St Lawrence River (Canada). *Aquatic toxicology,* 50**:**51-71.

Degger, N., Chiu, J. M. Y., Po, B. H. K., Tse, A. C. K., Zheng, G. J., Zhao, D.-M., Xu, D., Cheng, Y.-S., Wang, X.-H., Liu, W.-H., Lau, T. C. and Wu, R. S. S. 2016. Heavy metal contamination along the China coastline: A comprehensive study using Artificial Mussels and native mussels. *Environmental Management,* 180**:**238-246.

Degger, N., Wepener, V., Richardson, B. J. and Wu, R. S. S. 2011. Application of artificial mussels (AMs) under South African marine conditions: A validation study. *Marine Pollution Bulletin,* 63**:**108-118.

Del Rio, D., Stewart, A. J. and Pellegrini, N. 2005. A review of recent studies on malondialdehyde as toxic molecule and biological marker of oxidative stress. *Nutrition, metabolism and cardiovascular diseases,* 15**:**316-328.

Ek, K. H., Morrison, G. M. and Rauch, S. 2004. Environmental routes for platinum group elements to biological materials — a review. *Science of the Total Environment,* 334**:**21-38.

Forbes, V. E., Palmqvist, A. and Bach, L. 2006. The use and misuse of biomarkers in ecotoxicology. *Environmental toxicology and chemistry,* 25**:**272-280.

Frank, S. N., Singer, C. and Sures, B. 2008. Metallothionein (MT) response after chronic palladium exposure in the zebra mussel, *Dreissena polymorpha*. *Environmental research,* 108**:**309-314.

Genç, T. O., Po, B. H., Yılmaz, F., Lau, T.-C., Wu, R. S. and Chiu, J. M. 2018. Differences in metal profiles revealed by native mussels and artificial mussels in Sarıçay Stream, Turkey: implications for pollution monitoring. *Marine and Freshwater Research,* 69**:**1372-1378.

Giarratano, E., Duarte, C. A. and Amin, O. A. 2010. Biomarkers and heavy metal bioaccumulation in mussels transplanted to coastal waters of the Beagle Channel. *Ecotoxicology and Environmental Safety,* 73**:**270-279.

Gomes, S. I., Scott-Fordsmand, J. J. and Amorim, M. J. 2015. Cellular energy allocation to assess the impact of nanomaterials on soil invertebrates (enchytraeids): The effect of Cu and Ag. *Environmental Research and Public Health,* 12**:**6858-6878.

Gonzalez-Rey, M., Lau, T., Gomes, T., Maria, V., Bebianno, M. and Wu, R. 2011. Comparison of metal accumulation between ‘Artificial Mussel’ and natural mussels (*Mytilus galloprovincialis*) in marine environments. *Marine Pollution Bulletin,* 63**:**149-153.

Greenfield, R., Brink, K., Degger, N. and Wepener, V. 2014. The usefulness of transplantation studies in monitoring of metals in the marine environment: South African experience. *Marine Pollution Bulletin,* 85**:**566-573.

Haus, N., Zimmermann, S., Wiegand, J. and Sures, B. 2007. Occurrence of platinum and additional traffic related heavy metals in sediments and biota. *Chemosphere,* 66**:**619-629.

Hossain, M. M., Kibria, G., Nugegoda, D., Lau, T. and Wu, R. 2015. A Training Manual for Assessing Pollution (trace/heavy metals) in Rivers, Estuaries and Coastal waters-Using Innovative “Artificial Mussel (AM) Technology” - Bangladesh Model. *Research collaboration between scientists of the IMSF, University of Chittagong, Bangladesh, RMIT University, Australia, the City University of Hong Kong, and the University of Hong Kong.*

Howcroft, C., Amorim, M., Gravato, C., Guilhermino, L. and Soares, A. 2009. Effects of natural and chemical stressors on *Enchytraeus albidus*: Can oxidative stress parameters be used as fast screening tools for the assessment of different stress impacts in soils? *Environment international,* 35**:**318-324.

Kibria, G., Hossain, M. M., Mallick, D., Lau, T. and Wu, R. 2016a. Monitoring of metal pollution in waterways across Bangladesh and ecological and public health implications of pollution. *Chemosphere,* 165**:**1-9.

Kibria, G., Hossain, M. M., Mallick, D., Lau, T. and Wu, R. 2016b. Trace/heavy metal pollution monitoring in estuary and coastal area of Bay of Bengal, Bangladesh and implicated impacts. *Marine Pollution Bulletin,* 105**:**393-402.

Kibria, G., Lau, T. and Wu, R. 2012. Innovative ‘Artificial Mussels’ technology for assessing spatial and temporal distribution of metals in Goulburn–Murray catchments waterways, Victoria, Australia: Effects of climate variability (dry vs. wet years). *Environment international,* 50**:**38-46.

Leung, K. M. Y., Furness, R. W., Svavarsson, J., Lau, T. C. and Wu, R. S. S. 2008. Field validation, in Scotland and Iceland, of the artificial mussel for monitoring trace metals in temperate seas. *Marine Pollution Bulletin,* 57**:**790-800.

Marnett, L. J. 1999. Lipid peroxidation - DNA damage by malondialdehyde. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis,* 424**:**83-95.

Moldovan, M. 2007. Origin and fate of platinum group elements in the environment. *Analytical and bioanalytical chemistry,* 388**:**537-540.

Novais, S. C., Soares, A. M., De Coen, W. and Amorim, M. J. 2013. Exposure of *Enchytraeus albidus* to Cd and Zn – Changes in cellular energy allocation (CEA) and linkage to transcriptional, enzymatic and reproductive effects. *Chemosphere,* 90**:**1305-1309.

Rauch, S. and Fatoki, O. S. 2013. Anthropogenic platinum enrichment in the vicinity of mines in the Bushveld Igneous Complex, South Africa. *Water, Air, & Soil Pollution,* 224**:**1395.

Rauch, S. and Fatoki, O. S. 2015. Impact of Platinum Group Element Emissions from Mining and Production Activities. *In:* Zereini, F. and Wiseman, C. L. S. (eds.) *Platinum Metals in the Environment.* Heidelberg: Springer.

Rauch, S. and Peucker-Ehrenbrink, B. 2015. Sources of Platinum Group Elements in the Environment. *In:* Zereini, F. and Wiseman, C. L. S. (eds.) *Platinum Metals in the Environment.* Heidelberg: Springer.

Ravindra, K., Bencs, L. and Van Grieken, R. 2004. Platinum group elements in the environment and their health risk. *Science of the Total Environment,* 318**:**1-43.

Ruchter, N. and Sures, B. 2015. Distribution of platinum and other traffic related metals in sediments and clams (*Corbicula* sp.). *Water research,* 70**:**313-324.

Ruchter, N., Zimmermann, S. and Sures, B. 2015. Field studies on PGE in aquatic ecosystems. *In:* Zereini, F. and Wiseman, C. L. S. (eds.) *Platinum Metals in the Environment.* Heidelberg: Springer.

Ruiz-Fernández, A. C., Wu, R. S., Lau, T.-C., Pérez-Bernal, L. H., Sánchez-Cabeza, J. A. and Chiu, J. M. 2018. A comparative study on metal contamination in Estero de Urias lagoon, Gulf of California, using oysters, mussels and artificial mussels: Implications on pollution monitoring and public health risk. *Environmental pollution,* 243**:**197-205.

Schindl, R. and Leopold, K. 2015. Analysis of Platinum Group Elements in Environmental Samples: A Review. *In:* Zereini, F. and Wiseman, C. L. S. (eds.) *Platinum Metals in the Environment.* Heidelberg: Springer.

Singer, C., Zimmermann, S. and Sures, B. 2005. Induction of heat shock proteins (hsp70) in the zebra mussel (*Dreissena polymorpha*) following exposure to platinum group metals (platinum, palladium and rhodium): comparison with lead and cadmium exposures. *Aquatic toxicology,* 75**:**65-75.

Smolders, R., Bervoets, L., De Coen, W. and Blust, R. 2004. Cellular energy allocation in zebra mussels exposed along a pollution gradient: linking cellular effects to higher levels of biological organization. *Environmental pollution,* 129**:**99-112.

Sures, B., Ruchter, N. and Zimmermann, S. 2015. Biological effects of PGE on aquatic organisms. *In:* Zereini, F. and Wiseman, C. L. S. (eds.) *Platinum metals in the environment.* Heidelberg: Springer.

Sures, B. and Zimmermann, S. 2007. Impact of humic substances on the aqueous solubility, uptake and bioaccumulation of platinum, palladium and rhodium in exposure studies with *Dreissena polymorpha*. *Environmental pollution,* 146**:**444-451.

Wepener, V. 2008. Application of active biomonitoring within an integrated water resources management framework in South Africa. *Science,* 104**:**367-373.

Wepener, V. 2013. Active biomonitoring. *In:* Férard, J. F. and Blaise, C. (eds.) *Encyclopedia of Aquatic Ecotoxicology.* Dordrecht: Springer.

Wepener, V., Van Vuren, J., Chatiza, F., Mbizi, Z., Slabbert, L. and Masola, B. 2005. Active biomonitoring in freshwater environments: early warning signals from biomarkers in assessing biological effects of diffuse sources of pollutants. *Physics and Chemistry of the Earth, Parts A/B/C,* 30**:**751-761.

Wittmann, G. and Förstner, U. 1976. Heavy metal enrichment in mine drainage: 1. The Rustenburg platinum mining area. *Science,* 72**:**242-246.

Wu, R. and Lau, T. 1996. Polymer-ligands: a novel chemical device for monitoring heavy metals in the aquatic environment? *Marine Pollution Bulletin,* 32**:**391-396.

Wu, R. S., Lau, T., Fung, W. K., Ko, P. and Leung, K. M. 2007. An ‘artificial mussel’ for monitoring heavy metals in marine environments. *Environmental pollution,* 145**:**104-110.

Wu, R. S., Siu, W. H. and Shin, P. K. 2005. Induction, adaptation and recovery of biological responses: implications for environmental monitoring. *Marine Pollution Bulletin,* 51**:**623-634.

Zereini, F. and Wiseman, C. L. S. 2015. *Platinum metals in the environment,* Heidelberg, Springer.

Zimmermann, S., Alt, F., Messerschmidt, J., Von Bohlen, A., Taraschewski, H. and Sures, B. 2002. Biological availability of traffic-related platinum-group elements (palladium, platinum,

and rhodium) and other metals to the zebra mussel (*Dreissena polymorpha*) in water containing road dust. *Environmental toxicology and chemistry,* 21**:**2713-2718.

Zimmermann, S., Messerschmidt, J., Von Bohlen, A. and Sures, B. 2005. Uptake and bioaccumulation of platinum group metals (Pd, Pt, Rh) from automobile catalytic converter materials by the zebra mussel (*Dreissena polymorpha*). *Environmental research,* 98**:**203-209.

Zimmermann, S. and Sures, B. 2018. Lessons learned from studies with the freshwater mussel *Dreissena polymorpha* exposed to platinum, palladium and rhodium. *Science of the Total Environment,* 615**:**1396-1405.

Zimmermann, S., Sures, B. and Ruchter, N. 2015. Laboratory Studies on the Uptake and Bioaccumulation of PGE by Aquatic Plants and Animals. *In:* Zereini, F. and Wiseman, C. L. S. (eds.) *Platinum Metals in the Environment.* Heidelberg: Springer.

Chapter 3: Validation of the artificial mussel in platinum group element exposure studies

3.1 Introduction

Platinum, including the other platinum group elements (PGE), has many properties and characteristics that label it as a “critical metals” (Mudd *et al.*, 2018). Platinum is used mainly in automobile catalytic converters, 33% of Pt is allocated towards the jewellery industry, it is also used in Pt-based drugs in cancer treatment, as well as dental applications (Rauch and Peucker-Ehrenbrink, 2015). The chemical industry makes use of platinum as a catalyst for the production of nitric acid, silicone and benzene. The electronics industry uses platinum for computer hard disks and thermocouples, as well as optical fibres, turbine blades, spark plugs, pacemakers and dental fillings.

Most commercially produced platinum comes from South Africa, which entails 75% of the world’s Pt demand (Cawthorn, 2010). Platinum is sometimes prepared as a by-product of copper and nickel refining as well. Platinum group elements occur naturally at very low concentrations in the environment. It has been found that metals accumulate in the sediments, where these concentrations ranged between 3 – 14 ng/g Pt and 3 – 11 ng/g in 2002 (Ruchter *et al.*, 2015). Water concentrations for Pt ranged between 0.006 – 2.6 ng/L and Pd between 0.4 – 10.2 ng/L. Due to their increasing use in a number of applications some of the PGEs make their way into the environment, where even small additions to the natural concentrations may possibly have an effect on the environment.

It is necessary to monitor pollutants in the freshwater environment, since many of these pollutants can pose a threat to both humans and the aquatic ecosystem. Over the last couple of decades many forms of passive sampling methods have been implemented to determine the degree to which the aquatic environment is polluted. Passive sampling devices can overcome many problems that may possibly occur when using active biomonitoring methods and have been available since the 1970s which all work on the similar principle (Vrana *et al.*, 2005). The passive sampling devices are based on the free flow of analyte molecules, where diffusion is the main driving force (Namieśnik *et al.*, 2005).

With all of the passive sampling devices only the bioavailable fraction of the contaminants can be measured, which reflects the fractions that can be found in the water column (Vrana *et al.*, 2005). There are many chelating resins that can be used within these passive sampling devices. Wu and Lau (1996) evaluated different chelating resins against a selection of criteria. The desirable characteristics were identified as; being able to accumulate metals at environmental concentrations, both uptake and release is possible from the resin, not affected

by short term fluctuations and an equilibrium can be reached within 30 days. Their study found that Chelex®-100 resin responds to the bioavailable fraction of Cd, Pb and Zn and met most of the criteria. Based on these results a device was developed to monitor metal concentrations within the aquatic environment.

There are many passive sampling devices that are used to monitor metal contamination within the aquatic environment. Various studies have been conducted with various passive sampling devices. A summary of some of these studies can be found in Table 3-1. According to Namieśnik *et al.* (2005) semi-permeable membrane devices (SPMD) can also be used as a monitoring tool. This device has been used in several studies for several compounds indicating that the SPMD might be more effective than living organisms for monitoring trace contaminants in the water column of marine ecosystems (Namieśnik *et al.*, 2005). Another passive sampling device that has been used quite frequently is the DGT (diffusive gradient in thin film). This device makes use of gel layers that incorporates a binding agent permitting ion exchange to occur (Vrana *et al.*, 2005). The chelating resin can be found within the gel layer metals can move freely through this layer and then bind to the resin.

Table 3-1: Passive sampling devices used for monitoring metal concentrations in the aquatic ecosystem (adjusted from Namieśnik *et al*. (2005) and Vrana *et al*. (2005)).

Passive sampling device	Application	Reference
Artificial mussel (AM)	Marine and freshwater Al, Cd, Cr, Co, Cu, Fe, Hg, Mn, Ni, Pb, U and Zn	Wu *et al*. (2007) Leung *et al*. (2008) Degger *et al*. (2011) Gonzalez-Rey *et al*. (2011) Degger *et al*. (2016) Kibria *et al*. (2016a), Kibria *et al*. (2016b) Ruiz-Fernández *et al*. (2018) Genç *et al*. (2018) Kibria *et al*. (2012) Claassens *et al*. (2016) Dahms-Verster *et al*. (2018)
Semipermeable membrane devices (SPMD)	Marine – Sn	Følsvik *et al.* (2002) Følsvik *et al.* (2000)
Diffusion sampler – parallel plate dialyzer (PPD)	Surface water – Cr	Pressman and Aldstadt III (2003)
Stabilized Liquid Membrane Device (SLMD)	Grab and storm water – Cd, Co, Cu, Ni, Pb, Zn	Brumbaugh *et al.* (2002) Blom *et al.* (2002)
Permeation liquid membrane (PLM)	Freshwater – Cu, Pb, Zn and Cd	Parthasarathy and Buffle (1994) and Parthasarathy *et al.* (1997)
Kabis sampler, Hydra sampler, Discrete interval sampler, etc.	Ground water – As, Cd, Cr and Pb	Parker and Clark (2002)
Diffusive gradient in thin film (DGT)	Natural and synthetic freshwater – Cd, Cu, Fe, Mn, Ni, Pb and Zn	Vrana *et al.* (2005)

The artificial mussel (AM) is a passive sampling device that was developed by Wu *et al*. (2007) and can be used as a standardized method to detect metal contamination in aquatic environments. It consists of a non-permeable Perspex tubing, two semipermeable polyacrylamide gel layers and a polymer-ligand. Soluble metals from the environment have the ability to bind to the polymer-ligand, Chelex®-100 resin. During exposure water moves along a concentration gradient through the polyacrylamide gel into the cavity that contains the Chelex® beads, where the soluble metals bind to the Chelex® beads.

The AM was selected for this study since it can be deployed at any sampling site, in the field or in the laboratory, under any circumstances which includes anoxic and hypoxic environments as long as there is water available. Other advantages include: there is no power or energy required for this device to work, it is simple to handle, deploy, retrieve and to analyse (Kibria *et al.*, 2012, Hossain *et al.*, 2015). The main advantage is that the AM allows monitoring studies without having to sacrifice organisms.

The AM can be used as a standard tool for all types of water (i.e. fresh, marine, estuarine, recycled/waste water). This device proved to be effective for monitoring studies in freshwater and marine ecosystems (Claassens *et al.*, 2016, Degger *et al.*, 2016, Kibria *et al.*, 2016). The semipermeable gel of the device allows for the metal uptake and release to occur slowly over time. This limits short-term fluctuations of metals and simulates the uptake and release of metals by mussels (Wu *et al.*, 2007).

The AM was originally developed to monitor metal concentrations within the marine environment, these metals include Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, U and Zn (Wu *et al.*, 2007, Leung *et al.*, 2008, Degger *et al.*, 2011, Gonzalez-Rey *et al.*, 2011, Degger *et al.*, 2016, Kibria *et al.*, 2016, Genç *et al.*, 2018, Ruiz-Fernández *et al.*, 2018). This device has also been implemented in monitoring metals in the freshwater environment, e.g. Al, As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, U, V and Zn (Kibria *et al.*, 2012, Claassens *et al.*, 2016, Dahms-Verster *et al.*, 2018).

In order to use the artificial mussel to monitor PGE concentrations within the freshwater environment it needs to be validated. This was done to determine whether or not platinum has the ability to accumulate within the AM before it can be used in the environment. The aim of this chapter is to validate the AM as a tool that is able to take up bioavailable platinum concentrations in freshwater environments. This will be achieved through identifying the most suitable loading and elution solutions for Pt uptake and to determine the optimum exposure period required to ensure maximum Pt uptake by the AMs.

3.2 Materials and Methods

3.2.1 Artificial mussel design

The artificial mussel was originally developed by Wu *et al*. (2007). This device consists of a non-permeable Perspex tube (5 cm x 3 cm, with 1 cm x 2.5 cm spacer) that is capped by a layer of polyacrylamide gel at either end (Figure 3-1). In between these two gel layers, 200 mg Chelex® 100 (sodium form, 50-100 mesh, dry, Sigma-Aldrich) is suspended in 5 mL ultrapure water. The water diffuses through the polyacrylamide gel into the cavity that contains the Chelex® beads. Soluble metals move along a concentration gradient to the inside of the tube and bind to the Chelex® beads.

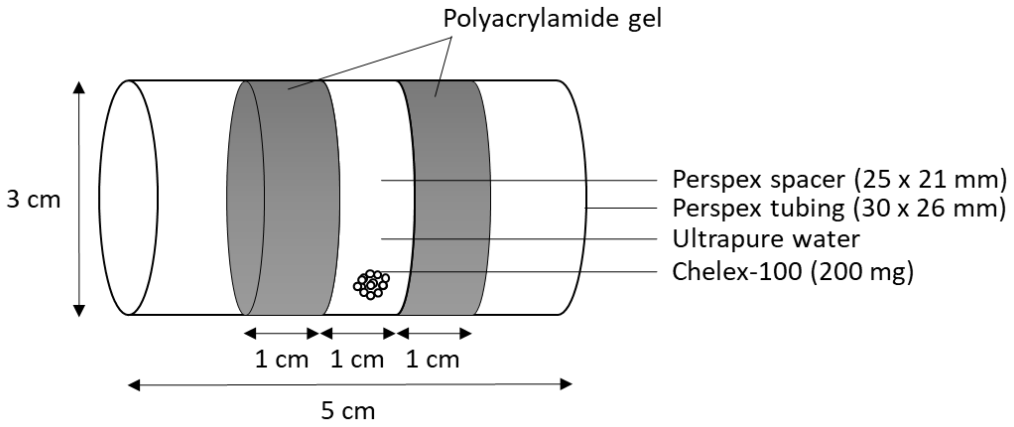

Figure 3-1: Modified design of the artificial mussel.

3.2.2 Assembling the artificial mussel

The protocol for assembling the artificial mussel was as follows. The end of each Perspex® tube was covered with parafilm to form a membrane to prevent the solutions for the gel layers from seeping out while it polymerizes. The gel consisted of three solutions: 15 g acrylamide (Acrylamide for electrophoresis, ≥99% (HPLC) powder, Sigma-Aldrich) and 0.5 g N,N-methylenebis-acrylamide (BioReagent, suitable for electrophoresis, 99%, Sigma-Aldrich) was dissolved in 100 mL ultrapure water for the first solution, of this 4 mL was pipetted into the plastic tubing. Where after 160 µL 10% ammonium peroxidisulfate (Reagent grade, 98%, Sigma-Aldrich) and 40 µL N,N,N′,N′-Tetramethylethylenediamine (BioReagent, for molecular biology, ≥99% (GC), Sigma-Aldrich) were added.

After the gel polymerized (usually within 5 minutes) the tubes with the gels were placed in a container filled with ultrapure water for 1 hour, this allows for the gels to swell. The gels were then carefully spaced within the tube, where after the Perspex® spacer, 200 mg Chelex® beads and 5 mL ultrapure water was added. The second gel was then carefully placed into the tube, making sure that no air bubbles were trapped in between the two gel layers. The design of the

AM can be seen in Figure 3-1 and Figure 3-2. After the AMs were assembled, they were stored in ultrapure water (Figure 3-3) until they were used for the exposure experiment.

Figure 3-2: Design of the Artificial Mussel.

Figure 3-3: Storage of the artificial mussels in ultrapure water before exposures.

3.2.3 Loading and elution of platinum from Chelex® beads

To determine if Chelex® beads have the ability to accumulate platinum (Pt) from their environment a short-term laboratory study was done to test (1) the binding of Pt to the Chelex® beads and (2) the most effective method of Pt elution from the Chelex® beads. The loading, washing and elution experiment was conducted to check the practicability and validity of the washing and elution procedure. To determine the validity (recovery and precision), Chelex® beads were loaded with a defined concentration of Pt to determine the loading of the beads. Furthermore, we wanted to confirm that Pt binds to the beads in ultrapure water as it is used in the AMs.

Loading of Chelex® beads with Platinum

The first part of this experiment was to test the loading of the Chelex® beads. For that 200 mg Chelex® beads were placed in 15 mL polypropylene tubes. In total 20 polypropylene tubes were used, 10 of these tubes were loaded with 4.95 mL ultrapure water and the other 10 contained 4.95 mL 0.5 M HCl (37%, suprapure, Merck). In each of these tubes 50 µL of 100 mg/L Pt standard solution (Pt standard for ICP TraceCERT®, 1000 mg/L, Pt(IV) in hydrochloric acid, Sigma-Aldrich diluted with 1% HNO_3, sub-boiled from 65%; p.a. quality, Merck, Germany) were added resulting in a Pt concentration of 1 mg/L. Samples were taken from the supernatant in regular time intervals to determine the loading kinetics of the Chelex® beads and their Pt concentrations were immediately analysed using a Perkin-Elmer model 4100ZL atomic absorption spectrometer (AAS) equipped with a Zeeman effect background correction system.

The samples were injected in a pyrolytic graphite furnace tube by the autosampler AS 70 and ran under the optimised operating parameters (Table 3-2). The calibration was performed by matrix adapted calibration where the concentrations in each sample were calculated by fitting a linear regression line to the points defined by the spiked concentration values.

Table 3-2: Optimised operating parameters for platinum analysis by AAS.

Temp (°C)	Ramp time (s)	Hold time (s)	Argon flow (psi)
110	1	5	250
140	10	60	250
600	20	1	250
1300	30	20	250
2200	0	3	0
2450	1	3	250

Washing

As soon as the loading of the Chelex® beads was finished, the beads went through three repeated washing steps. This was done to get rid of any unbound Pt that might have stayed in the tube or in the loading solution. The Chelex® beads were washed by adding 5 mL of ultrapure water and centrifugation (2 minutes at 1000 g). The centrifugation ensured that the beads settle at the bottom of the tube before removing the supernatant again, this was repeated two more times. The Pt concentrations in the supernatant of the washing steps were also analysed by AAS to determine any Pt loss that may occur. This is a new method for separating the beads from the solution. In the previous methods the Chelex® beads eluted twice with 12.5 mL 6 M HNO_3, filtered through a 0.45 µm cellulose nitrate filter paper and a sintered glass vacuum system and then made up to a known volume (Wu *et al.*, 2007). In more recent studies these beads were placed in canonical flasks on a shaker for 24 hours in 20 mL 6 M nitric acid and then filtered.

Elution procedure

After washing the beads the elution of the Pt from the Chelex® beads were tested with two different elution solutions. The first solution was used for half of the tubes consisting of 4.5 mL 6 M HNO_3 (sub-boiled from 65%; p.a. quality, Merck, Germany) and 0.5 mL HCl (37%, suprapure, Merck). The second solution was used on the other half of the tubes consisting of 5 mL 8 M HCl (37%, suprapure, Merck). Samples were taken from the supernatant in regular time intervals to determine the elution kinetics from the Chelex® beads. Samples were then immediately analysed by AAS as described above.

3.2.4 Long-term exposure of the artificial mussels to platinum

Experimental design

In order to validate the AM for Pt uptake, they were exposed to a series of increasing dissolved Pt concentrations under static conditions without water changes. The AMs were divided into six experimental groups (Table 3-3). The nominal Pt concentrations in the tank water were 0 µg/L, 0.1 µg/L, 1 µg/L, 10 µg/L, 100 µg/L and 1000 µg/L. The platinum was introduced to the exposure medium in the form of Pt standard solution (Pt standard for ICP TraceCERT®, 1000 mg/L, Pt(IV) in hydrochloric acid, Sigma-Aldrich).

Table 3-3: Experimental layout for Pt exposures.

	Experimental group	# AMs/ sampling	# AMs/ tank	addition of	
				standard	HCl (2 mol/L)
Tank 1	Control	7	42	none	10 mL
Tank 2	0.1 µg Pt/L	7	42	1 µL	10 mL
Tank 3	1 µg Pt/L	7	42	10 µL	10 mL
Tank 4	10 µg Pt/L	7	42	100 µL	9.9 mL
Tank 5	100 µg Pt/L	7	42	1 mL	9 mL
Tank 6	1000 µg Pt/L	7	42	10 mL	none

Figure 3-4: Experimental setup for Platinum exposure.

Six plastic tanks lined (300 x 750 mm) with polypropylene (PP) bags (Sarstedt, disposable bags, autoclavable, 600 x 780 mm) were prepared in advance (Figure 3-4). By lining the tanks with PP bags it is possible to reuse the plastic tanks. The tanks, with the plastic bags, were filled with deionised water ahead of time to ensure that there are no leakages and that the

bags can take their shape within the tank. The tanks were aerated, this also allowed for the continuous movement of the water and preventing the Pt from settling within the tank.

After a couple of days the water in the PP bags were discarded and replaced with exposure medium (10 L exposure medium), as well as the Pt standard solution according to Table 3-1. The bags were pre-conditioned to saturate the PP bags with Pt before the exposure begins. This pre-conditioning step was done to minimize the loss of Pt due to adsorption processes on the bag surfaces during the exposure. The exposure medium for this experiment consisted of reconstituted freshwater for molluscs which contained 0.043 g NaCl (for molecular biology, ≥98%, Sigma-Aldrich), 1.725 g KCl (for molecular biology, ≥99.0%, Sigma-Aldrich), 9.842 g $NaHCO_3$ (84.01, Merck), 18.734 g $MgSO_4.H_2O$ (BioUltra, ≥99.5%, Sigma-Aldrich) and 44.68 g $CaCl_2.H_2O$ (dihydrate, 147.02, Merck) in 100 L deionised water. After the pre-conditioning period of 24 hours, the water was completely discarded and replaced as described before. After equilibration of the Pt concentration in the exposure system a total of 42 AMs were added to each tank.

Water and artificial mussel sampling

Water samples were taken from each tank before the addition of the Pt, 10 min after the addition of the Pt standard solution, before the addition of the AMs and subsequently weekly before AMs were removed. The procedures were as follows: 10 mL tank water was removed and acidified with 10 µL HNO_3 (sub-boiled from 65%; p.a. quality, Merck, Germany). The conductivity, temperature and pH-values were measured each time water samples were taken. Artificial mussel samples (7 replicates) were taken once every week over a period of six weeks. The AMs were plugged at both ends with cotton that was soaked in ultrapure water. These samples were then placed in plastic bags, marked and stored at room temperature for metal analysis.

Metal analysis

For metal analysis the contents (including the Chelex® beads) of each individual AM were emptied into an acid pre-washed 15 mL polypropylene tube. These samples were then centrifuged, the supernatant was removed and the beads were rinsed with 5 mL ultrapure water. The supernatant was removed and the beads were eluted with 4.5 mL 6 M HNO_3 (sub-boiled from 65%; p.a. quality, Merck, Germany) and 0.5 mL HCl (37%, suprapure, Merck). The beads were left in the acid solutions for approximately 2 hours. This was based on the results obtained from the elution experiment, to ensure that all bound metals would be released from the beads. The supernatant was removed and placed in new polypropylene tubes for further analysis.

Accordingly, Pt concentrations in the water and AMs were determined by a quadrupole ICP-MS system (Perkin Elmer, Elan 6000) with an autosampler system (Perkin Elmer, AS-90). Between each measurement, the wash time was set to 30 s with 2% HNO_3 in order to avoid contamination. After every 10 samples a Pt standard solution (10 µg/L) was used to check the accuracy and stability of the measurements. Prior to measuring the Pt, samples were diluted 1:10 with an internal standard solution, consisting of 1% HNO_3 and 10 µg/L thulium (Certipur®, Merck). Calibration of the instrument was performed using a series of 11 dilutions of Pt standard solution. With this the concentrations of the sample analytes were calculated using regression lines with a correlation factor of ≥0.999.

There are many isotopic forms in which Pt can be found within the aquatic environment, for that reason Pt-194 was measured during this study. However, interferences occur and can have an effect on the Pt measured by the analytical instruments. For this reason Hafnium (Hf) was considered as an interference and calibrations of the instrument was performed using a series of 5 dilutions of Hf standard solution. For the water and AM samples the Hf interferences were below 2%, so that mathematical correction was not necessary.

Statistical analyses

Statistical analyses were performed using GraphPad Prism® 7 software. The significance in Pt concentrations between exposure period and concentrations were determined using a 2-way ANOVA, followed by multiple comparisons with Tukey's test. Statistical significance was set at $p<0.05$ for all comparisons. Homogeneity of variance was confirmed using the Kolmogorov-Smirnov test with Dallal-Wilkinson-Lilliefor P value.

3.3 Results

Loading and elution of platinum from Chelex® beads

The loading kinetics of the Pt concentrations of the two different loading solutions showed different patterns. The data on the graphs depict the Pt concentrations in the solutions (i.e. the Pt that was not taken up by the beads. The Pt concentration of loading solution 1 (ultrapure water) was relatively stable during the exposure period and contained about 45% of the Pt in the supernatant after 200 minutes exposure (Figure 3-5 A). In contrast, the Pt concentrations of loading solution 2 (0.5 M HCl) clearly decreased over time (Figure 3-5 B). Thus it can be assumed that most of the Pt bound to the Chelex® beads and that only 13% of the initial Pt concentration that was added remained in the loading solution.

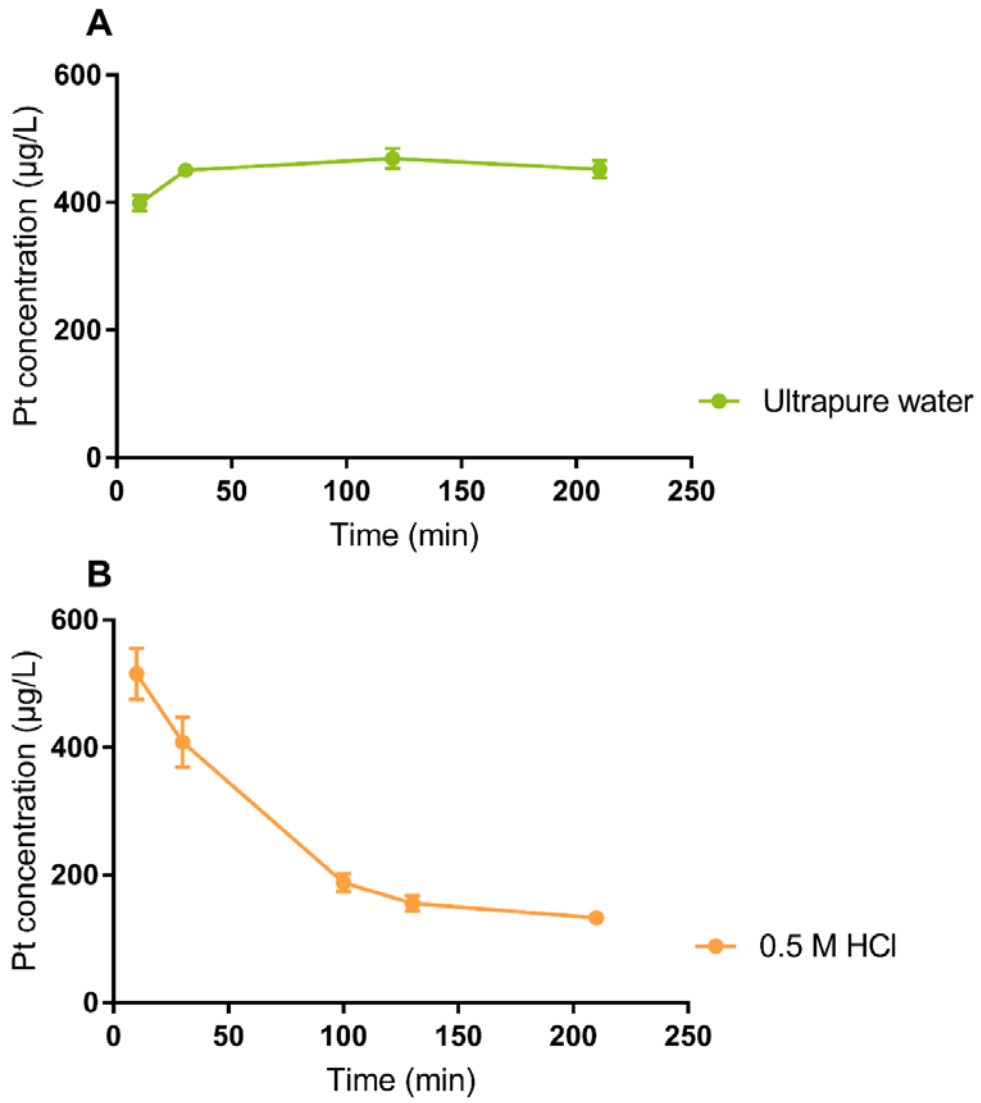

Figure 3-5: Loading kinetics with ultrapure water (A) and 0.5 M HCl (B) (mean ± SEM).

During the washing steps of the beads after the first washing step 1% of the added Pt was found, after the second washing step the concentrations decreased further. The elution kinetics was determined by adding the different elution solutions to the tubes and measuring the Pt concentrations in the supernatant. Only the tubes from the second loading solution were used for the elution experiment. In both Figure 3-6 A and Figure 3-6 B it can be seen that immediately after the elution solutions were added the Pt concentrations in the supernatant started to increase, and that the highest Pt concentrations were found after two hours. After this the Pt concentration within the solutions started to slightly decrease again. The elution rate of the first solution, i.e. a mixture of 4.5 mL 6 M HNO_3 and 0.5 mL HCl was much faster when compared with solution 2 (8 M HCl). Results indicated that approximately 98% Pt was recovered from elution solution 1 and 68% was recovered from elution solution 2 after 120 minutes.

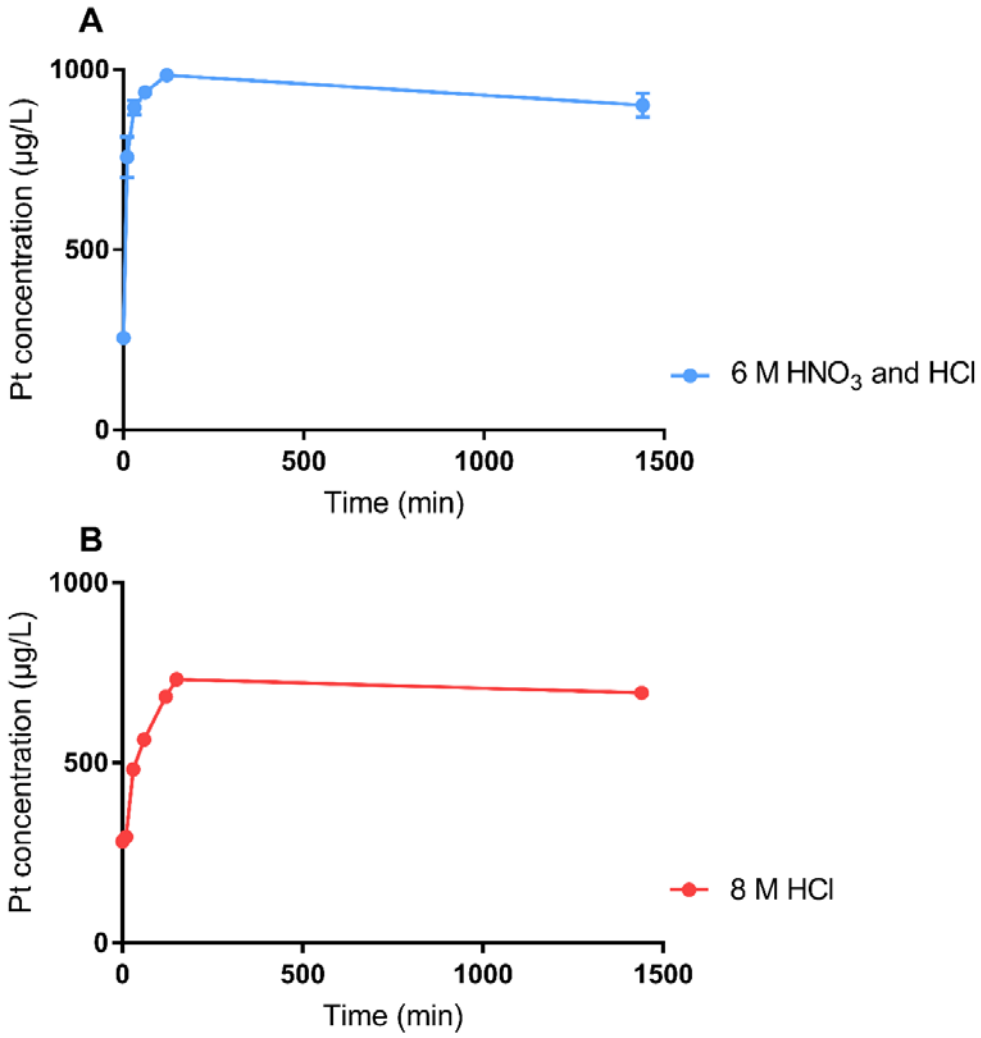

Figure 3-6: Elution kinetics with 6 M HNO_3 and HCl (A) and 8 M HCl (B) (mean ± SEM).

Long term exposure of the artificial mussels

The next step was to undertake long term exposures with the AMs to determine the optimum exposure period. The water quality parameters throughout all of the exposure tanks had a similar pattern during the exposure period regardless of the exposure concentration (Figure 3-7). The conductivity (Figure 3-7 A) increased within the tanks over the time, where the tank with the highest concentration had the highest conductivity readings. The pH (Figure 3-7 B) on the other hand decreased over time and indicated a negative relationship with the conductivity. During the 6 week exposure period the temperature was constant at 19.0 ± 0.66 ºC in all tanks.

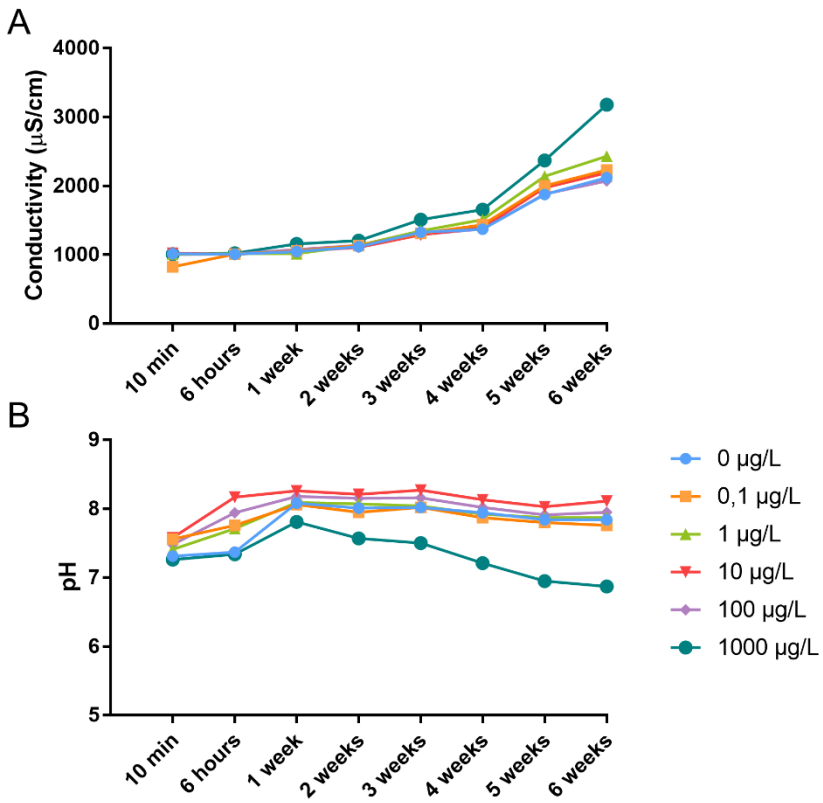

Figure 3-7: Mean conductivity (A) and pH (B) measurements at the different exposure concentrations during the six week platinum exposure period.

The Pt concentrations immediately increased in the exposure medium following the addition of the different exposure concentrations. Approximately 60% of the initially added Pt was found in the first water samples at 10 minutes. However this was not seen in the tank with the highest concentration, where approximately 10% was measured in the water. For all exposure concentrations there was an immediate peak followed by a slight decrease, this was followed by a general increase in Pt concentrations. It can be seen for Figure 3-8 A and B similar patterns could be seen in the concentrations over the six week exposure. In all experimental

groups the Pt concentrations increased in the 6 weeks of exposure. However, it can be seen that the 1000 µg/L had a significant increase in Pt concentration over the six week exposure period, where the Pt concentrations at the end of the exposure period exceeded the Pt concentration that was added.

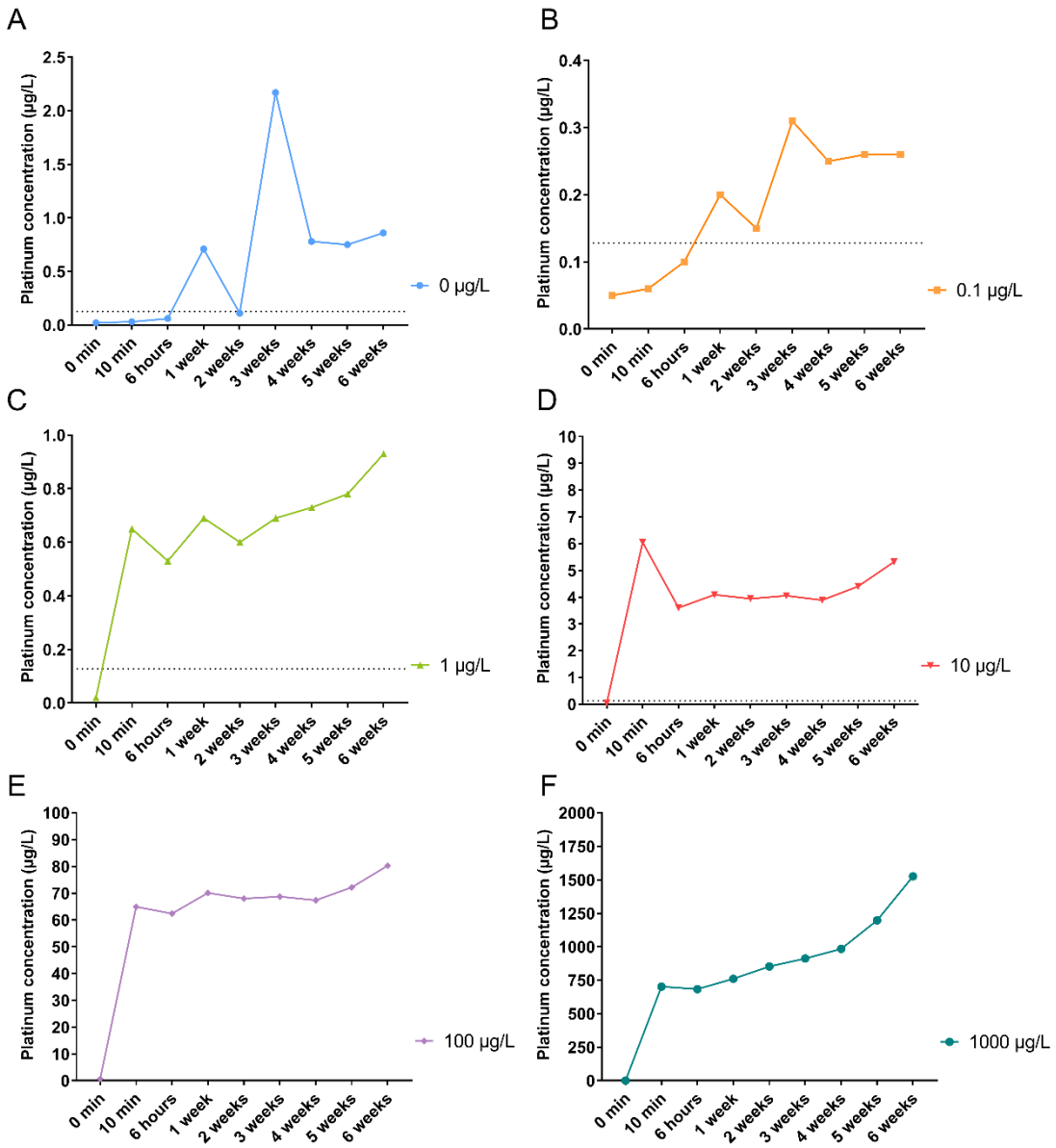

Figure 3-8: Mean platinum concentrations (µg/L) measured in the exposure media of the different exposure groups (detection limit indicated with dashed line = 3 x SD = 0.128 µg/L).

There is a time dependant increase in Pt concentrations in the AMs at all Pt exposure concentrations. After one week of exposure there is already a significant increase in Pt concentrations (Figure 3-9). Pt concentrations in the AMs increased from week 1 to 6 in all tanks, with the exception of the 1000 µg/L group. In this tank no significant difference in Pt

concentrations from week 1 and 6 occurred. The Pt concentration increased during the first 3 weeks of exposure, followed by a slight decrease during the next 3 weeks (Figure 3-9 F). From this it could be seen that of the initial concentrations that were added to the exposure medium 224% of the 1 µg/L, 138% of the 10 µg/L, 86% of the 100 µg/L and 56% of the 1000 µg/L were taken up by the AMs after 6 weeks.

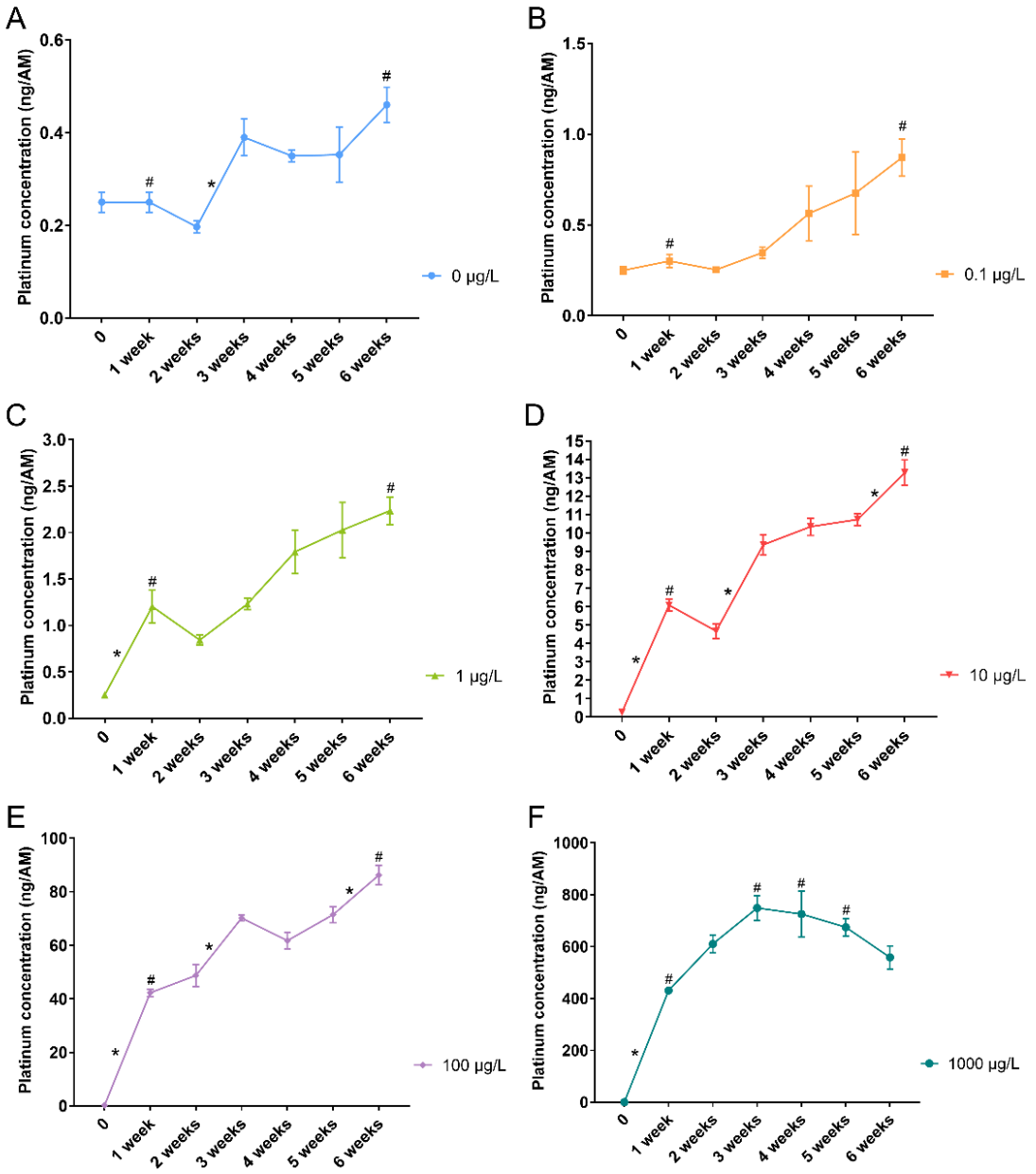

Figure 3-9: Platinum concentrations (mean ± SEM) expressed as ng/AM measured in the artificial mussels over the six week exposure period (significance was taken as p<0.05 with # indicative of a significant increase from week 1 to week 6, with the exception of F - 1000 µg/L, * is indicative of a significant increase between weeks sampled).

3.4 Discussion

Loading and elution of platinum from Chelex® beads

The AM was originally developed by Wu *et al*. (2007) for the marine environment, where after it was used in a number of studies in the freshwater environment. However it is not evident from these studies whether the AM was validated for freshwater. There are many methods that were used in previous studies for eluting the metals that were bound to the Chelex® beads (Wu *et al.*, 2007, Greenfield *et al.*, 2014, Claassens *et al.*, 2016, Dahms-Verster *et al.*, 2018). Since the AM has never been used for PGEs, it was decided to validate the uptake of Pt by the AMs through determining the kinetics behind the uptake and to optimize the elution of metals from the beads. It has been shown that Pt has the ability to bind to Chelex® beads (Samczyński *et al.*, 2000). Two solutions were used to test the loading of AMS, i.e. ultrapure water and hydrochloric acid. The ultrapure water was selected since the Chelex® beads are suspended within ultrapure water within the AM. The second solution consisted of hydrochloric acid (0.5 M HCl) and was used since it would ensure that the Pt would stay in its soluble form and that the Pt remains bound to the beads (Samczyński *et al.*, 2000).

For the ultrapure water, half of the Pt that was added to the loading solution remained in the solution for the duration of the experiment. Based on the results of the AM exposures it is clear that the Pt had bound to the Chelex® beads. In the second loading solution there was a definite time dependant decrease of the Pt concentrations. Thus, it was presumed that even if there was a loss of Pt due to other factors, the Chelex® beads had still accumulated the Pt. For this reason it was then decided to use only the tubes from the second loading solution for the elution experiment. There was an estimated 20% Pt still in solution at the end of the loading experiment. It could be seen that the concentrations within the solution was starting to reach equilibrium.

Before the elution experiment was initiated the Chelex® beads went through several washing steps. During these washing steps the supernatant was removed and analysed to determine if there was a loss of Pt from the beads. It was expected that there would be some Pt removal from the beads after the first washing step since it would rinse the tube and remove the rest of the loading solution. This was confirmed in that there were detectable (low) Pt concentrations in the first washing solution. However there was no Pt present in the second and third washing solutions, indicating that washing the beads by means of centrifugation is efficient and has advantages when compared to the traditional filtration method.

According to the literature all AM studies used the same elution solution (i.e. 6 M HNO_3) based on the (Wu *et al.*, 2007) protocol. However because Pt solutions are generally more stable

with HCl due to the formation of Pt chloro-complexes, HCl was included with 6 M HNO_3 in the first elution solution (Samczyński *et al.*, 2000). The second elution solution (8 M HCl) was used since it would ensure that the Pt would be eluted from the beads (Samczyński *et al.*, 2000). While both of the elution solutions were effective, it could definitely be seen that the mixture of nitric acid and hydrochloric acid had a higher elution rate and also resulted in recovery of higher Pt concentrations from the Chelex® beads. In both Pt concentrations in the elution solution started to decrease with increase in exposure period indicating that either the Pt started to bind to the Chelex® beads or to the walls of the PP exposure tubes.

For the elution the most effective solution was a mixture of 4.5 mL 6 M HNO_3 and 0.5 mL HCl. The Chelex® beads were kept in the elution solution for approximately 2 to 3 hours for the optimum release of Pt from the Chelex® beads. Two modifications to the original method are therefore recommended for use of AMs in monitoring PGEs, i.e. washing by centrifugation (it saves time and the use of filter paper) and using a mixture of acids to ensure the release of all bound Pt from the beads and that the elution remains stable once these metals are released in the solution.

Long term exposure of the artificial mussel

The artificial mussel has been deployed for monitoring of metals in marine (Wu *et al.*, 2007, Leung *et al.*, 2008, Degger *et al.*, 2011, Degger *et al.*, 2016) and freshwater ecosystems (Claassens *et al.*, 2016, Kibria *et al.*, 2016, Dahms-Verster *et al.*, 2018). The deployment period for these studies ranged between four to six weeks. However there are no data on the optimum deployment period required for PGEs to reach steady state conditions. Therefore the long term laboratory exposure was conducted to evaluate the length of deployment on Pt uptake by AMs at different exposure concentrations.

During the exposure period the measured water quality variables remained relatively stable throughout the exposure. During the exposure some evaporation occurred from the tanks during the five to six week exposure periods, which could have caused the increased conductivity particularly at the highest exposure concentration.

Changes in pH (i.e. a decrease) could have affected the Pt speciation, which may result in an increase in soluble Pt fraction (Charlatchka and Cambier, 2000). The pH can influence the adsorption properties of Pt to surfaces and, in this case, Pt bound to AMs and the Chelex® beads. Thus the more acidic exposure medium could have resulted in the Pt that was bound to the surface of the plastic bags, to be released back into solution. It is then also probable that some of the Pt that was bound to the AMs could be released into solution. This explains the increased Pt levels in the AMs with a corresponding decrease in pH at the high Pt exposure concentrations.

Except for the highest exposure group, the Pt concentrations in the AMs increased over the whole exposure period. Steady state conditions were not reached within the 6 week exposure period. This is an important factor to keep in mind for field studies where Pt concentrations are very low. There was however one exception, the tank with the highest concentration 1000 µg/L. There appeard that Pt levels reached saturation in week 3 after which the concentrations decreased in the AMs.

During this exposure the AMs were exposed to a single element, but during field exposures they will be exposed to a mixture of different elements. Some metals have a higher affinity to bind to Chelex® beads than others, thus it is possible that metals will compete for binding sites. It should also be kept in mind that AMs accumulate only the metal ion fraction that can cross the polyacrylamide gel layers. According to Bio-Rad Laboratories (Nobel, 2000) the selectivity and affinity of Chelex® beads to certain metals depend on the pH, ionic strength and other complex species. When specific cations were compared to a reference cation they found that Chelex® beads have the following affinity to a range of cations:

Table 3-4: Affinity for cations when compared to a reference cation (Zn) (Nobel, 2000).

Hg^{+2}	1060	Fe^{+2}	0.130
Cu^{+2}	126	Mn^{+2}	0.024
Ni^{+2}	4.40	Ba^{+2}	0.016
Pb^{+2}	3.88	Ca^{+2}	0.013
Zn^{+2}	1.00	Sr^{+2}	0.013
Co^{+2}	0.615	Mg^{+2}	0.009
Cd^{+2}	0.390	Na^{+1}	0.0000001

3.5 Conclusion

After considering all methods that can be used to elute metals from Chelex® beads, this study has assisted in developing an adapted method for using AMs to measure Pt (and other PGEs) in field studies. This method is easy to perform, has high recovery and high precision. The AM is a promising monitoring tool for Pt in the aquatic environment as it accumulates in a concentration dependant manner. From the results of the loading and recovery experiment, and the long term exposure, it can be seen that the threshold to which the AMs and Chelex® beads can accumulate Pt was not reached at the highest exposure concentration, i.e. 1000 µg/L that were tested in this study. This concentration is far above Pt concentrations which are expected in aquatic environments.

References

Blom, L. B., Morrison, G. M., Kingston, J., Mills, G. A., Greenwood, R., Pettersson, T. J. and Rauch, S. 2002. Performance of an in situ passive sampling system for metals in stormwater. *Environmental Monitoring,* 4**:**258-262.

Brumbaugh, W., Petty, J., Huckins, J. and Manahan, S. 2002. Stabilized liquid membrane device (SLMD) for the passive, integrative sampling of labile metals in water. *Water, Air, and Soil Pollution,* 133**:**109-119.

Cawthorn, R. G. 2010. The platinum group element deposits of the Bushveld Complex in South Africa. *Platinum Metals Review,* 54**:**205-215.

Charlatchka, R. and Cambier, P. 2000. Influence of reducing conditions on solubility of trace metals in contaminated soils. *Water, Air, and Soil Pollution,* 118**:**143-168.

Claassens, L., Dahms, S., Van Vuren, J. and Greenfield, R. 2016. Artificial mussels as indicators of metal pollution in freshwater systems: A field evaluation in the Koekemoer Spruit, South Africa. *Ecological Indicators,* 60**:**940-946.

Dahms-Verster, S., Baker, N. and Greenfield, R. 2018. A multivariate examination of 'artificial mussels' in conjunction with spot water tests in freshwater ecosystems. *Environmental monitoring and assessment,* 190**:**427.

Degger, N., Chiu, J. M. Y., Po, B. H. K., Tse, A. C. K., Zheng, G. J., Zhao, D.-M., Xu, D., Cheng, Y.-S., Wang, X.-H., Liu, W.-H., Lau, T. C. and Wu, R. S. S. 2016. Heavy metal contamination along the China coastline: A comprehensive study using Artificial Mussels and native mussels. *Environmental Management,* 180**:**238-246.

Degger, N., Wepener, V., Richardson, B. and Wu, R. 2011. Application of artificial mussels (AMs) under South African marine conditions: A validation study. *Marine Pollution Bulletin,* 63**:**108-118.

Følsvik, N., Brevik, E. M. and Berge, J. A. 2000. Monitoring of organotin compounds in seawater using semipermeable membrane devices (SPMDs)—tentative results. *Environmental Monitoring,* 2**:**281-284.

Følsvik, N., Brevik, E. M. and Berge, J. A. 2002. Organotin compounds in a Norwegian fjord. A comparison of concentration levels in semipermeable membrane devices (SPMDs), blue mussels (*Mytilus edulis*) and water samples. *Environmental Monitoring,* 4**:**280-283.

Genç, T. O., Po, B. H., Yılmaz, F., Lau, T.-C., Wu, R. S. and Chiu, J. M. 2018. Differences in metal profiles revealed by native mussels and artificial mussels in Sarıçay Stream, Turkey: implications for pollution monitoring. *Marine and Freshwater Research,* 69**:**1372-1378.

Gonzalez-Rey, M., Lau, T., Gomes, T., Maria, V., Bebianno, M. and Wu, R. 2011. Comparison of metal accumulation between 'Artificial Mussel' and natural mussels (*Mytilus galloprovincialis*) in marine environments. *Marine Pollution Bulletin,* 63**:**149-153.

Greenfield, R., Brink, K., Degger, N. and Wepener, V. 2014. The usefulness of transplantation studies in monitoring of metals in the marine environment: South African experience. *Marine Pollution Bulletin,* 85**:**566-573.

Hossain, M. M., Kibria, G., Nugegoda, D., Lau, T. and Wu, R. 2015. A Training Manual for Assessing Pollution (trace/heavy metals) in Rivers, Estuaries and Coastal waters-Using Innovative "Artificial Mussel (AM) Technology" - Bangladesh Model. *Research collaboration between scientists of the IMSF, University of Chittagong, Bangladesh, RMIT University, Australia, the City University of Hong Kong, and the University of Hong Kong.*

Kibria, G., Hossain, M. M., Mallick, D., Lau, T. and Wu, R. 2016a. Monitoring of metal pollution in waterways across Bangladesh and ecological and public health implications of pollution. *Chemosphere,* 165**:**1-9.

Kibria, G., Hossain, M. M., Mallick, D., Lau, T. and Wu, R. 2016b. Trace/heavy metal pollution monitoring in estuary and coastal area of Bay of Bengal, Bangladesh and implicated impacts. *Marine Pollution Bulletin,* 105**:**393-402.

Kibria, G., Lau, T. and Wu, R. 2012. Innovative 'Artificial Mussels' technology for assessing spatial and temporal distribution of metals in Goulburn–Murray catchments waterways, Victoria, Australia: Effects of climate variability (dry vs. wet years). *Environment international,* 50**:**38-46.

Leung, K. M., Furness, R. W., Svavarsson, J., Lau, T. and Wu, R. S. 2008. Field validation, in Scotland and Iceland, of the artificial mussel for monitoring trace metals in temperate seas. *Marine Pollution Bulletin,* 57**:**790-800.

Mudd, G. M., Jowitt, S. M. and Werner, T. T. 2018. Global platinum group element resources, reserves and mining–A critical assessment. *Science of the Total Environment,* 622**:**614-625.

Namieśnik, J., Zabiegała, B., Kot-Wasik, A., Partyka, M. and Wasik, A. 2005. Passive sampling and/or extraction techniques in environmental analysis: a review. *Analytical and bioanalytical chemistry,* 381**:**279-301.

Nobel, A. 2000. *Chelex ® 100 and Chelex 20 Chelating Ion Exchange Resin Instruction Manual* [Online]. Bio-Rad Laboratories. Available: http://www.bio-rad.com/webroot/web/pdf/lsr/literature/LIT200.pdf [Accessed 2 October 2018].

Parker, L. and Clark, C. 2002. Study of Five Discrete Interval-Type Groundwater Sampling Devices. ERDC. CRREL TR-02-12. Prepared for the US Army Environmental Center, Aberdeen Proving Ground, MD, USA. Google Scholar.

Parthasarathy, N. and Buffle, J. 1994. Capabilities of supported liquid membranes for metal speciation in natural waters: application to copper speciation. *Analytica chimica acta,* 284**:**649-659.

Parthasarathy, N., Pelletier, M. and Buffle, J. 1997. Hollow fiber based supported liquid membrane: a novel analytical system for trace metal analysis. *Analytica chimica acta,* 350**:**183-195.

Pressman, M. A. S. and Aldstadt III, J. H. 2003. A comparative study of diffusion samplers for the determination of hexavalent chromium by sequential injection spectrophotometry. *Microchemical,* 74**:**47-57.

Rauch, S. and Peucker-Ehrenbrink, B. 2015. Sources of Platinum Group Elements in the Environment. *In:* Zereini, F. and Wiseman, C. L. S. (eds.) *Platinum Metals in the Environment.* Heidelberg: Springer.

Ruiz-Fernández, A. C., Wu, R. S., Lau, T.-C., Pérez-Bernal, L. H., Sánchez-Cabeza, J. A. and Chiu, J. M. 2018. A comparative study on metal contamination in Estero de Urias lagoon, Gulf of California, using oysters, mussels and artificial mussels: Implications on pollution monitoring and public health risk. *Environmental pollution,* 243**:**197-205.

Samczyński, Z., Danko, B. and Dybczyński, R. 2000. Application of Chelex 100 ion exchange resin for separation and determiantion of palladium, platinum and gold in geological and industrial materials by neutron activation analysis. *Chemia Analityczna,* 45**:**843-857.

Vrana, B., Allan, I. J., Greenwood, R., Mills, G. A., Dominiak, E., Svensson, K., Knutsson, J. and Morrison, G. 2005. Passive sampling techniques for monitoring pollutants in water. *Trends in Analytical Chemistry,* 24**:**845-868.

Wu, R. and Lau, T. 1996. Polymer-ligands: a novel chemical device for monitoring heavy metals in the aquatic environment? *Marine Pollution Bulletin,* 32**:**391-396.

Wu, R. S., Lau, T., Fung, W. K., Ko, P. and Leung, K. M. 2007. An 'artificial mussel' for monitoring heavy metals in marine environments. *Environmental pollution,* 145**:**104-110.

Chapter 4: The application of artificial mussels in conjunction with active biomonitors to assess metal exposure in a platinum mining area

4.1 Introduction

During the last couple of years the concentration of platinum group elements (PGE) (platinum and palladium) have been increasing since they have been used more and more in a number of applications (Rauch and Peucker-Ehrenbrink, 2015). These applications include various chemical processes, the pharmaceutical industry and other production processes. One of the biggest contributors to PGEs in the environment is the use of automotive catalytic converters and mining activities. Mining activities in South Africa have been reported to greatly contribute to PGE emissions in the environment (Rauch and Peucker-Ehrenbrink, 2015). It is particularly in the platinum mining regions of the North-West Province of South Africa where PGE and associated metals show increased levels in the aquatic environment (Almécija *et al.*, 2017).

Bioaccumulation is an important process where living organisms take up toxicants at a greater rate than the rate at which it eliminates these substances (Zhou *et al.*, 2008). There are many factors that can influence the bioaccumulation of metals in the environment, such as temperature and salinity, as well as biotic factors such as age, body size and reproductive status of the organism. In addition there are also many factors that influence the bioavailability of metals such as pH and conductivity (Zhou *et al.*, 2008).

It is this process of bioaccumulation that has been applied in monitoring studies where organisms such as bivalves are used as indicators of e.g. metal exposure (Gupta and Singh, 2011). Traditionally resident bivalve populations have been used as indicator species (Farris and Van Hassel, 2006). However in instances where there are no resident species available, organisms that were collected from an unstressed or otherwise unpolluted population can be translocated to selected sites to determine the degree of pollution that occurs at that site (Wepener, 2008).

Suitable transplanted organisms are selected based on biological characteristics, such as tolerance and abundance. Transplanted organisms facilitate the investigation of areas where native specimens are absent or compensatory mechanisms occur in the native populations from contaminated areas (Giarratano *et al.*, 2010). For this study the freshwater clam, *Corbicula fluminalis africana,* was selected as bioindicator organism. Bivalves are excellent bioindicators since they are sensitive to contaminants, have a wide geographical distribution,

high abundance, sedentary, tolerant to environmental alterations, high bioconcentration factors and are sturdy enough for both laboratory and field studies (Zhou *et al.*, 2008).

Despite the widespread use of living organisms as indicators of metal bioaccumulation they have a number of disadvantages. The metal release and uptake in these organisms are affected by temperature, life cycles, size, depth and reproduction. It has been found that different species have different accumulation strategies, and that the indicator species may have different natural distribution patterns (Wepener, 2008). Every now and then the population may not be able to survive in certain environmental conditions. Indicator species may not always be present in all ecosystems that needs to be monitored, therefore it prevents the comparison between different geographical areas. Sometimes the kinetics behind the uptake and depuration of these metals are not well understood (Wu and Lau, 1996).

To overcome these disadvantages passive sampling devices such as the artificial mussel (AM) have been utilised. The advantages of the use of AMs for monitoring of metals are that they provide time-integrated estimates of metal concentrations within the aquatic ecosystems, it allows the comparison between water bodies that are not able to support a specific bio-indicator species, they also allow the monitoring of the water quality without having to kill organisms (Wu *et al.*, 2007, Kibria *et al.*, 2012). These devices are not affected by biotic and abiotic factors, thus it can be deployed in marine and freshwater ecosystems (Hossain *et al.*, 2015, Claassens *et al.*, 2016).

It was found that AMs can be used to compare the metal concentrations within the artificial monitoring devices and bioindicator species, since they accumulate metals within the same magnitude (Wu and Lau, 1996). There have been a couple of studies that included both AMs and transplanted organisms to monitor metal exposure. The results of these studies indicated that AMs are less affected by salinity and temperature than transplanted organisms (Wu *et al.*, 2007, Leung *et al.*, 2008, Degger *et al.*, 2011) and that AMs and bivalves accumulate metals at different levels but in a similar pattern (Degger *et al.*, 2011, Claassens *et al.*, 2016).

The aim of this chapter was to determine if the AM can be used as a tool to determine PGE and other metal exposure in the environment. The AM method for this study was described in Chapter 3 and will be tested in a field study alongside an established bivalve bioaccumulation indicator species to determine the degree of similarity in metal bioaccumulation.

4.2 Materials and methods

4.2.1 Study site and site selection

The Hex River flows through the city of Rustenburg, which is the most populated municipality in the North West Province of South Africa. Rustenburg is located on the western limb of the

Bushveld Igneous Complex where the main industrial activity is mining (Rauch and Fatoki, 2015). These mining activities include mining for platinum and chrome. Along the course of the Hex River there are impoundments, which can be affected by mining activities (Wittmann and Förstner, 1976).

Bospoort Dam was selected as a site downstream of the mining activities and would be the best indicator of any impacts from these activities. Olifantsnek Dam is situated well to the south of Rustenburg and is separated from the mining area by the Magaliesberg Mountain range, it was therefore chosen as the reference site (Wittmann and Förstner, 1976). A third site which is located in a tailings dam located on the premises of a platinum mine was also selected to serve as a reference site since high PGE and associated metals were expected to occur at this site.

Two sampling surveys during the low flow periods (dry seasons – May to June) in 2017 and 2018 were undertaken to the selected sites (Figure 4-1). The Tailings Dam site was only included in the second survey due to delays in obtaining the necessary permission from the mining company.

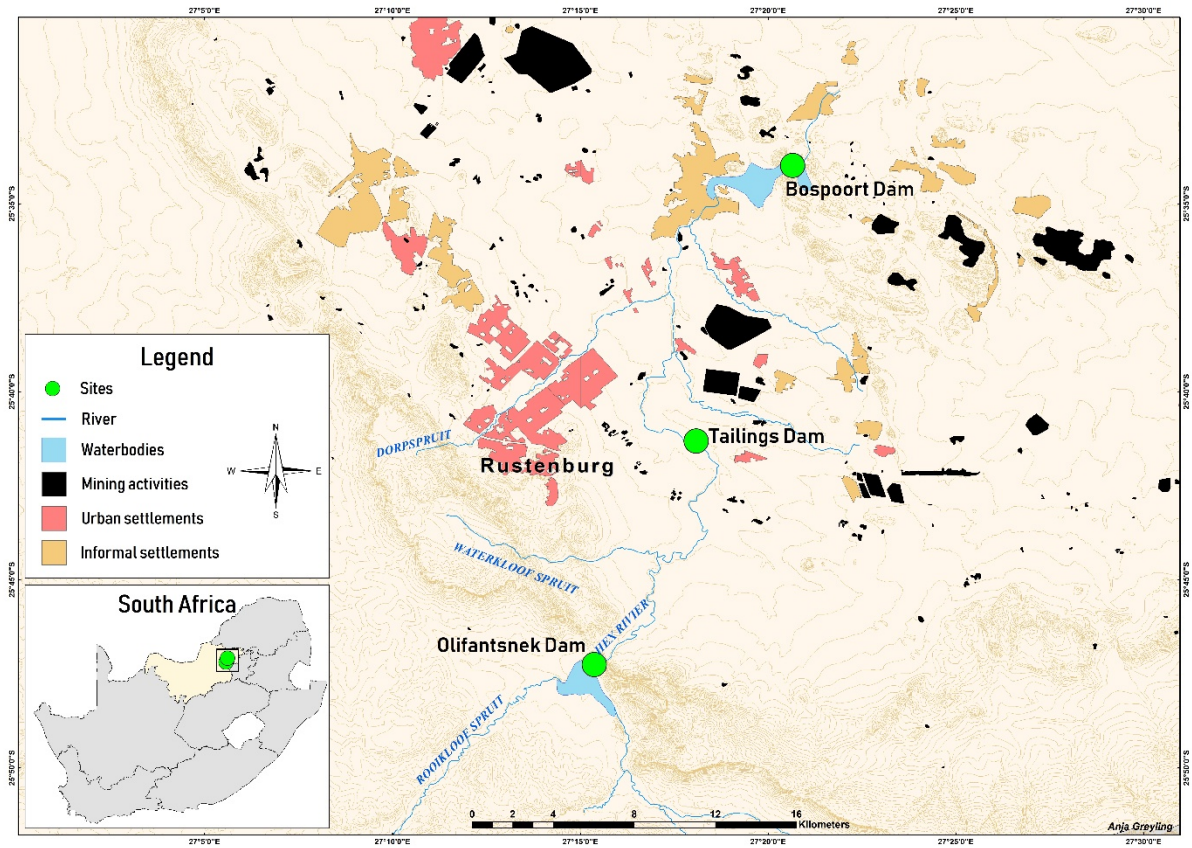

Figure 4-1: Map of the Hex River System in Rustenburg, North West Province, South Africa indicating the position of the sampling sites.

4.2.2 Description of the study area and sites

The Hex River originates south of Rustenburg, where the Olifantsnek Dam is situated close to the origin of the Hex River. According to Roux (2011) the part of the Hex River that joins with Olifantsnek Dam is in a good to fair ecological state. The main impacts on this impoundment include water abstraction and farming activities. The ecological category of this stretch of the river classifies it as largely natural/moderately modified. This impoundment is managed by private owners, where the main activity is recreation e.g. fishing and sailing.

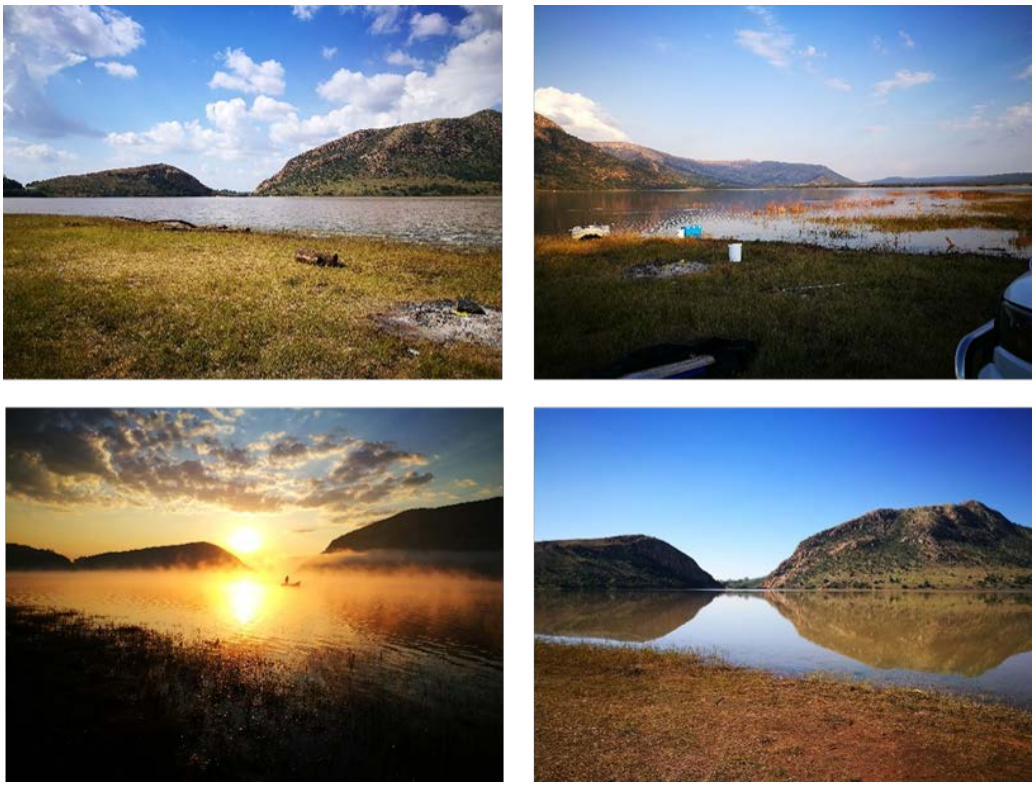

Figure 4-2: Olifantsnek Dam, Rustenburg.

Bospoort Dam is a small state-owned impoundment situated along the course of the Hex River in Rustenburg (Wittmann and Förstner, 1976). The land-uses in the catchment include urban developments, intensive mining activities and agricultural activities (Mogakabe and Van Ginkel, 2008). Bospoort Dam is also used for irrigation and in the past for domestic water supply. However due to a decrease in the water quality and increase in algal blooms that influenced the taste and odour of the water, Bospoort Water Treatment Works (WTW) ceased to operate. The Bospoort Dam is classified as being in a hypereutrophic state, where the possible influences include sewage treatment works, agricultural and urban run-offs, as well as recirculation of nutrients that can be found in the sediments. In 2008 a report was published on the water quality of Bospoort Dam where they stated that Cd, As and Pb in the water is of concern, even though these concentrations were low (Mogakabe and Van Ginkel, 2008).

Since the dam is also used for fishing activities, these concentrations might pose great risk for the aquatic life in this system. This would further result in the fish and water being unfit to be consumed by humans. The system also shows a high salt content, generally indicated by high conductivity values. Urban surface runoff and mining activities are possible sources of ions that contributed to the high conductivity in the Bospoort dam, while the possible sources of elevated metal concentrations include mining activities and runoff from agricultural fields. According to Roux (2011) the Hex River upstream of Bospoort Dam is classified as seriously to critically modified, whereas the river below the dam is in a moderate to largely modified category.

Figure 4-3: Bospoort Dam, Rustenburg.

4.2.3 Artificial Mussel (AM) deployment, retrieval and sampling

Artificial Mussel deployment

The AMs were assembled as described in Chapter 3 (Figure 4-4). Once the AMs were assembled the devices were placed in containers filled with ultrapure water and stored in the laboratory until it was time for deployment in the field. For the transportation of the AMs to the field, the devices were removed from the water and both ends of the tube were plugged with cotton soaked in ultrapure water. This prevents any damage that may occur during transportation.

Figure 4-4: The artificial mussel design (Wu *et al*., 2007).

In addition to the deployment of the AM, an established bioaccumulation indicator species was concomitantly deployed. The freshwater clam, *C. fluminalis africana,* with an average shell length of 28 ± 5 mm were collected from the Mooi River, Potchefstroom, South Africa (Figure 4-5). The clams were transported to the laboratory two weeks before the survey where they were kept in reconstituted freshwater to depurate any elements that it might have accumulated from the reference site. The transplanted clams were fed with *Spirulina*, cultured in the laboratory at the North-West University. Water was changed regularly throughout the two week depuration period. The transplanted clams were transported to the field in plastic containers filled with reconstituted freshwater and supplied with oxygen using a portable pump. A control group of transplanted organisms were prepared for metal analyses that served as an indication of the initial concentrations that could be found in the transplanted organisms before exposing them to the sampling sites.

Figure 4-5: Transplanted clams used in this study, *Corbicula fluminalis africana.*

The AMs and transplanted clams were deployed at each sampling site in two plastic baskets (Figure 4-6). Each basket was fitted with a mesh bag in the bottom of the basket and a mesh

covering for the top of the basket. Once arriving at the site the mesh bags were filled with sediment from that site, this was done to create a natural habitat for the clams for the exposure period. Thereafter 10 clams were transferred to each basket, in total 20 transplanted clams were deployed at each site. After transferring the clams the mesh bags were closed to prevent the organisms from escaping. The top half of each basket was then used to attach the AMs. Fifteen AMs were deployed at each site by fastening them to the side of the plastic baskets with cable ties. The baskets were covered with mesh to prevent any objects from damaging the AMs during the survey.

Figure 4-6: Steps taken to assemble the plastic baskets for the artificial mussels and transplanted clams.

Once the AMs and transplanted clams were secured, the baskets were tied together with rope and cable ties and secured to a weight (Figure 4-7). The weights ensured the submersion of the baskets within the water body. The baskets were secured to buoys and permanent structures within the impoundments and tailings dam on the mine site.

Figure 4-7: Deployment of the plastic baskets in Bospoort Dam.

Artificial mussel retrieval

Each batch of AMs and transplanted organisms were retrieved after the set exposure period (4 to 6 weeks) after deployment. Each AM was then rinsed with water from the site to remove any silt or algae that might have accumulated during the exposure period. Cotton pads were soaked in water from the site, and placed in both ends of the AM to form a plug. This ensured that the gel would not get damaged during transportation and to prevent the water and Chelex® beads from seeping from the AM. At the laboratory the containers with the AMs were placed in a cool area until further analysis.

The transplanted organisms were retrieved at the same time as the AMs. These organisms were removed from the mesh bags and placed in containers that were fitted with an oxygen pump and water from the site. They were transported back to the laboratory and were measured, weighed and then separated for metal and biomarker analysis. Wet tissue was dissected from the shells and samples for metal analysis were placed in acid pre-washed poly propylene Falcon tubes and frozen at -80°C freezer until further analysis. Wet tissue for biomarker analyses was transferred into tubes containing Henriksson's stabilising buffer (Henriksson *et al*., 1986) and placed in a -80°C freezer until further analyses

Water quality and sample collection

Water samples were collected at each site at the start and end of the field exposures. The sampling procedure was as follows; 10 mL water was removed and acidified with 10 µL HNO_3 (sub-boiled from 65%; p.a. quality, Merck, Germany). *In situ* water quality parameters were

measured at each site during deployment and retrieval of the AMs and transplanted organisms. These water quality parameters were pH, electrical conductivity, total dissolved solids, temperature and dissolved oxygen.

4.2.4 Metal analysis

For metal analysis the contents of each individual AM were emptied into a 15 mL polypropylene tube. These samples were then centrifuged (2 minutes at 1000 g), the supernatant was removed and the beads were rinsed with 5 mL ultrapure water. The supernatant was removed and the beads were eluted with 4.5 mL 6 M HNO_3 (sub-boiled from 65%, Merck, Germany) and 0.5 mL HCl (37%, suprapure, Merck). The beads were left in the acid solutions for approximately 2 hours to ensure that all bound metals would be released from the beads. The supernatant was removed and placed in polypropylene tubes at room temperature for further analysis.

Clam tissue samples were weighed prior to freeze drying. Replicate samples of at least 60 mg (dry weight) were weighed into 20 mL TFM® vessels (MarsXpress CEM, Germany). The digestion was carried out in a microwave digestion system (CEM, Mars 6) with 2.5 mL H2O2 (30%; Suprapur®, Merck, Germany) and 1.3 mL HNO_3 (sub-boiled from 65%; p.a. quality, Merck, Germany). Clear solutions were then transferred to 5 mL glass flasks and brought to volume with 1% HNO_3. The digested solutions were stored at room temperature in polypropylene tubes until metal analysis. Metals that were analysed included; As, Cd, Co, Cr, Ni, Pb, Pd, Pt, V and Zn.

Metal concentrations in the water, AMs and clam tissue were determined by means of a quadrupole ICP-MS system (Perkin Elmer, Elan 6000) with an autosampler system (Perkin Elmer, AS-90). Between each measurement, the wash time was set to 30 s with 2% HNO3 in order to avoid contamination. After every 10 samples a standard solution (10 µg/L), for all elements measured, was used to control the accuracy and stability of the measurements. Prior to measuring, samples were diluted 1:10 with an internal standard solution, consisting of 1% HNO3 and 10 µg/L thulium (Certipur®, Merck). Calibration of the instrument was performed using a series of 11 dilutions of standard solution. With this the concentrations of the sample analytes were calculated using regression lines with a correlation factor of ≥0,999.

4.2.5 Statistical analysis

Statistical analyses were performed using GraphPad Prism® 7 software. Homogeneity of variance was confirmed using the Kolmogorov-Smirnov test with Dallal-Wilkinson-Lilliefor P value. For metal analyses in water and sites 2-way ANOVAs were performed, followed by Tukey's multiple comparison test. For metal analyses of both the AMs and transplanted clams

the data were log transformed to compare all data sets since not all data passed normality. A two-way ANOVA was performed to determine significant differences between different surveys, different sites and different organisms. Where after Tukey's multi comparisons test was performed. Statistical significance was set at $p < 0.05$ for all comparisons. Principal component analyses (PCA) were conducted on the transplanted clams and AMs to plot metal uptake between the two bioindicators, Canoco 5 was used for this.

4.3 Results

From Table 4-1 it can be seen that the pH was relatively stable throughout both surveys. It can be seen that the temperature for the first survey was cooler than during the second survey, where this had an influence on the dissolved oxygen (DO) that was measured. During the second survey the DO was less than during the first survey, where Bospoort Dam had lower DO concentrations than Olifantsnek Dam. The EC and TDS of the three sites differed significantly from one another. Where Bospoort had significantly higher EC and TDS measurements than Olifantsnek Dam, still the tailings Dam had significantly higher measurements.

Table 4-1: Water quality parameters measured at each site during the first survey (2017) and the second survey (2018), results are average of parameters measured during a survey.

Site	pH	Temp (°C)	EC (µS/cm)	TDS (mg/L)	DO (mg/L)	DO (%)
Survey 2017						
Olifantsnek Dam	8.21	19.3	173.8	119.8	10.12	103.3
Bospoort Dam	8.46	19.45	910.5	570	9.69	81.4
Survey 2018						
Olifantsnek Dam	8.20	24.3	210	147	5.5	62.1
Bospoort Dam	7.65	28.3	1160	819	2.94	34.3
Tailings Dam	8.00	25.6	1775	1244	-	-

There was a significant difference in arsenic (As) concentrations of the water samples from Bospoort Dam between the beginning of the first survey and towards the end (Figure 4-8 A). It can also be noted that the tailings dam had significantly higher ($p<0.05$) As concentrations than in the two impoundments. The AMs and transplanted clams did not indicate a definite trend in As concentrations, with the exception of the transplanted clams from the first survey Figure 4-8 B). The concentrations found in these transplanted clams differed significantly from both the control and Bospoort Dam, as well as the transplanted clams from the second survey. The concentrations of As were higher in the AMs from Bospoort Dam during both surveys.

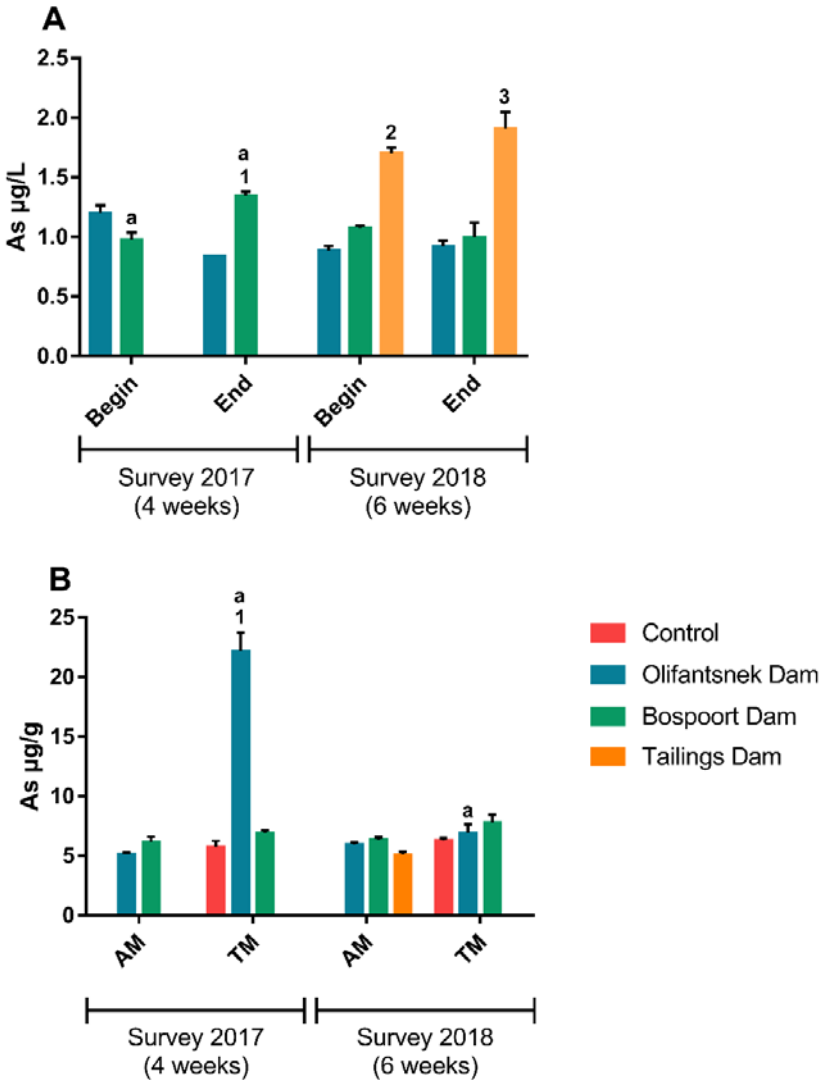

Figure 4-8: Arsenic concentrations (mean ± SEM) in water (A), and artificial mussels (AM) and transplanted clams (TM) during the two surveys (B). Concentrations are presented as µg/g dry mass for transplanted clams and µg/g Chelex® for the AMs. Bars with common numeral superscript represent significant differences between surveys while bars with common superscript letters represent significant differences within a specific survey. Significance was regarded as $p<0.05$.

The water samples from the three sites did not indicate a significant difference ($p>0.05$) in cadmium (Cd) concentrations (Figure 4-9 A), but there were some slight variation between the sites and the different surveys. There was no significant difference in the concentrations between the three different sampling sites for the AMs or the transplanted clams. The transplanted clams contained significantly higher Cd concentrations during both surveys than the AMs. During the second survey the AMs from both impoundments contained significantly lower ($p<0.05$) Cd concentrations. It can also be noted that there was a significant difference in Cd concentrations found in the control of the transplanted clams between the two surveys.

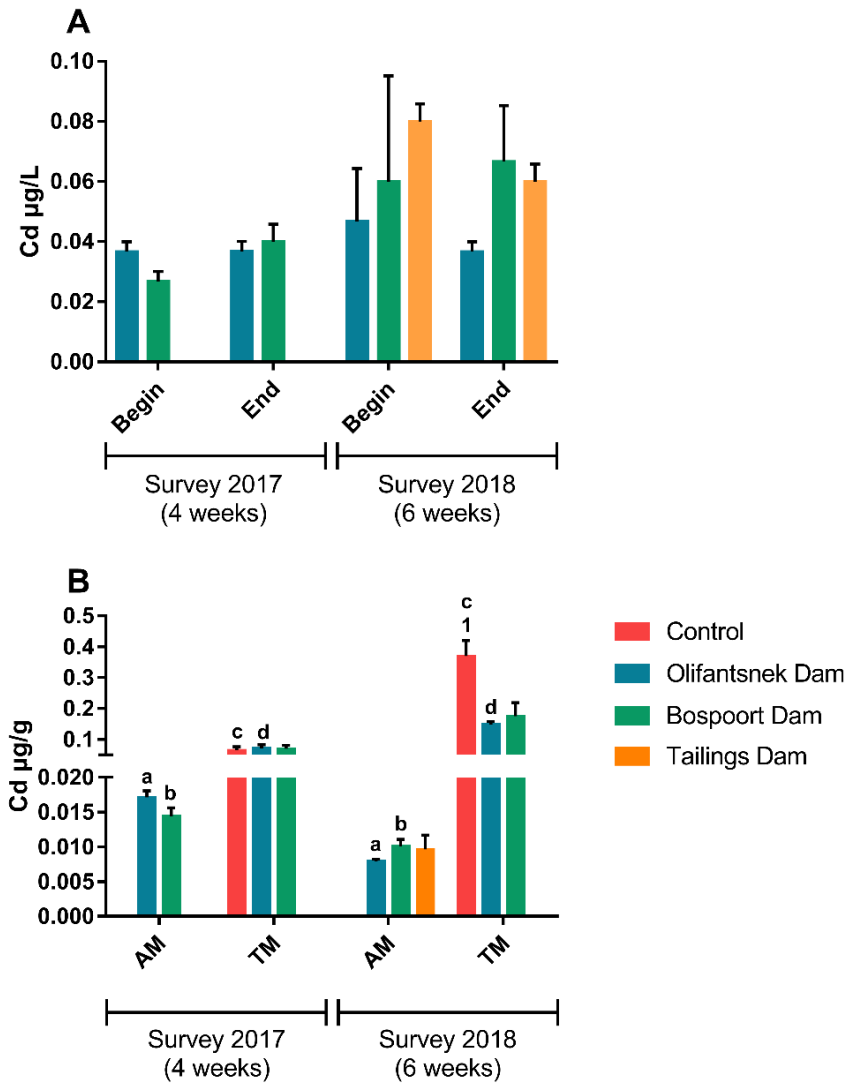

Figure 4-9: Cadmium concentrations (mean ± SEM) in water (A), and artificial mussels (AM) and transplanted clams (TM) during the two surveys (B). Concentrations are presented as µg/g dry mass for transplanted clams and µg/g Chelex® for the AMs. Bars with common numeral superscript represent significant differences between surveys while bars with common superscript letters represent significant differences within a specific survey. Significance was regarded as $p<0.05$.

The cobalt (Co) concentrations found in the water samples from the two impoundments indicated slight variations, while the tailings dam contained significantly higher ($p<0.05$) concentrations when compared to the concentrations during both surveys. From Figure 4-10 B it can be seen that AMs from Bospoort Dam contained significantly different Co concentrations during the first survey when compared to Olifantsnek Dam, there is also a significant difference when the AMs from Bospoort Dam are compared between the two surveys. The AMs from the tailings dam contained significantly lower Co concentrations when compared to the two impoundments. There was a significant difference in the control and the transplanted clams from Olifantsnek Dam between the two surveys. During the second survey the Co concentrations between the two impoundments differed significantly.

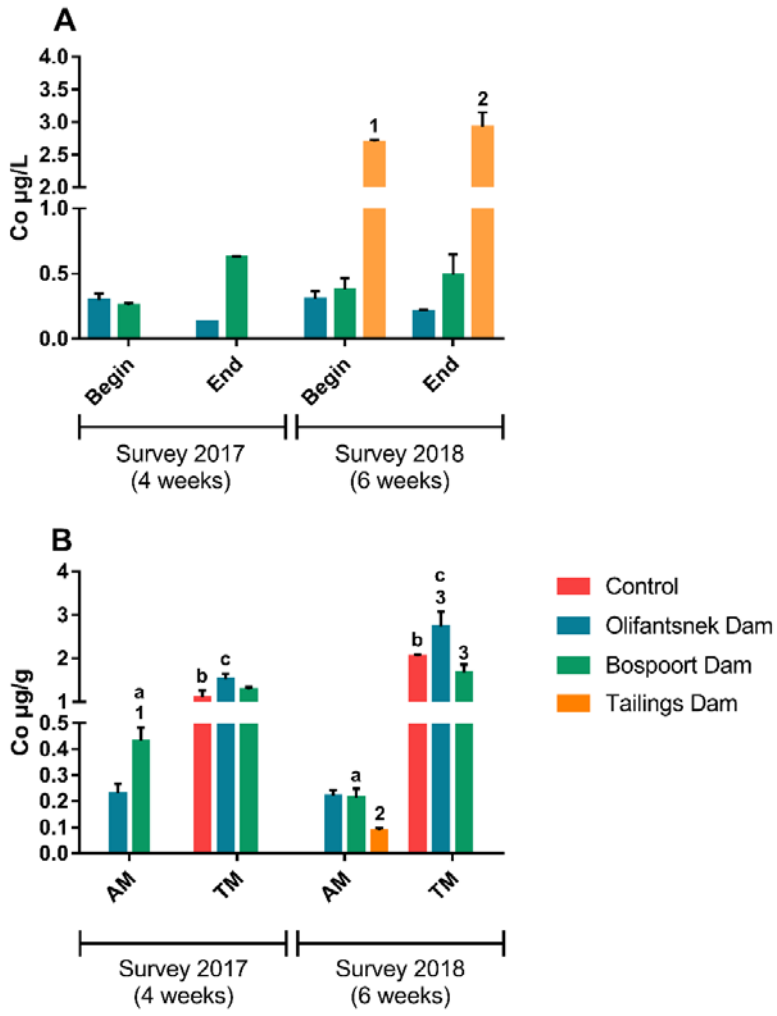

Figure 4-10: Cobalt concentrations (mean ± SEM) in water (A), and artificial mussels (AM) and transplanted clams (TM) during the two surveys (B). Concentrations are presented as µg/g dry mass for transplanted clams and µg/g Chelex® for the AMs. Bars with common numeral superscript represent significant differences between surveys while bars with common superscript letters represent significant differences within a specific survey. Significance was regarded as $p<0.05$.

In Figure 4-11 A it can be seen that there were slight variations in chromium (Cr) concentrations within the water samples, however the tailings dam contained significantly higher ($p<0.05$) concentrations at the end of survey 2. From these results it can be seen that during both surveys the transplanted clams from Olifantsnek Dam had significantly higher Cr concentrations than the control and Bospoort Dam (Figure 4-11 B).

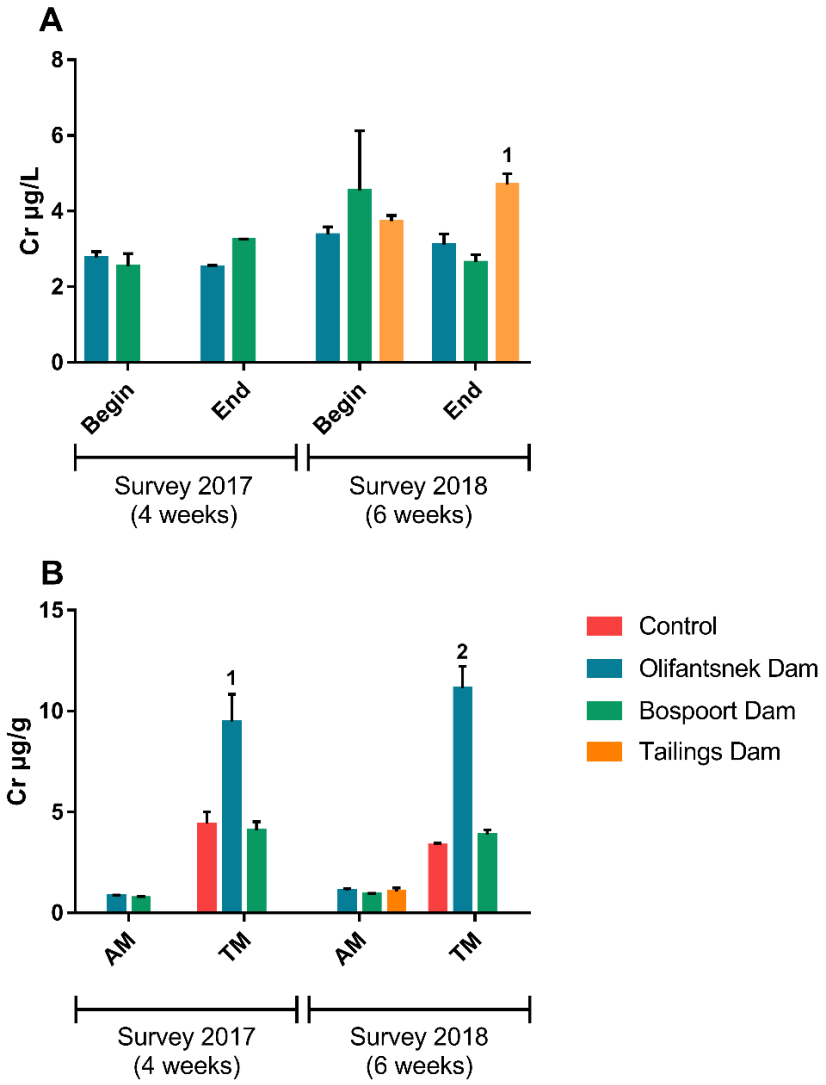

Figure 4-11: Chromium concentrations (mean ± SEM) in water (A), and artificial mussels (AM) and transplanted clams (TM) during the two surveys (B). Concentrations are presented as µg/g dry mass for transplanted clams and µg/g Chelex® for the AMs. Bars with common numeral superscript represent significant differences between surveys while bars with common superscript letters represent significant differences within a specific survey. Significance was regarded as $p<0.05$.

There was a significant difference in the water samples from Bospoort Dam from the beginning of the first survey to the end, where the concentrations in Bospoort Dam were significantly different from Olifantsnek Dam (Figure 4-12 A). The same trends were found in the water samples during the second survey, where the tailings dam contained significantly higher Ni concentrations ($p<0.05$). For the AMs it can be seen that each site differed significantly from one another for both of the surveys and in between sites during the same survey. The transplanted clams from the first survey differed significantly from one another, while these concentrations were similar in the second survey.

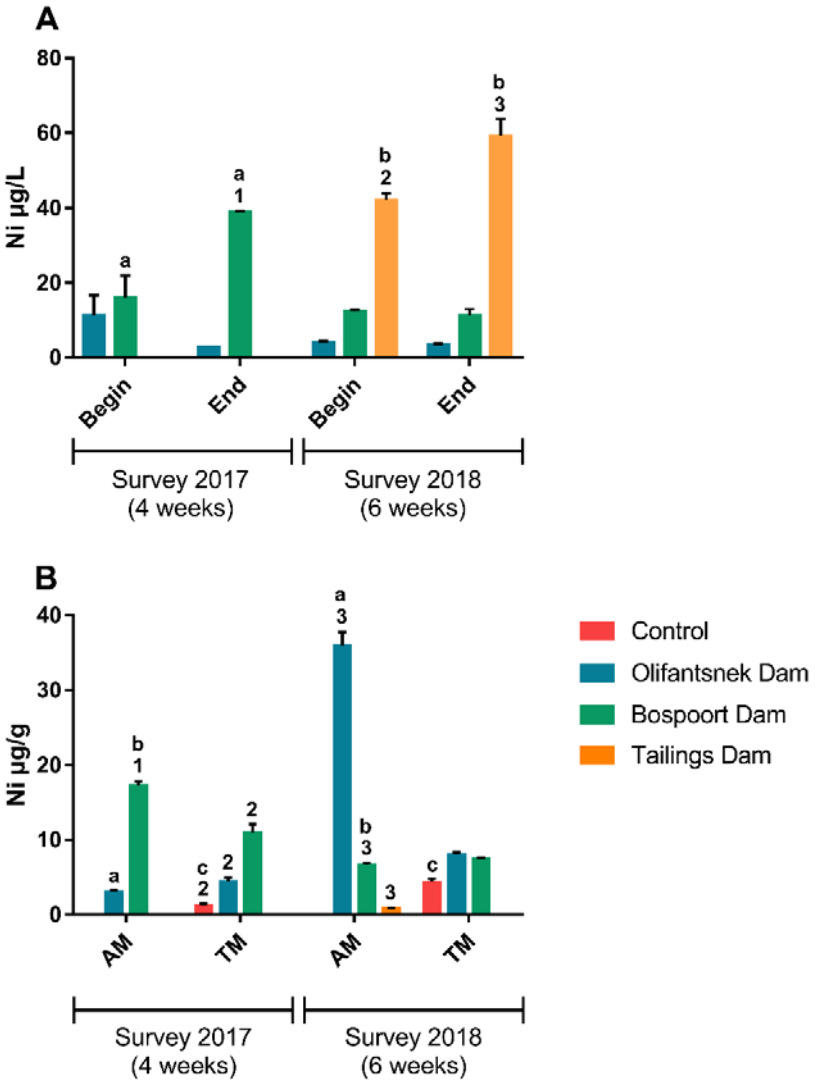

Figure 4-12: Nickel concentrations (mean ± SEM) in water (A), and artificial mussels (AM) and transplanted clams (TM) during the two surveys (B). Concentrations are presented as µg/g dry mass for transplanted clams and µg/g Chelex® for the AMs. Bars with common numeral superscript represent significant differences between surveys while bars with common superscript letters represent significant differences within a specific survey. Significance was regarded as $p<0.05$.

From the results obtained in Figure 4-13 A it can be seen that the water samples during both surveys did not indicate any significant differences (p>0.05) in lead (Pb) concentrations. On the other hand there was more variation in the concentrations from the AMs and the transplanted clams. During the first survey the AMs contained significantly higher concentrations than those from the second survey, for the second survey it can be seen that Bospoort Dam had significantly lower (p>0.05) concentrations than the other sites. It can be seen that the concentrations in the transplanted clams from Olifantsnek Dam differed significantly between the two surveys, in both surveys Bospoort Dam had significantly different concentrations from the other groups.

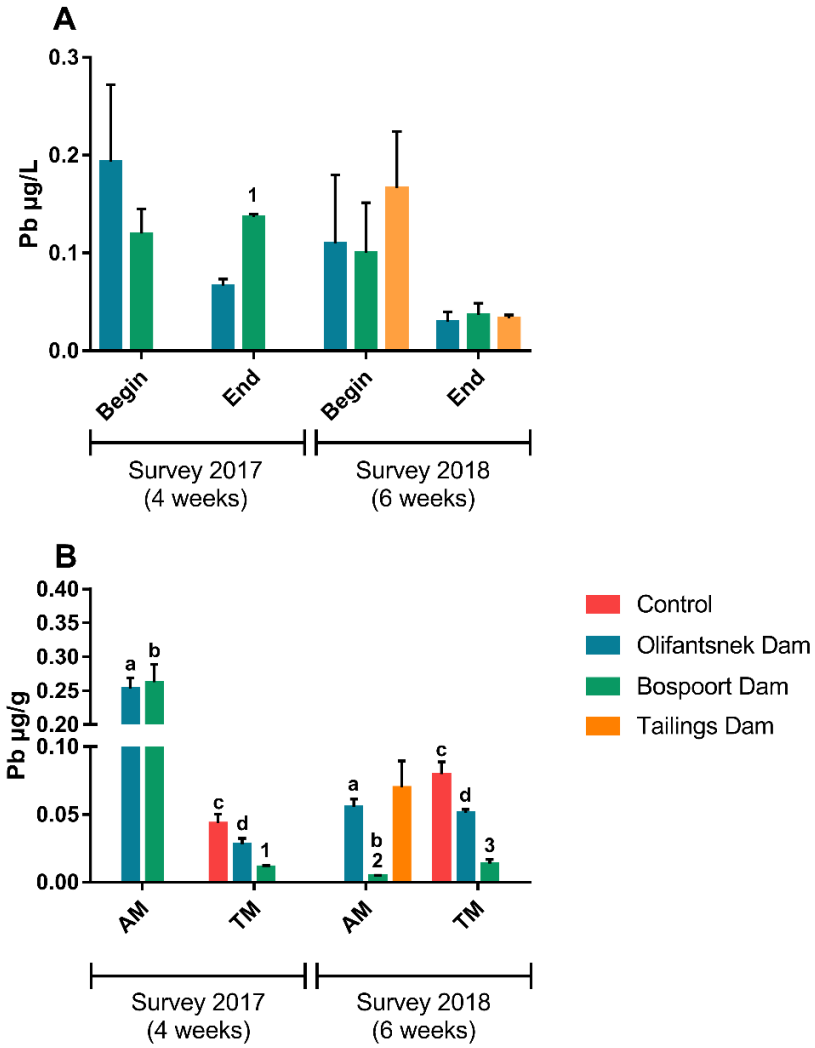

Figure 4-13: Lead concentrations (mean ± SEM) in water (A), and artificial mussels (AM) and transplanted clams (TM) during the two surveys (B). Concentrations are presented as µg/g dry mass for transplanted clams and µg/g Chelex® for the AMs. Bars with common numeral superscript represent significant differences between surveys while bars with common superscript letters represent significant differences within a specific survey. Significance was regarded as p<0.05.

From the results of the water data (Figure 4-14 A) it can be seen that the tailings dam contained significantly higher ($p<0.05$) palladium (Pd) concentrations than the other sites. At the end of the first survey the two impoundments differed significantly from one another, while at the beginning of the second survey the three sites differed significantly from each other. From the results of the AMs it can be seen that during the first survey Olifantsnek Dam contained significantly different Pd concentrations when compared to Bospoort Dam, while the concentrations from the first survey differed significantly from those in Bospoort Dam during the second survey. During the second survey the AMs contained significantly different Pd concentrations at each site. From Figure 4-14 it can be seen that the transplanted clams from Bospoort Dam and Olifantsnek Dam differed significantly from one another during the first survey.

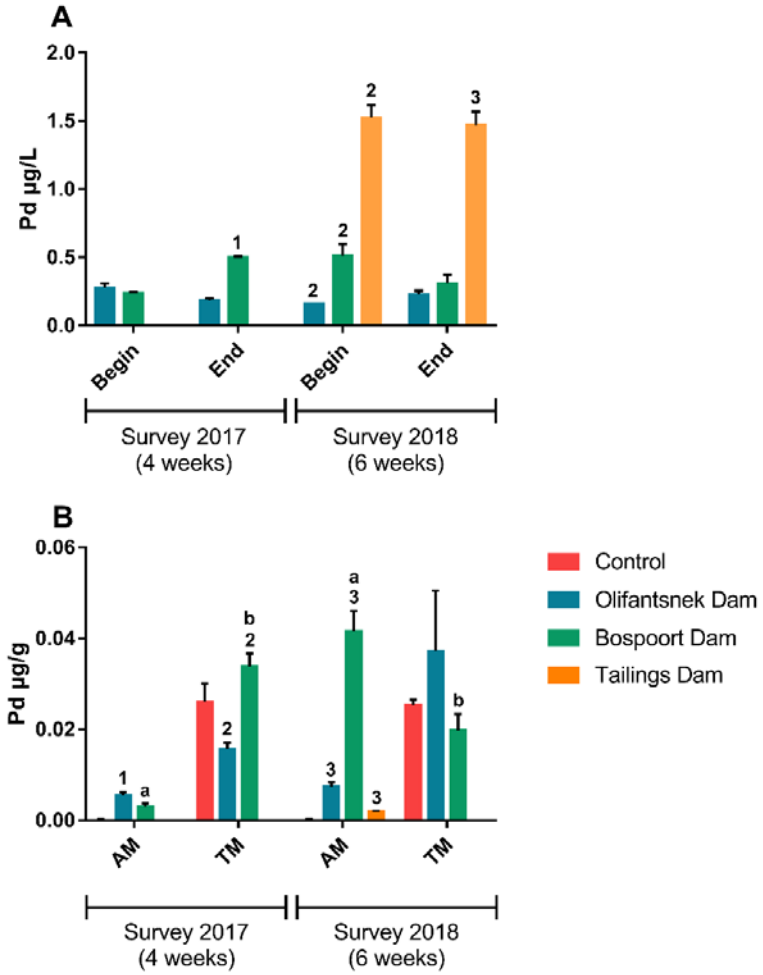

Figure 4-14: Palladium concentrations (mean ± SEM) in water (A), and artificial mussels (AM) and transplanted clams (TM) during the two surveys (B). Concentrations are presented as µg/g dry mass for transplanted clams and µg/g Chelex® for the AMs. Bars with common numeral superscript represent significant differences between surveys while bars with common superscript letters represent significant differences within a specific survey. Significance was regarded as $p<0.05$.

It can be seen that were no significant differences (p>0.05) in platinum (Pt) concentrations in the water from the two impoundments, while the tailings dam contained significantly higher concentrations Figure 4-15 A). It is apparent that the AMs contained significantly higher Pt concentrations than those found in the transplanted clams. During the first survey the Pt concentrations were below detection limit for the transplanted clams from Bospoort Dam. For the AMs it can be seen that there was a significant difference in the concentrations found at Bospoort Dam from the first to the second survey. It is apparent that the levels were relatively stable between the sites at each sampling time.

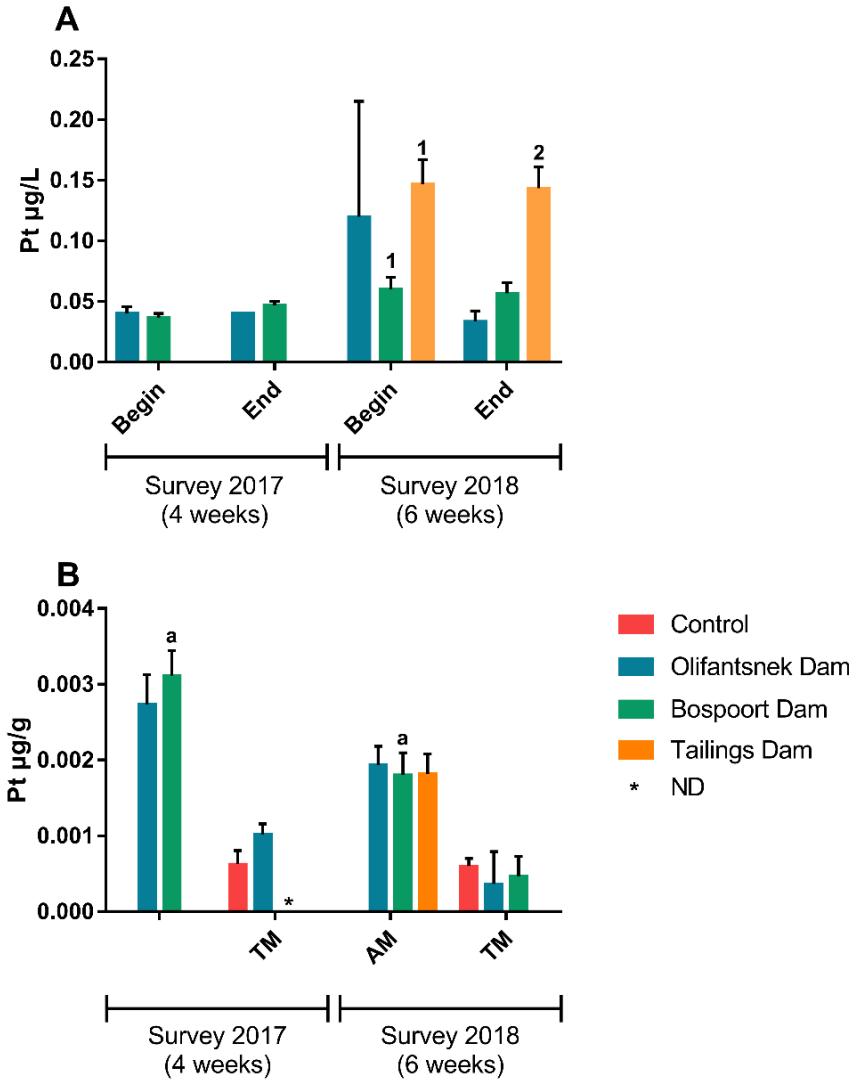

Figure 4-15: Platinum concentrations (mean ± SEM) in water (A), and artificial mussels (AM) and transplanted clams (TM) during the two surveys (B). Concentrations are presented as µg/g dry mass for transplanted clams and µg/g Chelex® for the AMs. Bars with common numeral superscript represent significant differences between surveys while bars with common superscript letters represent significant differences within a specific survey. Significance was regarded as p<0.05.

The water samples from Bospoort Dam (Figure 4-16) contained significantly different ($p<0.05$) vanadium (V) concentrations when compared to Olifantsnek Dam at the end of survey 1. The tailings dam contained significantly higher V concentrations during the second survey, where there was a significant difference from the beginning to the end of the survey. It can be seen that the AMs contained significantly higher V concentrations than the transplanted clams during both surveys, where there was a significant difference between the AMs from Bospoort Dam between the other sites and between the two surveys. The transplanted clams of the control group had significantly different V concentrations between the two surveys. It can be seen that during the first survey the transplanted clams from Bospoort Dam contained significantly different concentrations when compared to the other site, during the second survey Olifantsnek Dam were significantly different from the other sites.

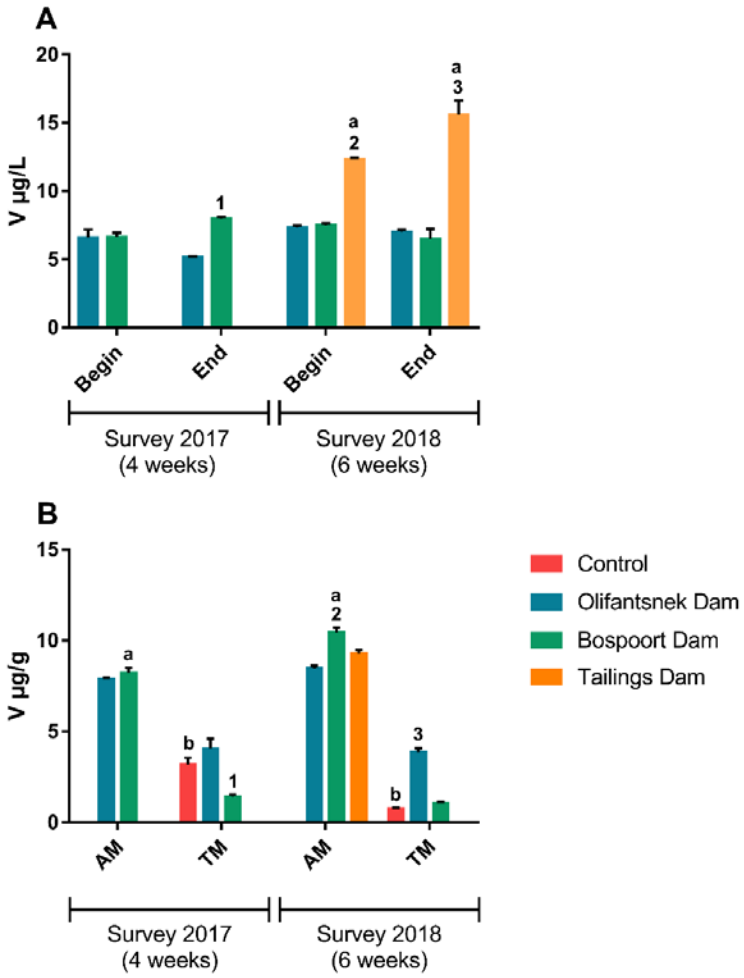

Figure 4-16: Vanadium concentrations (mean ± SEM) in water (A), and artificial mussels (AM) and transplanted clams (TM) during the two surveys (B). Concentrations are presented as µg/g dry mass for transplanted clams and µg/g Chelex® for the AMs. Bars with common numeral superscript represent significant differences between surveys while bars with common superscript letters represent significant differences within a specific survey. Significance was regarded as $p<0.05$.

It can be seen, from Figure 4-17, that the tailings dam contained significantly higher ($p<0.05$) zinc (Zn) concentrations when compared to the other sites sampled. It can be seen that the transplanted clams contained significantly higher Zn concentrations when compared to the AMs, there is a significant difference between the two control groups of the transplanted clams of the two surveys. It can be seen that there was a significant difference in the Zn concentrations measured in the AMs in the two impoundments between the two surveys. During the second survey the AMs from the tailings dam contained significantly lower Zn concentrations than those found at the other sites.

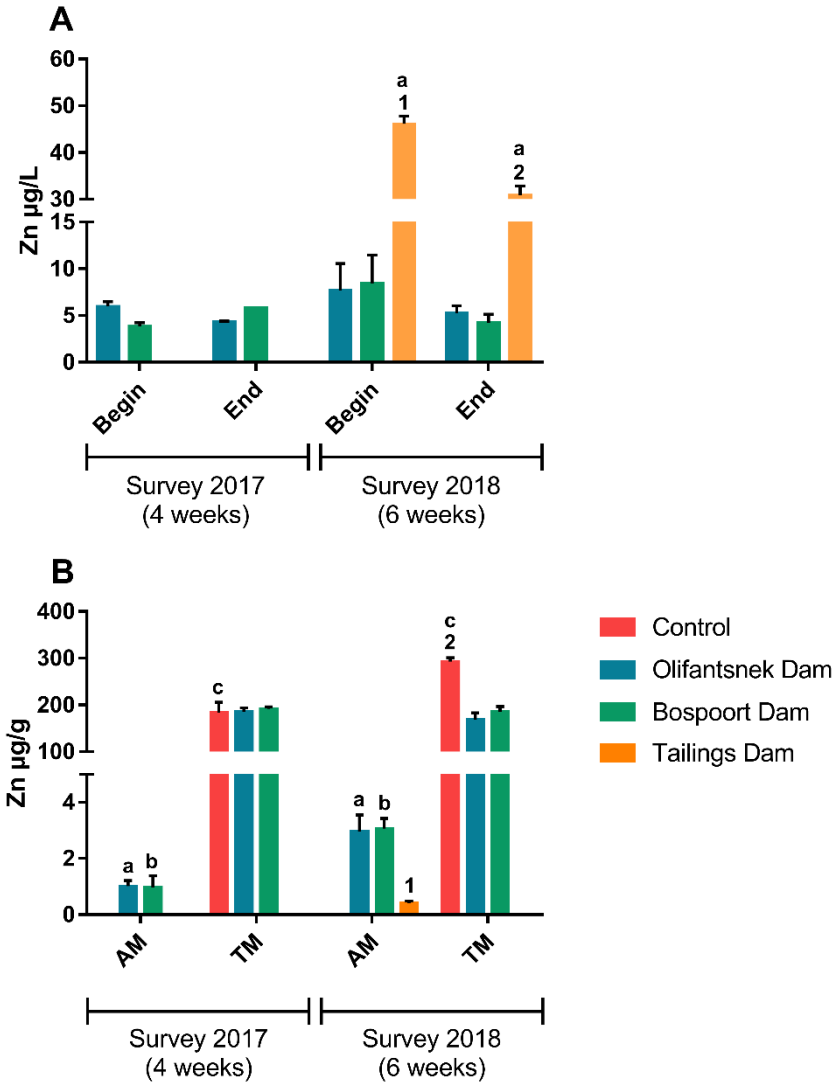

Figure 4-17: Zinc concentrations (mean ± SEM) in water (A), and artificial mussels (AM) and transplanted clams (TM) during the two surveys (B). Concentrations are presented as µg/g dry mass for transplanted clams and µg/g Chelex® for the AMs. Bars with common numeral superscript represent significant differences between surveys while bars with common superscript letters represent significant differences within a specific survey. Significance was regarded as $p<0.05$.

The AMs and transplanted organisms accumulated metals from the two impoundments in different patterns. This can be seen by the clear dissimilarity between the AMs and transplanted clams on the PCA (Figure 4-18). The AMs correlate strongly with Pb, Pt and V, while the transplanted clams correlate with Cd, Cr, Co, Zn and As. The Pd concentrations were very similar in AMs and transplanted clams. The groupings of metal accumulation in the AMs indicated site and survey-specific patterns, while the temporal and spatial groups were less obvious for the clams.

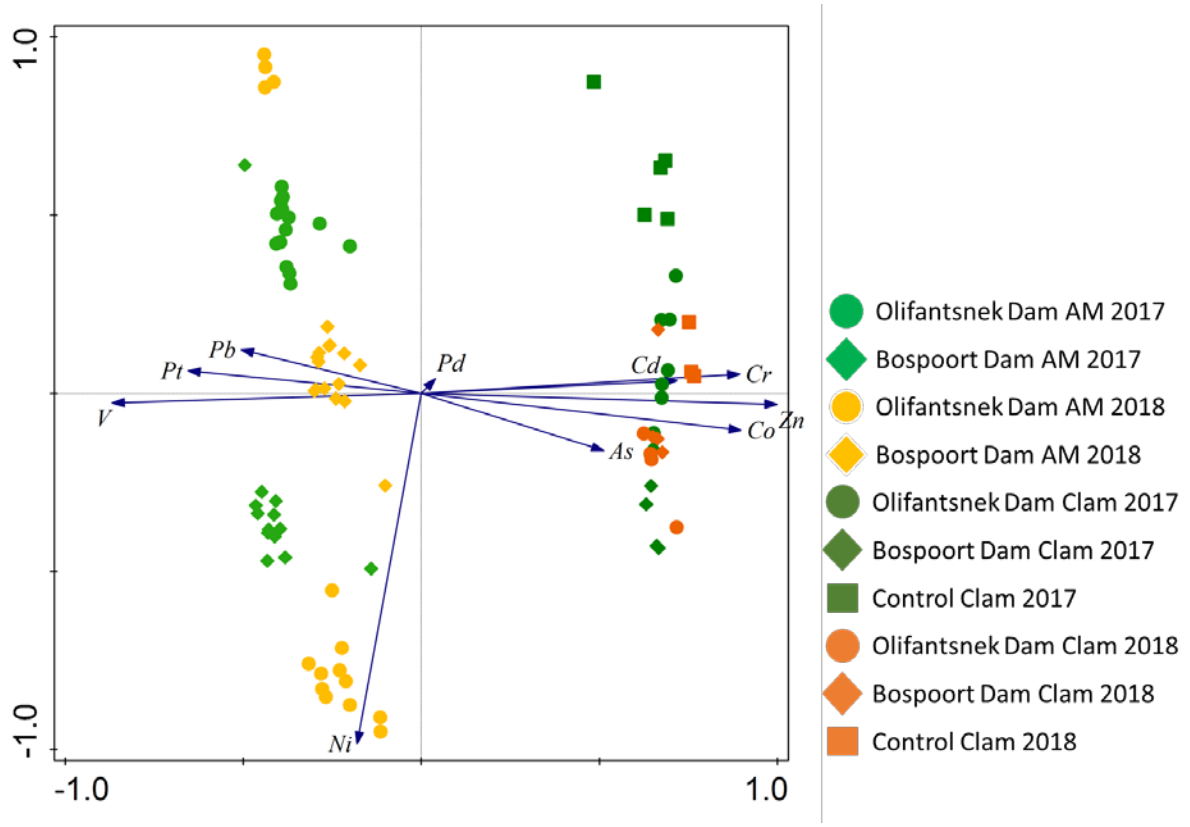

Figure 4-18: A principle component analysis (PCA) showing the grouping of AMs and transplanted clams based on similarity of metal bioaccumulation at the different sites and surveys. The ordination explains 94.8% of the total variation in the data with 82.9% on axis 1 and 11.9% on axis 2.

The metal concentrations within the AMs indicate that there was some groupings (Figure 4-19). The AMs from Olifantsnek Dam in 2017 correlated strongly with Cd, while it can be seen that in the survey conducted in 2017 there was a strong correlation within Pb and Pt in both impoundments. The metal concentrations within the second survey differed spatially. Olifantsnek Dam correlated with As, Co and Ni, while Pd and V correlated strongly with the clams from Bospoort Dam. When comparing this data to that found within the tailings dam, it could be seen that the AMs correlated with Cd.

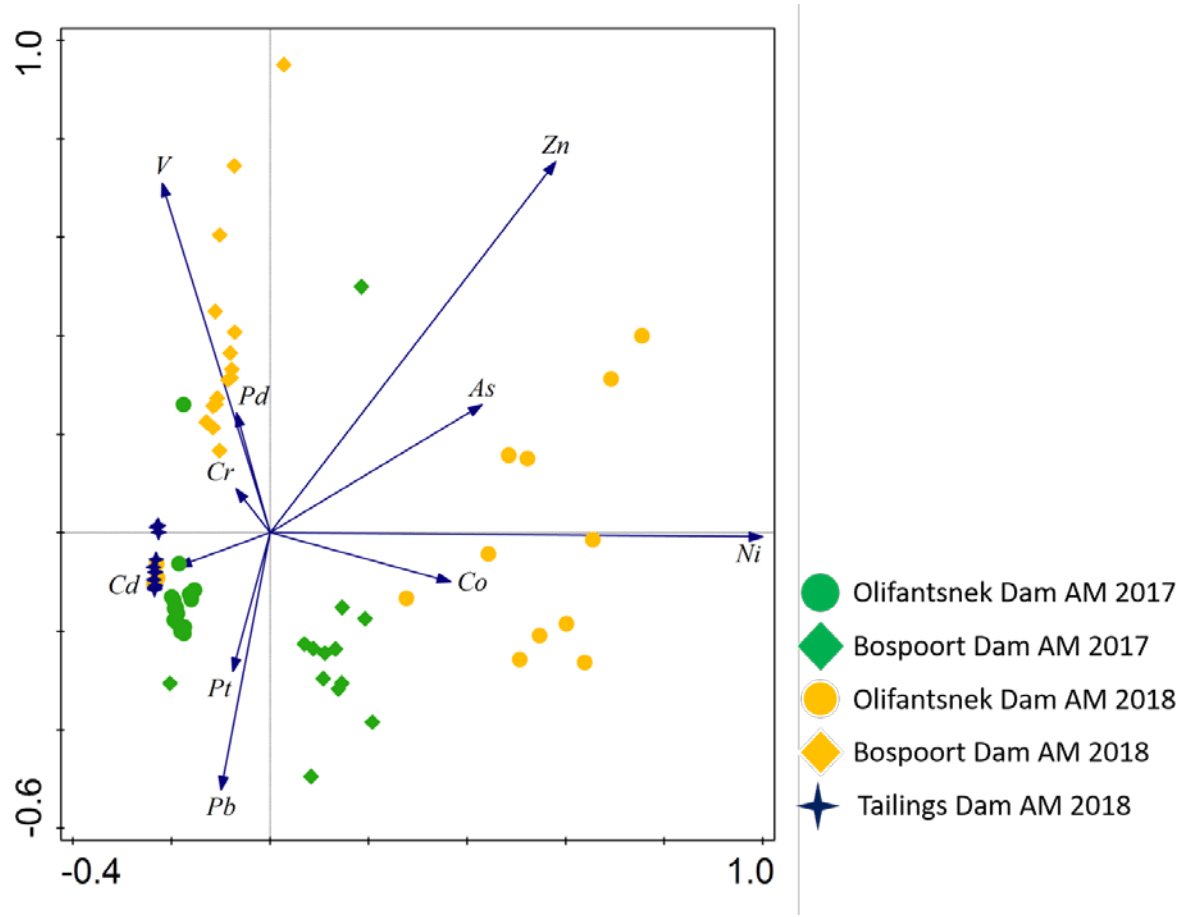

Figure 4-19: A PCA illustrating the grouping of AMs within the two impoundments during the two surveys based on similarity of metal bioaccumulation at the different sites and surveys. The ordination explains 98.8% of the total variation in the data with 97.2% on axis 1 and 1.6% on axis 2.

The Olifantsnek Dam clam bioaccumulation patterns during the 2017 were distinctly different from all the other surveys and correlated strongly with As, Cr, Pd, Pt and V. It should also be noted that the 2018 control clams correlated strongly with Cd, Pb and Zn. Nickel concentrations in the clams correlated strongly with Bospoort Dam during both surveys.

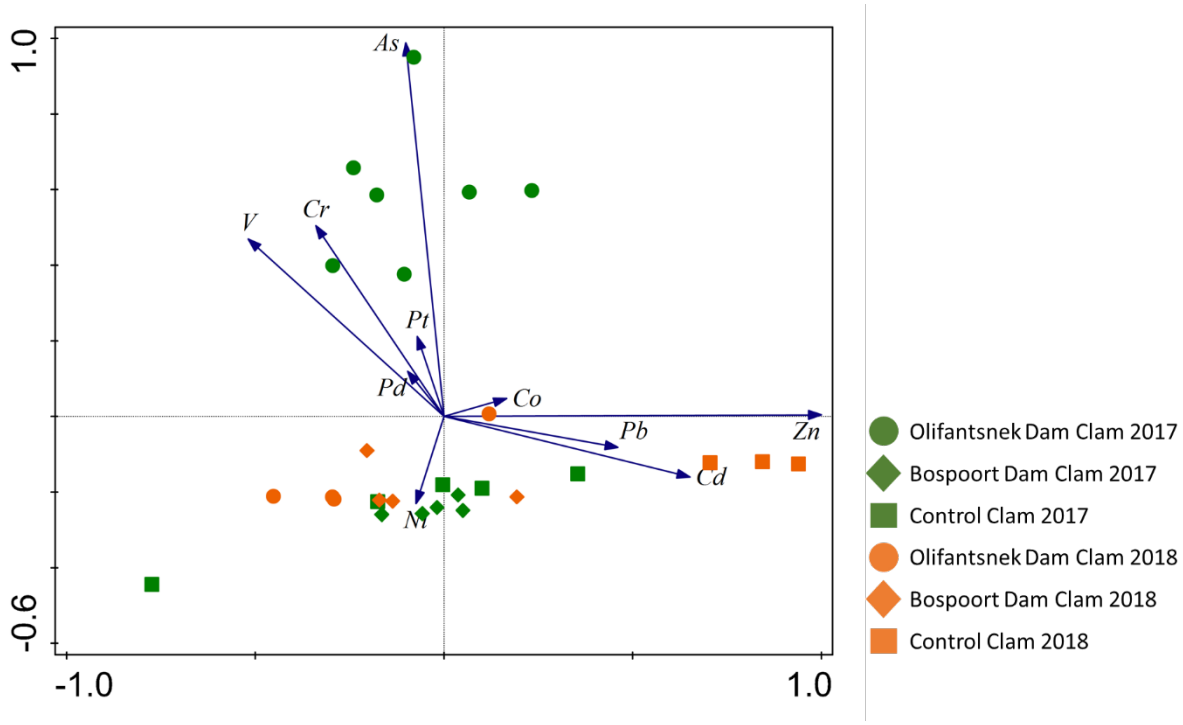

Figure 4-20: A PCA illustrating the grouping of transplanted clams within the two impoundments during the two surveys based on similarity of metal bioaccumulation at the different sites and surveys. The ordination explains 98.8% of the total variation in the data with 96.0% on axis 1 and 98.8% on axis 2.

4.4 Discussion

Emissions from mining and production activities are recognised as an important source of metals into the environment (Rauch and Fatoki, 2015). Platinum group elements are mined from primary deposits where they associate to other elements such as Cu and Ni and many other elements (Rauch and Fatoki, 2015). Elevated PGE concentrations have been reported in the vicinity of mining areas, these include elements that are associated to mining activities. Road dust was collected in four highly populated cities in South Africa during a study of Rauch and Fatoki (2009), it was found that Rustenburg had the most elevated Pt concentrations that are associated with mining and production activities. The Hex River, which flows through Rustenburg, drains one of the most important mining areas in the western limb of the Bushveld Igneous Complex (Almécija *et al.*, 2017). Rustenburg is located in a large basin which forms part of the Bushveld Igneous Complex, where the ore mainly consists of PGEs and is usually associated with chromite. In addition to the PGEs and chromite, the ore contains deposits of gold and sulphides of nickel, copper and iron (Wittmann and Förstner, 1976).

4.4.1 Metals in water

Two impoundments situated along the Hex River were studied during this study. Olifantsnek Dam is geographically separated from the mining area in Rustenburg, while Bospoort Dam is located in the vicinity of many informal settlements as well as many of the mines found in this area. From the results obtained it could be seen that Bospoort Dam was slightly more polluted than Olifantsnek Dam. This is due to the fact that the Hex River flows in a northerly direction through all the industrial and mining areas (Almécija *et al.*, 2017). Thus if any of these activities discharge wastes into the environment it will end up in the Hex River, where Bospoort Dam will act as a sink to accumulate all of the elements that can be found in the discharges. Since both of these impoundments are located in an area that contains geologically rich deposits it is assumed that some elements will occur naturally in high concentrations in both impoundments.

According to the weather data of Rustenburg region there was almost no rain during the first survey (Thorsen, 2017). Due to the absence of rainfall there was no run-off into the Hex River. This resulted in very low to no flow in the river between the two impoundments. The lower variation found *in situ* physico-chemical variables and metal concentrations can therefore be attributed to the lower flows. The rainfall and ensuing run-off was much greater and more variable between the start and ending of the second survey in 2018. This is also reflected in the far greater variation in physico-chemical parameters. This is supported by Prathumratana *et al.* (2008) who showed that rainfall and associated run-off has an influence on variations in site specific water quality.

The increase in the metal concentrations measured in Bospoort Dam compared to Olifantsnek Dam can also probably be linked to local point source contributions that can be found along the course of the Hex River and not from run-off from the greater catchment. Considering the differences in metal concentrations between the start and end of the first survey period (2017) the As, Ni, Pd and V concentrations in Bospoort Dam were significantly higher at the end of survey 1 than those measured in Olifantsnek Dam. In some instances Olifantsnek Dam had a decrease in metal concentrations. During the second survey it could be seen that there was more variation between the two impoundments from the beginning towards the end of the exposure, where it cannot be said that a specific site contained higher metal concentrations.

It could be seen that Bospoort Dam contained slightly higher Cd, Co and Ni concentrations than Olifantsnek Dam. In general metal concentrations were higher in Bospoort Dam when compared to Olifantsnek Dam. The water data showed that almost all of the metals were present in concentrations that are below the TWQR, for both aquatic ecosystems and domestic use (DWAF, 1996a, DWAF, 1996b). However, the Zn concentrations were above the TWQR for aquatic ecosystems. Both of the impoundments contained concentrations above the chronic effect value (3.6 µg/L), while the concentrations found in the tailings dam were far above the acute effect value (36 µg/L) (DWAF, 1996b).

The tailings dam from the mine contained significantly higher As, Co, Cr, Ni, Pt, Pd, V and Zn concentrations than both of the impoundments. These tailings dams are generally constructed to contain the slime and residues that are rejected from the ore (Wittmann and Förstner, 1976). Thus mines can dispose of various effluents, for example sludge and allows for precipitated solids to settle at the bottom of the dam while the solutions can evaporate. The main risk is the possibility that the wall of the dam can come apart and the slimes can flow over the mining areas, farmlands, roads and into aquatic ecosystems (Wittmann and Förstner, 1976). Therefore it is important to control the metal concentrations within these dams, since the tailings dam is located close to the river.

South Africa is a mineral resource-rich region and consequently metal concentrations are elevated in rivers that drain through mining regions. The Olifants River is generally regarded as the most metal-enriched system in South Africa and studies by Gerber *et al.* (2016) recorded average concentrations of 0.75 µg/L As, 7.41 µg/L Cd, 5.26 µg/L Co, 4.24 µg/L Cr, 1.43 µg/L Ni, 4.74 µg/L Pb and 2.68 µg/L Zn. During this study similar As, Co and Pb concentrations were found. The Cd concentrations were considerably higher and the highest Cr measured in the Olifants River was approximately double than those measured the in Olifantsnek and Bospoort dams. The Ni concentrations were 3:13:35 times higher in Olifantsnek Dam, Bospoort Dam and the tailings dam respectively. The Zn concentrations

were 2 times higher within the two impoundments, while the concentrations were 14 times higher in the tailings dam.

The Marico River system, which is the most pristine river system in South Africa (Kemp *et al.*, 2017) reported levels of 0.858 ± 0.1912 µg/L As, >0.0001 µg/L Cd, 0.693 ± 0.056 µg/L Co, 5.9 ± 0.154 µg/L Cr, 7.158 ± 0.586 µg/L Ni, 0.009 ± 0.007 µg/L Pb and 2.275 ± 1.33 µg/L Zn. The As concentrations within the two impoundments were similar to those measured in the Marico River, the tailings dam however contained double the amount that was found within the river. It was interesting to see that the Cr concentrations within the impoundments and the tailings dam were lower than those found in the Marico River. The Cd, Pb and Zn concentrations were higher at all three sites. Olifantsnek Dam contained lower Co and Ni, Bospoort Dam contained lower Co but higher Ni, while the tailings dam contained higher Co and Ni concentrations. Higher Cd, Pb, Zn concentrations observed upstream of the mining and other human activities may be the results of the geogenic input from the natural geology.

Platinum group elements occur at trace levels in the environment with the concentrations introduced to the environment due to human activities are relatively small. However, there has definitely been an increase in anthropogenic emissions during the last couple of decades (Hoppstock and Sures, 2004). It has been noted that the solubility of PGE in water from road dust decreases in the following order Pd > Pt ≥ Rh, this is the same pattern that these metals are bioavailable for plants and organisms in the environment (Zimmermann *et al.*, 2002, Hoppstock and Sures, 2004).

Studies on Pt concentrations in water recorded levels between 0.006 – 2.6 ng/L (Hoppstock and Alt, 2000, Moldovan *et al.*, 2003, Monticelli *et al.*, 2010, Cobelo-García *et al.*, 2013). In this study the concentrations found within the two impoundments ranged between 0.040 – 0.120 µg/L. The tailings dam had concentrations that were close to 0.145 µg/L, this is once again three times higher than those found in the two impoundments.

From literature it was found that Pd in water ranged from 0.0004 – 0.0102 µg/L (Eller *et al.*, 1989, Moldovan *et al.*, 2003), in this study the concentrations ranged between 0.160 – 0.513 µg/L in the impoundments and an average of 1.497 µg/L within the tailings dam. These concentrations are significantly higher than previous reports. Olifantsnek Dam contained Pd concentrations between 0.160 – 0.277 µg/L, while Bospoort Dam contained concentrations that ranged from 0.237 – 0.513 µg/L. From this it is clear that Bospoort Dam had significantly higher Pd concentrations than Olifantsnek Dam. The tailings dam contained concentrations that were threefold higher than those in Bospoort Dam.

4.4.2 Transplanted organisms

Over the last few decades live organisms has been used as biological indicators, organisms used to monitor pollutants include various sentinel organisms. The use of living organisms to indicate the levels of pollutant exposure in the environment has been used since the inception of the Mussel Watch programme in the 1970s (Goldberg, 1975). These sessile organisms record changes of a range of both organic and inorganic pollutants occurring in the water, food or sediment. Bivalves are useful since they are available worldwide, they are abundant, sedentary and they have the ability to bioaccumulate pollutants (Goldberg, 1975).

In the event where there are no suitable resident organism to act as bioaccumulation bioindicators or when resident organisms may have undergone evolutionary adaptation to metal exposure, the use of transplanted organisms from a clean or reference site has been implements (Kibria *et al.*, 2016). The use of organisms that are collected from unstressed and generally unpolluted populations are often translocated to selected unpolluted sites (Wepener, 2008). The chemical and biological changes within these organisms can then be monitored in space and time (Wepener, 2008).

During this study the clam data indicated that during both surveys there was a significant difference in Cr, Pb and V concentrations, which was significantly higher in Olifantsnek Dam. During the 2017 survey Ni and Pd was significantly higher in Bospoort Dam. The metal concentrations between surveys did not vary that much, Cd, Co and Pb were higher during the first survey in Olifantsnek Dam. During the transplantation studies some of the control clams contained higher metal concentrations than the transplanted clams. This includes, Cd, Pt and Zn during the second survey when compared to both impoundments and Pb during both surveys. Co and Pd were higher during the second survey, as well as Pt and V during the first survey when compared to Bospoort Dam. Pd was higher when compared to Olifantsnek Dam during the first survey.

During studies that make use of transplanted organisms it is difficult the compare the data found to those found in literature. Transplanted organisms do not always indicate the same accumulation patterns than that found in the water column. These organisms are exposed for longer periods, while water samples only indicate what the concentrations are at that specific time. Transplanted organisms have different accumulation strategies, as well as mechanisms that can eliminate some of the metals. In a study by Claassens *et al.* (2016) they made use of transplanted organisms at different localities within the Koekemoer Spruit, they found that there is no specific pattern when comparing the concentrations found within the transplanted organisms and the water.

Likewise, in this study it could be seen that there is no correlation between exposure and accumulated concentrations. There are many factors that can influence this, seasonality, bioavailability of metals and regulating of metals (Greenfield *et al.*, 2014). The pre-exposure of the reference transplanted organisms can result in the reference having higher metal concentrations or responses than the exposed sites. The effects of upwelling and a change in habitat of these organisms can have an effect on the initial concentrations in the transplanted clams (Greenfield *et al.*, 2014).

When comparing the metal accumulated within the soft tissue with those found in snails transplanted to a gold mining area it could be seen that similar Co concentrations were measured. The As, Cr, Ni, V and Zn measured in the soft tissue of the snails were higher when compared, while the Cd and Pb was much lower (Claassens *et al.*, 2016).

When PGEs accumulate in the soft tissue of bivalves, e.g. *Dreissena polymorpha*, it was found that biological availability of metals decreased in the following order: Zn > Cu > Pd > Sb > Pb > Pt > Rh = Sn (Hoppstock and Sures, 2004). Platinum concentrations in bivalves from other studies ranged between 0.00001 – 0.0013 µg/g (Zimmermann *et al.*, 2002, Ruchter, 2012), whereas the transplanted clams from this study ranged between 0.001 – 0.003 µg/g. Palladium concentrations in a previous study by Zimmermann *et al.* (2002) were close to 0.001 µg/g. In the current study the transplanted clams accumulated concentrations that ranged between 0.016 – 0.037 µg/g. Therefore the results from this study showed an increase in Pt and Pd bioaccumulation compared to the other studies reported in the literature.

4.4.3 Artificial mussels

Artificial mussels have successfully been used as monitoring tools for metal bioaccumulation in the aquatic environment (Kibria *et al.*, 2012, Claassens *et al.*, 2016, Dahms-Verster *et al.*, 2018, Genç *et al.*, 2018). Many of these studies endorse the use of AMs during biomonitoring studies, since they are not affected by the water quality conditions that would otherwise greatly effect a bioindicator. In previous studies it has been stated that temperature does not have an effect on the bioaccumulation strategy of AMs (Wu *et al.*, 2007). In another study by Degger *et al.* (2011) it was noted that there might be other factors that can influence the uptake of metals by AMs.

The water metal concentrations indicated that Bospoort Dam contained higher concentrations than those found in Olifantsnek Dam, while the AMs from the first survey showed that Bospoort Dam has slightly higher metal concentrations. During the second survey there were some significant differences but these results (AMs) did not give an indication towards a specific site having higher concentrations. Claassens *et al.* (2016) found that the AM accumulated higher Cd, Pb, V and Zn concentrations than the transplanted organisms. From the data obtained

during this study it could be seen that the AMs accumulated higher Pb, Pd, Pt and V concentrations, which correlates well with results obtained from Claassens *et al.* (2016). It could also be seen that the AMs and transplanted clams accumulated similar As and Ni concentrations.

From other studies it can be seen that there is variation in data. Artificial mussels that were used in the gold mining region (Claassens *et al.*, 2016) accumulated higher Cr, Co, Cd, Pb, V and Zn concentrations, while AMs exposed to potentially less impacted sites along the Nyl River (Dahms-Verster *et al.*, 2018) contained either similar or higher concentrations compared to this study. From these results it can be seen that there are no recorded data for any of the PGEs.

4.4.4 Comparison of Artificial Mussels and transplanted clams

For almost all of the metals, it could be seen that the transplanted clams contained significantly higher metal concentrations than the AMs. According to Wu *et al.* (2007) it is possible that the AM and any bioindicator species bioaccumulate different metal fractions during field exposures. This can be related to metals occurring in the environment in different complexes and there are many factors that can influence their speciation. It is possible that the transplanted clams can ingest different metal concentrations from the food that is available within the environment and it can be regulated by these organisms (Wu *et al.*, 2007). The characteristics of the permeability of the AM and the Chelex® beads can also have an influence (Wu *et al.*, 2007, Degger *et al.*, 2011). Where a combinations of all of these factors could also lead to these results.

From other studies it could be seen that AMs and transplanted organisms accumulate metals in similar patterns but at different concentrations (Claassens *et al.*, 2016, Degger *et al.*, 2016). When comparing the metal concentrations in the transplanted clams from the impoundments and the control group, which indicates the initial concentrations, it could be seen that the metal concentrations did not indicate great variation from the initial concentrations found within the clam tissue. There were exceptions; As, Cr, Ni and V, while all the other metals were either similar to the initial concentrations or significantly lower. The accumulation patterns within the AMs indicated significant accumulation when compared to the initial concentrations. When regarding this statement it could then be said the AMs work much better than the transplanted clams for metal biomonitoring studies.

During the second survey, over a period of six weeks, there was a massive water hyacinth (*Eichhornia crassipes*) bloom that occurred in Bospoort Dam. These water plants covered more than 50% of the impoundment at the end of the exposure period. These water plants could have had an effect on the reduction of bioavailable metals for the AMs and the

transplanted clams to take up. Farago and Parsons (1994) showed that the water hyacinth can accumulate high levels of metals from solutions. In their study they found that the hyacinths were capable of recovering PGEs from dilute solutions in the following order: Pt^{2+} > Pd^{2+} > Os^{4+} = Ru^{3+} > Ir^{3+} > Rh^{3+}. When considering the biomass of the water hyacinth that covers such a great area of the impoundment the total metal accumulation can be significant. This could serve as an explanation to why the concentrations in the water of Bospoort Dam weren't that significantly different from those found in Olifantsnek Dam during the second survey and why the concentrations during the first survey were that much higher in Bospoort Dam.

During the second survey transplanted clams and AMs were deployed in the tailings dam on the mine ground, but due to many factors none of the transplanted clams survived. This made it impossible to determine the metal concentrations that could have been accumulated within the clams to compare with the AMs. It is presumed that the transplanted clams did not perish due to the metal concentrations found in the water of the tailings dam but due to a layer of oil that could be found on the water and due to the water level dropping over the six week period. The oil found in the water layer could have impaired the filtering mechanism of the clams and would definitely have an effect on the oxygen available.

It could be seen that even though the metal concentrations within the water from the tailings dam was significantly higher, the AMs indicated the exact opposite. Where the concentrations found the AMs were either similar to those found from the impoundments or it was significantly lower. The AMs from the tailings dam contained significantly lower Co, Ni, Pd and Zn concentrations when compared to concentrations accumulated in the AMs from the two impoundments. An explanation for this could be that the oil layer that could have obstructed the metals from diffusing through the gel layers to bind to the Chelex® beads.

4.5 Conclusion

Different bioindicator species have different accumulation strategies, they are affected by many other factors within the environment and have the ability to regulate the internal metal concentrations through other processes (Degger *et al.*, 2011, Claassens *et al.*, 2016). The difference between the results of the AMs and the transplanted clams should not be seen as a shortcoming and was expected based on results from previous studies. From this study it can be seen that the transplanted clams and AMs accumulated different metals at different concentrations and indicated accumulation of metals in different forms and differing metal bioavailability. From the results it could be seen that the AMs and transplanted clams accumulated similar As and Pd concentrations, while the AMs preferentially accumulated Pb, Ni, Pt and V.

The activities in the catchment and subsequent run-off into the Hex River contributes to the higher metal loads that end up in Bospoort Dam. Thus many of the metal concentrations that are significantly higher than those from Olifantsnek Dam can be attributed to mining activities. The higher water temperatures recorded in Bospoort Dam may have resulted in the metabolism of the transplanted clams being higher than in Olifantsnek Dam since increased temperature and resulting metabolism results in aquatic organisms accumulating more metals (van Heerden *et al.*, 2006).

The significant difference in metal concentrations from the two impoundments at the end of the first survey can be attributed to the change in season, where there was almost no rainfall during this period. During this period metal concentrations within the water became more concentrated due to low in and out flow of the impoundment and water evaporation. During the second survey the metal concentrations within the two impoundment were more similar, where it is presumed that the water hyacinth bloom had a big influence on the metal concentrations. These water plants could have had an effect on the reduction of bioavailable metals for the AMs and the transplanted clams to take up. Farago and Parsons (1994) showed that the water hyacinth can accumulate high levels of metals from solutions.

Metals can be found in various forms within the aquatic environment, the semi-permeable membrane of the AM permits the bioavailable metal ions to pass through and bind to the Chelex® beads. Some metal ions have a stronger binding affinity towards the Chelex® beads, where it is possible that these ions compete for binding sites within the AMs. For that reason it is possible that some metals can be more abundant in the environment but the AMs will not be able to indicate these concentrations due to competition by other metals. Nonetheless, in this study it was shown that the AM successfully accumulated PGEs such as Pt and metals such as Ni, Pb and V. Traditional bioindicator bivalves were more successful in indicating

exposure to metals such as As, Cd, Co, Cr and Zn. Thus the combination of AMs and bioaccumulation indicator organisms will provide a holistic assessment of metal exposure in aquatic environments associated with mining activities.

References

Almécija, C., Cobelo-García, A., Wepener, V. and Prego, R. 2017. Platinum group elements in stream sediments of mining zones: the Hex River (Bushveld Igneous Complex, South Africa). *African Earth Sciences,* 129**:**934-943.

Claassens, L., Dahms, S., Van Vuren, J. and Greenfield, R. 2016. Artificial mussels as indicators of metal pollution in freshwater systems: A field evaluation in the Koekemoer Spruit, South Africa. *Ecological Indicators,* 60**:**940-946.

Cobelo-García, A., López-Sánchez, D. E., Almécija, C. and Santos-Echeandía, J. 2013. Behavior of platinum during estuarine mixing (Pontevedra Ria, NW Iberian Peninsula). *Marine Chemistry,* 150**:**11-18.

Dahms-Verster, S., Baker, N. and Greenfield, R. 2018. A multivariate examination of 'artificial mussels' in conjunction with spot water tests in freshwater ecosystems. *Environmental monitoring and assessment,* 190**:**427.

Degger, N., Chiu, J. M. Y., Po, B. H. K., Tse, A. C. K., Zheng, G. J., Zhao, D.-M., Xu, D., Cheng, Y.-S., Wang, X.-H., Liu, W.-H., Lau, T. C. and Wu, R. S. S. 2016. Heavy metal contamination along the China coastline: A comprehensive study using Artificial Mussels and native mussels. *Environmental Management,* 180**:**238-246.

Degger, N., Wepener, V., Richardson, B. and Wu, R. 2011. Application of artificial mussels (AMs) under South African marine conditions: A validation study. *Marine Pollution Bulletin,* 63**:**108-118.

Department of Water Affairs and Forestry. 1996a. South African Water Quality Guidelines (second edition). Volume 1: Domestic Use.

Department of Water Affairs and Forestry. 1996b. South African Water Quality Guidelines. Volume 7: Aquatic Ecosystems.

Eller, R., Alt, F., Tölg, G. and Tobschall, H. 1989. An efficient combined procedure for the extreme trace analysis of gold, platinum, palladium and rhodium with the aid of graphite furnace atomic absorption spectrometry and total-reflection X-ray fluorescence analysis. *Fresenius' Zeitschrift für analytische Chemie,* 334**:**723-739.

Farago, M. and Parsons, P. 1994. The effects of various platinum metal species on the water plant Eichhornia crassipes (MART.) Solms. *Chemical Speciation & Bioavailability,* 6**:**1-12.

Farris, J. L. and Van Hassel, J. H. 2006. *Freshwater bivalve ecotoxicology,* Boca Raton, CRC Press.

Genç, T. O., Po, B. H., Yılmaz, F., Lau, T.-C., Wu, R. S. and Chiu, J. M. 2018. Differences in metal profiles revealed by native mussels and artificial mussels in Sarıçay Stream, Turkey: implications for pollution monitoring. *Marine and Freshwater Research,* 69**:**1372-1378.

Gerber, R., Smit, N. J., Van Vuren, J. H. and Wepener, V. 2016. Metal concentrations in *Hydrocynus vittatus* (Castelnau 1861) populations from a premier conservation area: relationships with environmental concentrations. *Ecotoxicology and Environmental Safety,* 129**:**91-102.

Giarratano, E., Duarte, C. A. and Amin, O. A. 2010. Biomarkers and heavy metal bioaccumulation in mussels transplanted to coastal waters of the Beagle Channel. *Ecotoxicology and Environmental Safety,* 73**:**270-279.

Goldberg, E. 1975. The mussel watch — a first step in global marine monitoring. *Marine Pollution Bulletin,* 6**:**111.

Greenfield, R., Brink, K., Degger, N. and Wepener, V. 2014. The usefulness of transplantation studies in monitoring of metals in the marine environment: South African experience. *Marine Pollution Bulletin,* 85**:**566-573.

Gupta, S. K. and Singh, J. 2011. Evaluation of mollusc as sensitive indicator of heavy metal pollution in aquatic system: a review. *IIOAB,* 2**:**49-57.

Henriksson, J., Chi, M., Hintz, C., Young, D., Kaiser, K., Salmons, S., and Lowry, O. (1986). Chronic stimulation of mammalian muscle: Changes in enzymes in six metabolic pathways. *American Journal of Physiology*, 251:614-632.

Hoppstock, K. and Alt, F. 2000. Voltammetric determination of ultratrace platinum and rhodium in biological and environmental samples. *In:* F, Z. and F, A. (eds.) *Anthropogenic platinum-group element emissions.* Heidelberg: Springer.

Hoppstock, K. and Sures, B. 2004. Platinum-Group Metals. *In:* Merian, E., Anke, M., Ihnat, M. and Stoeppler, M. (eds.) *Elements and Their Compounds in the Environment: Occurrence, Analysis and Biological Relevance.* Second ed. Weinheim: WILEY-VCH Verlag GMBH & Co. KGaA.

Hossain, M. M., Kibria, G., Nugegoda, D., Lau, T. and Wu, R. 2015. A Training Manual for Assessing Pollution (trace/heavy metals) in Rivers, Estuaries and Coastal waters-Using Innovative “Artificial Mussel (AM) Technology” - Bangladesh Model. *Research collaboration between scientists of the IMSF, University of Chittagong, Bangladesh, RMIT University, Australia, the City University of Hong Kong, and the University of Hong Kong.*

Kemp, M., Wepener, V., De Kock, K. and Wolmarans, C. 2017. Metallothionein Induction as Indicator of Low Level Metal Exposure to Aquatic Macroinvertebrates from a Relatively Unimpacted River System in South Africa. *Bulletin of environmental contamination and toxicology,* 99**:**662-667.

Kibria, G., Hossain, M. M., Mallick, D., Lau, T. and Wu, R. 2016. Monitoring of metal pollution in waterways across Bangladesh and ecological and public health implications of pollution. *Chemosphere,* 165**:**1-9.

Kibria, G., Lau, T. and Wu, R. 2012. Innovative ‘Artificial Mussels’ technology for assessing spatial and temporal distribution of metals in Goulburn–Murray catchments waterways, Victoria, Australia: Effects of climate variability (dry vs. wet years). *Environment international,* 50**:**38-46.

Leung, K. M., Furness, R. W., Svavarsson, J., Lau, T. and Wu, R. S. 2008. Field validation, in Scotland and Iceland, of the artificial mussel for monitoring trace metals in temperate seas. *Marine Pollution Bulletin,* 57**:**790-800.

Mogakabe, D. and Van Ginkel, C. 2008. The water quality of the Bospoort Dam. Internal Report No. N/0000/00/DEQ/0108 for the Department of Water Affairs and Forestry, South Africa.

Moldovan, M., Gomez, M. M. and Palacios, M. A. 2003. On-line preconcentration of palladium on alumina microcolumns and determination in urban waters by inductively coupled plasma mass spectrometry. *Analytica chimica acta,* 478**:**209-217.

Monticelli, D., Carugati, G., Castelletti, A., Recchia, S. and Dossi, C. 2010. Design and development of a low cost, high performance UV digester prototype: Application to the determination of trace elements by stripping voltammetry. *Microchemical,* 95**:**158-163.

Prathumratana, L., Sthiannopkao, S. and Kim, K. W. 2008. The relationship of climatic and hydrological parameters to surface water quality in the lower Mekong River. *Environment international,* 34**:**860-866.

Rauch, S. and Fatoki, O. S. 2009. Platinum and lead in South African road dust. *In:* Rauch, S., Morrison, G. and Monzón, A. (eds.) *Highway and urban environment.* Dordrecht: Springer.

Rauch, S. and Fatoki, O. S. 2015. Impact of Platinum Group Element Emissions from Mining and Production Activities. *In:* Zereini, F. and Wiseman, C. L. S. (eds.) *Platinum Metals in the Environment.* Heidelberg: Springer.

Rauch, S. and Peucker-Ehrenbrink, B. 2015. Sources of Platinum Group Elements in the Environment. *In:* Zereini, F. and Wiseman, C. L. S. (eds.) *Platinum Metals in the Environment.* Heidelberg: Springer.

Roux, H. 2011. Status of the yellowfish populations & River health Programme in NW Province. *15th Yellowfish Working Group Conference,* 18 - 20 February 2011. Mpumalanga, South Africa.

Ruchter, N. 2012. *Ecotoxicology of traffic related Platinum in the freshwater environment.* Universität Duisburg-Essen, Fakultät für Biologie.

Thorsen, S. 2017. *Past Weather in Rustenburg, South Africa — May 2017* [Online]. Available: https://www.timeanddate.com/weather/south-africa/rustenburg/historic?month=5&year=2017 [Accessed 1 October 2018].

Van Heerden, D., Van Rensburg, P. J., Nikinmaa, M. and Vosloo, A. 2006. Gill damage, metallothionein gene expression and metal accumulation in *Tilapia sparrmanii* from selected field sites at Rustenburg and Potchefstroom, South Africa. *African Journal of Aquatic Science,* 31**:**89-98.

Wepener, V. 2008. Application of active biomonitoring within an integrated water resources management framework in South Africa. *Science,* 104**:**367-373.

Wittmann, G. and Förstner, U. 1976. Heavy metal enrichment in mine drainage: 1. The Rustenburg platinum mining area. *Science,* 72**:**242-246.

Wu, R. and Lau, T. 1996. Polymer-ligands: a novel chemical device for monitoring heavy metals in the aquatic environment? *Marine Pollution Bulletin,* 32**:**391-396.

Wu, R. S., Lau, T., Fung, W. K., Ko, P. and Leung, K. M. 2007. An ‘artificial mussel’ for monitoring heavy metals in marine environments. *Environmental pollution,* 145**:**104-110.

Zhou, Q., Zhang, J., Fu, J., Shi, J. and Jiang, G. 2008. Biomonitoring: an appealing tool for assessment of metal pollution in the aquatic ecosystem. *Analytica chimica acta,* 606**:**135-150.

Zimmermann, S., Alt, F., Messerschmidt, J., Von Bohlen, A., Taraschewski, H. and Sures, B. 2002. Biological availability of traffic-related platinum-group elements (palladium, platinum, and rhodium) and other metals to the zebra mussel (*Dreissena polymorpha*) in water containing road dust. *Environmental toxicology and chemistry,* 21**:**2713-2718.

Chapter 5: The effect of mining activities on the biological response of transplanted clams

5.1 Introduction

In the last decade there has been a growing interest in determining Platinum group elements (PGE) in different environmental compartments. It has been demonstrated that mining activities in South Africa contribute to elevated PGE concentrations in the environment, as well as an increase in metals that are associated with mining activities (Rauch and Peucker-Ehrenbrink, 2015). The aquatic environment is expected to be influenced more than other environmental compartments since it is an important sink for PGE and other elements that are discharged from these mining and production activities (Ruchter *et al.*, 2015).

Road runoff and industrial effluents contribute to elevated concentrations of these elements. These elements accumulate in the sediments of any water system and can be transferred throughout flowing water systems and eventually end up in lentic water ecosystems (Ruchter *et al.*, 2015). When elevated metal concentrations are introduced into the aquatic ecosystem the biota come in contact with it (Zimmermann *et al.*, 2015). It is known that PGEs may have a range of negative effects on the biota (Sures *et al.*, 2015), these responses will change the organism's physiology.

The chemical analysis of the tissue of these organisms make it possible to determine the magnitude to which these organisms accumulate metals from the environment. As a result of metal bioaccumulation the deviation of the biological responses from the normal physiological homeostasis can be measured (Sures *et al.*, 2015). These deviations can then be used to indicate the effect of pollutants on an organism and are called biomarkers. Biomarkers are biochemical and physiological changes of an organism in response to toxic substance exposure (de Lafontaine *et al.*, 2000). The use of biomarkers to demonstrate the effect of metal exposure has successfully been used in studies conducted on the Olifants (Gerber *et al.*, 2016) and Vaal (Wepener *et al.*, 2011) rivers, which are both metal contaminated systems.

There are biomarkers of exposure and biomarkers of effect. Biomarkers of exposure are e.g. metallothioneins (MTs), which are non-enzymatic proteins with a low molecular weight and high cysteine content (Amiard *et al.*, 2006). Metallothioneins play a central role in regulating essential metal concentration in the tissue of organisms, it is also involved in detoxification of non-essential toxic metals (Frank *et al.*, 2008). Generally MTs control the homeostasis of essential metals such as zinc and copper (Amiard *et al.*, 2006). When non-essential metals enter the organism there is competition between them and the essential metals to bind to metalloproteins (Amiard *et al.*, 2006). For this reason MTs are regarded as a specific

biomarker for metal pollution, where an increase in MTs will be stimulated in the presence of metal exposure (Gerber *et al.*, 2018).

Biomarkers of effect include endpoints that indicate oxidative stress, i.e. catalase and malondialdehyde (MDA). Catalase (CAT) is regarded as an important and sensitive biomarker of oxidative stress as it reveals biological effects on the redox status of aquatic organisms (Van der Oost *et al.*, 2003, Gerber *et al.*, 2018). Catalase is one of the cellular defense mechanisms against the harmful effects that oxyradicals may have on the biological reactions within an organism and breaks down H_2O_2 into non-toxic H_2O and H_2 (Kim *et al.*, 2010). Malondialdehyde formation is a biomarker of lipid peroxidation due to oxidative stress (Gerber *et al.*, 2018). Cellular energy allocation (CEA) is an indicator of cellular energy utilization during stressful conditions (Smolders *et al.*, 2004). During stressful conditions the CEA will decrease due to additional sources of energy being required (Gerber *et al.*, 2018).

Transplantation studies using molluscs have been applied successfully in freshwater studies (Damiens *et al.*, 2007, Greenfield *et al.*, 2014, Claassens *et al.*, 2016). Transplantations make use of organisms that are collected from an unstressed population, which are then translocated to polluted sites. The aim of this study was to use the four biomarkers to assess the effects that PGE exposure may have on transplanted clams in an aquatic ecosystem within an intensive platinum mining region.

5.2 Materials and methods

5.2.1 Study design

The study site and site selection were described in Chapter 4. Transplanted freshwater clams were deployed in two impoundments that are located along the Hex River system. Bospoort Dam is located directly below extensive mining activities while the Olifantsnek Dam is situated upstream of the mining area.

The freshwater clam, *Corbicula fluminalis africana* (average shell length of 28 ± 5 mm), were collected from the Mooi River, Potchefstroom, South Africa (Figure 5-1). The transplanted clams were kept in reconstituted freshwater for two weeks prior to deployment to allow them to depurate metals accumulated from the reference sampling site. The transplanted clams were regularly fed with *Spirulina*, cultured in the laboratory at the North-West University, throughout the two weeks. Holding water was changed after each feeding. The clams were transported to the field in plastic containers filled with reconstituted freshwater fitted with an oxygen pump. A control group of clams were retained in the laboratory to serve as control organisms.

Figure 5-1: Clams that were used as transplant bioindicators for this study, *Corbicula fluminalis africana.*

The clams were deployed at the two sampling sites in duplicate polypropylene containers (Figure 5-2). The procedure followed for the deployment and retrieval of these containers were discussed in Chapter 4. Briefly, each container was fitted with a mesh bag and filled with sediment from the sampling site. Two replicate containers each containing 10 clams (i.e. total of 20 transplanted clams) was deployed at each site. After transferring the clams the mesh bags were closed to prevent the organisms from escaping. Two sampling surveys were conducted during the study in 2017 and 2018. Following an exposure period of four weeks the transplanted clams were retrieved. These organisms were removed from the mesh bags and placed in containers that were aerated with an oxygen pump and water from the site. They were transported back to the laboratory. In the laboratory the clams were measured, weighed and then separated for metal and biomarker analysis. Samples for metal analysis were stored at -21 °C and tubes containing Henriksson's stabilizing buffer with tissue for biomarkers were placed in the -80 °C freezer until further analysis

Figure 5-2: Deployment of the plastic containers.

5.2.2 Metal analysis

Tissue samples of the clams were weighed prior to freeze drying. Clam samples of 60 mg (dry weight) were weighed into 20 mL TFM® vessels (MarsXpress CEM, Germany). The digestion

were carried out in a microwave digestion system (CEM, Mars 6) with 2.5 mL H_2O_2 (30%; Suprapur®, Merck, Germany) and 1.3 mL HNO_3 (sub-boiled from 65%; p.a. quality, Merck, Germany). Clear solutions were then transferred to 5 mL glass flasks and brought to volume with 1% HNO_3. The digested solutions were stored at room temperature in polypropylene tubes until metal analysis. Metals that were analysed includes; As, Cd, Cr, Co, Ni, Pb, Pd, Pt, V and Zn.

Metal concentrations in the water and clam digestions were determined by means of a quadrupole ICP-MS system (Perkin Elmer, Elan 6000) with an autosampler system (Perkin Elmer, AS-90). Between each measurement, the wash time was set to 30 s with 2% HNO_3 in order to avoid contamination. After every 10 samples a standard solution (10 µg/L), for all elements measured, was used to control the accuracy and stability of the measurements. Prior to measuring, samples were diluted 1:10 with an internal standard solution, consisting of 1% HNO_3 and 10 µg/l thulium (Certipur®, Merck). Calibration of the instrument was performed using a series of 11 dilutions of standard solution. With this the concentrations of the sample analytes were calculated using regression lines with a correlation factor of ≥0,999.

5.2.3 Biomarker analysis

Metallothioneins (MTs)

For the determination of MT, clam tissue was homogenized within 0.25 M sucrose solution (Merck) in 1:4 w/v ratio. The homogenate was centrifuged at 20 000 g for 20 min at 4 °C. From the supernatant 300 µL was processed for MT analysis by the silver saturation method (Scheuhammer and Cherian, 1986) with slight modifications (Frank *et al.*, 2008). Samples were incubated with 500 µL 20 mg/L silver standard solution (standard for ICP, 1000 mg/L, Fluka Analytical) for 20 minutes at room temperature. This permitted the silver to saturate the metal binding sites of the MTs. The excess silver ions were removed by adding 100 µL bovine red blood cell hemolysate (Red blood cells, Glutaraldehyde stabilized, sheep, 10% haematocrit) followed by heat treatment in a water bath (100°C for 10 minutes). The heat treatment caused the precipitation of silver-bound haemoglobin and other proteins, except the MTs which are heat stable. The denaturised proteins were removed by centrifugation at 1000 g for 5 minutes. The addition of the hemolysate, heat treatment and centrifugation was repeated three times, where the final supernatant was centrifuged for 15 minutes at 16 000 g. The silver concentration in the final supernatant, which is proportional to the amount of MT, was determined by means of an ICP-MS. In addition to the silver, Pt concentrations were also measured to determine the levels of Pt in the supernatant since the binding affinity of Pt to MT

is higher than that of Ag. Thus the levels of MTs bound to unidentified metals were determined as well as the MTs that was bound to Pt.

Catalase activity (CAT)

Catalase activity was determined by using methods described in Cohen *et al.* (1970). Sample homogenates were obtained by homogenizing the soft tissue in 1:10 (tissue weight:buffer volume) in phosphate buffer (0.01 M, pH 7.0). Homogenised tissue were then centrifuged for 10 minutes at 10 000 g and 4ºC. The supernatant was used for both CAT and protein analysis. The assay consisted of a blank, samples and phosphate buffer as a standard. The assay mixture comprised of 10 µL sample, 93 µL H_2O_2 (6 mM, kept at 4 ºC), 19 µL H_2SO_4 (95-99%, Merck) and 130 µL $KMnO_4$ (analytical reagent, Rochelle Chemicals). Eight samples were analysed at a time, in triplicate per microplate. Absorbance was measured at 490 nm within 30-60 seconds. Catalase activity was calculated as µmol H_2O_2/min/mg protein.

Malondialdehyde (MDA)

Lipid peroxidation was used to measure MDA contents with methods described in Ohkawa *et al.* (1979) as modified by Üner *et al.* (2006). The tissue was homogenized in 0.25 M Sucrose buffer (pH 7.4) (1:5), where after it was centrifuged at 9 500 g at 4 ºC for 10 minutes. The supernatant was used for both MDA and protein analysis. The reaction mixture consisted of 12.5 µL homogenate, 25 µL 8.1% SDS (Merck), 187.5 µL 20% acetic acid (analytical reagent, Rochelle Chemicals), 187.5 µL 0.8% thiobarbituric acid (≥98%. Sigma-Aldrich) and 87.5 µL ultrapure water. Then place in a boiling water bath (95 ºC) for 30 minutes, after the samples were allowed to cool down 125 µL ultrapure water and 625 µL n-butanol (for HPLC, 99.8%, Fluka Analytical) and pyridine (anhydrous, 99.8%, Sigma-Aldrich) (15:1) solution were added. The samples were then centrifuged at 1000 g for 10 minutes, the absorbance was measured at 540 nm. 1,1,3,3-Tetramethoxypropane (Sigma-Aldrich) was used as a standard.

Cellular Energy Allocation (CEA)

Energy available – Ea

Available energy reserves were measured by determining the total protein, carbohydrate and lipid content at each time point spectrophotometrically and transforming them into energetic equivalents using enthalpy combustion (24 000 mJ/mg proteins, 17 500 mJ/mg glycogen and 39 5000 mJ/mg lipids) as described in De Coen and Janssen (1997), De Coen and Janssen (2003), Rasouli *et al.* (2014) and Rasouli *et al.* (2015).

Clam tissues (pooled samples of two to three clams) were homogenised in deionised water for protein and lipid measurements. Protein content was determined according to methods

described in Bradford (1976), 5 µL homogenate and 245 µL Bradford reagent (Sigma-Aldrich) was added to a microplate in triplicate. After 5 min the absorbance was read at 595 nm, using bovine serum albumin as a standard.

Total lipids were extracted by the method described by Bligh and Dyer (1959), 250 µL homogenate was used, where after 500 µL chloroform (CHROMASOLV®, for HPLC, ≥99.8%, Sigma-Aldrich), 500 µL methanol (analytical reagent, Rochelle Chemicals) and 250 µL deionised water was added. After centrifugation (5 min at 3000 g at 4 ºC) 500 µL H_2SO_4 (95-99%, Merck) were added to 100 µL of lipid extract (organic bottom phase) and incubated at 200 ºC for 15 min. One mL deionised water was added to each tube and allowed to cool down. Total lipid content was determined in a microplate, where 245 µL sample was measured in triplicate at 360 nm with Tripalmitin as standard and chloroform as a blank.

The phenol-sulfuric acid microassay was used to determine the glycogen content of each sample, it was performed in small test tubes and then transferred to and read in a 96-well microplates following the method of Rasouli *et al.* (2015) for the preparation of the samples and Rasouli *et al.* (2014) for the glycogen determination. Fifty mg of tissue was weighed and transferred to 2 mL test tubes with 200 µL KOH 30%, where after the test tubes were placed in a water bath at 95 ºC for 10 minutes. The samples were allowed to cool down, where after 275 µL ethanol (55%, Sigma-Aldrich) was added. Samples were mixed and centrifuged at 1700 g at room temperature for 10 minutes. The supernatant was decanted off and the pellet re-suspended in 2 mL ultrapure water. From this 50 µL sample, 50 µL ultrapure water, 100 µL 6.5% phenol (Sigma-Aldrich) and 500 µL H_2SO_4 (95-99%, Merck) was added to 2 mL test tubes. Samples were vortexed and allowed to stand for 30 minutes, afterwards absorbance was measured at 492 nm with glycogen (Type II from oyster, Sigma-Aldrich) as standard.

Energy consumed – Ec

The consumed energy (consumed oxygen rate) was determined based on the measurement of the electron transport system activity following the methodology described by De Coen and Janssen (1997) and De Coen and Janssen (2003). Each replicate (two to three organisms) was homogenised in electron transport (ETS) homogenising buffer (1:5). The ETS homogenising buffer consists of Tris-HCl (Trizma® hydrochloride, Sigma-Aldrich), 0.2% Triton X-100 (for electrophoresis, Sigma-Aldrich), 15% Poly Vinyl Pyrrolidone and $MgSO_4$ (analytical reagent, Sigma-Aldrich). After centrifugation, 10 min at 3000 g at 4 ºC, 25 µL supernatant was transferred to a microplate. Where after 75 µL buffered substrate solution, consisting of 0.3% Triton X-100 and Tris-HCl, were added. Thereafter 25 µL NADPH solution (NADH and NADPH) were added. To start the reaction 50 µL p-IodoNitroTetrazolium violet/chloride was

added. The absorbance was measured at 490 nm at 20 ºC at 1 min time intervals over a period of 5 minutes.

Cellular Energy Allocation – CEA

These energy reserves were transformed into energetic equivalents, 17500 mJ/mg glycogen, 24000 mJ/mg protein and 39500 mJ/mg lipid. The total Ea value was calculated by integrating the change in the different energy reserve fractions over the exposure periods. The Ec value was calculated based on the theoretical relationship that for each 2 µmol of formazan formed, 1 µmol of O_2 was consumed in the ETS system. The CEA, representing the total net energy budget, was calculated for each survey as described in (De Coen and Janssen, 1997).

5.2.4 Statistical analysis

Statistical analyses were performed using GraphPad Prism® 7 software. For biomarker analyses all results were log transformed (Y = Log(Y)). Multiple comparisons with 1-way ANOVA was performed to determine significant differences between the control group and clam samples collected from each impoundment during each survey (

Table 5-1). Where after Dunnett's multi comparisons test was performed. Statistical significance was set at $p < 0.05$ for all comparisons. Statistical analyses of metal bioaccumulation and water concentrations were presented in Chapter 4. Principal component analyses (PCAs) were performed on the data to determine the relationship between biomarker responses and metal bioaccumulation using a multivariate approach by using Canoco 5.

5.3 Results

Metal concentrations within the transplanted clams were determined as described in Chapter 4: The application of artificial mussels in conjunction with active biomonitors to assess metal exposure in a platinum mining area. Metal concentrations measured in the clams from the two surveys can be seen in Table 5-1.

Table 5-1: Metal concentrations measured in the transplanted clams from two surveys conducted in Bospoort Dam and Olifantsnek Dam, where the control refers to concentrations in reference clams that were maintained in the laboratory. Concentrations are measured in µg/g (mean ± SEM, ND = Not detected) (an asterisk (*) indicates significant differences of a site between surveys, whereas common superscript letters indicate significant differences between sites.

	Survey 1 (2017)			Survey 2 (2018)		
	Control	Olifantsnek Dam	Bospoort Dam	Control	Olifantsnek Dam	Bospoort Dam
As	5.8 ± 0.51^{a}	22.2 ± 1.57*^{ab}	6.9 ± 0.23^{b}	6.3 ± 0.18	6.9 ± 0.70*	7.8 ± 0.67
Cd	0.064 ± 0.012*	0.0714 ± 0.0114*	0.0701 ± 0.0114	0.370 ± 0.05*^{ab}	0.148 ± 0.0111*^{a}	0.175 ± 0.0441^{b}
Co	1.1 ± 0.17*	1.5 ± 0.12*	1.3 ± 0.06	2.1 ± 0.044*	2.8 ± 0.34*^{a}	1.7 ± 0.18^{a}
Cr	4.4 ± 0.58^{a}	9.5 ± 1.34^{ab}	4.1 ± 0.42^{b}	3.4 ± 0.101^{a}	11.1 ± 1.09^{ab}	3.9 ± 0.22^{b}
Ni	1.2 ± 0.23*^{a}	4.4 ± 0.58^{a}	11.1 ± 1.102^{a}	4.3 ± 0.44*	8.1 ± 0.29	7.5 ± 0.18
Pb	0.044 ± 0.007*^{a}	0.028 ± 0.004*^{b}	0.011 ± 0.001^{ab}	0.079 ± 0.009*^{a}	0.051 ± 0.003*^{b}	0.014 ± 0.003^{ab}
Pd	0.026 ± 0.004	0.016 ± 0.001^{a}	0.034 ± 0.003*^{a}	0.025 ± 0.001	0.037 ± 0.013	0.02 ± 0.004*
Pt	0.0006 ± 0.0002	0.0010 ± 0.0001	ND	0.0006 ± 0.0001	0.0004 ± 0.0004	0.0005 ± 0.0003
V	3.2 ± 0.37*^{a}	4.1 ± 0.52^{b}	1.4 ± 0.10^{ab}	0.78 ± 0.020*^{a}	3.7 ± 0.22^{ab}	1.1 ± 0.08^{b}
Zn	183.5 ± 22.31*	185.6 ± 8.12	191.6 ± 4.58	292.8 ± 8.01*^{ab}	168.4 ± 14.44^{a}	185.8 ± 10.82^{b}

There were no statistical differences in the CAT levels measured between the control and the clams transplanted in the two impoundments during both surveys (Figure 5-3). There is a significant difference in the CAT activity between the two surveys.

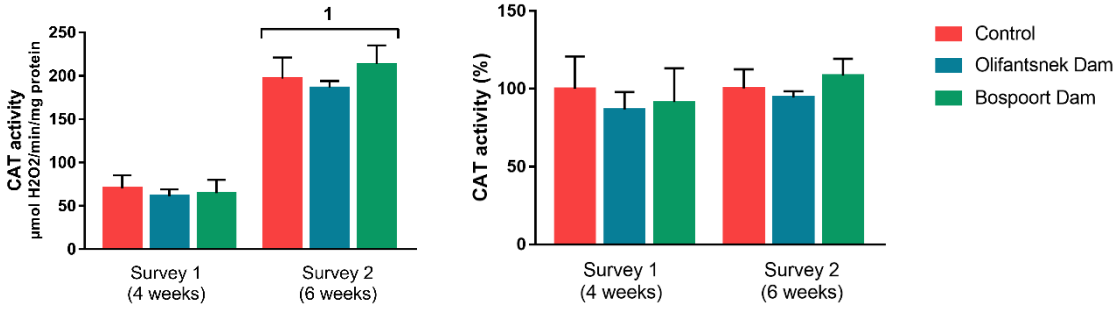

Figure 5-3: Comparison of catalase activity (CAT) measured in the transplanted clams from two impoundments (mean ± SEM, where bars with number is significantly different from other survey).

The MDA activity measured in the transplanted clams indicated no significant difference from the control during the first survey. The MDA response in Olifantsnek Dam was slightly higher. However, when the results were normalized relative to the control group it could be seen that there was a significant difference in the MDA response during the second survey for the clams from Bospoort Dam (Figure 5-4).

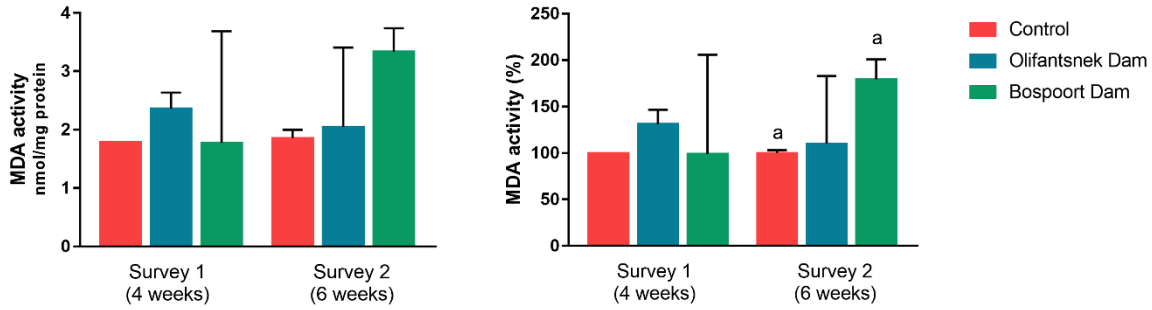

Figure 5-4: Comparison of Malondialdehyde activity (MDA) measured in the transplanted clams from two impoundments (mean ± SEM, bars with common superscript indicate significant difference).

The significant compounds responsible for the available energy between the surveys were protein concentrations, where both impoundments were significantly different between surveys (Figure 5-5 C). One difference was found in lipid concentrations (Figure 5-5 B) between surveys for Olifantsnek Dam, whereas there were no significant differences in glycogen concentrations (Figure 5-5 A). The glycogen levels in the second survey was slightly higher in both impoundments. Protein concentrations differed temporally in both

impoundments, while only Bospoort Dam was significantly lower during the second survey when compared to the control.

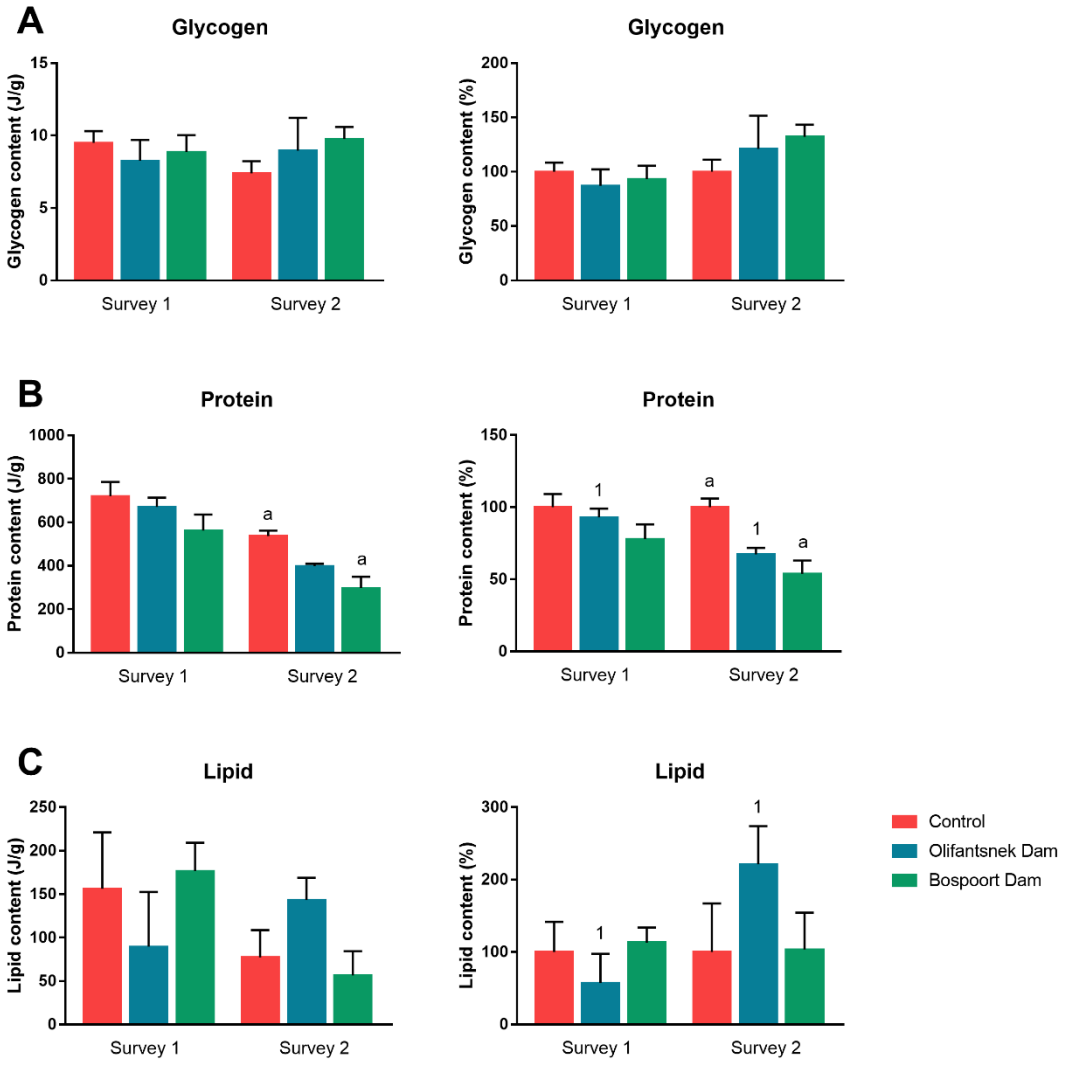

Figure 5-5: Comparison of A) glycogen, B) lipid and C) protein contents measured to determine energy available (Ea) in the transplanted clams from two impoundments (mean ± SEM) Bars with common superscript indicate significant difference between sites (indicated by letters) and between surveys (indicated by numbers).

When considering the Ea the slight decreases were observed over time for both surveys, where the same trends could be seen for the total energy budget (Figure 5-6 A and C). The transplanted clams from Bospoort Dam indicated significantly lower energy available when compared to the control during the second survey. There is also a significant difference in the Ea in Bospoort Dam between the two surveys. During the first survey the Ec was slightly higher within the two impoundments however during the second survey there was significantly lower energy consumed by the transplanted clams in both the impoundments when compared to the

control (Figure 5-6 B). The Ec also indicated differences between the surveys, where the second survey indicated significantly lower Ec. The overall Ea and CEA followed similar trends, with the CEA during both surveys were lower than that measured in the control. It can therefore be seen that the CEA of the transplanted clams form Bospoort Dam were significantly lower during the second survey (Figure 5-6 C).

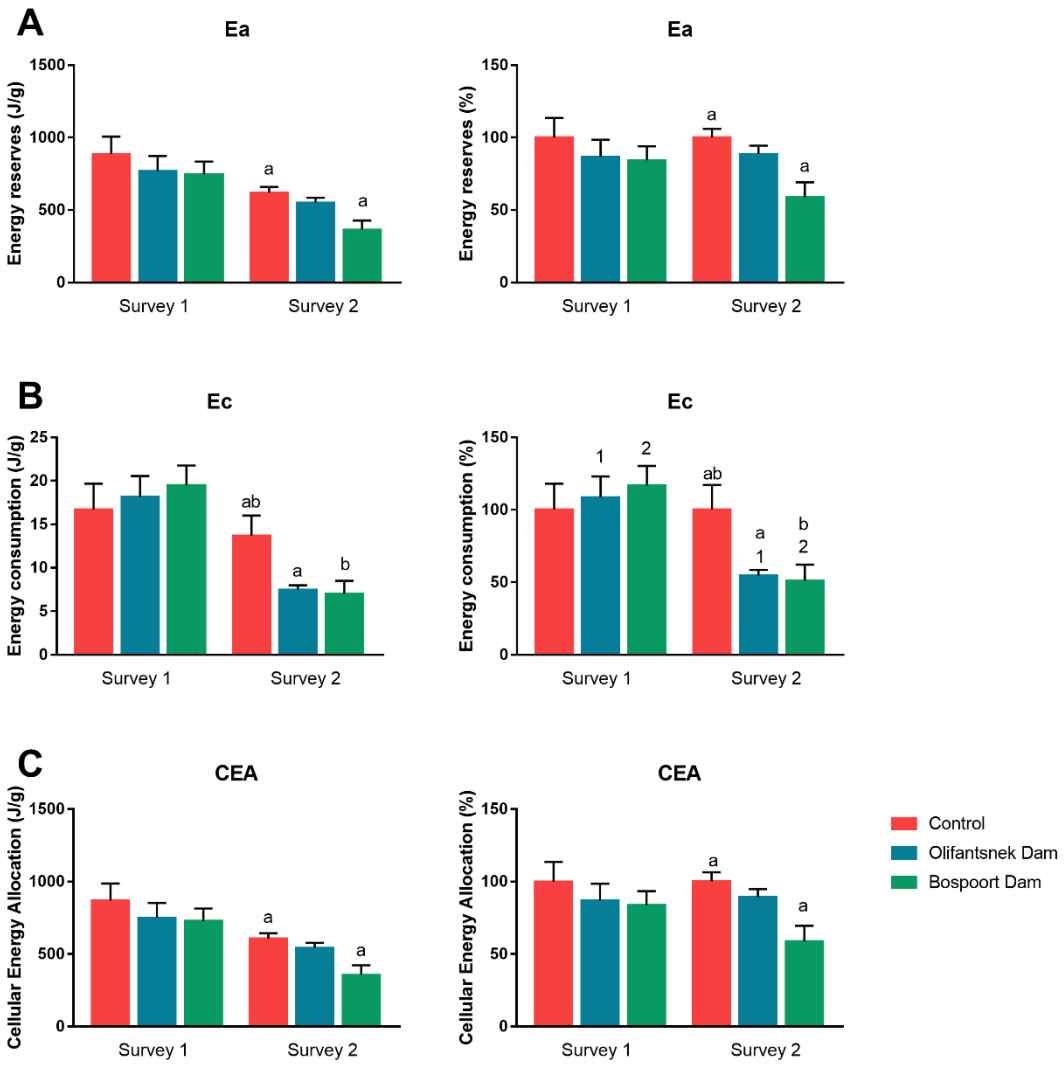

Figure 5-6: Comparison of A) energy available, B) energy consumed and C) total energy levels measured in the transplanted clams from two impoundments (mean ± SEM). Bars with common superscripts indicate statistical significant difference between sites (indicated by letters) and between surveys (indicated by numbers).

There was no significant difference in Ag-Mt when compared to the control group with Olifantsnek Dam being highest during both surveys. (Figure 5-7). In Figure 5-7 B it can be seen that the Pt-MT from the two impoundments were significantly lower when compared to the control group during both surveys. It should also be noted that the Pt-MT concentrations

are significantly lower during the second survey, while Ag-MT concentrations are significantly higher during the second survey.

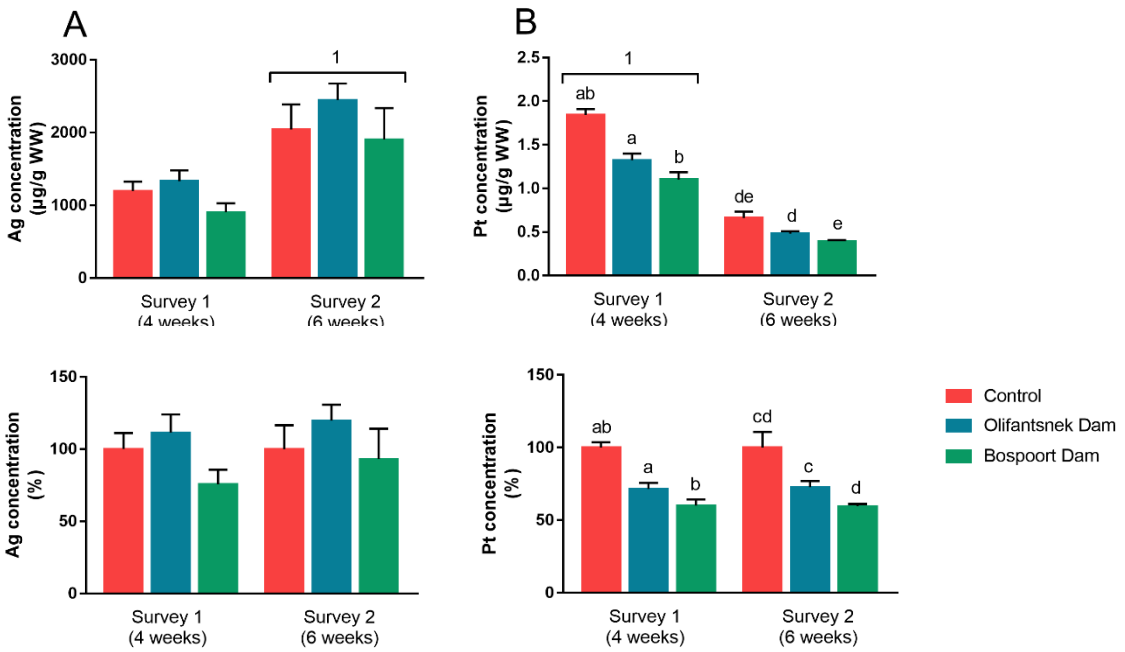

Figure 5-7: Comparison between Ag-MT and Pt-MT measured in transplanted clams from two impoundments (mean ± SEM) (bars with common superscripts indicate statistical significant differences).

The RDA (Figure 5-8) explains 35.1% of the variation in the data on axis 1 and 2. From this analysis it could be seen that there was no specific response elicited in the translocated clams by metal exposure. However it can be seen that higher energy reserves were available during the first survey, which also indicates a depletion of energy reserves during the second survey. The Cd, Co, Cr, Pb and Pd correlated strongly with the biomarker responses in clams from Olifantsnek Dam during the second survey. With this it could be seen there was an increase in CAT activation during the second survey, however there was no correlation with MDA in any of the surveys.

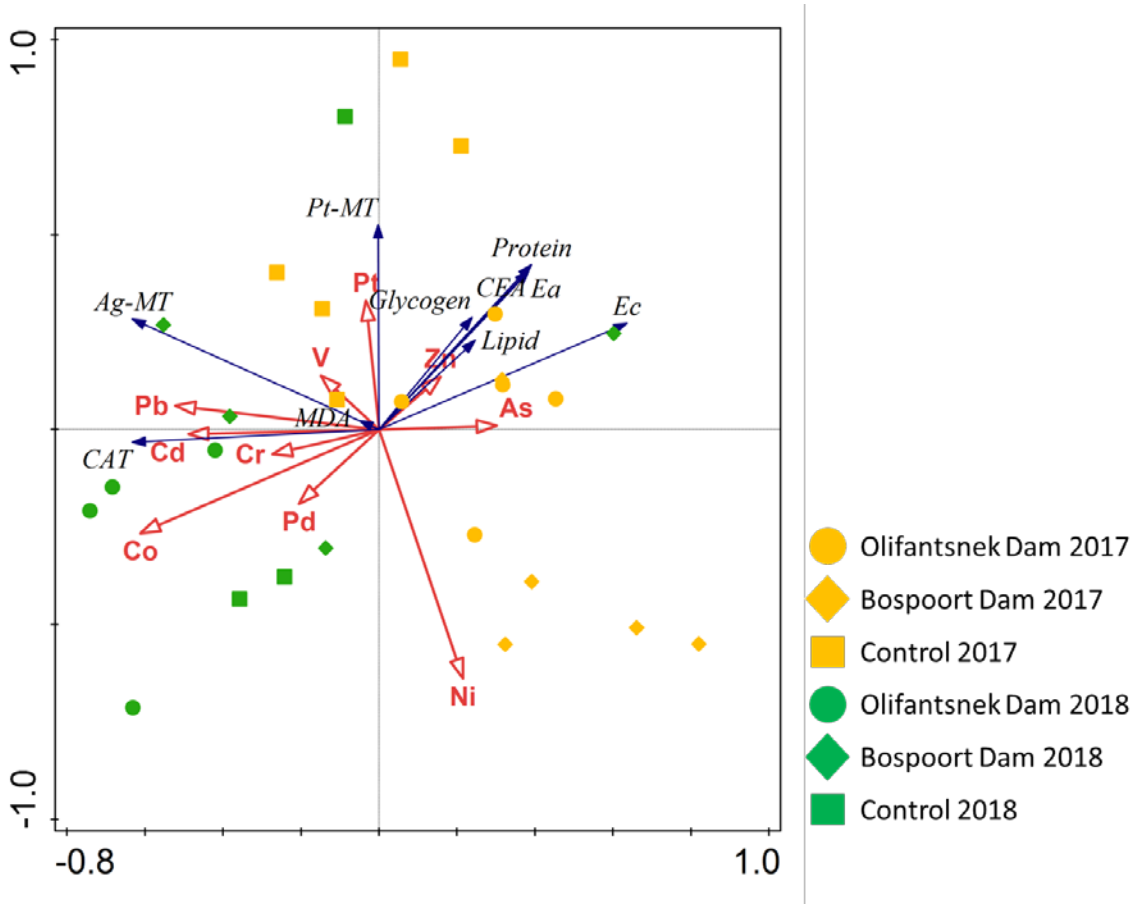

Figure 5-8: Canonical variates derived from a redundancy analysis (RDA) using metal concentrations found in the tissue of the transplanted clams and the biomarker responses. Function 1 (24.8%) and 2 (10.3%) explains a total of 35.1% of the variation in the data.

5.4 Discussion

Different stressors in the environment will elicit different biochemical responses in organisms, these stressors can produce alterations which can be quantified by estimating biological responses (biomarkers) (Gerber *et al.*, 2018). There is a wide variety of stressors in the environment, therefore specific biomarkers can reflect a response to a specific contaminant. Bivalve molluscs are filter feeding sedentary species that have been used extensively as bioindicators for pollutants. Many pollutants generally associate with oxidative stress and oxidative damage. There is however variability in the anti-oxidant defence mechanisms of these organisms and ensuing protein damage, lipid peroxidation and estimation of energy reserves.

The catalase system is known as the first line of defence when it comes to oxidative stress. Catalase activity is biologically important since it is responsible for the removal of H_2O_2, this is a highly reactive and toxic form of reactive oxygen species (Kim *et al.*, 2010). An increase in CAT activity will be due to an increase in oxidative stress, whereas an inhibition can suggest a transitory response to acute pollution (Vlahogianni *et al.*, 2007).

From these results it can be seen that there were no significant differences between the CAT activity in the control and transplanted clams for both surveys. This would imply that there were no anti-oxidant protective measures initiated at any of the sites. However there was an increase in CAT activity during the second survey. The first survey was conducted during May/June 2017 where the water temperature in Olifantsnek Dam and Bospoort Dam had an average of 19.3ºC and 19.45ºC respectively. During the second survey (March/April 2018) the temperature in the two impoundments was relatively higher, where Olifantsnek Dam had an average of 24.3ºC and Bospoort Dam 28.3ºC during the second survey (2018). Khessiba *et al.* (2005) and Orbea *et al.* (2002) reported that CAT activity increases in response to increased temperature. It is therefore that seasonal variations elicited a variation in CAT activity in the clams.

In addition food availability can also have an effect on the CAT activity where CAT activity will decrease when food is available (Khessiba *et al.*, 2005). When food is available for animals, they will have better physiological conditions and there may be a decrease in their anti-oxidant defences. During the second survey a massive water hyacinth (*Eichhornia crassipes*) bloom occurred in Bospoort Dam. These water plants covered more than 50% of the impoundment at the end of the exposure, it is possible that the hyacinths had an influence on the availability of food sources during the second survey in this impoundment. This could explain why the CAT activity was slightly higher in the transplanted clams from Bospoort Dam.

Anti-oxidant defence mechanisms are induced by various environmental pollutants, initially the concentrations of these anti-oxidants will increase to respond to the oxidative stress (Vlahogianni *et al.*, 2007). Long term exposure will cause a depletion of the anti-oxidants or lead to the damage of proteins, DNA and lipid peroxidation (Van der Oost *et al.*, 2003, Vlahogianni *et al.*, 2007). Metals such as Cd, Ni, Cr and Pb have the potential to cause oxidative stress, while other metals are essential to these organisms but it can be harmful if the concentrations exceed the concentrations that are needed (Vlahogianni *et al.*, 2007). From the results it can be seen that there was no correlation with the MDA response of the transplanted clams.

Increased energy requirements due to the activation of protective mechanisms other than CAT in Bospoort Dam as a result of exposure to stressors such as pollutants or other environmental factors can lead to diminished energy reserves (carbohydrates, lipids and proteins) (Gerber *et al.*, 2018). Protein and lipid damage can occur within an organism in the presence of oxidants, these oxidants are then further broken down through protective mechanisms of anti-oxidants such as CAT (Van der Oost *et al.*, 2003). Previous studies have demonstrated that energy related measurements can be a sensitive indicator of environmental stress. Together, the lipid, protein and glucose content of the organism is used to determine the energy reserves available (Ea) (De Coen and Janssen, 1997, De Coen and Janssen, 2003).

The glycogen had no influence on the energy available during both surveys, however the lipid content during the first survey in Olifantsnek Dam was slightly lower while these levels were elevated during the second survey. It has been reported that when organisms are going through starvation periods an increase in lipid content can be linked to increased survival (De Coen and Janssen, 1997). During the first survey there was a slight increase in lipid peroxidation, when comparing to the control, which correlates with the decrease in lipid contents in Olifantsnek Dam. During the second survey the lipid concentrations were much higher, it may be that the organisms were increasing their lipid contents to increase the chances of survival.

It can be seen that there was a decrease in protein levels within the transplanted clams during both surveys. There was a significant decrease in the protein levels within the clams from both impoundments, where these levels were significantly lower during the second survey. It has been suggested that a decrease in protein contents can be linked to an impairment in growth (De Coen and Janssen, 1997). The decrease in protein contents can be linked to the activation of protective mechanisms to oxidative stress. It can be seen that less energy was available for the organisms during the second survey, this can possibly be attributed to organisms needing less energy during summer months than during the winter months and to the organisms using their energy reserves to survive. During the winter months these organisms need higher

energy reserves to endure the colder temperatures, to ensure that their metabolism and protective mechanisms stay active and to be able to survive during periods where less food is available.

The significantly lower Ea in Bospoort Dam and during the 2018 survey may be due to the organisms using more energy to cope with oxidative stress and exposure to pollutants. It may also be a result of a depletion in energy reserves caused by an increase in metabolic activity or a decrease in energy uptake. The Ec is estimated by measuring the electron transport activity (De Coen and Janssen, 1997). The slightly higher Ec levels during the first survey can be attributed to organisms consuming more energy to survive the winter months and exposure to pollutants. During the second survey significantly lower energy was consumed. This can be attributed to lower energy levels being available, less energy is consumed during warmer months and may lead to a reduction in food ingested by the clams. Similar trends could be seen for the proteins, Ec and CEA.

The total energy budget was slightly decreased in both impoundments during both surveys, where there was a significant decrease in the CEA within Bospoort Dam when compared to the control and when compared to the CEA measured during the first survey. A decrease in the energy budget of an organism indicates that the organism is responding to stressors in the environment, where the organism requires additional energy sources. An increase in the energy budget indicates that there are additional energy sources available for the organism (Van der Oost *et al.*, 2003, Gerber *et al.*, 2018). Depleted energy reserves can affect tissue growth, reproduction and the overall health of the organism.

Metallothioneins are used as an early indicator of exposure to metals in the environment, which is a very important for this study since mining activities are the main source of pollution within the Hex River system. For the determination MTs the silver saturation method was used, where Ag(I) can displace metals with lower affinities for MT, the Ag-MT levels could then be used to determine non-Pt-induced MT levels (Scheuhammer and Cherian, 1986). When considering the Ag-MT it can be seen that there is a significant difference between the two surveys. During the two surveys there were slight variation in metal concentrations, concentrations in Olifantsnek Dam was slightly higher than the first survey while Bospoort Dam was rather similar. It is possible that the metals were more bioavailable for the clams to accumulate during the second survey, which could stimulate MT synthesis. However it can be seen that when the clams had higher Pt-MT concentrations, lower Ag-MT concentrations were observed.

The induction of MT synthesis by metal contaminants has been observed for many species and in a wide range of studies (Amiard *et al.*, 2006). There are contradictions and

inconsistencies for the induction of MTs. In some studies it has been found that where metals are bioavailable and at high concentrations some species do not indicate an increase in MT concentration (Amiard *et al.*, 2006). There have been cases were experiments have indicated that MT responses were induced after a very short time, while field studies have indicated no significant rise in MT concentrations (Amiard *et al.*, 2006). Two studies with *Corbicula fluminea* indicated no induction of MT after being exposed to Pb, Cu, Zn and Cd after 15 days (Rainglet, 1998), Cd and Zn for 21 days where at some sites MT response was induced at one site after 21 days and at the other site after 150 days (Baudrimont *et al.*, 1999). In studies that involved *Dreissena polymorpha* de Lafontaine *et al.* (2000) found that in the field Cu and Se induced MT response, while Lecoeur *et al.* (2004) found that Cd induced MT response Cu did not.

According to Amiard *et al.* (2006) some organisms might be less sensitive to an increase in metal concentrations and therefore a rise in MT concentration can be linked to a decrease in the sensitivity to metal exposure. Since MTs are responsible for the detoxification processes it is important to keep in mind if the transplanted clams are exposed to metal contamination in their natural environment no response might be stimulated at the translocated sites (Amiard *et al.*, 2006).

During both surveys it could be seen that the Pt-MT concentrations were lower than those observed in the control. It is possible that the source of the clams used in the translocation study were already exposed to metals at the source site, which resulted in MT induction. There was a significant difference in the Pt-MT concentrations between the surveys, the transplanted clams contained significantly lower Pt-MT concentrations. The lower Pt-MT concentrations found in the translocated clams indicates that there was no induction of MT binding, the initially high MT concentrations in the control could be due to the possible protective role that is needed to survive in their natural habitat. It has been found that seasonal changes have an influence on MT concentrations, this can be associated with fluctuations in weight, reproductive status and changes in food availability (Geffard *et al.*, 2005). According to Amiard *et al.* (2006) MTs have other roles, which include protection against ionizing radiation and more generally antioxidant defense. It was proposed that organisms that are exposed to metals beforehand can resist oxidative stress more effectively, where the induction of MTs seems to limit the effects of the reactive oxygen species.

In polluted environments the organisms are exposed to a mixture of different metals, this generally makes it impossible to attribute MT induction to a single element (Amiard *et al.*, 2006). With the method used in this study it is possible to determine the total MT concentrations (Ag-MT), which are bound to a mixture of metals as well as the fraction that is bound to Pt to determine if these organisms were exposed to these metals. Based on the

results obtained it was clear that the MT induction found was not the result of Pt exposure but rather exposure to other MT-inducing metals such as Ni, Pb and Zn.

5.5 Conclusion

The variation in metal concentrations between the control and the transplanted clams from the impoundments elicited different biomarker responses. In this study, it was found that protective mechanisms, CAT, was activated against oxidative stress during the second survey. During the 2017 survey higher energy reserves were available, while this could also indicate a depletion of energy reserves in 2018. The activation of the protective mechanisms against oxidative stress damaged the lipids and proteins in the clams, which in turn lead to a depletion of energy reserves. During the second survey this caused significant depletion of Ea in Bospoort Dam. Overall less energy reserves were available during the second survey. There seemed to be no effects due to metal exposure, however other factors influence the responses of the transplanted clams. During colder/winter periods organisms are known to use more energy to survive the colder temperatures and to be able to survive when less food is available. During the second survey of this study it was found that significant energy was utilised. This can be due to the increase in metabolic activities, more energy being consumed, less energy being available and exposure to pollutants within this study. It is presumed that during the second survey less food was available in Bospoort Dam due to the water hyacinth bloom. In addition to the other factors; i.e. less energy being available, warmer temperatures, oxidative stress and exposure to pollutants, it was clear that the clams were subjected to multiple stressors and not just metals at the exposure sites.

References

Amiard, J.-C., Amiard-Triquet, C., Barka, S., Pellerin, J. and Rainbow, P. 2006. Metallothioneins in aquatic invertebrates: their role in metal detoxification and their use as biomarkers. *Aquatic toxicology,* 76**:**160-202.

Baudrimont, M., Andrès, S., Metivaud, J., Lapaquellerie, Y., Ribeyre, F., Maillet, N., Latouche, C. and Boudou, A. 1999. Field transplantation of the freshwater bivalve *Corbicula fluminea* along a polymetallic contamination gradient (river Lot, France): II. Metallothionein response to metal exposure. *Environmental Toxicology and Chemistry: An International Journal,* 18**:**2472-2477.

Bligh, E. G. and Dyer, W. J. 1959. A rapid method of total lipid extraction and purification. *Biochemistry and Physiology,* 37**:**911-917.

Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical biochemistry,* 72**:**248-254.

Claassens, L., Dahms, S., Van Vuren, J. and Greenfield, R. 2016. Artificial mussels as indicators of metal pollution in freshwater systems: A field evaluation in the Koekemoer Spruit, South Africa. *Ecological Indicators,* 60**:**940-946.

Cohen, G., Dembiec, D. and Marcus, J. 1970. Measurement of catalase activity in tissue extracts. *Analytical biochemistry,* 34**:**30-38.

Damiens, G., Gnassia-Barelli, M., Loquès, F., Roméo, M. and Salbert, V. 2007. Integrated biomarker response index as a useful tool for environmental assessment evaluated using transplanted mussels. *Chemosphere,* 66**:**574-583.

De Coen, W. M. and Janssen, C. 1997. The use of biomarkers in *Daphnia magna* toxicity testing. IV. Cellular energy allocation: a new methodology to assess the energy budget of toxicant-stressed *Daphnia* populations. *Aquatic Ecosystem Stress and Recovery,* 6**:**43-55.

De Coen, W. M. and Janssen, C. R. 2003. The missing biomarker link: Relationships between effects on the cellular energy allocation biomarker of toxicant-stressed *Daphnia magna* and corresponding population characteristics. *Environmental toxicology and chemistry,* 22**:**1632-1641.

De Lafontaine, Y., Gagné, F., Blaise, C., Costan, G., Gagnon, P. and Chan, H. 2000. Biomarkers in zebra mussels (*Dreissena polymorpha*) for the assessment and monitoring of water quality of the St Lawrence River (Canada). *Aquatic toxicology,* 50**:**51-71.

Frank, S. N., Singer, C. and Sures, B. 2008. Metallothionein (MT) response after chronic palladium exposure in the zebra mussel, *Dreissena polymorpha*. *Environmental research,* 108**:**309-314.

Geffard, A., Amiard-Triquet, C. and Amiard, J.-C. 2005. Do seasonal changes affect metallothionein induction by metals in mussels, *Mytilus edulis*? *Ecotoxicology and Environmental Safety,* 61**:**209-220.

Gerber, R., Smit, N., Van Vuren, J. H., Ikenaka, Y. and Wepener, V. 2018. Biomarkers in tigerfish (*Hydrocynus vittatus*) as indicators of metal and organic pollution in ecologically sensitive subtropical rivers. *Ecotoxicology and Environmental Safety,* 157**:**307-317.

Gerber, R., Smit, N. J., Van Vuren, J. H. and Wepener, V. 2016. Metal concentrations in *Hydrocynus vittatus* (Castelnau 1861) populations from a premier conservation area: relationships with environmental concentrations. *Ecotoxicology and Environmental Safety,* 129**:**91-102.

Greenfield, R., Brink, K., Degger, N. and Wepener, V. 2014. The usefulness of transplantation studies in monitoring of metals in the marine environment: South African experience. *Marine Pollution Bulletin,* 85**:**566-573.

Khessiba, A., Roméo, M. and Aïssa, P. 2005. Effects of some environmental parameters on catalase activity measured in the mussel (*Mytilus galloprovincialis*) exposed to lindane. *Environmental pollution,* 133**:**275-281.

Kim, J., Kim, S., An, K. W., Choi, C. Y., Lee, S. and Choi, K. 2010. Molecular cloning of *Daphnia magna* catalase and its biomarker potential against oxidative stresses. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology,* 152**:**263-269.

Lecoeur, S., Videmann, B. and Berny, P. 2004. Evaluation of metallothionein as a biomarker of single and combined Cd/Cu exposure in *Dreissena polymorpha*. *Environmental research,* 94**:**184-191.

Ohkawa, H., Ohishi, N. and Yagi, K. 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical biochemistry,* 95**:**351-358.

Orbea, A., Ortiz-Zarragoitia, M., Solé, M., Porte, C. and Cajaraville, M. P. 2002. Antioxidant enzymes and peroxisome proliferation in relation to contaminant body burdens of PAHs and PCBs in bivalve molluscs, crabs and fish from the Urdaibai and Plentzia estuaries (Bay of Biscay). *Aquatic toxicology,* 58**:**75-98.

Rainglet, F. 1998. *Biomarqueurs specifiques de pollution: evaluation de l'exposition et des effets precoces des dechets solides (ordures menageres, machefers) a l'egard du mollusque bivalve Corbicula fluminea (Muller, 1774).* These de Doctorat, Universite de Nantes.

Rasouli, M., Ostovar-Ravari, A. and Shokri-Afra, H. 2014. Characterization and improvement of phenol-sulfuric acid microassay for glucose-based glycogen. *Medical and Pharmocological Sciences,* 18**:**2020-2024.

Rasouli, M., Shokri-Afra, H. and Ostovar-Ravari, A. 2015. A new protocol for separation of acid soluble and insoluble fractions from total glycogen and simultaneous measurements. *Medical and Pharmocological Sciences,* 19**:**1785-1789.

Rauch, S. and Peucker-Ehrenbrink, B. 2015. Sources of Platinum Group Elements in the Environment. *In:* Zereini, F. and Wiseman, C. L. S. (eds.) *Platinum Metals in the Environment.* Heidelberg: Springer.

Ruchter, N., Zimmermann, S. and Sures, B. 2015. Field studies on PGE in aquatic ecosystems. *In:* Zereini, F. and Wiseman, C. L. S. (eds.) *Platinum Metals in the Environment.* Heidelberg: Springer.

Scheuhammer, A. and Cherian, M. G. 1986. Quantification of metallothioneins by a silver-saturation method. *Toxicology and applied pharmacology,* 82**:**417-425.

Smolders, R., Bervoets, L., De Coen, W. and Blust, R. 2004. Cellular energy allocation in zebra mussels exposed along a pollution gradient: linking cellular effects to higher levels of biological organization. *Environmental pollution,* 129**:**99-112.

Sures, B., Ruchter, N. and Zimmermann, S. 2015. Biological effects of PGE on aquatic organisms. *In:* Zereini, F. and Wiseman, C. L. S. (eds.) *Platinum metals in the environment.* Heidelberg: Springer.

Üner, N., Oruç, E. Ö., Sevgiler, Y., Şahin, N., Durmaz, H. and Usta, D. 2006. Effects of diazinon on acetylcholinesterase activity and lipid peroxidation in the brain of *Oreochromis niloticus*. *Environmental toxicology and pharmacology,* 21**:**241-245.

Van Der Oost, R., Beyer, J. and Vermeulen, N. P. 2003. Fish bioaccumulation and biomarkers in environmental risk assessment: a review. *Environmental toxicology and pharmacology,* 13**:**57-149.

Vlahogianni, T., Dassenakis, M., Scoullos, M. J. and Valavanidis, A. 2007. Integrated use of biomarkers (superoxide dismutase, catalase and lipid peroxidation) in mussels *Mytilus galloprovincialis* for assessing heavy metals' pollution in coastal areas from the Saronikos Gulf of Greece. *Marine Pollution Bulletin,* 54**:**1361-1371.

Wepener, V., Van Dyk, C., Bervoets, L., O'brien, G., Covaci, A. and Cloete, Y. 2011. An assessment of the influence of multiple stressors on the Vaal River, South Africa. *Physics and Chemistry of the Earth, Parts A/B/C,* 36**:**949-962.

Zimmermann, S., Sures, B. and Ruchter, N. 2015. Laboratory Studies on the Uptake and Bioaccumulation of PGE by Aquatic Plants and Animals. *In:* Zereini, F. and Wiseman, C. L. S. (eds.) *Platinum Metals in the Environment.* Heidelberg: Springer.

Chapter 6: Overall conclusion and Recommendations

6.1 Conclusions

The knowledge of PGE contamination in freshwater systems is important for the risk assessment for aquatic health and water resource management. From this study it could be seen that laboratory bioaccumulation studies alone are not sufficient since organisms in natural environments are affected by different environmental conditions that influence the bioavailability of metals to organisms. PGE concentrations in living organisms from field studies give valuable information on the metals that are associated with the mining activities, the bioavailable PGE fraction and the effective PGE concentrations inside of the organisms.

The aim of this study was to develop the artificial mussel is a novel method to detect bioavailable PGEs from freshwater ecosystems. So to revisit the hypotheses formulated at the beginning of this study:

Hypothesis 1: When the artificial mussel is exposed to freshwater containing platinum (Pt) this metal will be accumulated by the AM device. It is posited that the Pt concentration accumulated by the AMs will correlate with the external Pt concentrations provided the binding capacity of the AM is not reached.

In Chapter 2 the design of the AM was validated and optimised in the laboratory for uptake of PGEs. An optimum loading and elution protocol was developed for Pt. It was demonstrated that the Chelex® beads and the AMs accumulate Pt in a dose-response relationship and reflect the bioavailable environment concentrations. From this it was also found that when the beads are exposed to Pt it will bind the metal immediately with maximum Pt uptake from the exposure medium within week. Therefore this hypothesis is accepted.

Hypothesis 2: When the AM is exposed to environmental PGEs there will be a gradient of PGE exposure along the Hex River system, implying that there will be higher PGE levels closer to the mining areas.

When the AMs were exposed to the tailings dam and the two impoundments different PGE concentrations could be found. The AMs accumulated significantly higher Pd concentrations in Bospoort Dam during the second survey, while these concentrations were similar in the two impoundments during the first survey. For Pt it could be seen that these concentrations were

similar in both impoundments during the second survey and Bospoort Dam contained slightly higher Pt concentrations during the first survey. However the AMs from the tailings dam contained significantly lower Pd concentrations while the Pt concentrations were similar to those measured in the impoundments. The water concentrations reflect that there is a gradient of Pd along the Hex River, while the Pt were similar in the two impoundments, and the tailings dam contained significantly higher PGE concentrations. Therefore this hypothesis is accepted. Even though this hypothesis is accepted for PGEs, it did not show an exposure gradient for some of the other metals. It was presumed that since the metal concentrations in the water from the tailings dam were high, the AMs would reflect that as well. But this was not the case for all of the metals, for most of the metals it could be seen that AMs accumulated significantly lower concentrations. This could be attributed to a higher conductivity, which could have reduced the bioavailability of metals and the oil layer that could have prevented the diffusion of ions through the gel layers of the AM.

Hypothesis 3: During the concomitant exposure of transplanted organisms the uptake patterns within the AMs and transplanted organisms will be similar.

The accumulation patterns within in the AMs and the transplanted clams indicated different uptake patterns. The transplanted clams probably indicated different bioaccumulation patterns due to differences in the metal forms that they were exposed to. Clams will take up metals from the water both in the dissolved form and as complexes bound to particulate matter. Thus the physical and chemical conditions at the different sites and during the different surveys could influence the form of the metal available for uptake by the clams. On the other hand the AMs showed great accumulation patterns for all metals. The AMs correlated well with the concentrations found in the water column, which indicated that Bospoort Dam had higher metal concentrations, while the transplanted clams indicated that Olifantsnek Dam had higher concentrations. Therefore this hypothesis is rejected. However it should be kept in mind that organisms do not only take up metals in the dissolved form but that there are other exposure routes as well, e.g. through food that is ingested or particulate bound metals. Thus for a holistic exposure assessment both dissolved metal (AMs) and particular metal (bioindicator organisms) bioaccumulation should be determined.

Hypothesis 4: The biological responses in the form of biomarkers will reflect a dose-response relationship with increased PGE exposure.

The transplanted clams could not be used to determine the biological responses in the tailings dam since they did not survive the exposure period. It was found that the clams from the

reference site were exposed to high metal concentrations at the source site prior to deployment. It is therefore highly likely that the responses following deployment in the study sites were masked by the pre-exposed conditions. The possible reason for the lack of significant biomarker responses observed between the control and transplanted groups. Mechanisms against oxidative stress were observed with increased CAT activities (non-significant increases) when exposed to conditions in Olifantsnek Dam. These responses in turn caused a depletion in energy reserves and damage to lipid and protein content of the clams. Metallothionein induction (based on total metals) was higher in Bospoort Dam, while the Pt-MT concentrations were higher in the reference clams and to a degree in Olifantsnek Dam, which correlated with Pt concentrations found in the clam tissues. Based on the fact that environmental conditions experienced by the transplanted clams were reflected in biomarker responses (even though not significantly different from the reference clams) the hypothesis is accepted.

This study provided some insight into PGE concentrations that can be found in PGE mining areas in South Africa, in areas that are not impacted (Olifantsnek Dam) by these activities to areas that are heavily impacted (tailings dam). The results from this study contribute towards a better understanding of the risks that are involved and during this study a newly developed method was formulated to elute metals from AMs.

6.2 Recommendations

For future laboratory studies it could be useful to do exposures with the AMs and other PGEs, and conduct exposure studies with mixtures of these elements to determine if there is competition for binding sites on the Chelex® beads. The influence of changing physico-chemical parameters on metal uptake by AMs should also be investigated to understand possible environmental interactions in the field. It is also worthwhile to do exposures with other organisms to determine how PGEs are accumulated and the responses that PGEs may elicit and to do some exposure studies with the water hyacinths. In the mining area there is a lot of road dust on the roads, during the rainy season it is possible that the dust can make its way in to the river systems. Therefore laboratory-based studies on road dust would provide some insight into the contribution of road dust as a rout of Pt exposure into aquatic organisms.

For future field studies it would be necessary to determine effects of the high metal concentrations found in the tailings dam on indicator organisms. This could be done by conducting serial dilutions of the tailings dam water and testing the survival and biomarker

responses in suitable indicator organisms. The high metal concentrations in the tailings dam do not pose a risk at the moment, but if the wall of the tailings dam is breached it could have detrimental effects on the aquatic environment in the vicinity. During the second survey a hyacinth bloom occurred. It was postulated that these floating plants accumulated metals from the water column thereby reducing the bioavailable fractions for up take by both the AMs and transplanted clams. To verify this, water hyacinth samples from different areas of Bospoort Dam should be collected and analysed for PGEs and other metals in the different parts i.e. roots, stem and leaves.