

The partitioning of platinum in aquatic ecosystems and disruption to aquatic communities

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ABSTRACT

Due to the increasing presence of platinum (Pt) in the environment, it becomes crucial to identify its toxic potential in species at risk of being exposed, especially those found in aquatic environments where metal pollutants tend to accumulate. Comprehensive characterisation of possible adverse effects of Pt exposure on aquatic organisms remains vague. To accurately determine the impact on the aquatic environment, the compartmentalisation of Pt in the aquatic environment needs to be understood. However, the various environmental influencing factors and the binding characteristics of PGEs identified in recent studies concluded that laboratory studies (controlled environment) alone do not provide sufficient information concerning the behaviour of PGEs in the aquatic environment. As a result of the various environmental influencing factors, field-based studies cannot provide laboratory “controlled” information on the behaviour of PGEs as it can be tough to correlate field-based results to that of laboratory studies. Therefore, it is crucial to address the void between these two scientific approaches in the PGE study area and broaden our knowledge on the effects of Pt in the aquatic ecosystem. Using 18 microcosms, mimicked natural aquatic communities were established. These communities were then exposed to a range of standard Pt concentrations (0.1, 1, 10, 100, and 500 µgPt/L) for eight weeks. Samples were collected on three separate occasions: 48 hours (acute), four weeks (chronic), and eight weeks (chronic). These samples were then analysed to determine the compartmentalisation of Pt in the aquatic environment. Platinum concentrations in the different aquatic compartments (water, sediment, macrophytes, and artificial mussels) were quantified using an Atomic Absorption Spectrometer (AAS) and an Inductively Coupled Plasma-Mass Spectrometer (ICP-MS) to determine the different Pt concentrations within each of the compartments. The separate compartments were divided into bioavailable environmental accumulation (water and sediment) and bioaccumulation (macrophytes and artificial mussels).

Platinum concentrations in the water column decreased over time. Similarly, the sediment, macrophytes and, artificial mussels (AM) showed higher initial accumulated Pt followed by a progressive decrease over time. Two macrophyte species were identified (*Fontinalis antipyretica* and *Charophyta sp*) and both species accumulated Pt over time. Interestingly, the accumulation levels between the two species varied, where the *F. antipyretica* reported a higher accumulation level than the *Charophyta sp*. The difference in accumulation can be explained by the different habitat preferences of the species in the ecosystem, and their differences in Pt absorption and metabolism since macrophyte species rooted in the sediment are known to be more sensitive to metal pollution than suspended macrophytes (Gupta *et al.*, 2013).

Epiphytic and benthic diatom community assemblages were sampled 48-hours (acute) and eight weeks (chronic) after exposure to assess the impact of different Pt exposures on diatom community assemblages. Initial observations reported that diatom communities showed little to no response after the 48-hours; however, all the samples collected from the aquatic habitat indicated the presence of pollution tolerant taxa after a chronic eight-week Pt exposure. Diatom deformities were also observed, although these deformities occurred at Pt levels higher than those present in the environment. A total of 25 type 1 (morphological abnormalities of the frustule) teratologies were identified from four 500 µgPt/L chronic exposure samples. Resulting in 1.025% of teratologies identified for the epiphytic diatom community and 1.875% of teratologies for the benthic diatom community. This teratology indicates a possible disruptive potential of increased Pt concentrations on aquatic communities.

Keywords: Platinum, Aquatic ecosystems, Diatoms, Disruption of aquatic communities, Microcosms, Partitioning of platinum, Environmental compartments, Accumulation, Artificial mussels, Aquatic health,

ABBREVIATIONS AND ACRONYMS

AAS	Atomic Absorption Spectrometer
AM	Artificial Mussel
ANOVA	Analysis of Variance
Au	Gold
BDI	Biological Diatom Index
BIC	Bushveld Igneous Complex
CAT	Catalase
Cd	Cadmium
Cl	Chlorine
COD	Chemical Oxygen Demand
Cr	Chrome
Cu	Copper
DIC	Differential Interface Contrast
EC	Electrical Conductivity
Fe	Iron
GDI	Generic Diatom Index
HCl	Hydrochloric acid
Hf	Hafnium
HNO ₃	Nitric acid
ICP-MS	Inductively Coupled Plasma-Mass Spectrometer
IPS	Pollution Sensitivity Index
Ir	Iridium
KMnO ₄	Potassium permanganate
METSI	Mooi River Ecosystem Trials for Scientific Investigation
MI	Macroinvertebrates
Ni	Nickel
NMDS	Non-Metric Multidimensional Scaling
NO ₃	Nitrate
NWA	National Water Act
Os	Osmium
PCA	Principal Component Analyses
Pd	Palladium
PE	Pre-Exposure
PET	Polyethylene terephthalate

PGEs	Platinum Group Elements
ppm	Parts Per Million
PRC	Principal Response Curve
Pt	Platinum
PT%	Pollution Tolerant Taxa
Rh	Rhodium
PICT	Pollution Induced Community Tolerance
ROS	Reactive Oxygen Species
Ru	Ruthenium
SO ₄	Sulphate
TDI	Trophic Diatom Index
TDS	Total Dissolved Solids
Zn	Zinc

TABLE OF CONTENTS

CHAPTER 1: INTRODUCTION.....	1
1.1 Background.....	1
1.2 Problem statement and rationale for the study	2
1.3 Research aims and objectives.....	3
1.4 Structure and outline of the dissertation	4
CHAPTER 2: LITERATURE REVIEW	6
2.1 Platinum Group Elements and their sources.....	6
2.2 Platinum exploitation in South Africa and the rest of the world	7
2.3 Platinum Group Elements in the aquatic environment.....	9
2.4 Biomonitoring and aquatic ecosystem health.....	10
2.5 Bioindicator organisms	12
2.6 Using microcosms to assess PGEs in the aquatic environment	14
2.7 Metal exposures in microcosms	15
2.8 Compartmentalisation of PGEs in microcosms	17
CHAPTER 3: METHODOLOGY.....	19
3.1 Experimental design	19
3.2 Field sampling	22
3.3 Sample preparation.....	23
3.4 Metal detection and Quality Control	24
3.5 Diatom sampling, preparation, and index assessments	26

3.6	Data analyses.....	27
3.6.1	Metal analyses	27
3.6.2	Diatom Community analyses	27
CHAPTER 4: RESULTS.....		29
4.1	Physicochemical parameters and nutrient levels in the water of the microcosms	29
4.2	Platinum concentration in the water column.....	33
4.3	Platinum accumulation in the sediment	33
4.4	Platinum in macrophytes	34
4.5	Platinum accumulation in Artificial Mussels (AM)	35
4.6	Diatom diversity and community assemblages	36
4.7	Diatom Biological indices	43
4.8	Principal Component Analysis (PCA).....	51
4.9	Principal Response Curve (PRC)	55
4.10	Diatom deformities after eight weeks of Pt exposure.....	56
CHAPTER 5: DISCUSSION.....		58
5.1	Water Quality	58
5.2	The accumulation of Pt in the water and sediment.....	59
5.3	The accumulation of Pt in the macrophytes and artificial mussels.....	60
5.4	Bioavailability of Platinum to aquatic biota.	62
5.5	Diatom assemblages.....	63
CHAPTER 6: CONCLUSION AND RECOMMENDATIONS.....		67
6.1	Conclusion	67

6.2	Recommendations for future studies	68
	BIBLIOGRAPHY.....	70
	ANNEXURE A: Physicochemical water variables	89
	ANNEXURE B: Diatom taxa and counts for each of the sites for initial and chronic sample times.....	96

LIST OF TABLES

Table 1: Experimental layout of Pt exposures and sample count.....	21
Table 2: Optimised operating parameters for Platinum analysis on the Atomic Absorption Spectrometer.....	24
Table 3: Range of diatom indices classification values and their meaning (Eloranta & Soininen 2002; Taylor, 2004; Dumnicka <i>et al.</i> , 2006).	28

LIST OF FIGURES

Figure 1: The study area where the microcosms were built and left during the project. (A) Google maps of where the site is situated in relation to Potchefstroom in South Africa. (B) The complex where the microcosms were built and left. (C) Gerhard Minnebron stream where sediment, rocks, and macroinvertebrates were collected. 19

Figure 2: Layout and numbering of the eighteen different microcosms and their respective concentrations..... 20

Figure 3: The basic model design of the microcosm with a width of 0.95 meters, length of 1.5meters, and height of 0.4 meters. Note the positioning of the rocks, sandblasted microscope slides placed at a 45-degree angle, AMs and the five-centimetre-thick sediment layer. Environmental components were sourced from the nearby Gerhard Minnebron. Macrophyte *Fontinalis antipyretica* are known to grow on rocks and were thus introduced into the microcosms along with the rocks. Macrophyte *Charophyta sp.* germinated from seeds in the sediment during the eight-week colonisation period prior to the exposures..... 21

Figure 4: (A) The METSI research facility of the Unit for Environmental Sciences and Management of the North-West University and the microcosm layout. (B) Field sampling of water quality samples from one of the microcosms. 23

Figure 5: The optimised calibration curve used to calibrate the atomic absorption Spectrometer with 0 value of 0.0001, 1 value of 0.0004, 5 value of 0.0012, 10 value of 0.0023, 20 value 0.0044, 25 value of 0.0056, 50 value 0.0108 and 100 value of 0.0215..... 25

Figure 6: The optimised calibration curve used to calibrate the Inductively coupled plasma mass spectrometer with 0 value of 58.642, 1 value of 4796.637, 5 value of 24721.24, 10 value of 46412.1, 20 value 96799.71, 50 value 271025.1, and 100 value of 510829.80. 25

Figure 7: Line graphs illustrating the physicochemical water quality parameters for each microcosm exposure group over the experimental time from May 2019 to July 2019. (A) pH, (B) Salinity, (C) Total dissolved solids (TDS), (D) Electrical conductivity, (E) Temperature, (F) Turbidity..... 31

Figure 8: Illustration of the changes in water nutrients of each microcosm exposure group for the duration of the experiment. (A) Turbidity, (B) Total Hardness (TH), (C) Ammonium (NH_4^+),

(D) Nitrate (NO_3), (E) Nitrite (NO_2), (F) Chloride (Cl), (G) Phosphate PO_4 , (H) Chemical oxygen demand (COD)..... 32

Figure 9: Mean Pt concentration found in the water column in each exposure concentration throughout the experiment at the three different sample times, i.e., 48-hours, four weeks, eight weeks (n = 54). Significant differences over time are indicated with an asterisk (*). 33

Figure 10: Mean Pt concentration found in the sediment throughout the experiment for the three different sample times, i.e., 48-hours, four weeks, eight weeks (n = 54). Significant differences over time are indicated with an asterisk (*). 34

Figure 11: Mean Pt concentration found in *Charophyta* sp. (Musk grass) and *F. antipyretica* (Lesser water moss) throughout the experiment, in the form of three different sample times 48-hours, four weeks, eight weeks (n = 108). Supplementary information can be found in Table B-3 in the annexure. Significant differences over time are indicated with an asterisk (*). 35

Figure 12: Mean Pt concentration found in artificial mussels throughout the experiment, in the form of three different sample times 48-hours, four weeks, eight weeks (n = 54). Supplementary information can be found in Table B-3 of the annexure. 36

Figure 13: Pielou's Evenness Index (J') indicates the evenness of the diatom communities identified on the microscope slides. (A) acute epiphytic diatom samples (n = 18), (B) chronic epiphytic diatom samples (n = 18), (C) acute benthic diatom samples (n = 18), (D) chronic benthic diatom samples (n = 18), and (E) acute benthic diatom samples over time (n = 18), (F) chronic benthic diatom samples over time (n = 18). Significant differences over time are indicated with an asterisk (*). 37

Figure 14: Shannon Index $H'(\log_e)$ indicates the diversity of the diatom communities identified on the microscope slides. (A) acute epiphytic diatom samples (n = 18), (B) chronic epiphytic diatom samples (n = 18), (C) acute benthic diatom samples (n = 18), (D) chronic benthic diatom samples (n = 18), (E) acute benthic diatom samples (n = 18), and (F) chronic benthic diatom samples (n = 18) over time. Significant differences over time are indicated with an asterisk (*). 38

Figure 15: Simpson diversity index indicates the number and abundance of diatom species found on the microscope slides for (A) acute epiphytic diatom samples (n = 18), (B) chronic epiphytic diatom samples (n = 18), (C) acute benthic diatom samples (n = 18), (D) chronic benthic diatom samples (n = 18), (E) acute benthic diatom samples (n = 18), and (F) chronic

benthic diatom samples (n = 18) over time. Significant differences over time are indicated with an asterisk (*). 40

Figure 16: Total number of diatom species(S) indicating the diversity of diatom species found on the microscope slides for (A) acute epiphytic diatom samples (n = 18), (B) chronic epiphytic diatom samples (n = 18), (C) acute benthic diatom samples (n = 18), (D) chronic benthic diatom samples (n = 18), (E) acute benthic diatom samples (n = 18), and (F) chronic benthic diatom samples (n = 18) over time. Significant differences over time are indicated with an asterisk (*). 41

Figure 17: Species richness(d) indicating the diversity of the diatom community found on the microscope slides of (A) acute epiphytic diatom samples (n = 18), (B) chronic epiphytic diatom samples (n = 18), (C) acute benthic diatom samples (n = 18), (D) chronic benthic diatom samples (n = 18), (E) acute benthic diatom samples (n = 18), and (F) chronic benthic diatom samples over time (n = 18). Significant differences over time are indicated with an asterisk (*). 42

Figure 18: Biological Diatom index (BDI) illustrating the OMNIDIA diatom community results obtained for (A) acute epiphytic diatom samples (n = 18), (B) chronic epiphytic diatom samples (n = 18), (C) acute benthic diatom samples (n = 18), (D) chronic benthic diatom samples (n = 18), (E) acute benthic diatom samples (n = 18), and (F) chronic benthic diatom samples (n = 18) over time. 44

Figure 19: Generic Diatom Index (GDI) illustrating the OMIDIA diatom community results obtained for (A) acute epiphytic diatom samples (n = 18), (B) chronic epiphytic diatom samples (n = 18), (C) acute benthic diatom samples (n = 18), (D) chronic benthic diatom samples (n = 18), (E) acute benthic diatom samples (n = 18), and (F) chronic benthic diatom samples (n = 18) over time. 45

Figure 20: Trophic Diatom Index (TDI) illustrating the OMIDIA diatom community composition results obtained for (A) acute epiphytic diatom samples (n = 18), (B) chronic epiphytic diatom samples (n = 18), (C) acute benthic diatom samples (n = 18), (D) chronic benthic diatom samples (n = 18), (E) acute benthic diatom samples (n = 18), and (F) chronic benthic diatom samples (n = 18) over time. 48

Figure 21: Pollutant Tolerant Taxa Index (% PT) indicating the OMIDIA diatom community results on the presence pollution tolerant taxa for (A) acute epiphytic diatom samples (n = 18), (B) chronic epiphytic diatom samples (n = 18), (C) acute benthic diatom samples (n = 18), (D)

chronic benthic diatom samples (n = 18), (E) acute benthic diatom samples (n = 18), and (F) chronic benthic diatom samples (n = 18) over time. Significant differences over time are indicated with an asterisk (*). 49

Figure 22: Pollution Sensitivity Index (IPS) indicating the OMIDIA diatom community results for (A) acute epiphytic diatom samples (n = 18), (B) chronic epiphytic diatom samples (n = 18), (C) acute benthic diatom samples (n = 18), (D) chronic benthic diatom samples (n = 18), (E) acute benthic diatom samples (n = 18), and (F) chronic benthic diatom samples (n = 18) over time. 50

Figure 23: Principal Component Analyses (PCA) of the 48 hour (black) and eight week (blue) epiphytic diatom communities. The biplot explains the diatom species composition throughout the exposure time with 39 species. The first axis defines 26.98% of the data variation, and the second axis explains 16.98%. With T indicating the sample time, K indicating the respective exposure concentrations, and M indicating that the sample was collected from the macrophytes. Full species names for respective abbreviations used in this figure can be found in the supplementary information in Table B1 in the annexure. 52

Figure 24: Principal Component Analyses (PCA) of the 48 hour (black) and eight week (blue) benthic diatom communities. The biplot presents the diatom species composition throughout the exposure time with 50 species. The first axis defines 45.70% of the data variation, and the second axis explains 15.80%. Full species names for respective abbreviations used in this figure can be found in the supplementary information in Table B1 in the annexure. 53

Figure 25: Principal Component Analyses (PCA) of the 48 hour (black) and eight week (blue) benthic diatom communities. The biplot presents the diatom species composition throughout the exposure time with 53 species. The first axis defines 38.86% of the data variation, and the second axis explains 15.53%. Full species names for respective abbreviations used in this figure can be found in the supplementary information in Table B1 in the annexure. 54

Figure 26: Principal Component Analyses (PCA) of the integrated multivariate analysis for the 48-hour and eight-week samples. The biplot presents the diatom species composition throughout the exposure time with 80 species. The first axis defines 55.09% of the data variation, and the second axis explains 11.88%. Full species names for respective abbreviations used in this figure can be found in the supplementary information in Table B1 in the annexure. 55

Figure 27: Principal response curve (PRC) indicating the effects of the different Pt concentrations on the Diatom community. The first axis explains 20.1% of the variance with the 25 best fitting Species. A complete species list can be found in Table B5 with supplementary information in table B2 of the annexure..... 56

Figure 28: Diatom teratologies that were identified in the epiphytic and benthic chronic 500 µgPt/L Pt exposure groups with blue arrows indicating frustule teratologies for (A) unidentifiable pennate species, (B) deformed *Navicula tripunctata*, (C) deformed *Navicula tripunctata*, (D) deformed *Fragilaria biceps*, (E) unidentifiable pennate species when compared to, (F) normal appearance of *Fragilaria biceps* and (G) normal symmetric pennate specimen. 57

CHAPTER 1: INTRODUCTION

1.1 Background

Even though Platinum Group Elements (PGEs) has been researched for over two decades, the behaviour of PGEs in the aquatic environment remains a topic of interest for researchers. In particular, there is limited research on the bioaccumulation, disruptive effects, compartmentalisation, and distribution of PGEs in the aquatic environment. These limitations also apply to the methods used to determine and effectively monitor PGE levels in the aquatic environment. Ultimately resolving these limitations and updating the methods used to determine and effectively monitor PGE levels in the aquatic environment will lead to a more holistic understanding of PGE behaviour in the aquatic environment (Labuschagne *et al.*, 2021). With several research studies reporting a steady increase of PGEs in the environment, only a few provide data on the characterisation and partitioning. Platinum group elements continuously enter the aquatic environment via natural and anthropogenic events; therefore, knowledge of PGEs in freshwater ecosystems are important for risk assessment of aquatic ecosystem health, water resource management, and ultimately human health (Ruchter *et al.*, 2015; Zereini and Wiseman, 2015; Zimmerman and Sures, 2018). Platinum group elements consist of six rare precious metals that can only be found in trace concentrations in the earth's crust. These six rare metals are, ruthenium (Ru), rhodium (Rh) palladium (Pd), osmium (Os) iridium (Ir), and platinum (Pt) (Peucker-Ehrenbrink and Jahn, 2001; Hoppstock and Sures, 2004; Singer *et al.*, 2005; Bossi and Gediga, 2017; Buchspies *et al.*, 2017; Mudd *et al.*, 2018). These elements occur naturally in the sub microgram per kilogram ($\mu\text{g/kg}$) (Rh: 0.06 $\mu\text{g/kg}$; Pd: 0.4 $\mu\text{g/kg}$; Pt: 0.4 $\mu\text{g/kg}$) range (Wedepohl, 1995).

However, as limited and discrete as PGEs may be in the environment, there are a few sites in the world where deposits of PGEs form part of noble metal-rich ores and form a crucial part of mining industries (Bossi and Gediga, 2017). These deposits can be found in the Bushveld Igneous Complex (BIC) in South Africa and the Norilsk/Talnakh complex in Russia (Godel *et al.*, 2008; Rauch and Fatoki, 2015). South Africa has some of the world's largest PGE mines: Anglo-American Platinum, Impala Platinum, Sibanye, and Lonmin (Rauch and Fatoki, 2013; Johnson Matthey, 2018; Johnson Matthey, 2021). From a global perspective, South Africa is considered the largest producer of Pt in the world, with an estimated 66.6% of the world's Pt deposits located in the BIC (Mudd *et al.*, 2018) and an average Pt production of 94 to 152 metric tons per year between the years of 2010 to 2020 (Johnson Matthey, 2018; Garside, 2020; Johnson Matthey, 2021). In the second place, Russia had an average Pt production of 22 to 23 metric tons per year between 2010 and 2019 (Garside, 2020). The third-largest Pt producer in the world is Zimbabwe producing around half that of Russia. Lastly, Canada and the United States are fourth and fifth

largest, respectively, averaging four to nine metric tons of Pt per year between 2010 and 2019 (Rauch and Fatoki, 2015; Johnson Matthey, 2018; Garside, 2020). The discovery of the platinum-bearing reef in Rustenburg, situated in the North West province of South Africa, in 1925 marked the beginning of the exploitation of PGEs from the world's largest PGE deposit (Wittmann and Förstner, 1976, Cawthorn, 2010). As a result, PGE concentrations in the vicinity of mine shafts, processing plants, transport roadways, and the surrounding environments have increased considerably (Pawlak *et al.*, 2014; Ruchter and Sures, 2015; Zereini and Wiseman, 2015; Diaz-Morales *et al.*, 2021). South Africa produces large amounts of PGEs in the BIC area. The intensive mining activities require large volumes of water (190 - 1100 m³) to sustain mining production and remove waste products (Glaister and Mudd, 2010; Haggard *et al.*, 2015). Although mining legislation encourages water reuse, leaks and spills from pipelines and sludge dams are inevitable, resulting in an unregulated influx of industrial water into the terrestrial and aquatic environment. Thus, the impact of PGEs on the environment is a unique and essential field of study, especially in South African aquatic and terrestrial ecosystems.

1.2 Problem statement and rationale for the study

Due to the various environmental influencing factors and the binding characteristics of Pt to plastics and different substrates, the effective detection of Pt is challenging in the aquatic environment. In recent studies conducted on PGEs, it was concluded that laboratory studies (controlled environment) alone are not sufficient. Organisms in natural settings are affected by different environmental conditions that influence the bioavailability of metals to these organisms (Zimmermann *et al.*, 2015; Brand *et al.*, 2019; Erasmus *et al.*, 2020; Labuschagne *et al.*, 2021). Due to the variability of influencing factors, field-based studies cannot provide laboratory "controlled" information on the behaviour of PGEs as it can be tough to correlate field-based results to that of laboratory studies. However, field-based studies are a vital research method that reflects the real-time metals present in the study area. Due to the void between these two scientific approaches in the PGE study area, a link must be found to integrate the two approaches. It is surprising to learn that after two decades of research on PGEs, there are still so many questions about the characteristics and adverse effects of PGEs on the aquatic environment.

In this study, partitioning refers to the natural division of the initial Pt dose concentration after being administered to the water column based on the affinity/ability of Pt to adsorb/distribute in the controlled aquatic environment. Compartmentalisation refers to the fraction of the total Pt dose concentration found in the individual environmental compartments after the exposure period. (e.g. water column, sediment, macrophytes and AMs). Until now, most of the field-based research completed on the ecotoxicology of Pt was focused on the Pt contamination from automobile catalytic converters, resulting in limited research on other sources of PGEs in the aquatic

environment. This research is specifically in bioaccumulation, effects, toxicity, compartmentalisation, distribution impacts on aquatic communities, and other important sources of contamination (Moldovan *et al.*, 2002; Moldovan *et al.*, 2003; Ruchter and Sures, 2015; Kibria *et al.*, 2016; Diaz-Morales *et al.*, 2021). These limitations also apply to the methods for determining acceptable/safe PGE levels in the aquatic environment. This indicates the need to further investigate the role of PGEs in the aquatic environment to expand our knowledge on PGEs and the potential risks posed to aquatic communities.

1.3 Research aims and objectives

To date, limited research is available on the amount of Pt pollution contributed by platinum mines, even more so the behaviour and potential effects of Pt in the aquatic environment. The available literature on the ecotoxicological behavioural characteristics of Pt is either lab-based or field-based, with no way of effectively characterising the behaviour of Pt in the aquatic environment. This study aimed to understand the characteristics and behaviour of Pt when adding a known Pt concentration to a semi-controlled aquatic environment and the potential effects Pt pollution may have on the aquatic ecosystem by assessing the environmental compartments, including epiphytic and benthic diatom communities. The relevance of the Pt concentrations and results could link the concentrations found in the environment and contribute to practical risk assessment for aquatic ecosystem health.

Hypotheses:

1. The compartmentalisation and accumulation of Pt in the sediment, water, macrophytes, and artificial mussels (AM) will be dependent on the Pt concentration introduced to the aquatic environment.
2. An increase of Pt in the aquatic environment will result in a decrease in pollution-sensitive diatom taxa and an increase in pollution tolerant diatom taxa, causing a disruptive effect on the aquatic diatom community assemblages.

Aims and Objectives

Aim 1

To mimic the aquatic environment by constructing microcosms, seeding these microcosms, and allowing colonisation of aquatic plants and aquatic communities to occur.

Objectives related to Aim 1

- Build microcosms at the METSI research facility.

- Seed the microcosms with sediment and rocks from the Gerhard minnebron stream and fill the microcosms with borehole water.
- Allow colonisation to occur over eight weeks.

Aim 2

Determine partitioning and accumulation of Pt in a semi-controlled environment by exposing the established aquatic ecosystems to a range of pure Pt (0.1, 1, 10, 100, and 500 µgPt/L) and collecting samples at three separate time intervals (48 hours (acute), four weeks (chronic) and eight weeks (chronic)) over an eight-week exposure period.

Objectives related to Aim 2

- Expand established aquatic communities to a standard Pt solution range (0.1, 1, 10, 100, and 500 µgPt/L).
- Collect samples from the sediment, water column, macrophytes and AMs on three different sampling times over eight weeks.
- Determine the Pt concentration of each environmental compartment during each sampling interval.

Aim 3

Determine the disruptive effect caused by different Pt concentrations on established aquatic diatom communities through the use of univariate and multivariate analysis methods to assess the possible changes in aquatic community traits and structure.

Objectives related to Aim 3

- Sample benthic and epithetic diatom communities from each microcosm on the 48 hour (acute) and eight week (chronic) sample times.
- Prepare diatom samples for identification and identify the diatoms to species level where possible.
- Assess the diatom community assemblage making use of univariate and multivariate analyses.

1.4 Structure and outline of the dissertation

Chapter 1 - General introduction explaining the study's rationale and the established hypothesis and aims.

Chapter 2 - Literature review on Pt, serving as the background information and identifying potential gaps in the Pt research field.

Chapter 3 - Materials and methods, detailing the experimental design, setup, and maintaining of microcosms, methods used for sampling the sediment, water column, macrophytes, artificial mussels, and diatom communities, as well as the methods and indices used to analyse all the collected samples, quality assurance, and statistical analysis.

Chapter 4 - Results obtained after the eight-week exposure of Pt regarding:

- Changes in physicochemical water quality and water nutrient levels over the exposure period.
- Accumulation of Pt in the environmental compartments (Water column and sediment).
- Accumulation of Pt in the Biological compartments (Macrophytes and artificial mussels).
- Assessing the effect of Pt on aquatic diatom communities using diatom indices, PCA, and Principle Response Curves analyses.

Chapter 5 - In-depth discussion of the findings and their possible implications.

Chapter 6 - Conclusions drawn from the study and the recommendations for future studies on the ecotoxicology and community assemblages based on the effects of Pt in aquatic ecosystems.

CHAPTER 2: LITERATURE REVIEW

This literature review will mainly focus on the available literature for determining the partitioning, mobility, and bioavailability of Pt in aquatic ecosystems and the effects thereof. This review will discuss the following aspects to understand PGEs:

- Platinum group elements and their sources.
- Biomonitoring and PGEs (metal) exposures in the aquatic environment.
- Bioindicator organisms commonly used for metal ecotoxicology.
- Microcosms and why do we use them?
- Exposures in microcosms.
- Effects of PGEs on aquatic biota.

2.1 Platinum Group Elements and their sources

Over the last few decades, studies have reported that PGE concentrations in the surrounding environments of urban, roadside, mining shafts, smelters, and processing plants have steadily increased as a result of anthropogenic activities (Maboeta *et al.*, 2006; Rauch and Fatoki, 2013; Pawlak *et al.*, 2014; Rauch and Fatoki, 2015; Okorie *et al.*, 2015; Zereini and Wiseman, 2015; Almécija *et al.*, 2017; Erasmus *et al.*, 2020). From a global perspective, the primary uses of PGEs are in automotive catalytic converters (37%), industrial chemical processing (25%), glass industries (16%), electrical (12%), as well as jewellery industry, and medical and dental practices (10%) (Cawthorn, 2010; Zereini and Wiseman, 2015; Johnson Matthey, 2018; Johnson Matthey, 2021). To date, most studies have focused on automobile catalysts as one of the significant sources of PGEs in the natural environment (Pawlak *et al.*, 2014; Zereini and Wiseman, 2015). 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; Garside, 2020). According to Rauch and Peucker-Ehrenbrink (2015), catalysts are the most potent sources of PGEs because Pt, Pd, and Rh are the main active components (Ravindra *et al.*, 2004, Singer *et al.*, 2005; Garside, 2020). However, in South Africa, the primary source of PGE pollution is the result of large-scale mining and processing of PGE rich ores (Mudd *et al.*, 2018). This makes PGE pollution in South Africa unique to the rest of the world since high volumes of PGEs are mined, transported and processed locally.

2.2 Platinum exploitation in South Africa and the rest of the world

South Africa is a semi-arid country, and as a result, water scarcity is an increasing reality making the effective management of water resources a crucial economic driving factor (NWA, 1998). As a result, the South African National Water Act (Act 36 of 1998) (NWA) and the 1997 Water Services Act ensure the sustainable use of ground and surface water resources to ensure future economic and socio-economic development. The mining and industrial sector is a significant water user with an estimated demand of roughly 5% of available water resources in South Africa (NWRS2, 2013). According to Prosser *et al.*, (2011), there are many reasons to justify the usage of water during mining processes, including but not limited to:

- 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.

A report published by the Department of Water and Sanitation (DWS) in 2016 on the “Benchmarks for water conservation and water demand management in the mining sector” stated that the mining sector of South Africa is currently expanding into new areas (mainly coal and Pt mining). This expansion will lead to an increase in future water demand (DWS, 2016). Mining activities need substantial amounts of water for processing plants and can significantly impact surface and groundwater resources (Mudd, 2008), resulting in increased attention towards mining pollutants, water issues, and the implementation of strict regulations governing water rights and responsibilities. According to a study conducted by Mudd (2008) and Northey *et al.*, (2019), over the past two decades, the focus in the mining sector has been shifted to the reuse of embodied water to improve water management and the potential risks associated with mining to the environmental and hydrological water resources.

The discovery of the platinum-bearing reef in Rustenburg, situated in the North West province of South Africa, in 1925 marked the beginning of the exploitation of platinum group elements from the world's largest PGE deposit (Wittmann and Förstner, 1976, Cawthorn, 2010). In the mineral sector, a specific area, the BIC, is recognised for its deposits of PGEs (Cawthorn, 2010). The BIC is a large, layered geological formation found in the North West of South Africa (Cawthorn, 2010). Mined primarily from deposits that usually consist of magnetic or igneous minerals, PGEs are associated with the other elements found in the igneous rocks (Rauch and Fatoki, 2015). The

BIC in South Africa and the Norilsk/Talnakh complex in Russia contain these deposits (Rauch and Fatoki, 2015).

Studies in urban settings suggest that multiple sources contribute to PGE fluxes in the environment. In pharmaceutical drugs (Pt-based), Pt is used for cancer treatment and drug research in the medical field, where it can enter the environment through the excretion of administered drugs. Patients that have undergone these types of treatments can excrete Pt traces for a period lasting up to eight years after treatment (Kümmerer *et al.*, 1999; Rauch and Peucker-Ehrenbrink, 2015). Even though these sources contribute marginally to the overall PGE levels, the combination thereof with other applications will still contribute to PGEs released in the environment. These other applications, as mentioned earlier, include electrical, chemical industry, jewellery, glass, and dental applications (Rauch and Fatoki, 2015). Industrial activities are also associated with PGE emissions. Due to the low natural occurrence of PGE in the environment, concentration ratios can differentiate between point sources of PGEs pollution in the environment (Rauch and Fatoki, 2015, Erasmus *et al.*, 2020). Different PGE ratios can characterise industrial and automobile emissions. The activity in the vicinity of sampling points can influence the proportions of PGE pollution from industrial sources. For instance, a study conducted by Rauch and Fatoki, (2015) found that smelters have much higher concentrations of PGEs than the underground shafts or ore processing plants. Indicating that the type of activity in an industrial setting can influence the level of PGE pollution found in the surrounding environment (Rauch and Fatoki, 2013; Rauch and Fatoki, 2015).

Sewage and urban runoff are secondary sources of PGEs in the environment resulting from drug treatment in medical facilities and the accumulation of PGEs on-road surfaces (Rauch and Peucker-Ehrenbrink, 2015). Urban runoff is channelled into storm drains during rainfall events and forms part of the sewage systems. Discharges from the water treatment plant can result in an influx of PGE into the aquatic environment depending on the sewage treatment method used. Therefore, anthropogenic PGE emissions accumulate from a range of anthropogenic activities. Many of these emissions, although small, are equally important sources of PGEs, and need to be assessed and identified as to which sources contribute to PGEs in the environment (Rauch and Fatoki, 2015). Metal production is an important source of metal influxes into the environment, but the emission of PGEs from metal processing plants has generally received little attention (Rauch and Fatoki, 2015).

Since 1925, an increase in the use of PGEs in developing new technologies has led to a rise in mining activities. Inevitably this influences the release of PGEs into the environment to such an extent that the anthropogenic PGE influxes exceed the natural fluxes at the earth's surface (Rauch and Fatoki, 2015; Diaz-Morales *et al.*, 2021). These sources emit the PGEs into the

water, soils, atmosphere and ultimately enter plants, animals, and humans. All environmental compartments should be studied to determine how these elements affect organisms (Zereini and Wiseman, 2015).

2.3 Platinum Group Elements in the aquatic environment

When aiming to characterise Pt in the aquatic environment, it is essential first to understand what it means to characterise a metal from an ecotoxicological point of view. Firstly, the characterisation of a metal entails collecting information based on the functional interaction of different metal concentrations of the same metal with the aquatic ecosystem and its compartments and the overall impact of said metal concentration on the ecosystem (Sures *et al.*, 2015). This characterisation answers questions such as where, what, when, and how Pt interacts with each compartment whilst exposed to the natural elements and water quality parameters. Secondly, how the aquatic environment reacts to being exposed to Pt. Thirdly, understand how the change of a single environmental parameter can influence the behaviour of Pt in the aquatic environment. Characterisation of metals will indicate how the individual compartments of the ecosystem will respond to the change in Pt concentration once such parameter changes. Lastly, to depict the concentrations that had a negative effect in each compartment and the level of Pt concentration that negatively impacted the overall ecosystem.

Research on metal exposures has revealed that environmental factors such as temperature, water hardness, pH, salinity, and conductivity of the water body plays a key role and can alter the behaviour and bioavailability of a metal in an aquatic environment (Zimmerman *et al.*, 2017, Mutlu and Kurnaz, 2018, Labuschagne *et al.*, 2021). Gaps in the knowledge on PGEs have shown that there are still many questions concerning the characteristics and adverse effects of PGEs on the aquatic environment. Until now, the results of field-based and laboratory studies could not accurately compare characteristics due to environmental influencing factors not being present in laboratory studies and vice versa, with field-based studies having a variety of environmental influencing factors that could not be controlled in the natural environment (Zimmermann *et al.*, 2015; Brand *et al.*, 2019; Erasmus *et al.*, 2020; Labuschagne *et al.*, 2021).

However, most of the studies done in the past focused on PGE pollution sources. Metals are known to persist in the aquatic and terrestrial environments for long periods and thus may have the potential to adversely affect these environments and their ecosystems (Gagnon *et al.*, 2006; Nouri *et al.*, 2008; Nouri *et al.*, 2011). As a result, a challenge in the study field was created to bridge the gap and successfully characterise the compartmentalisation, accumulation, bioaccumulation, and disruptions caused in and to aquatic communities after exposure to various Pt concentrations.

The knowledge of PGE contamination in freshwater systems is essential for the risk assessment of aquatic animals, human health, and water resource management. However, early experiments regarding PGE levels in aquatic ecosystems focused on marine/saltwater environments (Lee 1983; Hodge *et al.*, 1986). Few studies focus on the chemical behaviour, characteristics, and disruption of PGEs to ecosystems in lakes, rivers, and streams (Haus *et al.*, 2010). To date, the main focus of these studies was to determine the level of PGEs in aquatic sediment samples (Zereini and Wiseman, 2015; Diaz-Morales *et al.*, 2021). Since ecologically relevant data is hard to come by, scientists reverted to characterising PGEs in laboratory-based tests; however, the results obtained proved to have limited realism in the natural aquatic environment (Labuschagne *et al.*, 2021). Since organisms in natural conditions are influenced by all the environmental factors that affect the bioavailability of PGEs, laboratory bioaccumulation studies are insufficient (Breure *et al.*, 2003; Ek *et al.*, 2004; Balcerzak, 2011; Labuschagne *et al.*, 2021). Thus, the knowledge of PGE behaviour in the aquatic environment remains elusive as it is complicated to characterise a metal in an uncontrolled environment. According to research done by Zimmermann *et al.*, (2015), most published studies made use of semi-static or static tests. Static exposure tests entail that the test organisms are exposed to the same test solution for the test duration. The exposure substance is administered at the beginning of the exposure period and will not be renewed during the rest of the study period (Brand *et al.*, 2019). It is also important to note that PGE concentrations can be affected in many ways before the exposure has even started. Most aquatic exposure studies have shown that PGE concentrations measured within the system are far less than those applied (Zimmermann *et al.*, 2015; Brand *et al.*, 2019). Thus, one of the biggest problems during aquatic exposure studies involving Pt is the loss of dosed metal concentrations from the water phase (Zimmermann and Sures, 2018; Brand *et al.*, 2019). This phenomenon can be explained by dynamic partitioning and uptake by biotic factors and abiotic processes that affect Pt concentrations in the exposure media, including precipitation and adsorption of Pt to the surface of the tank, rocks, and exoskeletons of organisms (Sures and Zimmermann, 2007; Miranda *et al.*, 2022).

2.4 Biomonitoring and aquatic ecosystem health

Scientists use biomonitoring to assess the impact of natural and anthropogenic activities on living biota found in aquatic environments (Oertel and Salánki, 2003; Herman and Nejadhashemi, 2015). Bio-indicators are organisms that live in the given ecosystem that respond accordingly when their environments experience stress from internal and external sources (Breure *et al.*, 2003). It is essential to add that each aquatic ecosystem may have its own unique set of bioindicators depending on its location and set of ecological drivers. Biomonitoring is valuable since it uses the composition of the rich diversity of fauna and flora found in an aquatic ecosystem,

including algae, diatoms, macrophytes, macroinvertebrates, and fish (Krajenbrink *et al.*, 2019; Levin *et al.*, 2019). These methods utilise the information gathered to reflect the effects and changes in time, biotic interaction, anthropogenic stressors, geomorphological impacts, pollution, and seasonal changes (Costanza *et al.*, 1998; Oertel and Salanki, 2003; Krajenbrink *et al.*, 2019).

Physical, biological, and chemical components determine the health of an ecosystem. These components and their interrelationships are important to ensure that the ecosystem is classified as a healthy system. The ecosystem composition must be resilient enough to withstand stressors and changes. A healthy system can further be identified if there is no abnormal decline or lack of abundance of endemic biota, no organic pollutants and hazardous chemicals and no human interference on landscapes and ecological processes (Costanza *et al.*, 1998; Seadoc). When referring to aquatic ecosystems, water quality and the abundance of biota are the main points of interest. They play a crucial role in determining the integrity of an aquatic ecosystem.

Biomonitoring field studies of PGE concentrations and living organisms reflect valuable information on the bioavailable fraction of PGEs and their subsequent effect on the community structure of organisms. Platinum is one of the rarest elements on earth (Oertel and Salanki, 2003; Hoppstock and Sures, 2004) and has exceptional characteristics like its high melting point, corrosion, and oxidation resistance (Zereini and Wiseman, 2015; Brand *et al.*, 2019). Apart from the use of Pt in anti-cancer drugs, details on the biological and accumulation effects thereof in organisms and the environment remain elusive (Giarratano *et al.*, 2010) however, the effects of Pt have been studied on the molecular, cellular, and organismic levels using several aquatic organisms (Sures *et al.*, 2015). Revealing that Pt might potentially induce detrimental effects on aquatic plants and animals (Brand *et al.*, 2019). This point will be discussed further under the section regarding the impact of Pt on aquatic biota.

Water quality deterioration has become a common issue in South Africa. Water monitoring has been taking place in various forms, and multiple methods that incorporate chemical and biological monitoring are used to resolve these issues (Breure *et al.*, 2003; Soininen and Könönen, 2004; Claassens *et al.*, 2016). Bioindicators are used to determine the status of an ecosystem as water quality parameters alone do not give sufficient data on habitat stability, integrity, acute and chronic effects of pollutants on biological organisms (Taylor *et al.*, 2007; Claassens *et al.*, 2016). The use of the unique biota, otherwise known as bioindicators, in monitoring programs, has increased drastically in the last few decades as these organisms are selected to represent the resilience and integrity of the aquatic ecosystem (Wu and Lau, 1996). For an ecosystem to be healthy, the unique biota also needs to be healthy (Ruchter *et al.*, 2015). During biomonitoring studies, the accumulation of substances within an organism is measured to determine the environmental

status. The same principle applies to pollutants and harmful substances that will influence the community integrity and occurrence of certain bioindicator organisms in the area of interest.

Aquatic organisms are sensitive to fluctuations in heavy metal concentrations and often reflect the ambient metal concentrations of the environment and are widely utilised for these types of studies (Morin *et al.*, 2012, Claassens *et al.*, 2016; Morin *et al.*, 2016). During such assessments, numerous factors can influence the mechanisms in which organisms are affected and the degree to which they can accumulate these pollutants (Forbes *et al.*, 2006). According to Sures *et al.* (2015), free-living organisms are threatened by environmental stressors such as PGEs, that may alter their physiology. Organisms affected by these substances can accumulate metals inside their bodies and develop chronic side effects. When organisms are exposed to pollutants, a biochemical response may be induced, resulting in specific metabolic proteins indicative of stress (Brand *et al.*, 2019). Monitoring programs involving metal concentrations often use biomarkers to detect stressors that may influence aquatic organisms (Brand *et al.*, 2019).

The concentration of metals found in organisms is affected by many biological and physical factors. The metal release and uptake in these organisms are affected by temperature, life cycles, size, depth, water quality, and reproduction (Zhou *et al.*, 2008; Zimmerman *et al.*, 2017; Mutlu and Kurnaz, 2018, Labuschagne *et al.*, 2021). It was found that different species have different accumulation pathways and that the indicator species may have other natural distribution patterns (Wepener, 2008; Greenfield *et al.*, 2014).

2.5 Bioindicator organisms

When looking at bioindicators, it is vital to note that different organisms have different mechanisms when bioaccumulating pollutants, resulting in additional water quality needs and pollution tolerances. The choice of indicator organism can thus influence the relevance, success, and interpretation of the results (Oertel and Salanki, 2003; Wepener, 2008; Bervoets *et al.*, 2016). Therefore, a list of criteria was compiled to recognise an organism as a prospective bioindicator (Gerhardt, 2002; Wepener, 2008, Wepener, 2013). First, the organisms should be abundant so that the ecosystem's origin is not influenced negatively. A suitable organism should commonly be immobile or site-specific to represent the study area. The organisms should accumulate pollutants to levels similar to those present in the environment, and they should be relatively tolerant of pollutants (Deborde *et al.*, 2016; Ojija *et al.*, 2017). However, when assessing and characterising an unknown metal, indicator organisms sensitive to pollutants are preferable as even the slightest effect or changes can be significant (Kunz *et al.*, 2010; Bervoets *et al.*, 2016; Boudou and Ribeyre, 2018).

A few other characteristics can include that they should be easy to identify, handle, collect, and they should be able to survive for a relatively long period. It is vital to use organisms of reasonable size, ensuring that there is enough tissue sample to be used for analyses (Labuschagne *et al.*, 2021). Along with this, a sufficient number of organisms should be used at each sample site. When an organism meets the requirements of this list, it can be used as a bioindicator organism (Wepener, 2008; Labuschagne *et al.*, 2021). Bioindicator organisms can be obtained from various sources, such as a hatchery, a laboratory, or even unpolluted reference sites located within the same system. Different organisms such as fish, macroinvertebrates, macrophytes, algae (including diatoms) have been used as bioassessment tools (Stevenson *et al.*, 2010). According to a study done by Greenfield *et al.* (2014), relocated mussels can accumulate metal concentrations at much higher concentrations than the indigenous mussels since resident organisms can adapt to exposed concentrations (Wepner, 2013). Exposure periods are also significant since the relocated mussels can regulate metals (Balcerzak, 2011; Labuschagne *et al.*, 2021).

On the other hand, ecosystem health can also be determined by using existing communities as indicator organisms because they are found within the study area and reflect the habitat's real-time viability. Diatoms, also known as single-cell algae, belong to the class *Bacillariophyceae* and are the most diverse and ecologically relevant primary producers in the aquatic environment (Taylor *et al.*, 2007, Barragán *et al.*, 2018; Pandey *et al.*, 2018, Rivera *et al.*, 2018). They are sensitive to environmental changes and respond rapidly to changes in their immediate environment because of their short two-to-three-week life cycle (Pandey *et al.*, 2018). Due to diverse species, diatoms can also be found in a wide variety of environmental conditions (Pandey *et al.*, 2018). Individual species have different preferences and environmental requirements resulting in a cosmopolitan presence in water bodies (Dixit *et al.*, 1992; Stevenson *et al.*, 2010). A range of diatom-based indices have been developed and are used to assess general water quality and overall ecosystem health (Lavoie *et al.*, 2006, 2014). Diatoms are the perfect monitoring tool for determining aquatic chemical status, given their sensitivity to contaminants and environmental stressors (Stevenson *et al.*, 2010). Studies have found that environmental variables such as temperature, water quality, substrate type, light availability, flow velocity, nutrients, and physicochemical parameters have the potential to influence the distribution of diatom communities in a given aquatic ecosystem (Tornés *et al.*, 2015, Barragán *et al.*, 2018; McGowan *et al.*, 2018; Pandey *et al.*, 2018, Rivera *et al.*, 2018, Kock *et al.*, 2019; Mirzahasanlou *et al.*, 2019 & 2020).

2.6 Using microcosms to assess PGEs in the aquatic environment

Microcosms are small experimental units that replicate aspects of the natural environment as closely as possible, thus allowing more ecological realism regarding a biological system (Alldredge *et al.*, 1995; Dupont *et al.*, 2010; Cadmus *et al.*, 2016). In this way, microcosm studies provide a valuable link between field surveys and controlled laboratory experiments (Dupont *et al.*, 2010). These units can be purpose-built to be small (microcosms) or medium to large (mesocosms), ranging from 1 to 10,000 L, and contain multiple trophic levels of interacting organisms (Bohan *et al.*, 2013). In contrast to laboratory experiments, microcosm and mesocosm studies are typically conducted outdoors to incorporate natural climate and environmental variation (Dupont *et al.*, 2010). It should also be noted that microcosm studies may be performed in an enclosure small enough for key variables to be altered to ensure constant control or by field-collecting critical components of the natural environment for further experimentation (Bohan *et al.*, 2013). Ecologists have used mesocosms extensively to gain a mechanistic understanding of ecosystem responses to various variables based on the assumption that these systems can replicate the critical responses of natural assemblages in the environment (Dupont *et al.*, 2010; Lozano *et al.*, 2018). Microcosms have some vital advantages over laboratory-based microcosm studies. Despite typically providing more extensive replication and control, research done with microcosms provides the ability to make direct evaluations of responses across replicated communities under identical environmental conditions and across at least one generation of the longest living organism (Bohan *et al.*, 2013). Some studies that can be conducted in microcosms may include but are not limited to:

- Influence of extreme events (e.g., heavy rainfalls and cyclones) on fauna and flora.
- Trophic level interaction.
- Effects of climate change on aquatic communities.
- Predator-prey interaction.
- Community structures of aquatic macroinvertebrates and vertebrates.
- Bioremediation.
- Toxicity effects of different substances on the aquatic communities.

The limitations and potential shortcomings of the microcosm approach are mostly related to the logistical constraints, which mean they are restricted and sometimes limited in terms of scales and levels of biological complexity (Bohan *et al.*, 2013). Although the ability to replicate treatments is a clear advantage of microcosms, their ecological realism to whole freshwater

systems has also been questioned (Bohan *et al.*, 2013). Dupont *et al.*, (2010) assessed the water quality and community composition of replicate microcosms, reporting that acceptable levels of realism may be attained in large outdoor systems (Dupont *et al.*, 2010).

2.7 Metal exposures in microcosms

The aquatic environment can be considered a PGE sink, and the accumulation of PGEs is expected to occur in sediment, where the biota are exposed to these metals (Bowles and Gize, 2005; Diaz-Morales *et al.*, 2021). According to Wang *et al.*, (2019), high carbon and nitrogen ratios in the aquatic environment can be associated with high concentrations of organic agents. Research on the possible influences of organic complexing agents found that the aqueous solubility of metallic Pt increased in the presence of humic acids (Lustig *et al.*, 1998; Bowles and Gize, 2005). However, other parameters such as pH, the concentration of Pt, cation exchange, type of soil or sediment should also be considered when characterising the behaviour of Pt in the environment (Kotze *et al.*, 2019).

Platinum is known as an inducer of Reactive Oxygen Species (ROS), as demonstrated in toxicity studies with human lung epithelial cells exposed to Pt (II) and Pt (IV), respectively (Schmid *et al.*, 2007; Brand *et al.*, 2019). Furthermore, a study by Vannini *et al.* (2011) focused on the physiological effects of Pd on an aquatic unicellular alga (*Pseudokirchneriella subcapitata*) and has shown that K_2PdCl_4 has a stress effect on the algae, even in low concentrations (0,1 mg/L). Palladium caused toxic effects on the primary metabolism, which caused reduced growth, pigment- and photosynthetic- modification (Vannini *et al.*, 2011). Plants react to heavy metals by producing cisplatin and cysteine-rich peptides, such as glutathione and phytochelatins. This reaction is also true in aquatic plants, as determined by Supalkova *et al.*, (2008). The overall growth of *Lemna minor* was inhibited when exposed to cisplatin, and visible signs of chlorosis were observed on the leaves of exposed plants (Supalkova *et al.*, 2008).

Brand *et al.*, (2019) conducted a lab-based study characterising the possible adverse effects of Pt on freshwater Zebra mussels (*Dreissena polymorpha*). Zebra mussels with a shell length of 25mm were exposed to a range (0.1, 1, 10, 100, and 1000 $\mu\text{g/L}$ of Pt) of standard platinum solution over four days. Results indicated that an accumulation of Pt within these mussels showed possible impairment reaction on metabolic functions. Mussels could not form Glutathione S-transferase-related defence mechanisms, which are used to remove toxins within the body when exposed to high concentrations of Pt (Brand *et al.*, 2019). Also, catalase (CAT) activity in mussels increased when exposed to Pt to reduce ROS production due to metal toxicity, damaging cellular structures (Brand *et al.*, 2019). Overall, zebra mussels had increased Pt-Metalllothioneins (MT) levels, inhibiting MT from binding to essential metals (Brand *et al.* 2019). Higher exposures

overwhelmed the antioxidant defence mechanisms and finally caused lethal effects when exposed to levels of 1000 µg/L of Pt (Brand *et al.*, 2019).

Zimmerman *et al.*, (2017) tested the effects of Pt, Pd, and Rh on a planktonic crustacean (*Daphnia magna*). The toxicity of the three metals to the tested daphnids decreased in order: Pd (II) > Pt (IV) > Rh (III). When daphnids were exposed to 110 µgPt/L (EC₅₀), the metal caused immobilization after 48-hours of exposure. On the other hand, Pd had an EC₅₀ (48-hours) value of 13µg.L⁻¹ and Rh a value of 12300µg.L⁻¹. Furthermore, Pd seemed to cause severe effects much faster than Pt and Rh when comparing the time between immobility to lethality. Even though different toxicities were found between metals, all three metals have similar modes of action and caused the production of heat shock proteins, which indicates stressful conditions (Zimmerman *et al.*, 2017). An interesting observation is that, in combination, PGEs could cause synergistic effects, which could cause more severe consequences than a single PGE exposure. This consequence could be because one metal inhibits an organism's defence- and repair mechanisms, while another could freely bind and act with endogenous biological ligands (Zimmerman *et al.*, 2017). However, this has not yet been tested, and future studies are needed to confirm the suspicion that multiple metal exposures cause higher toxicities.

Other tests done on zebrafish (*Danio rerio*) showed that Pt is more bioavailable than Pd. The high concentrations of Pt had extreme toxic effects on zebrafish embryogenesis, leading to lower hatch rates, cardiac malformation, pericardial oedema, increased production of stress proteins, and lower heartbeat rates within the embryos (Osterauer *et al.*, 2011). Osterauer *et al.*, (2011) also concluded that when zebrafish were exposed to PtCl₂, increased levels of histopathological defects within the liver and gut could be observed. Exposure concentrations of 1 µg/L caused inflammation of the liver, causing dilations of the capillary spaces, increases in macrophage numbers, and variability of cytoplasm and nuclei density. Higher concentrations led to vacuolation of the cytoplasm, swelling of hepatocytes, and karyopyknosis (Osterauer *et al.*, 2011). Further, histopathological research indicated that gut cells showed ablation from the basal lamina, asymmetrical compartmentalisation, and increased mucus cells. Ramshorn snails (*Marisa cornuarietis*) were also affected, showing changes in their hepatopancreas and gills. Exposure to Pt in a similar study even showed that Pt prevented external shell formation, resulting in an internal shell being developed when embryos were exposed (Osterauer *et al.*, 2011).

In ecotoxicology, diatoms are more commonly known as bioindicators of water quality and the environmental state of aquatic environments, reflecting ecosystem integrity through the toxic effects of metal pollutants through cell wall deformities (Bere and Tundisi, 2012; Morin *et al.*, 2012). To study the relation between metal pollutants and diatom deformities, microcosms have been used extensively over the past two decades to assess the impact of a variety of metals (e.g.,

Cu, Fe, Cd, Pd, Zn, Au, and Ni) on diatom communities (Bertrand *et al.*, 2001; Meylan *et al.*, 2004; Pandey *et al.*, 2015; Vendrell-Puigmitja *et al.*, 2020)

2.8 Compartmentalisation of PGEs in microcosms

In a natural, uncontrolled environment, it might be challenging to study the effect of PGEs in all possible environmental components. Microcosm studies offer the ability to replicate experiments while maintaining many of the critical dimensions of the structure and functioning of natural systems (Clements, 2004; Cleveland *et al.*, 2012). Microcosms are particularly useful when the behaviour and distribution of certain animals and substances are difficult to observe under natural conditions; however, microcosms also have their limitations. As one gains experimental control by using such systems, the boundaries of small-scale experiments can complicate the interpretation of results due to limited variation in flow regimes, nutrient cycling, invertebrate colonisation, and other factors (Maine *et al.*, 2019). Some of these factors can be experimentally manipulated; for instance, variation in flow regimes can be mimicked by modulating the circulation velocity created by a motor in a microcosm. The spatial and temporal scales of experiments must be relevant to the short-term variation experienced (Cleveland *et al.*, 2012). Overcoming the challenges of realism in experimental systems is crucial to achieving a more robust and holistic understanding of natural systems (Labuschagne *et al.*, 2021).

Now that the concept and function of a microcosm are understood, it is possible to explore its relevance to this study. This can be done by looking at the available knowledge on substance exposures in microcosms and the results obtained thus far. To date, an extensive amount of microcosm studies have been conducted to evaluate how organisms and communities might react to environmental change. Variables can be manipulated deliberately to mimic environmental change, such as increased oxygen, temperature, metals, chemical concentrations, salinity/pH levels, turbidity, and carbon dioxide.

It is essential to understand the behaviour of metals and PGEs in aquatic ecosystems to evaluate the potential impact and risks of these elements. Accumulated pollutants usually have chronic adverse effects on the exposed organisms (Duarte *et al.*, 2004; Brand *et al.*, 2019). As mentioned earlier, recent studies on the bioaccumulation of PGEs in the aquatic environment have shown that different aquatic biota has different accumulation potentials or tolerances for PGEs (Duarte *et al.*, 2004; Zimmermann *et al.*, 2005 & 2015). On the other hand, short-term laboratory experiments have limited realism in understanding how complex multispecies systems react across different organisational levels (Labuschagne *et al.*, 2021). For this reason, microcosm exposure experiments are crucial for studies, as surveys alone will not be able to replicate the conditions observed in nature. Therefore field-based microcosm experiments represent an

optimal approach to study different characteristics to ultimately form predictive models (Iwasaki *et al.*, 2012 & 2013; Barlow *et al.*, 2015).

To fully understand how ecosystems respond to extreme events in general, controlled manipulations are needed (Forbes *et al.*, 2006), including microcosm experiments to explore the community and functional responses to substances (accumulation), chemicals (accumulation), and natural events (Mette *et al.*, 2011; Iwasaki *et al.*, 2013). Platinum can enter the aquatic environment via road runoff (Ruchter and Sures, 2015) and accumulate in sediments, reaching concentrations of up to 85 ng/g (Ruchter *et al.*, 2015). However, Pt concentrations detected in river sediments are reported to be lower in orders of magnitude, e.g., a maximum of 3 ng/L was measured by Sures *et al.*, (2015). However, due to the limited knowledge surrounding the partitioning of Pt, these field studies could be underreporting concentrations due to the limited bioavailability of Pt in the aquatic environment.

Different factors should be considered when determining PGEs' toxicity to ensure that accurate results are obtained during a microcosm exposure study. For instance, many test parameters can influence the uptake and accumulation of PGEs by biota in laboratory and field exposure studies (Bonzom *et al.*, 2007; Iwasaki *et al.*, 2013; Bonzom *et al.*, 2017; Labuschagne *et al.*, 2021). These parameters include test systems, PGEs sources, route of exposure, exposure concentration, test organisms, exposure medium, exposure period, and temperature (Zimmermann *et al.*, 2015). According to Allen *et al.*, (1980) and Niyogi and Wood (2004), the bioavailability and toxicity of metals in an aquatic environment can also be dependent on water pH, water hardness, and the amount of dissolved organic matter.

The determination of PGEs such as Pt, Pd, and Rh in plants, sediment, and water samples in recent studies has confirmed the increased contamination of urban and natural sites with these metals (Ruchter and Sures, 2015). Considering the results from several exposure experiments combined with monitoring studies of PGEs in the environment and collected samples from the field suggest that differences in the mobility and transformation behaviour of the three elements in the environment are significant for their bioavailability (Zimmermann *et al.*, 2015). Exposure studies revealed that uptake rates in plants are highest for Rh, followed by Pt, and lowest for Pd (Zimmermann *et al.*, 2015; Labuschagne *et al.*, 2021). However, field studies indicate that Pd accumulation in plants is most distinct (Gupta *et al.*, 2013). On the other hand, studies confirmed that Pt in the aquatic environment is adsorbed to the sediment layer due to the organic matter found in the sediment (Dias-Morales *et al.*, 2021). But these results are still inconclusive when looking at the overall impact and compartmentalisation of PGEs.

CHAPTER 3: METHODOLOGY

3.1 Experimental design

Outdoor microcosms were set up at the METSI research facility of the Unit for Environmental Sciences and Management of the North-West University. Located in the North West province of South Africa (-26,490427, 27,132394) close to the eye of Gerhard Minnebron during Austral Autumn (March) of 2019 (Figure 1).

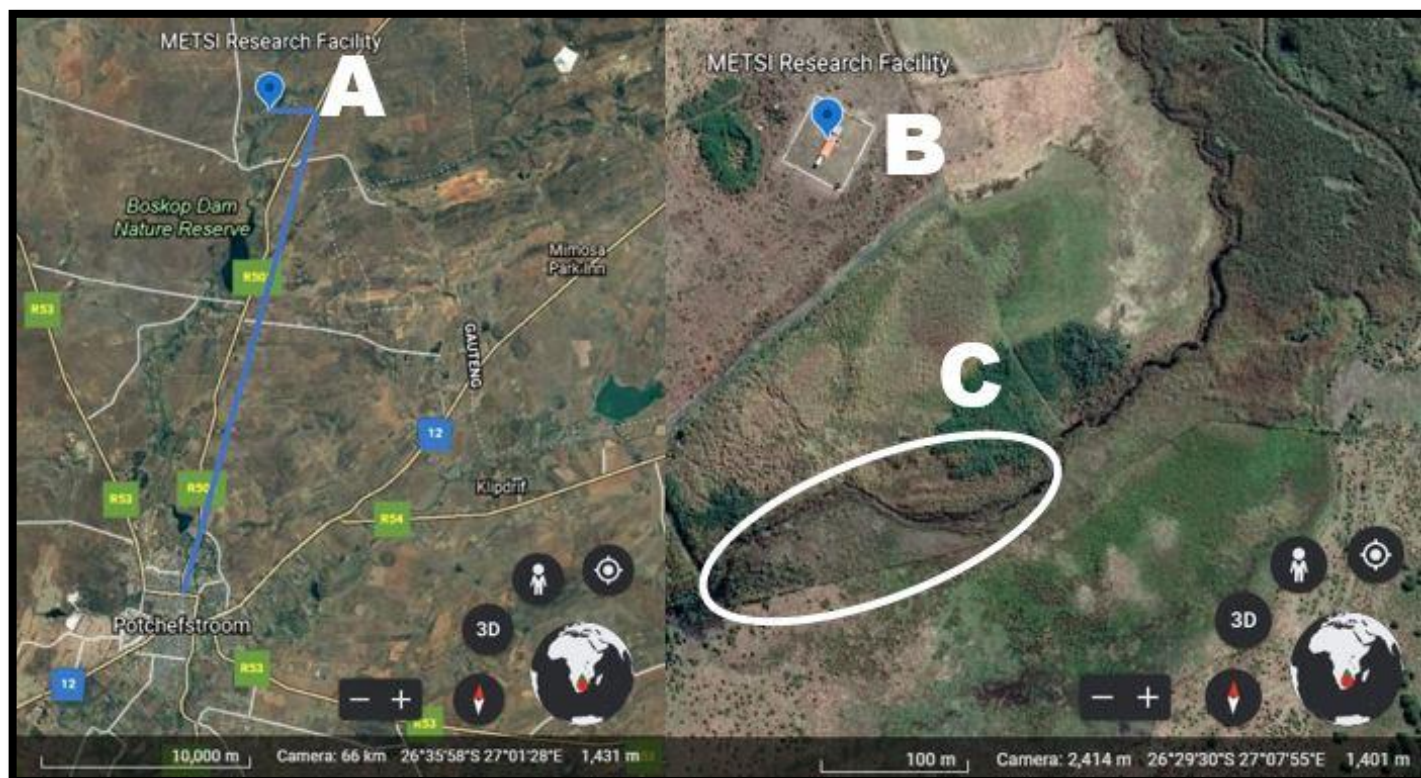


Figure 1: The study area where the microcosms were built and left during the project. (A) Google maps of where the site is situated in relation to Potchefstroom in South Africa. (B) The complex where the microcosms were built and left. (C) Gerhard Minnebron stream where sediment, rocks, and macroinvertebrates were collected.

For the ecotoxicological characterisation of Pt, a lentic microcosm study without any water changes was selected to determine the behaviour and potential effect of Pt in the aquatic environment. The experimental design consisted of 18 low-density polyethylene microcosms, each 0.4 m deep, 1.5 m in length, and 0.95 m in width with a 200 L water capacity. Microcosms were arranged in a grid pattern of two rows of five and two rows of four roughly 600 mm apart (refer to Figure 2 and Figure 3, respectively).

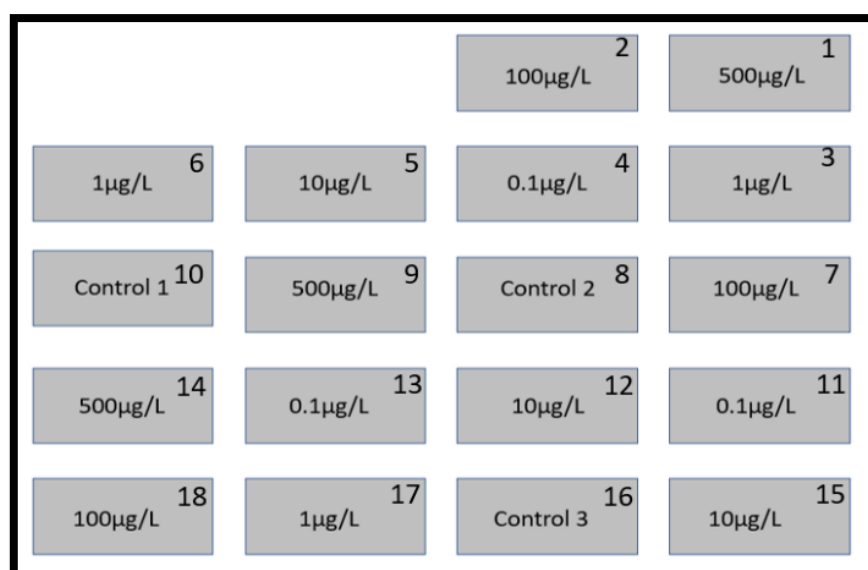


Figure 2: Layout and numbering of the eighteen different microcosms and their respective concentrations.

Due to the binding characteristics of Pt, each microcosm was lined with polyethylene plastic (black) sheeting. Sediment was collected from the instream sediment deposits found in the Gerhard minnebron stream using shovels and 20L buckets. Each microcosm was filled with a five- to seven-centimetre-thick sediment (sand and mud mixture) layer and three rocks (10-20 cm in diameter) covered with Lesser Water Moss (*F. antipyretica*) collected from the nearby Gerhard Minnebron stream. The microcosms were filled with 200 L borehole water. The water level was maintained within a 10% range of this volume throughout the experiment with the same water source to simulate a natural lentic (static) microcosm environment. To assess the bioavailability of Pt in the aquatic environment, passive sampling devices known as Artificial mussels were used. Artificial mussels are known to accumulate soluble and bioavailable metals from both marine and aquatic environments, do not need any power, are not affected by biotic or abiotic factors, and are easy to handle, deploy, recover and analyse (Wu et al., 2007). Artificial Mussels were assembled using a Perspex® tube, a Perspex® spacer, two 15 g acrylamide gel plates and 200 mg of Chelex® beads according to the protocol used by Labuschagne *et al.*, (2021). Three AMs were placed into each microcosm and suspended one centimetre above the sediment layer using black cable ties to ensure that the gel layers were not damaged or blocked with sediment or organic material during the experimental time.

Furthermore, six sand blasted microscope slides were placed into each of the 18 microcosms at a 45-degree angle against the rocks to minimize silt and sediment precipitation on the slide surfaces. Following the eight-week ecological colonisation period, the 18 microcosms were separated into six groups, namely untreated control (C), exposure one (K1), exposure two (K2), exposure three (K3), exposure four (K4), and exposure five (K5) each having three replicates.

The location of the different exposure concentrations was based on a random allocation method. The Pt 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) using the Four E's Scientific 20-200 µL and a 100-1000 µL pipets in exposure concentrations of 0.1 µgPt/L for exposure one, 1 µgPt/L for exposure two, 10 µgPt/L for exposure three, 100 µgPt/L for exposure four, and 500 µgPt/L for exposure five (Brand et al., 2019).

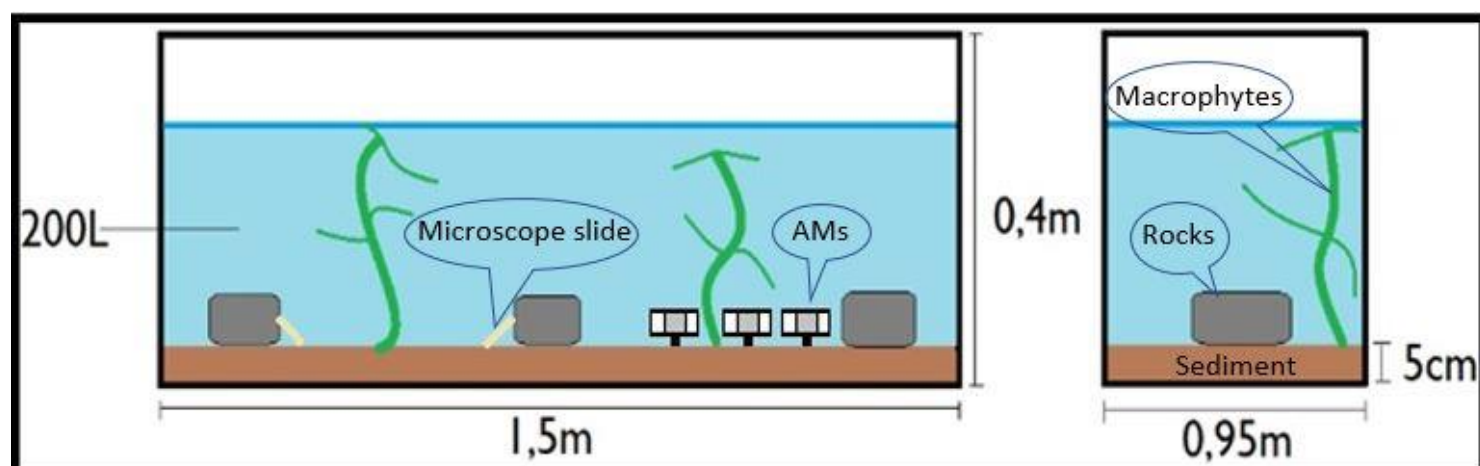


Figure 3: The basic model design of the microcosm with a width of 0.95 meters, length of 1.5meters, and height of 0.4 meters. Note the positioning of the rocks, sandblasted microscope slides placed at a 45-degree angle, AMs and the five-centimetre-thick sediment layer. Environmental components were sourced from the nearby Gerhard Minnebron. Macrophyte *Fontinalis antipyretica* are known to grow on rocks and were thus introduced into the microcosms along with the rocks. Macrophyte *Charophyta* sp. germinated from seeds in the sediment during the eight-week colonisation period prior to the exposures.

Microcosms were constructed, maintained, and sampled based on the guidelines set by the Organisation for Economic Co-operation and Development (OECD) (2006). All the microcosms were subjected to the same environmental elements (i.e., UV radiation, temperature, precipitation, and seasonal changes). Since this study did not remove any microcosms after sample times, each of the 18 microcosms were used to determine the immediate, acute and chronic effects and the characteristics of Pt in the aquatic environment. Table 1 summarises the experimental layout and the number of samples collected for the study from each microcosm.

Table 1: Experimental layout of Pt exposures and sample count.

Microcosm number#	Experimental group	Sediment samples #	Water samples #	Macrophytes samples #	Artificial Mussels samples #	Standard solution added
8,10,16	Control	9	9	18	2	0 mL
4,11,13	0.1 µgPt/L	9	9	18	2	0.02 mL
3,6,17	1 µgPt/L	9	9	18	2	0.2 mL

5,8,12	10 µgPt/L	9	9	18	2	2 mL
2,7,18	100 µgPt/L	9	9	18	2	20 mL
1,9,14	500 µgPt/L	9	9	18	2	100 mL

The Faculty of Natural and Agricultural Sciences Ethics Committee (FRASREC) of the North-West University reviewed the ethical considerations for this study. It was determined that the nature of the study posed no risk to human or animal health and was classified as a “No-Risk” study, hereby granting authorisation and ethics number NWU-01657-20-A9 valid from 01/02/2020 to 31/12/2022.

3.2 Field sampling

All 18 microcosms were used throughout the study to determine the characteristics and behaviour of Pt in the aquatic environment. Microcosms were sampled after 48 hours, four weeks and eight weeks after Pt exposure. In order to ensure a standard level of consistency, all samples were collected at 9 am throughout the experiment. Environmental samples were taken in a particular order to minimize the over disturbance to each microcosm. Every sample day started with the measurement of the physicochemical parameters, namely: electrical conductivity, salinity, total dissolved solids (TDS), pH, temperature (using the EXTECH ExStick II EC500 instrument), and dissolved oxygen (using ExStick II EC600 instrument) *in situ* for each of the 18 microcosms. After collecting the physicochemical parameters, the various environmental compartments were sampled in triplicate following the specific order of sampling the water, macrophytes, AMs, and lastly sediment, respectively. Water samples for water nutrient analyses were placed in pre-cleaned 50mL polyethylene containers (Figure 4B). Water samples collected for metal analyses were not filtered but were placed in pre-cleaned 500 mL polyethylene-terephthalate (PET) bottles acidified with HNO₃ (65% supra pure quality, Merck) to an acid concentration of 1%. One whole plant of both *Charophyta sp* and *F. antipyretica* were collected from each microcosm and placed into separate pre-cleaned Ziplock bags with no water. One AM was collected from each of the 18 microcosms and placed into individual pre-cleaned Ziplock bags with approximately 10 mL of water from each sample site to ensure that the gel is not damaged and prevent water and Chelex® beads from seeping out. Sediment samples were collected from the upper three-centimetre layer using 50 mL falcon tubes marked at three centimetres, each with a small ventilation hole. The tubes were inserted into the sediment up to the three-centimetre mark after the small hole was closed to create a vacuum. The sediment sample was removed and placed into pre-cleaned polyethylene containers. This sampling method resulted in minimal sediment disruption and was done two times for each of the 18 microcosms to obtain the desired sediment sample size at each of the three sampling events. All samples except for the AMs (stored in a refrigerator at 4 °C)

were stored in freezers (-16 °C) until analysis. In order to keep the labelling of the samples as short and specific as possible, collected samples were given a unique code consisting of the sample time, treatment value, and where the sample was taken. Thus, as with the different dose concentrations, the three sample times and the specific environmental compartment samples were abbreviated as follow, 48 hours (T1), four weeks (T2), eight weeks (T3), sediment (S), Rocks (R) macrophytes (M), water column (W) and AMs (AM). For example, sample T1K1S was collected on the first sampling time (T1) for the 0.1 µgPt/L exposure (K1) from the sediment (S).



Figure 4: (A) The METSI research facility of the Unit for Environmental Sciences and Management of the North-West University and the microcosm layout. (B) Field sampling of water quality samples from one of the microcosms.

3.3 Sample preparation

Water samples for nutrient analyses were left to defrost and reach room temperature to allow for filtration and further analyses with the relevant Merck test kits and following the standard protocols with a spectrophotometer (Spectroquant® Pharo 300, Merck). Water variables and test kits used were ammonium ($\text{NH}_4^+\text{-N}$, 114752), chloride (Cl^- , 114897), chemical oxygen demand (COD, 101796), sulphate (SO_4^{2-} , 114791), nitrite (NO_2^-N , 114776), nitrate (NO_3^- , 109713), ortho-phosphate (PO_4^{3-}P , 114848) and total hardness (TH, 100961). Water samples for metal analysis were defrosted and placed into 50 mL falcon tubes in preparation for analysis. The two macrophyte species and sediment samples were placed inside the -80°C freezer and left overnight. The next day the samples were placed inside the vacuum freeze-dryer (FreeZone® 6, Lab-conco) and left to dry over a minimum two-day period until all moisture was extracted from the samples. The dried sediment and macrophyte samples were then weighed accurately (~ 0.2 g dry weight) and placed into separate 20 mL TMF® vessels. Each macrophyte sample contained roots, leaves, and stems that were crushed to a powder. To fully digest the range of samples,

different HNO₃ and HCl ratios were used. Sediment samples were digested using 2.5 mL HNO₃ and 7.5 mL HCl, while macrophytes were digested using 7.5 mL HNO₃ and 2.5 mL HCl (Labuschagne *et al.*, 2021). After adding the different digestion mixtures, the TMF[®] vessels were placed into a microwave digester (CEM, Mars 6). Artificial mussels were disassembled, and the 200 mg Chelex[®] beads were collected and washed in 4.5 mL HNO₃ and 0.5 mL HCl acid solution. The supernatant was extracted and placed in 15 mL falcon tubes (Wu *et al.*, 2007; Labuschagne *et al.*, 2021). Following the digestion, the supernatant solution of each sample was transferred to a 50 mL volumetric flask and brought to volume with MilliQ[®] water. All the samples were transferred to 50m falcon tubes and were stored at room temperature until metal analyses were performed.

3.4 Metal detection and Quality Control

Samples with higher dose concentrations (100 and 500 µgPt/L) were analysed using a Perkin-Elmer model 4100ZL atomic absorption spectrometer (AAS) equipped with a Zeeman effect background correction system. The samples were injected into a pyrolytic graphite furnace tube by the autosampler AS 70 and ran under the optimised operating parameters (Table 2). Calibration was performed using a matrix adapted calibration where the concentrations in each sample were calculated by fitting a linear regression line to the points defined by spiked concentration values. Dose concentrations below 100 µgPt/L were analysed by using an Inductively Coupled Plasma-Mass Spectrometer (ICP-MS). All samples were diluted using MiliQ[®] water to a 1:5 ratio. The ICP-MS operated at 1000 W plasma power, 14 L/min plasma gas flow, 0.95 L/min nebulizer gas flow, and a sample flow rate of 1 mL/min regulated by a peristaltic pump. Calibration of the ICP-MS was performed using a series of 11 dilutions of a multi-element standard solution. With this calibration, the concentrations of the elements were calculated in the samples using corresponding regression lines with a correlation factor of ≥ 0.999. As concentrations of Pt determined by ICP-MS can be affected by interferences, the potential mass interferences during the detection of Pt were assessed. Following the measurement series, a matrix-adapted addition with hafnium (Hf) was performed as described by Ek *et al.*, (2004). Importantly, obtained interference rates for water, sediment, AM, and plastic liner were less than 2%, and therefore, no Hf-correction was applied. All Pt concentration assessments were done on the ¹⁹⁴Pt.

Table 2: Optimised operating parameters for Platinum analysis on the Atomic Absorption Spectrometer.

Temp (°C)	Ramp time (s)	Hold time (s)	Argon flow (psi)
110	1	5	250
140	10	60	250
1300	50	20	250

2500	0	3	0
2600	1	3	250

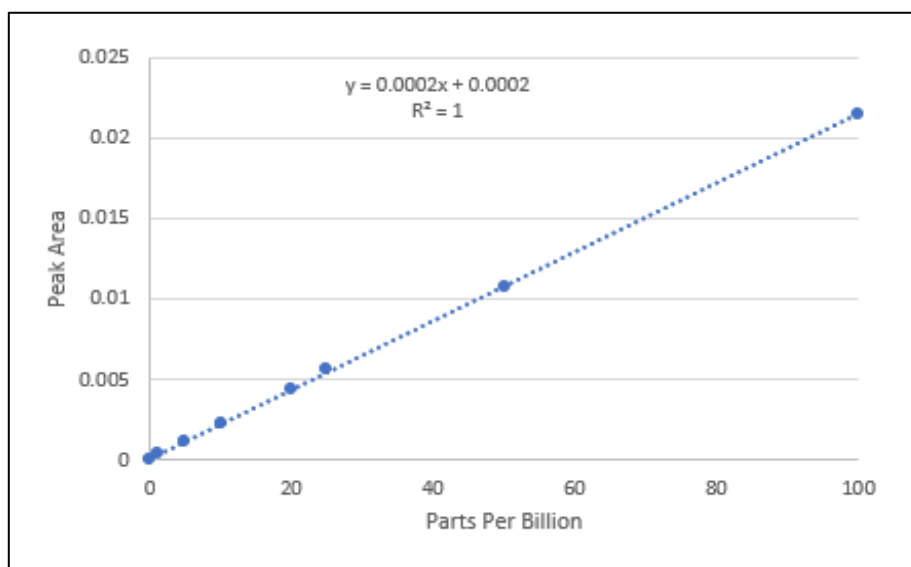


Figure 5: The optimised calibration curve used to calibrate the atomic absorption Spectrometer with 0 value of 0.0001, 1 value of 0.0004, 5 value of 0.0012, 10 value of 0.0023, 20 value 0.0044, 25 value of 0.0056, 50 value 0.0108 and 100 value of 0.0215.

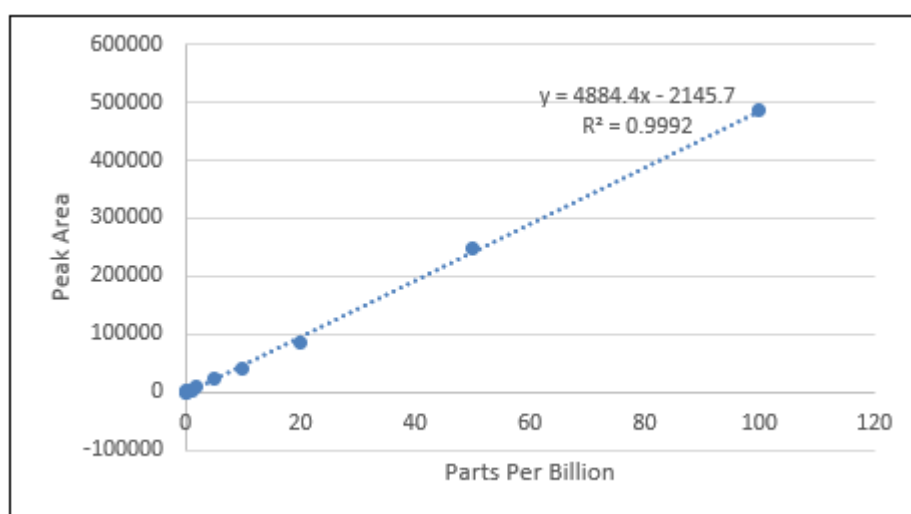


Figure 6: The optimised calibration curve used to calibrate the Inductively coupled plasma mass spectrometer with 0 value of 58.642, 1 value of 4796.637, 5 value of 24721.24, 10 value of 46412.1, 20 value 96799.71, 50 value 271025.1, and 100 value of 510829.80.

The risk of contamination during the collection and storage of environmental samples is extremely low as PGEs are not abundant in these samples. Samples, such as rock or soil, are collected with plastic equipment and stored after drying in polypropylene vessels (Whiteley and Murray, 2005; Wichmann *et al.*, 2007; Tsogas *et al.*, 2008). Liquid samples, such as surface waters or wastewaters, are typically acidified after sampling to keep the PGEs in solution and prevent adsorption onto vessel walls (Zimmermann and Sures, 2018).

3.5 Diatom sampling, preparation, and index assessments

Epiphytic and benthic diatoms were collected on two of the three sample times (48 hours – acute and eight weeks – chronic). Epiphytic diatoms were concurrently collected from the two macrophyte species whilst sampling for metal analyses. A whole specimen of *Charophyta sp.* and *F. antipyretica* macrophytes respectively were removed from the microcosm and placed in separate pre-cleaned Ziplock bags along with water from the specific sample site. Each bag containing a *Charophyta sp.* sample was gently rubbed to dislodge diatoms from the leaves and stems. After rubbing, the water of each sample was decanted into pre-cleaned polyethylene containers. During the microcosm setup, six sandblasted microscope slides were placed in each of the 18 microcosms. Sandblasted slides were used as a less destructive and easier sampling method of the benthic diatom community. As a precautionary measure, two sandblasted slides were sampled at each sampling time to ensure that the sample collected represented the real-time benthic diatom community assemblage found on the rocks. A pipette was dragged 10 to 15 centimetres over the plastic lining and sediment (in triplicate) for each of the 18 microcosms to sample the benthic diatoms present on the sediments. All of the samples collected for the macrophytes (M), rock (R), and sediment (S) samples, along with water from the specific sample sites, were collected and placed into pre-cleaned polyethylene containers and sealed off to prevent spilling during transport to the laboratory. Seventy percent ethanol was added to the diatom samples directly after sampling to preserve the samples until further analysis (Taylor *et al.*, 2007a). The samples were then brought to the laboratory and left to precipitate at room temperature

Due to the high levels of organic matter in South African diatom samples, the preferred method of slide preparation is the hot hydrochloric acid (HCl) and potassium permanganate (KMnO₄) method of Taylor *et al.*, (2007a). All diatom samples were sorted and left to precipitate over 24 hours (Taylor *et al.*, 2007a). After 24 hours, the excess water and 70 % ethanol mixture were decanted from each sample container. The concentrate of each sample was then resuspended and placed into marked test tubes with 2-3 mL of KMnO₄ and HCl (32%). Samples were then brought to boiling point in a hot water bath and boiled until the sample cleared. A single drop of hydrogen peroxide was added to each sample to determine if all the organic matter was removed. Boiled samples were then washed through four washing cycles with distilled water and then pipetted onto cleaned coverslips and left overnight to dry. Coverslips were mounted to microscope slides using Pleurax (refraction index, 1.73).

The diatom slides were then analysed under a Nikon 80i compound microscope equipped with differential interface contrast (DIC) using a 100x 1.4N oil immersion objective. Diatoms were identified to species level where possible and counted until 400 diatom valves, or the entire

microscope slide were counted. Identification of diatoms was made using “An illustrated guide to some common diatom species from South Africa” (Taylor *et al.*, 2007b). Data obtained were used to assess diatom communities' potential effects and responses to the different Pt exposure concentrations. Results obtained after analysis were interpreted according to Class limit values imposed on diatom index scores to indicate pollution levels as per a Finland study (Eloranta and Soinen, 2002). These values (Table 3) were also used for the interpretation of the scores yielded by the various indices (See section 3.6.2) (Taylor, 2004).

3.6 Data analyses

3.6.1 Metal analyses

The results obtained from experimentally determining the partitioning and accumulation of Pt in an aquatic ecosystem were tested for normality and homogeneity of variance using the D'Agostino and Pearson omnibus normality test and Shapiro-Wilk normality test, respectively (Pyrzrak, 2016). One-way analysis of variance (ANOVA) was used with the Dunnett T3 post hoc method for normally distributed data, and Kruskal Wallis method for non-normally distributed data along with Tukey's multiple comparison test, to test for significant differences in metal concentrations within environmental compartments across dose concentrations (GraphPad Prism v7). The level of significance was set at $p < 0.05$.

3.6.2 Diatom Community analyses

Diatom communities were assessed via OMNIDIA software and analysed using PRIMER 6 and GraphPad Prism v 7. Data obtained were tested for normality using the Shapiro-Wilk test. A parametric one-way ANOVA analysis was used to test the significant differences between normally distributed data across concentrations. For non-normally-distributed data, significant differences were determined using nonparametric one-way ANOVA. Significant differences were set at $p < 0.05$ to evaluate the effects (if any) of the different metal concentrations on the individual diatom indices. Statistical analyses made use of multiple univariate and multivariate analyses methods. Paired T-tests were used to compare the diversity and diatom community from the same microcosms over time. Univariate analyses included the diversity indices known as Pielou's evenness index (evenness), which indicates whether there is a dominance of individuals in the community at each site (Pielou, 1971), Shannon-Wiener diversity index (Shannon diversity), which integrates both Margalef and Pielou's indices to determine overall diversity of each site (Shannon and Weaver, 1963; Clarke and Warwick, 2001) and Simpson's index, which determines the probability that two individuals, drawn at random from a community, would belong to the same species. If the probability is high, the diversity is low (Simpson, 1949). The total species and

species richness were also determined. A suite of diatom indices was used to assess the community responses. These indices are known as the Generic Diatom Index (GDI) (Coste and Ayphassorho, 1991), the Biological Diatom Index or BDI (Lenoir and Coste, 1996), the Trophic Diatom Index or TDI (Kelly and Whitton, 1995), the Pollution Tolerant taxa (%PT) (Kelly and Whitton, 1995), and the Pollution Sensitive Index (IPS) (Cemagref, 1982). For all the above indices, except for %PT and TDI (maximum value of 100), the maximum value of 5 (converted to 20 by the software package OMNIDIA; Lecoite *et al.*, 1993) indicates pristine water.

Table 3: Range of diatom indices classification values and their meaning (Eloranta & Soininen 2002; Taylor, 2004; Dumnicka *et al.*, 2006).

Index score	Water quality class	Ecological status	Trophic state	Trophic Diatom Index and Pollution Tolerant taxa
>17	I	High quality	Oligotrophic	<35
15 to 17	II	Good quality	Oligo/mesotrophic	35 to 50
12 to 15	III	Moderate quality	Mesotrophic	50 to 60
9 to 12	IV	Poor quality	Eutrophic	60 to 75
<9	V	Bad quality	Hypertrophic	>75

Multivariate analyses were carried out to assess the possible effects of Pt on the diatom community over the exposure time. The significance level was set at $p = 0.05$ to evaluate the diatom community response to the different Pt exposures over time. Principal Component Analyses (PCA) are used to assess the effect of the different Pt concentrations on species composition. The various diatom sample sites (Sediment (S), Macrophytes (M), Rocks (R)) data sets obtained for the 48-hour and eight weeks of exposure were analysed with the Principal Response Curve (PRC) method using the CANOCO version 5, software package (Van den Brink and ter Braak, 1999; ter Braak and Smilauer 2012). The PRC method is a specific type of redundancy analysis (RDA) and thus can analyse the variation within a community assemblage as stress observed in the total variation.

CHAPTER 4: RESULTS

4.1 Physicochemical parameters and nutrient levels in the water of the microcosms

The physicochemical water variables measured at each microcosm indicated visible temporal differences (Figure 7). The pH remained relatively constant throughout the study, with observable changes in pH in the first two weeks and the last two weeks of the experiment. Variation in pH (Figure 7A) was also observed between the individual exposure groups as it was noted that the 100 and 500 $\mu\text{gPt/L}$ exposure groups had an overall lower pH than the other exposure groups.

Overall salinity (Figure 7B) steadily increased from an average minimum of 275 uS.cm^{-1} measured at the first sample time (48 hours after exposure) to a maximum of 467 uS.cm^{-1} measured on the final sampling event. The Total Dissolved Solids (TDS) (Figure 7C) and Electrical Conductivity (EC) (Figure 7D) showed a similar increasing trend over the experimental period. With TDS increasing from an average minimum of 400 ppm to a maximum of 671 ppm, and EC increasing from an average minimum of 468 uS.cm^{-1} to a maximum of 962 uS.cm^{-1} after the eight weeks of exposure. As indicated by TDS and EC, it was observed that salinity tended to increase as Pt concentrations increased. The lowest values for all three variables were recorded for the control microcosms, and the highest values were recorded for the 500 $\mu\text{gPt/L}$ microcosms, except for the four-week sampling time. Temperature (Figure 7E) fluctuations occurred throughout the experimental time leading to a 10°C difference between the 48 hour and four-week sample times and a 4°C difference between the four- and eight-week sampling times. The turbidity (Figure 7F) for all the microcosms varied over the experimental time. The lowest average turbidity was recorded after four weeks of exposure, and the highest average turbidity was recorded after eight weeks.

Temporal variation in the nutrient levels was evident between the collected samples (Figure 8). However, total hardness (Figure 8A) remained constant throughout the experiment, with a visible increase in water hardness for all of the exposures over an eight-week exposure period except for the 500 $\mu\text{gPt/L}$ exposure. The ammonium (Figure 8B) in all the microcosms concentrations shows a noticeable decline between the 48 hour and four-week samples. The eight-week sample indicated increased ammonium levels for the control, 0.1, 1, and 100 $\mu\text{gPt/L}$ microcosms. The nitrate (Figure 8C) concentrations increased over the eight-week exposure period, with the highest nitrate concentration observed in the 100 $\mu\text{gPt/L}$ exposure. Nitrite (Figure 8D) concentrations over time varied considerably across the exposure range. With 0.1, 1, and 10 $\mu\text{gPt/L}$ microcosms indicating a decrease in nitrite, whereas the control, 100, and 500 $\mu\text{gPt/L}$ microcosms indicated an increase in nitrite over the eight-week exposure period. In all the microcosms, chloride (Figure 8E) concentrations increased over time, with the highest

concentrations in the eight-week water samples. Phosphate (Figure 8F) concentrations also varied between the different sample times with no visible trends. However, it was noted that the average phosphate concentrations decreased with increasing Pt exposures and did not change over time. The average COD (Figure 8G) remained constant in all the microcosms except the 10 $\mu\text{gPt/L}$. It was noted that the control, 0.1, and 1 $\mu\text{gPt/L}$, indicated a decrease, while exposures 100 and 500 $\mu\text{gPt/L}$ showed an increase in COD.

--- Control --- 0.1ug/L --- 1ug/L --- 10ug/L --- 100ug/L --- 500ug/L

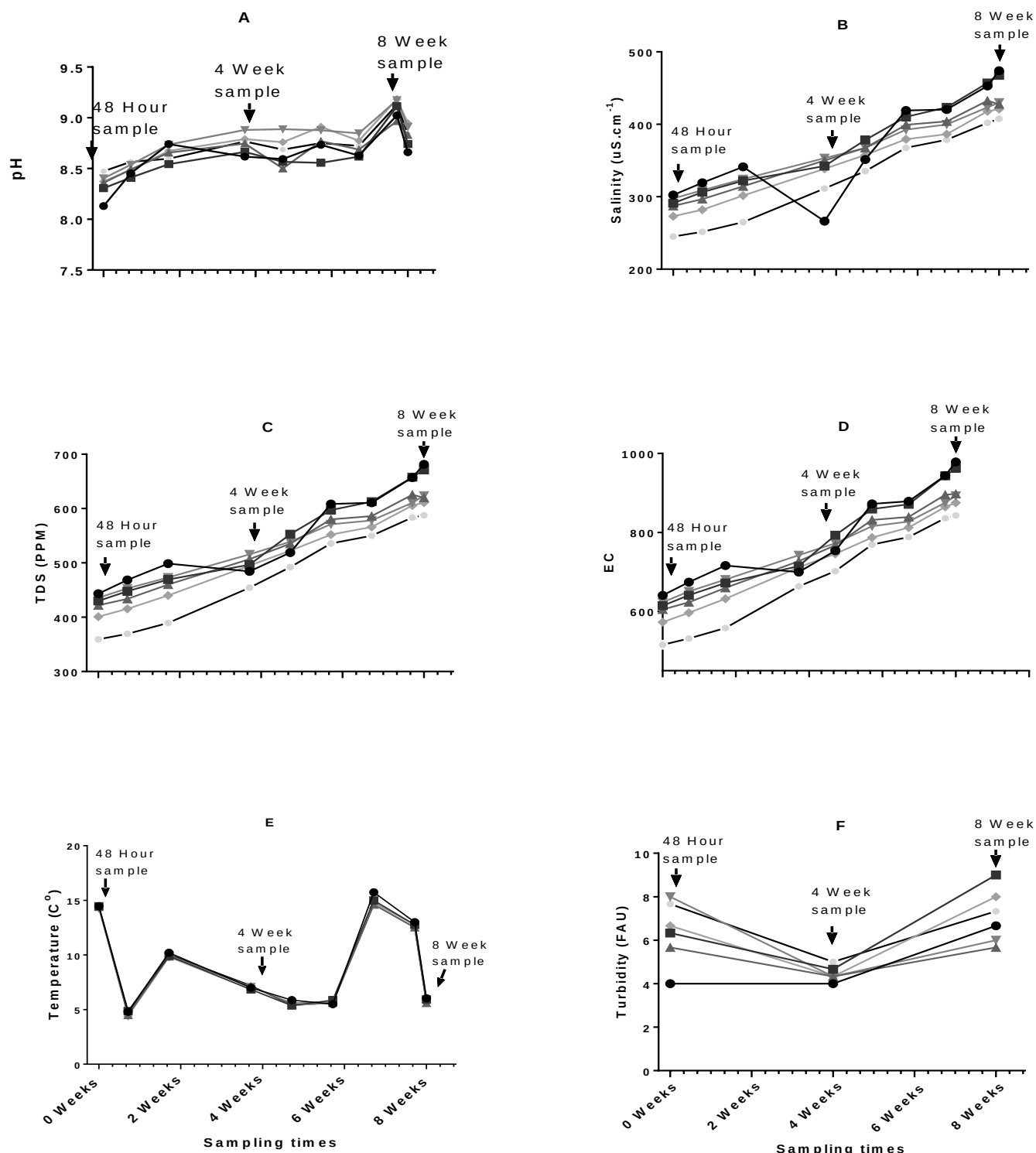


Figure 7: Line graphs illustrating the physicochemical water quality parameters for each microcosm exposure group over the experimental time from May 2019 to July 2019. (A) pH, (B) Salinity, (C) Total dissolved solids (TDS), (D) Electrical conductivity, (E) Temperature, (F) Turbidity.

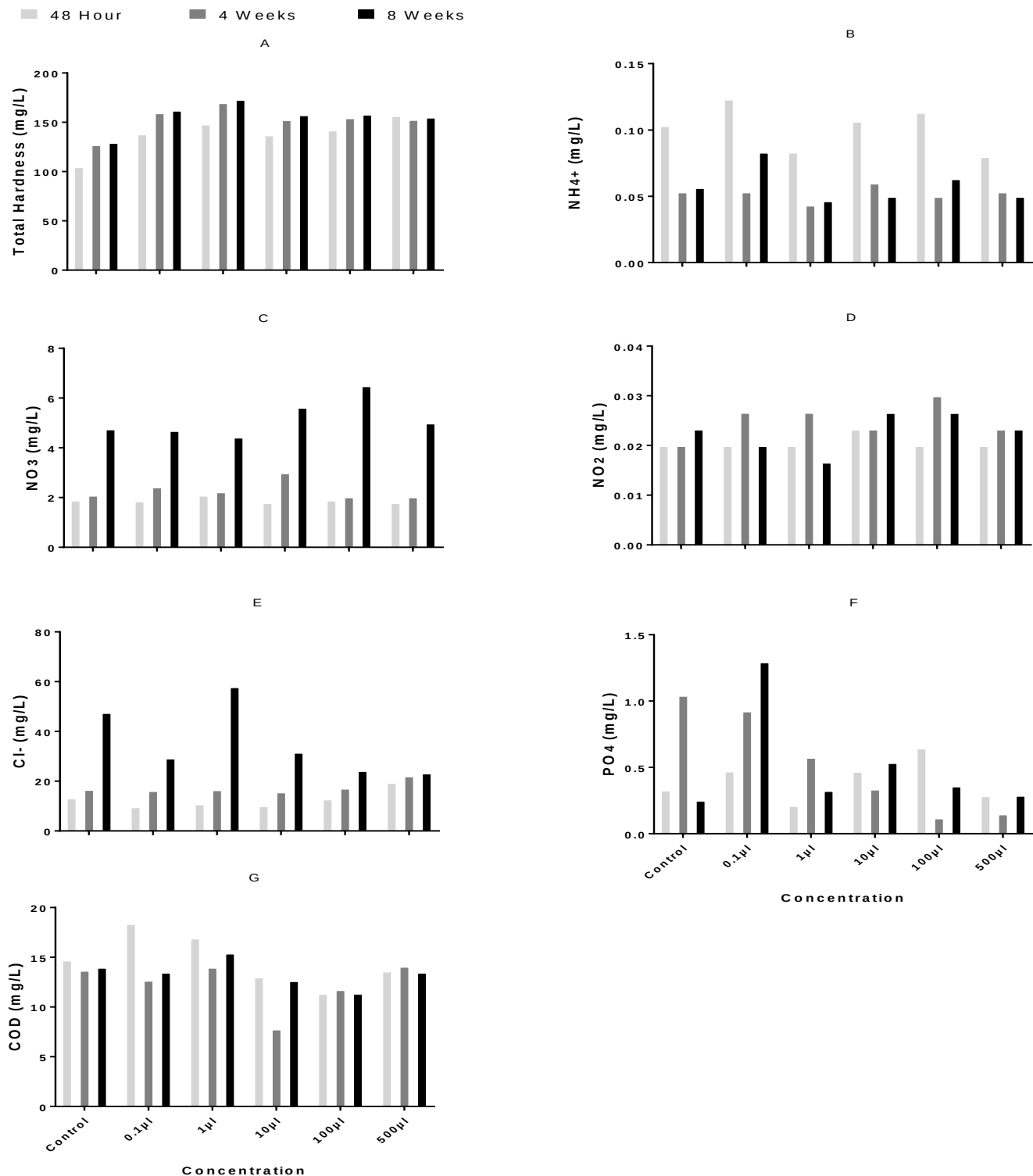


Figure 8: Illustration of the changes in water nutrients of each microcosm exposure group for the duration of the experiment. (A) Turbidity, (B) Total Hardness (TH), (C) Ammonium (NH₄⁺), (D) Nitrate (NO₃), (E) Nitrite (NO₂), (F) Chloride (Cl), (G) Phosphate PO₄, (H) Chemical oxygen demand (COD).

4.2 Platinum concentration in the water column

After metal detection, Pt concentrations found in the water column indicated a lower Pt concentration than the dosed concentrations administered to the microcosms (Figure 9). Platinum concentration decreased over time, with the 48-hour sample having the highest, and the eight-week sample the lowest concentration for each of the exposure concentrations except for the four-week 0.1 µgPt/L exposure. One-way ANOVA analyses indicated that the 0.1 ($p = <0.0001$), 10 µgPt/L ($p = <0.0001$) and 100 µgPt/L ($p = 0.0001$) did indicate a significant difference in Pt concentration in the water column over time. Supplementary information can be found in Table B-3 of the annexure. For the 500 µgPt/L exposure, 37.00%, 4.87% and 3.59% of the dose concentration were recovered after 48 hours, four weeks and eight weeks, respectively.

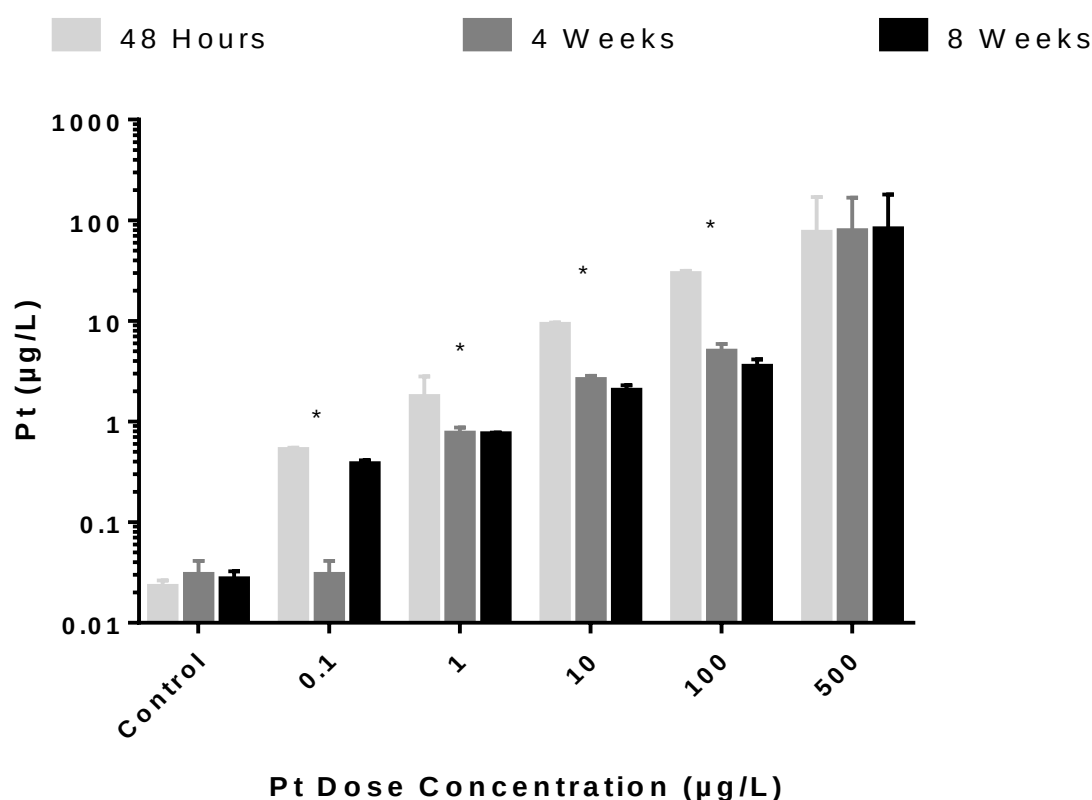


Figure 9: Mean Pt concentration found in the water column in each exposure concentration throughout the experiment at the three different sample times, i.e., 48-hours, four weeks, eight weeks (n = 54). Significant differences over time are indicated with an asterisk (*).

4.3 Platinum accumulation in the sediment

Platinum concentrations found in the sediment was lower than the dose concentrations as it was found that Pt concentrations for the 0.1, 1, and 10 µgPt/L exposures indicated little to no Pt accumulation, while noticeable changes in sediment Pt levels could be seen in the 100 and 500 µgPt/L exposures (Figure 10). No changes were observed over time for the 100 µgPt/L exposure

concentration. For the 500 µgPt/L concentration, the 48-hour (acute) samples had higher Pt concentrations than the four-week (chronic) or eight-week (chronic) samples. One-way ANOVA analyses indicated that only the 100 µgPt/L ($p = 0.0001$) indicated a significant difference in Pt concentration in the sediment over time. For the 500 µgPt/L exposure, 37.00%, 4.40% and 2.81% of the dose concentration were recovered after 48 hours, four weeks and eight weeks, respectively.

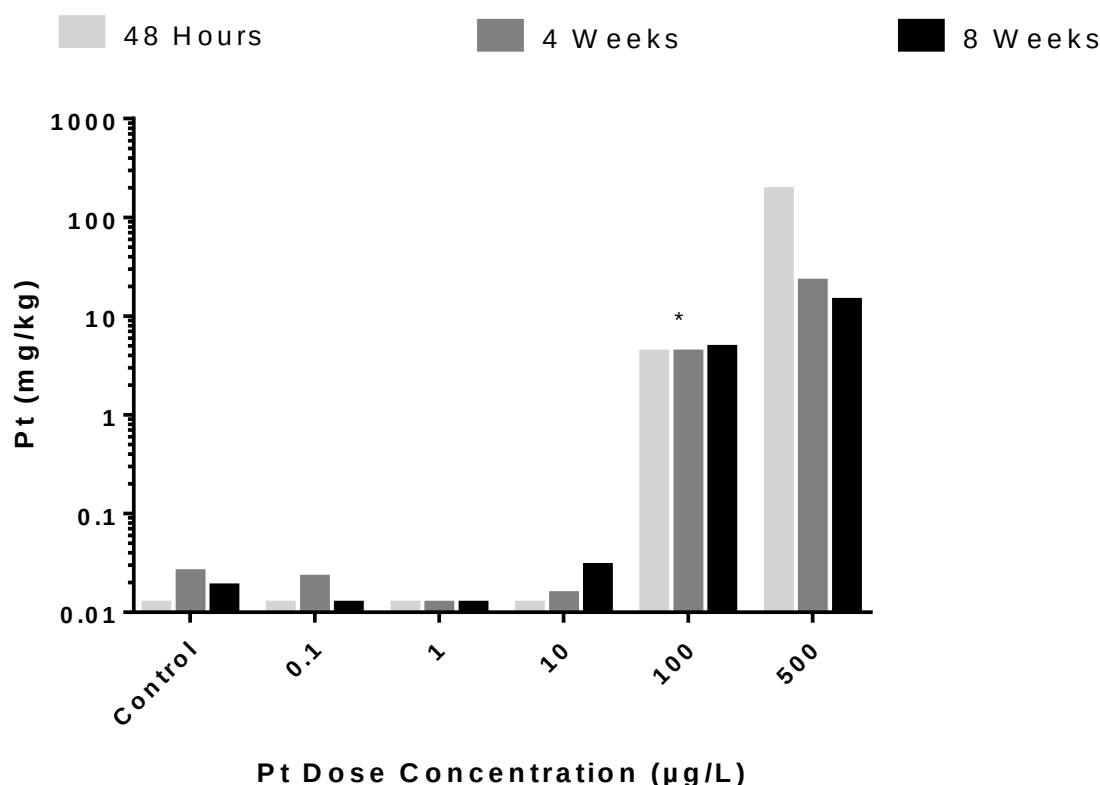


Figure 10: Mean Pt concentration found in the sediment throughout the experiment for the three different sample times, i.e., 48-hours, four weeks, eight weeks ($n = 54$). Significant differences over time are indicated with an asterisk (*).

4.4 Platinum in macrophytes

After metal analyses, results indicated that both macrophyte species had detectable concentrations of Pt. As expected, it was found that the Pt exposure microcosms of 10, 100, and 500 µgPt/L had higher Pt concentrations than that of 0.1 and 1 µgPt/L. It was observed that the *F. antipyretica* tended to accumulate higher Pt concentrations than that of the *Charophyta sp.* However, One-way ANOVA analyses indicated that only the 1 ($p = 0.0160$) and 500 µgPt/L ($p = 0.0152$) indicated a significant difference in Pt concentration in the *Charophyta sp* over time. For

the *Charophyta* sp. 500 µgPt/L exposure, a total of 83.09%, 54.75% and 21.83%, and for the *F. antipyretica* 500 µgPt/L exposure, a total of 113.84%, 172.83% and 106.63% of the dose concentrations were recovered after 48 hours, four-weeks and eight-weeks respectively.

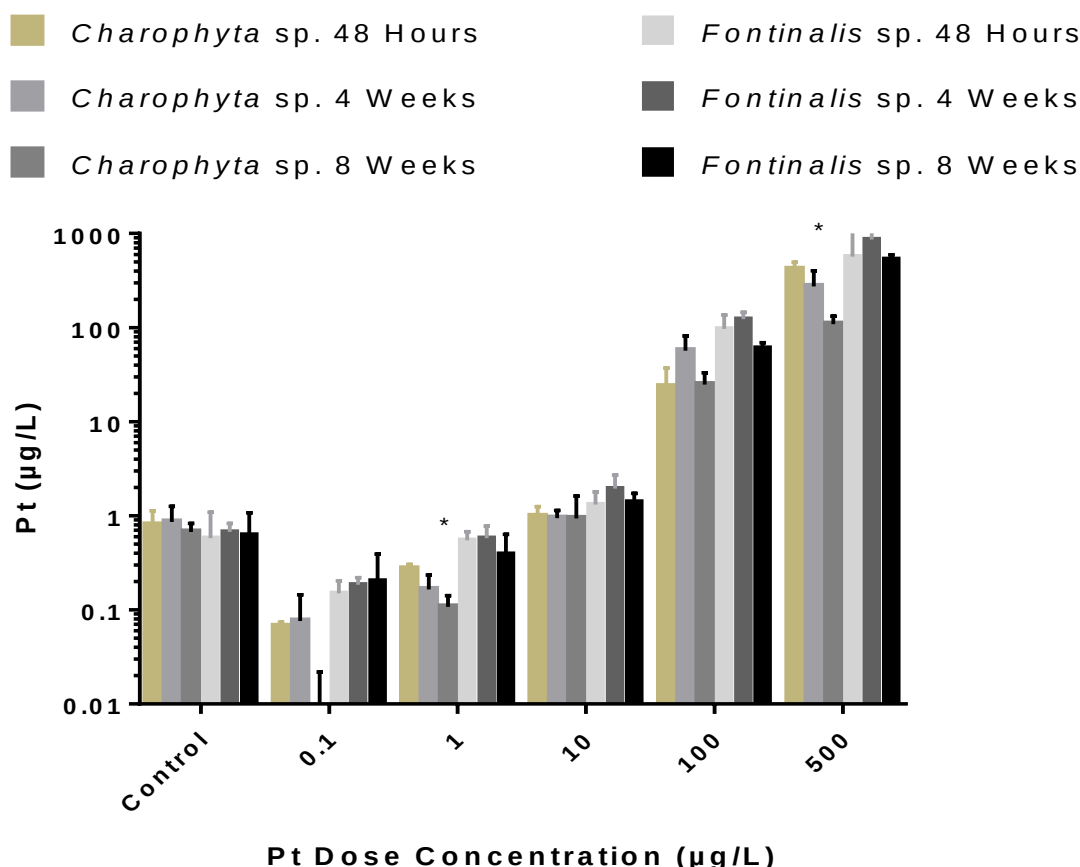


Figure 11: Mean Pt concentration found in *Charophyta* sp. (Musk grass) and *F. antipyretica* (Lesser water moss) throughout the experiment, in the form of three different sample times 48-hours, four weeks, eight weeks (n = 108). Supplementary information can be found in Table B-3 in the annexure. Significant differences over time are indicated with an asterisk (*).

4.5 Platinum accumulation in Artificial Mussels (AM)

The Pt concentrations found in the AM revealed a visible increase in partitioned Pt concentrations for both the 48-hour and eight-week samples. An increasing trend of Pt concentrations for these sample times was associated with the increased dose concentrations. The 100 and 500 µgPt/L concentrations showed the highest Pt concentration for all three sampling times, with 48-hours being the highest, four weeks second-highest, and eight weeks having the lowest Pt concentration, respectively. The 500 µgPt/L concentration showed no difference between the 48-hours, four weeks, and eight weeks Pt concentrations. One-way ANOVA analyses indicated no significant differences in Pt concentration over time. For the 500 µgPt/L, a total of 33.21%, 18.74%

and 7.54% of the dose concentrations were recovered after 48 hours, four weeks and eight weeks, respectively.

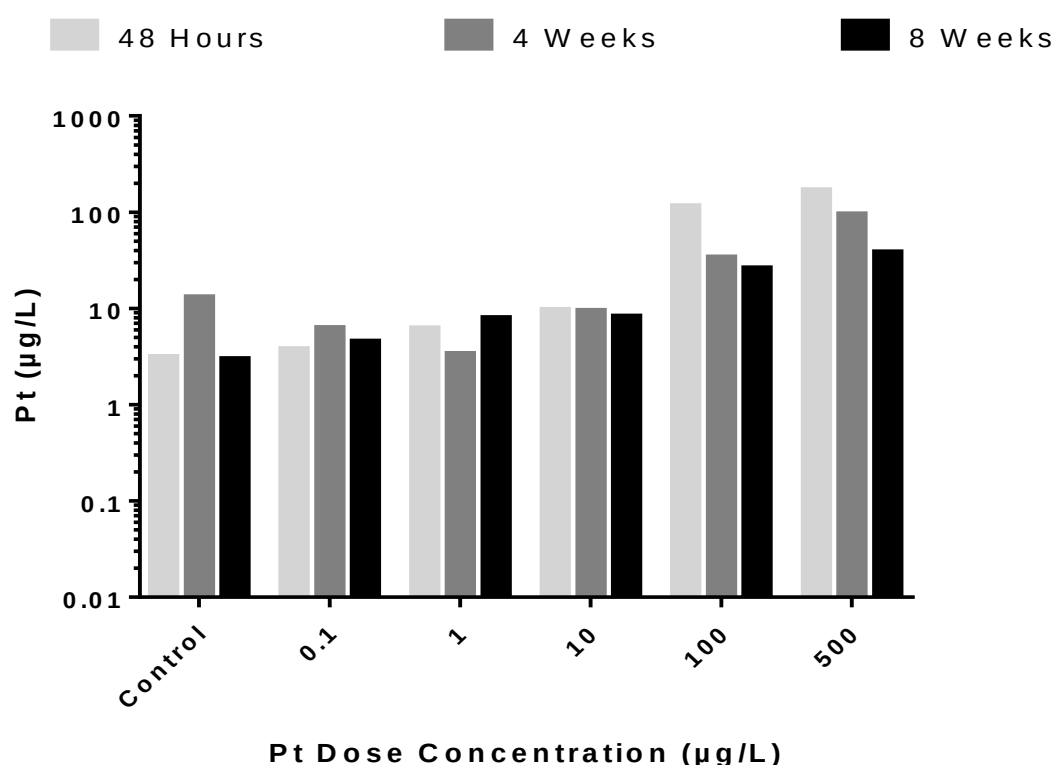


Figure 12: Mean Pt concentration found in artificial mussels throughout the experiment, in the form of three different sample times 48-hours, four weeks, eight weeks (n = 54). Supplementary information can be found in Table B-3 of the annexure.

4.6 Diatom diversity and community assemblages

Pielou's evenness (Figure 13) indicated that the epiphytic and benthic diatom communities were evenly distributed during both the acute and chronic exposure periods. However, a visual difference between the 48 hour (acute) and eight week (chronic) benthic diatom communities sampled from the sediment was observed with a difference between the two sediment sample groups' evenness scores. Paired T-tests indicated significant differences over time for the epiphytic diatoms (Figure 13 A and B $p < 0.05$) and benthic diatoms (Figure 13 C and D and E and F $p < 0.05$). Supplementary information can be found in Table B-4 of the annexure.

The Shannon diversity index (Figure 14) results indicated that epiphytic and benthic diatom communities showed a similar amount of diversity of species throughout the experiment. The chronic exposures showed a negligible difference compared to the acute samples. No change in community diversity was observed for the acute and chronic exposure groups to the control groups. However, for the benthic diatom community, a visual change in the community assemblage was noticed between the acute and chronic exposure groups (Figure 14). Paired T-

tests indicated no significant differences over time for the epiphytic diatoms (Figure 14 A and B $p > 0.05$) and benthic diatoms (Figure 14 C and D $p > 0.05$) except for the benthic diatom community sampled from the sediment (Figure 14 E and F $p < 0.05$). Supplementary information can be found in Table B-4 of the annexure.

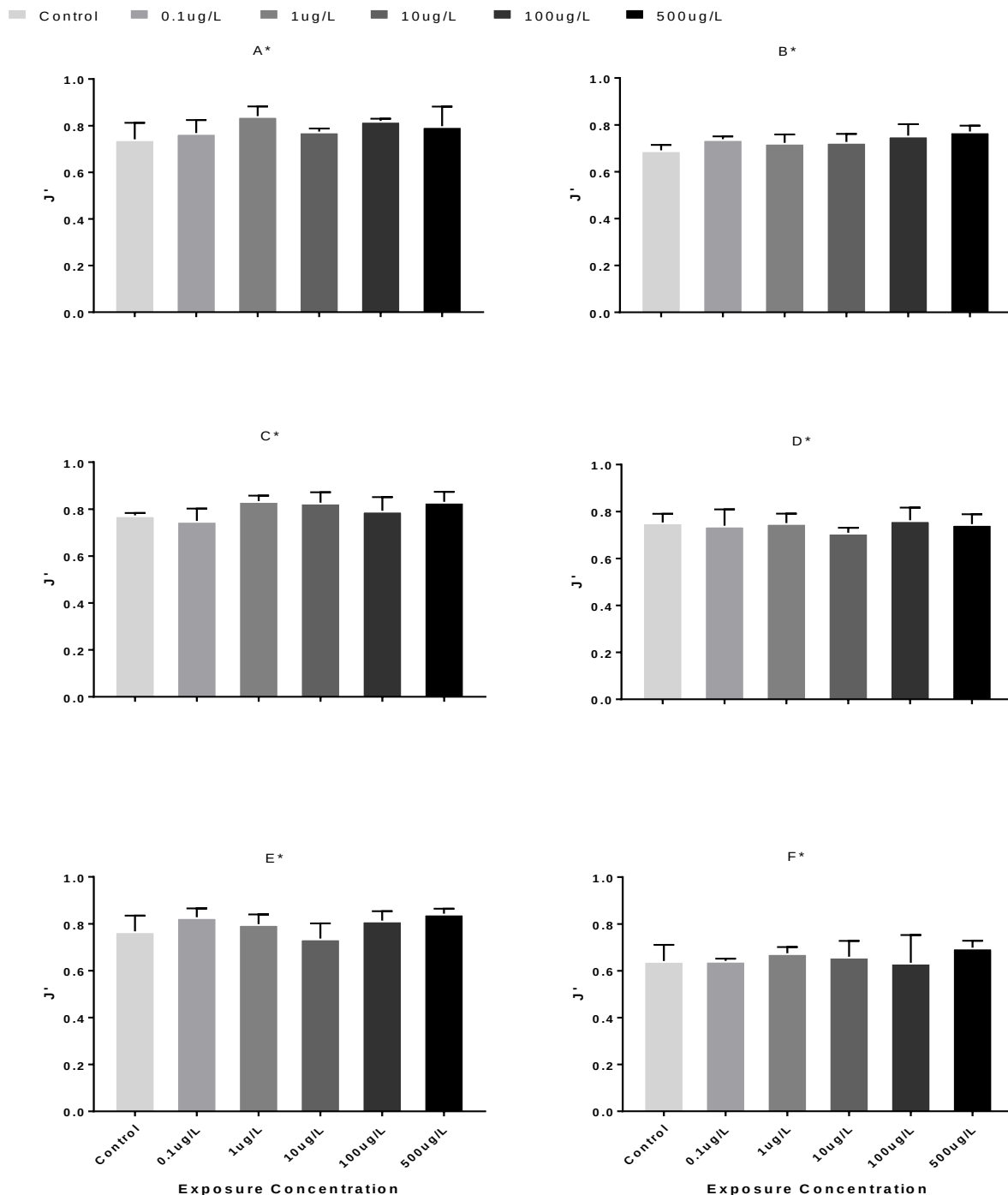


Figure 13: Pielou's Evenness Index (J') indicates the evenness of the diatom communities identified on the microscope slides. (A) acute epiphytic diatom samples ($n = 18$), (B) chronic epiphytic diatom samples ($n = 18$), (C) acute benthic diatom samples ($n = 18$), (D) chronic benthic diatom samples ($n = 18$), and (E) acute benthic diatom samples over time

(n = 18), (F) chronic benthic diatom samples over time (n = 18). Significant differences over time are indicated with an asterisk (*).

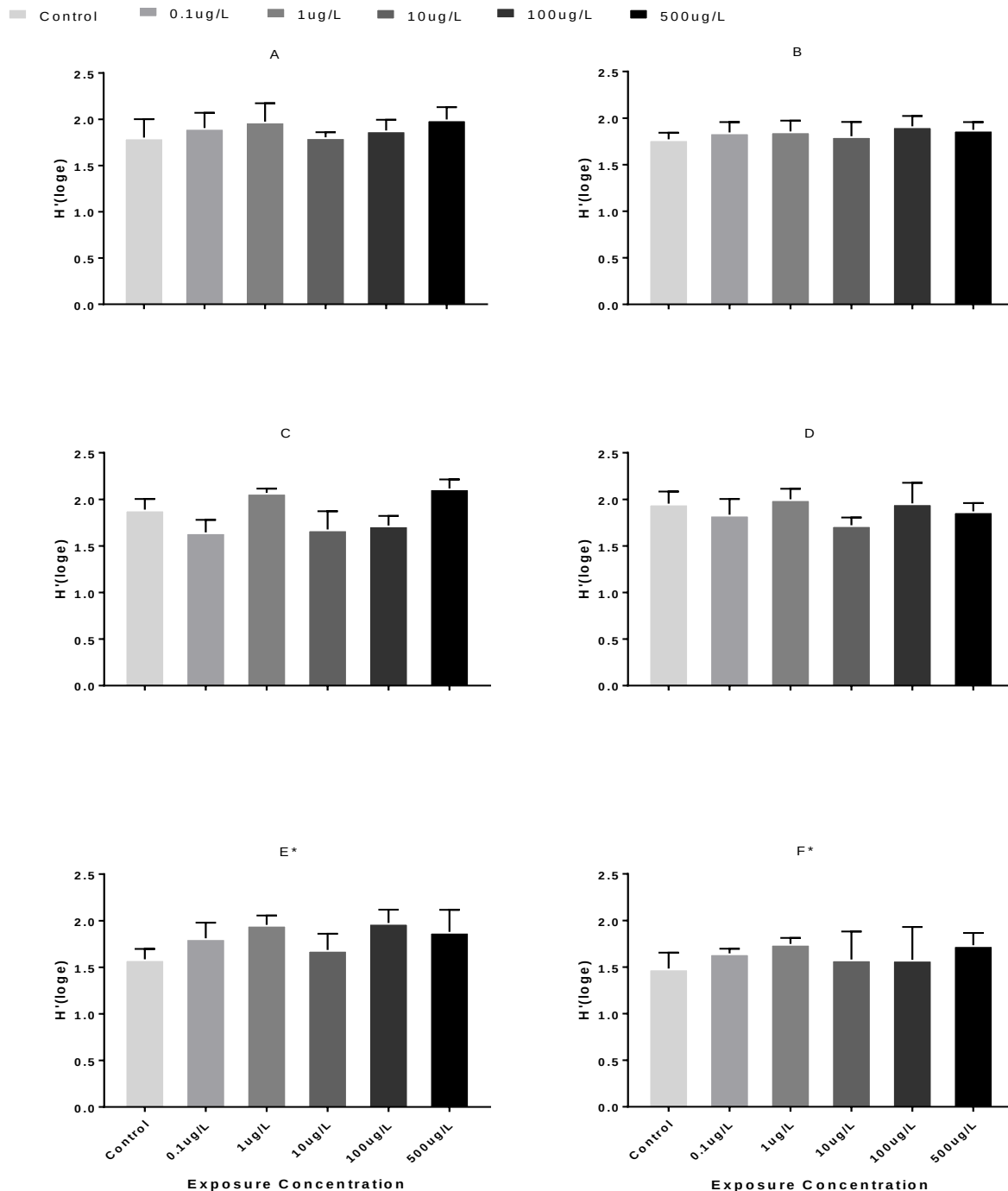


Figure 14: Shannon Index $H'(\log_e)$ indicates the diversity of the diatom communities identified on the microscope slides. (A) acute epiphytic diatom samples (n = 18), (B) chronic epiphytic diatom samples (n = 18), (C) acute benthic diatom samples (n = 18), (D) chronic benthic diatom samples (n = 18), (E) acute benthic diatom samples (n = 18), and (F) chronic benthic diatom samples (n = 18) over time. Significant differences over time are indicated with an asterisk (*).

The Simpson diversity index (Figure 15) results for the epiphytic and benthic (sampled from the rocks) diatom communities indicated that the acute and chronic exposure results showed a similar index with a negligible difference between the sample times with an index just lower than 0.8. However, for the benthic diatom community sampled from the sediment, the acute exposure group showed an average Simpson index just lower than 0.8. The chronic exposure group showed an average index value of 0.7 for diatoms sampled from the sediment. Paired T-tests indicated no significant differences over time for the epiphytic diatoms (Figure 15 A and B $p > 0.05$) and benthic diatoms (Figure 15 C and D $p > 0.05$) except for the benthic diatom community sampled from the sediment (Figure 15 E and F $p < 0.05$). Supplementary information can be found in Table B-4 of the annexure.

The total species (Figure 16) and species richness (Figure 17) indicated similar trends in acute and chronic exposure groups. With visible changes in the epiphytic and benthic diatom communities. The epiphytic diatom community results indicated that the community assemblage showed a decreasing trend as the exposure concentrations increased for both acute and chronic exposures, except the acute 500 $\mu\text{gPt/L}$ exposure (Figure 16 and 17 (A & B)). The most apparent differences between the benthic diatoms' acute and chronic exposure groups were observed. For C and D of Figures 16 and 17, the results indicated an increase in the total species over time, especially for the 0.1, 10, and 100 $\mu\text{gPt/L}$ exposure groups. For E and F of Figures 16 and 17, a visible increase of benthic diatom species over time was observed for all exposure concentrations. For the total species and species richness, paired T-tests indicated no significant differences for the epiphytic diatoms (A and B $p > 0.05$). However, paired T-tests did show a difference for the benthic diatoms between the 48 hour and eight-week sample groups (C and D $p < 0.05$, E and F $p < 0.05$). Supplementary information can be found in Table B-4 of the annexure.

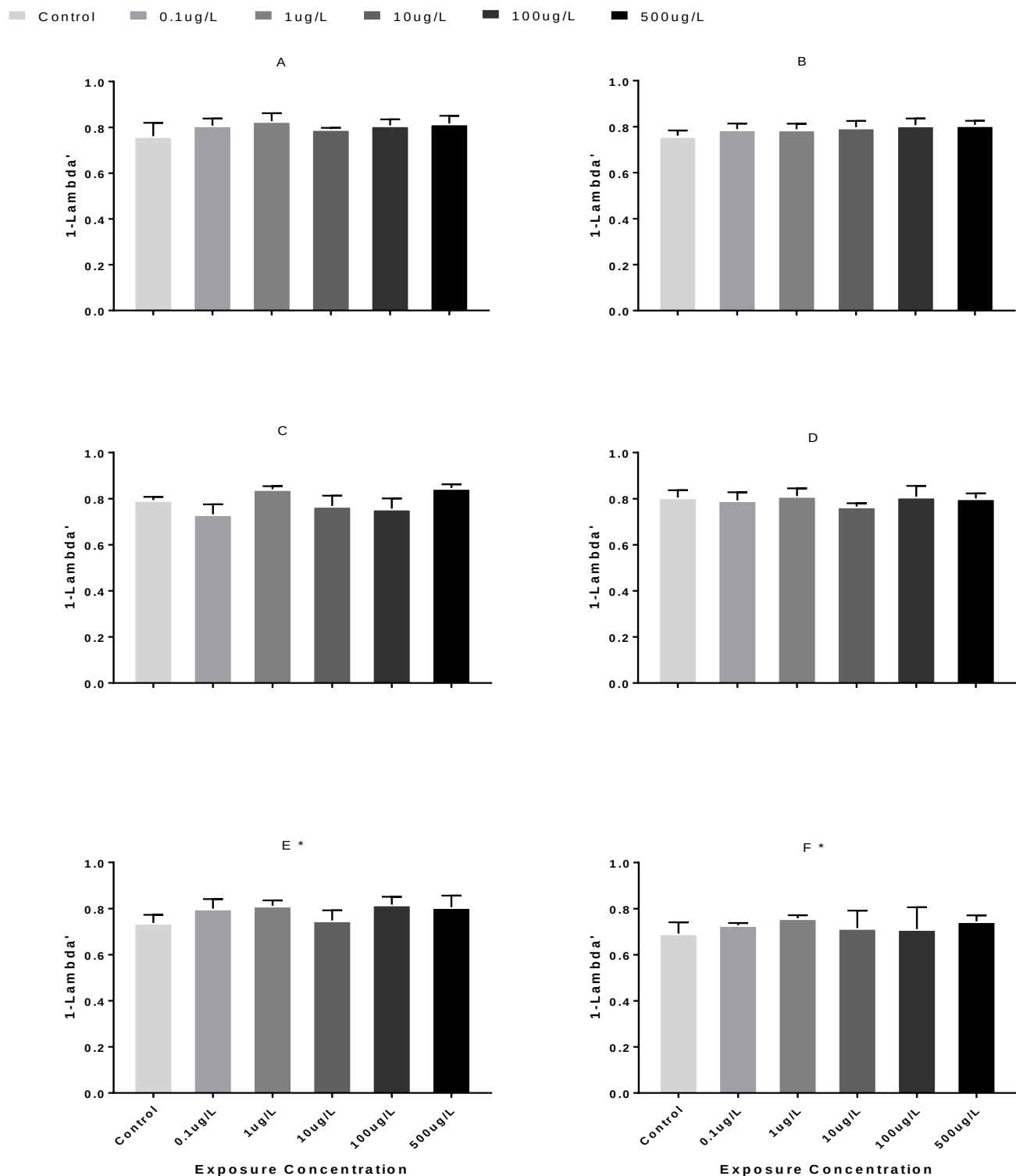


Figure 15: Simpson diversity index indicates the number and abundance of diatom species found on the microscope slides for (A) acute epiphytic diatom samples (n = 18), (B) chronic epiphytic diatom samples (n = 18), (C) acute benthic diatom samples (n = 18), (D) chronic benthic diatom samples (n = 18), (E) acute benthic diatom samples (n = 18), and (F) chronic benthic diatom samples (n = 18) over time. Significant differences over time are indicated with an asterisk (*).

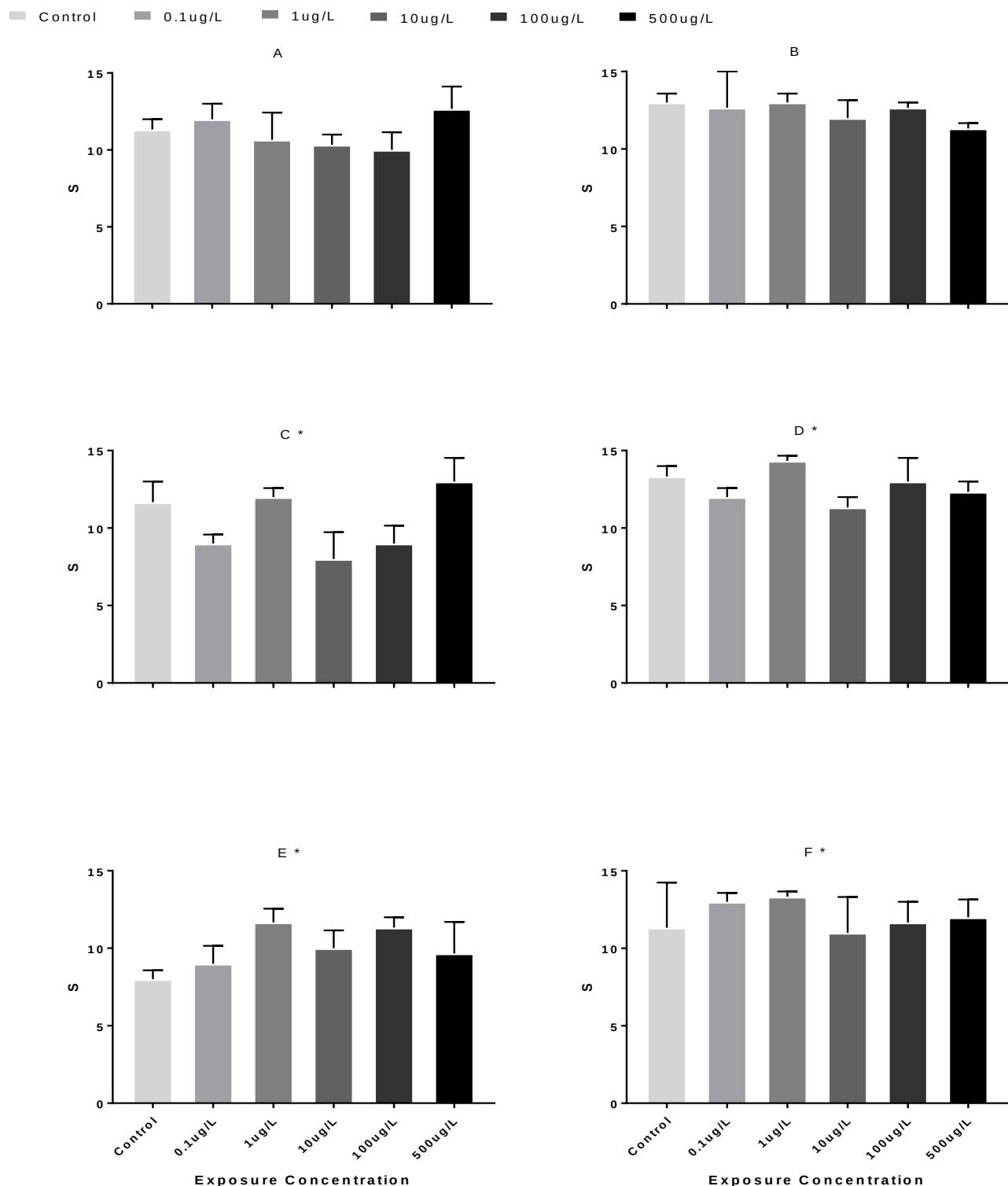


Figure 16: Total number of diatom species(S) indicating the diversity of diatom species found on the microscope slides for (A) acute epiphytic diatom samples (n = 18), (B) chronic epiphytic diatom samples (n = 18), (C) acute benthic diatom samples (n = 18), (D) chronic benthic diatom samples (n = 18), (E) acute benthic diatom samples (n = 18), and (F) chronic benthic diatom samples (n = 18) over time. Significant differences over time are indicated with an asterisk (*).

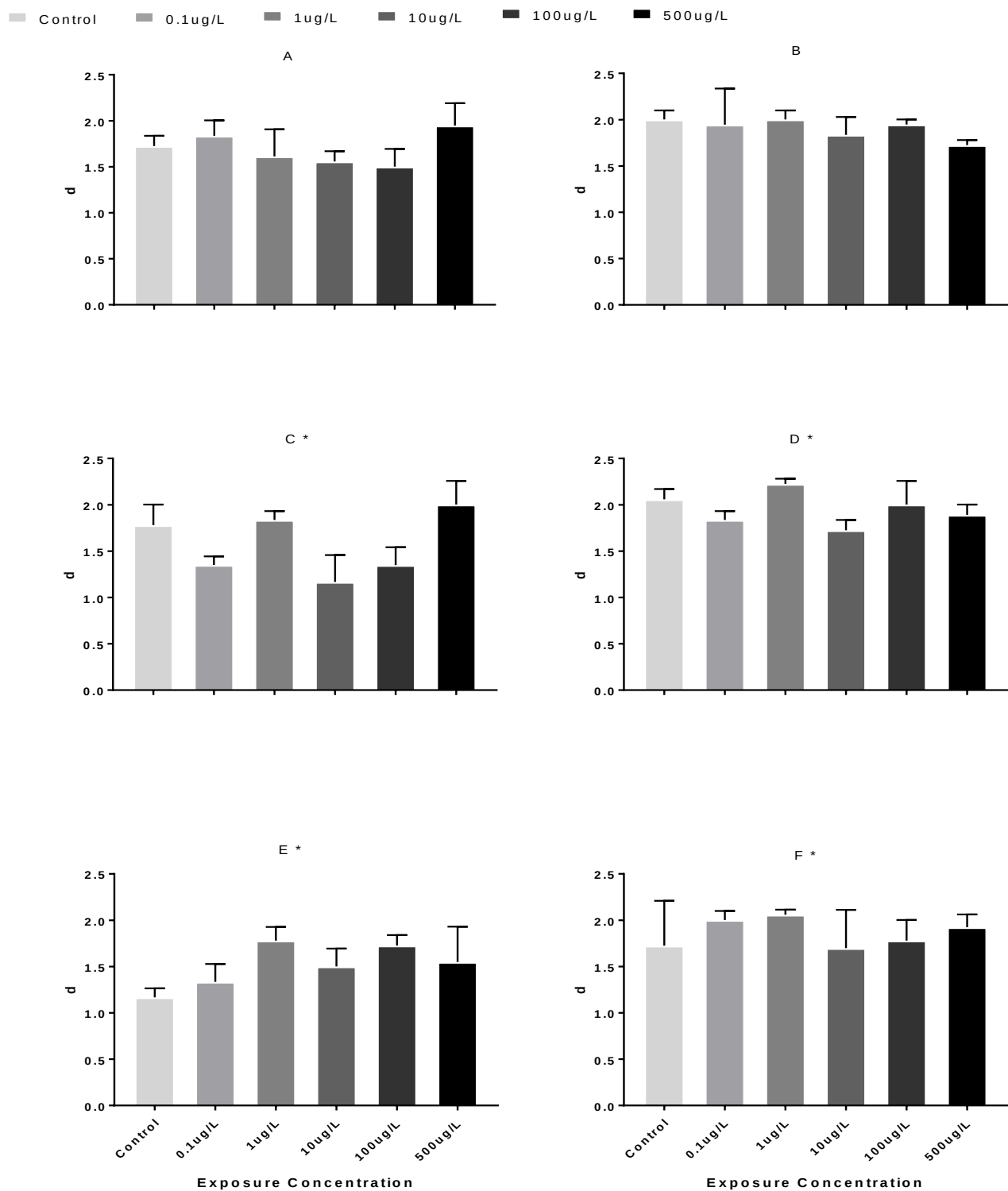


Figure 17: Species richness(d) indicating the diversity of the diatom community found on the microscope slides of (A) acute epiphytic diatom samples ($n = 18$), (B) chronic epiphytic diatom samples ($n = 18$), (C) acute benthic diatom samples ($n = 18$), (D) chronic benthic diatom samples ($n = 18$), (E) acute benthic diatom samples ($n = 18$), and (F) chronic benthic diatom samples over time ($n = 18$). Significant differences over time are indicated with an asterisk (*).

4.7 Diatom Biological indices

After analysing the BDI, results indicated low index scores of a deteriorating environmental state (Figure 18 A, B, C & D), except for the benthic (sediment) diatom samples (Figure 18 E & F). The sediment group showed a higher average score than the epiphytic and benthic (rock) diatom samples (Figure 18). Index scores indicated that both the epiphytic and benthic diatom groups had a decreasing average score between the acute and chronic exposure groups. Paired T-tests results did not reveal any significant differences between the 48-hour (acute) and eight-week (chronic) diatom communities. Supplementary information can be found in Table B-4 of the annexure.

The GDI results indicated a high average score for both the epiphytic and benthic diatom communities representing a moderate to good environmental state. The results (Figure 19) observed that the chronic epiphytic and benthic diatom communities tended to have a higher average score and more even index scores across the exposure range than acute communities, irrespective of the dose concentrations. Paired T-tests results did not indicate significant differences between the acute and chronic diatom communities. Supplementary information can be found in Table B4 of the annexure.

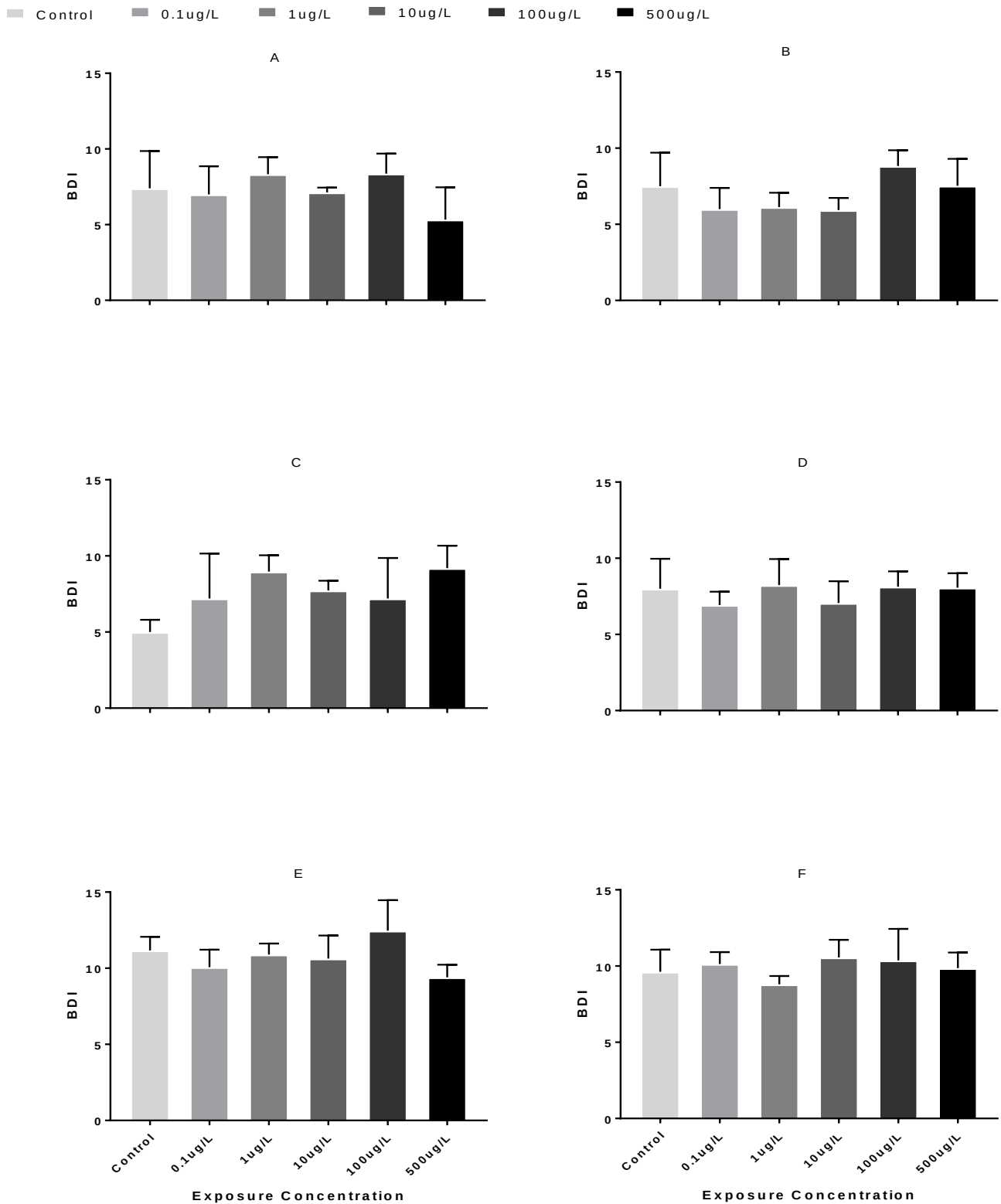


Figure 18: Biological Diatom index (BDI) illustrating the OMNIDIA diatom community results obtained for (A) acute epiphytic diatom samples (n = 18), (B) chronic epiphytic diatom samples (n = 18), (C) acute benthic diatom samples (n = 18), (D) chronic benthic diatom samples (n = 18), (E) acute benthic diatom samples (n = 18), and (F) chronic benthic diatom samples (n = 18) over time.

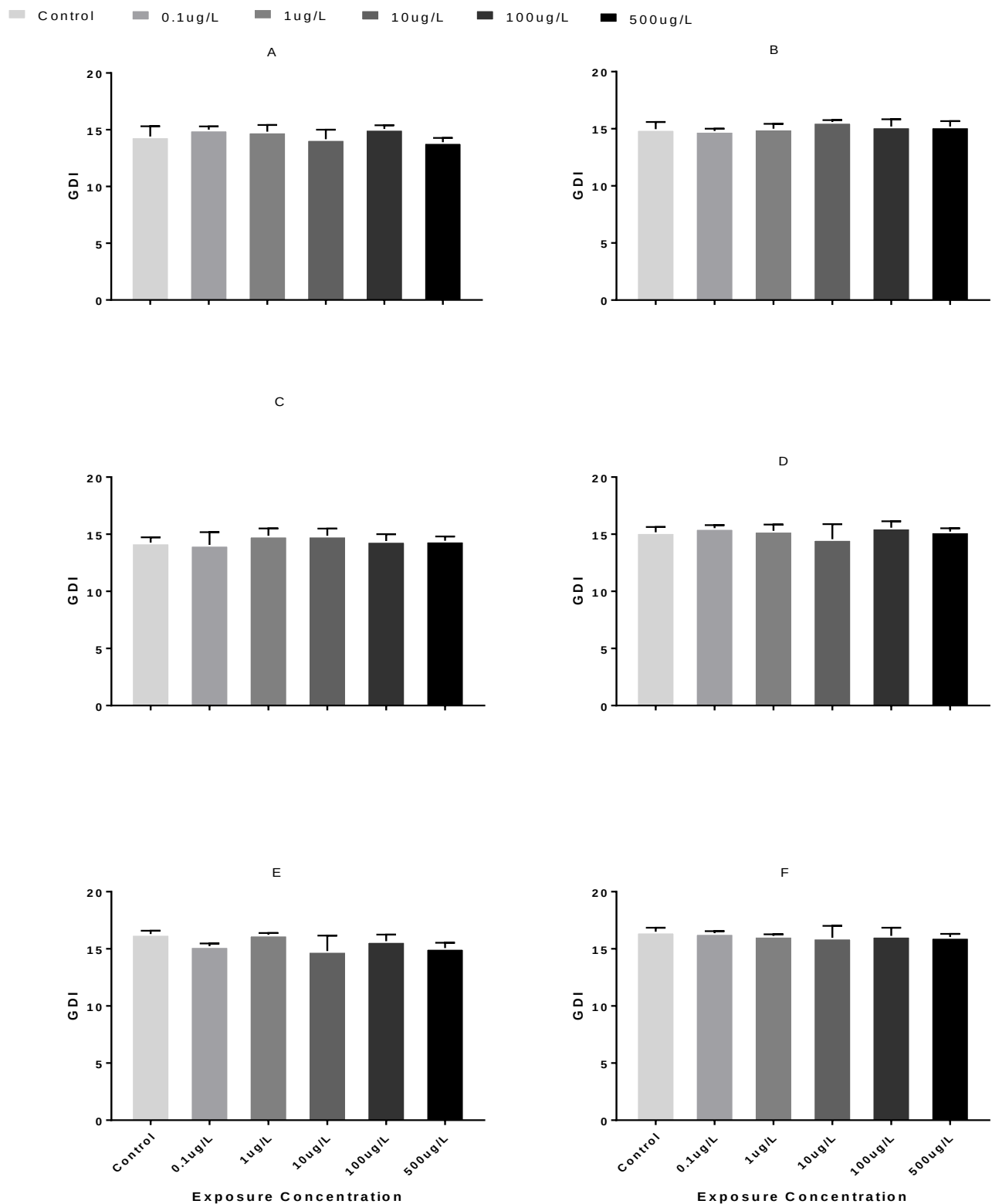


Figure 19: Generic Diatom Index (GDI) illustrating the OMIDIA diatom community results obtained for (A) acute epiphytic diatom samples (n = 18), (B) chronic epiphytic diatom samples (n = 18), (C) acute benthic diatom samples (n = 18), (D) chronic benthic diatom samples (n = 18), (E) acute benthic diatom samples (n = 18), and (F) chronic benthic diatom samples (n = 18) over time.

Index scores obtained for the TDI indicated a poor environmental state. As with the GDI, the TDI analyses (Figure 20) revealed that both the epiphytic and benthic diatom communities for the eight-week exposure groups tended to have overall higher index scores across the exposure range than the 48-hour exposure groups. However, paired T-tests results did not indicate significant differences between the acute and chronic diatom communities. Supplementary information can be found in Table B4 of the annexure.

The %PT results obtained for the epiphytic diatom community (Figure 21 A & B) indicated that for the acute exposure groups, exposures 0.1, 1, and 10 $\mu\text{gPt/L}$ had little to no pollution tolerant taxa present after 48-hours of exposure. In contrast, the 100 and 500 $\mu\text{gPt/L}$ exposure groups showed a higher presence of pollution tolerant taxa after 48-hours of exposure. The opposite was observed for the chronic exposure epiphytic diatom communities. The 0.1 and 1 $\mu\text{gPt/L}$ exposure groups showed higher levels of pollutant tolerant taxa than that of the 10, 100, and 500 $\mu\text{gPt/L}$ after eight weeks of exposure. Paired T-tests indicated that the 1 $\mu\text{gPt/L}$ ($p < 0.05$) showed an increase, and the 500 $\mu\text{gPt/L}$ ($P < 0.05$) showed a decrease over time. The benthic diatoms sampled from the rocks (Figure 21 C & D) showed a presence of pollutant tolerant taxa after 48-hours of exposure. However, after eight weeks of exposure, only the 1 and 10 $\mu\text{gPt/L}$ exposures showed much higher levels of pollutant tolerant taxa than that of the control. When comparing individual benthic diatom communities sampled from the rocks, paired T-tests indicated a significant difference for the 0.1, 10, 100 $\mu\text{gPt/L}$ ($p < 0.05$) exposure concentrations, respectively with 0.1 $\mu\text{gPt/L}$ showing a decrease and the 10 and 100 $\mu\text{gPt/L}$ showing an increase in pollution tolerant taxa over time. The chronic exposure results of the benthic diatom community sampled from the sediment (Figure 21 E & F); indicated that all the exposure concentrations except for the 1 $\mu\text{gPt/L}$ exposure showed a considerable increase in pollution tolerant taxa after the eight-week exposure period. Paired T-tests indicated that 0.1, 10, 100, and 500 $\mu\text{gPt/L}$ all had p values of $p < 0.05$, indicating a significant increase in pollution tolerant taxa over time.

The pollution sensitivity results showed that the acute epiphytic diatom community (Figure 22 A & B) IPS scores varied throughout the exposure ranges, with the 0.1, 10, and 500 $\mu\text{gPt/L}$ exposures being lower than the 1 and 100 $\mu\text{gPt/L}$ exposures concentrations. The chronic epiphytic diatom communities showed a trend where pollution sensitivity increased as the exposure concentrations increased. However, the paired T-tests indicated no significant differences for the epiphytic diatom communities over time. Supplementary information can be found in Table B4 of the annexure.

Results for the benthic diatom community sampled from the rocks and sediment reflected the opposite of the effect found for the epiphytic community. For the diatom community sampled from the rocks (Figure 22 C & D), the acute exposure results showed that the pollution sensitivity

increased as the exposure concentrations increased. After eight weeks of exposure, a variation in pollution sensitivity was observed, with the 0.1, 10, and 500 µgPt/L exposures being lower than that of the 1 and 100 µgPt/L exposures concentrations. It was also noted that the benthic diatom community sampled from the sediment indicated that the acute exposure community showed an overall higher index score than that of the chronic exposure community. Paired T-tests indicated no significant differences over time for any benthic diatom communities. Supplementary information can be found in Table B4 of the annexure.

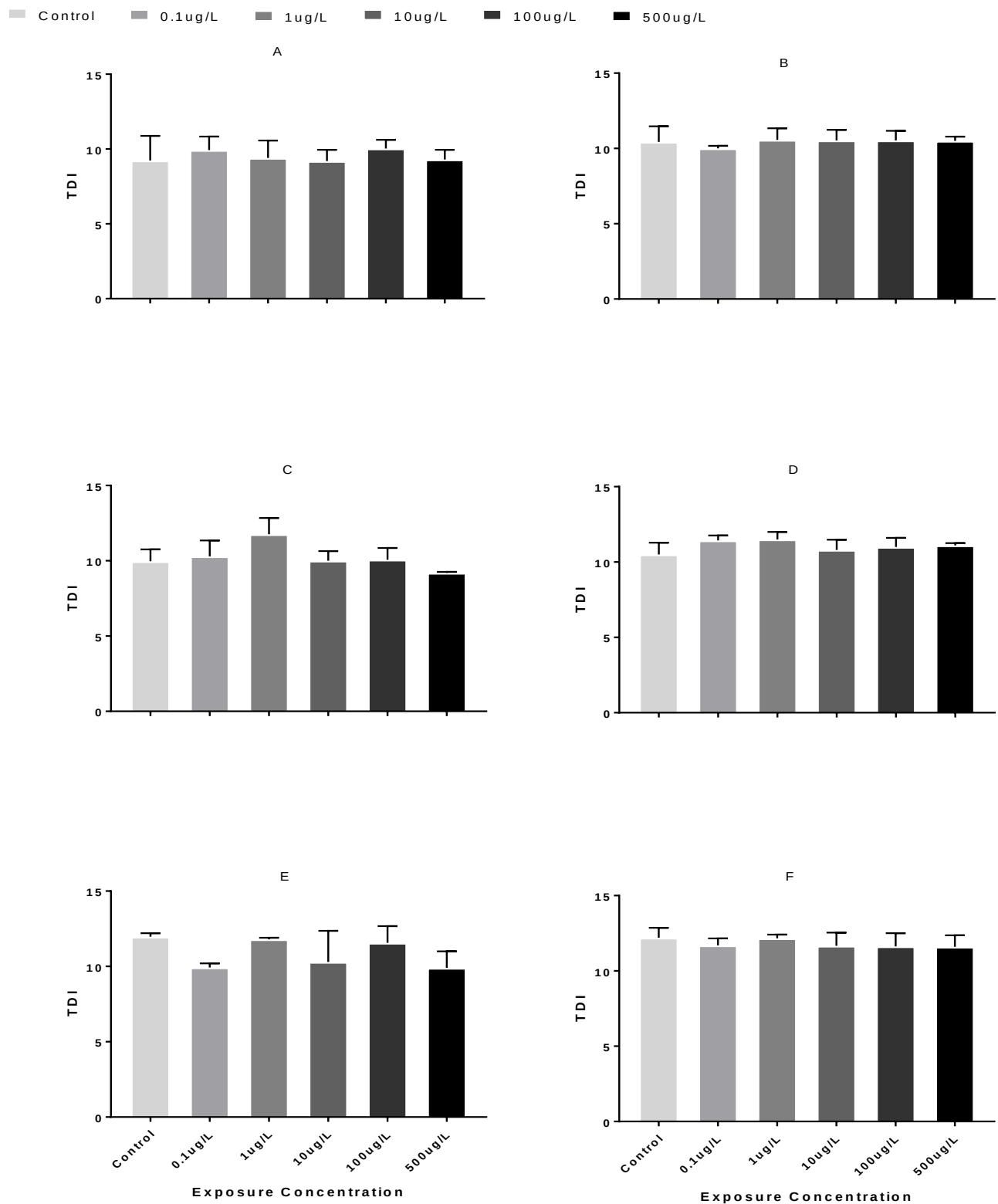


Figure 20: Trophic Diatom Index (TDI) illustrating the OMIDIA diatom community composition results obtained for (A) acute epiphytic diatom samples (n = 18), (B) chronic epiphytic diatom samples (n = 18), (C) acute benthic diatom samples (n = 18), (D) chronic benthic diatom samples (n = 18), (E) acute benthic diatom samples (n = 18), and (F) chronic benthic diatom samples (n = 18) over time.

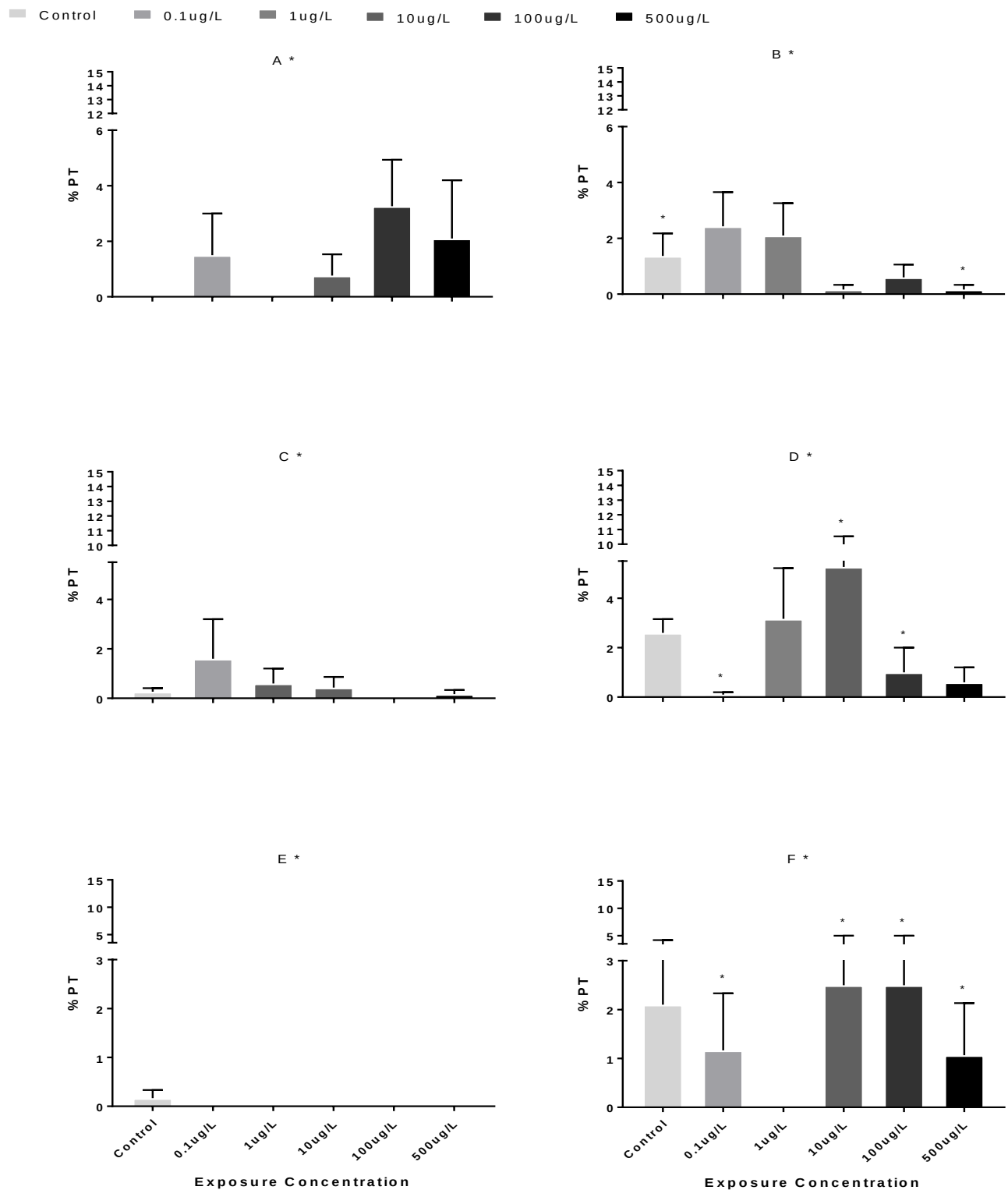


Figure 21: Pollutant Tolerant Taxa Index (% PT) indicating the OMIDIA diatom community results on the presence pollution tolerant taxa for (A) acute epiphytic diatom samples (n = 18), (B) chronic epiphytic diatom samples (n = 18), (C) acute benthic diatom samples (n = 18), (D) chronic benthic diatom samples (n = 18), (E) acute benthic diatom samples (n = 18), and (F) chronic benthic diatom samples (n = 18) over time. Significant differences over time are indicated with an asterisk (*).

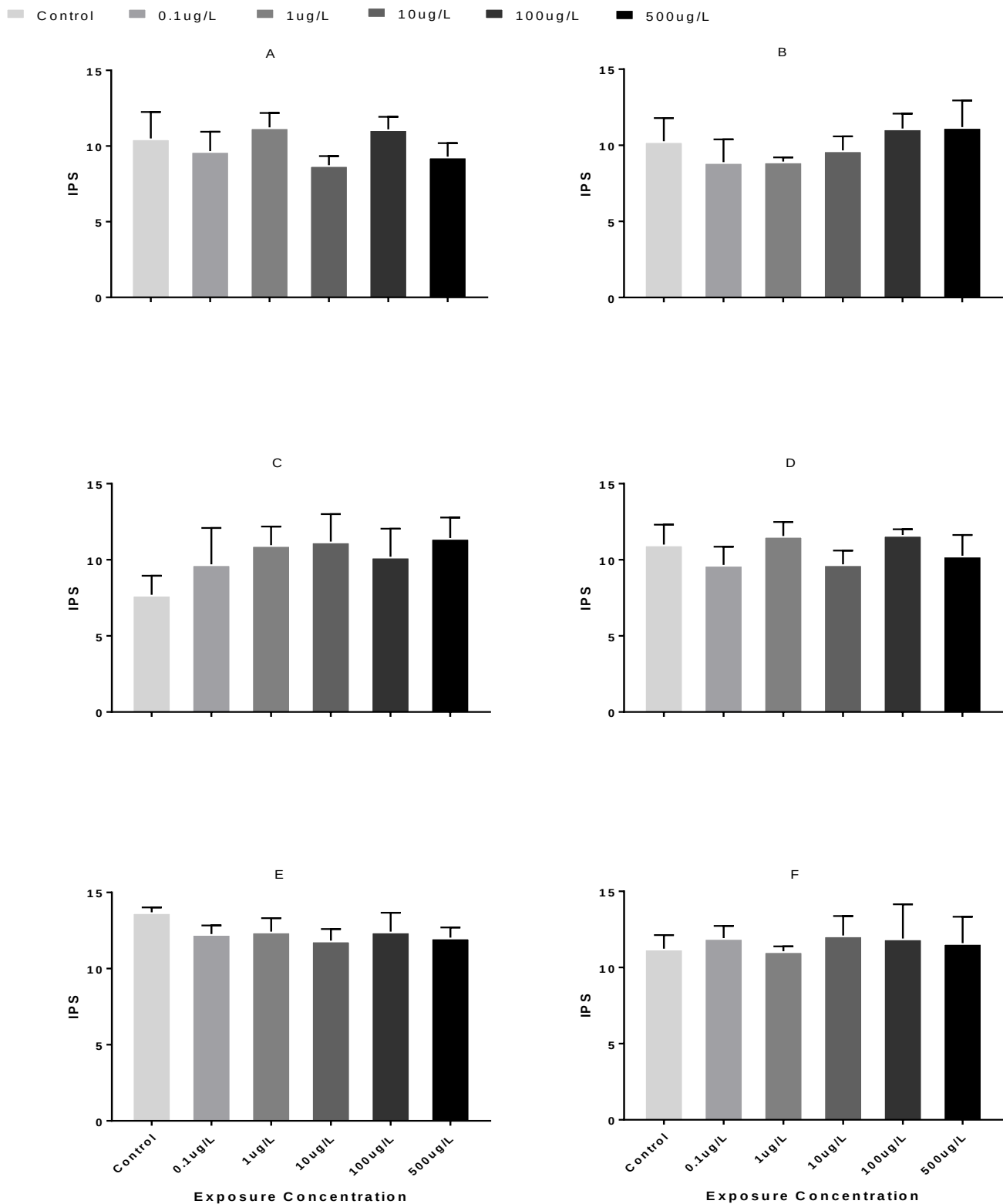


Figure 22: Pollution Sensitivity Index (IPS) indicating the OMIDIA diatom community results for (A) acute epiphytic diatom samples (n = 18), (B) chronic epiphytic diatom samples (n = 18), (C) acute benthic diatom samples (n = 18), (D) chronic benthic diatom samples (n = 18), (E) acute benthic diatom samples (n = 18), and (F) chronic benthic diatom samples (n = 18) over time.

4.8 Principal Component Analysis (PCA)

Results of the PCA analysis found that both the epiphytic (first axis = 26.98% and second axis = 16.98%) (Figure 23) and the benthic (rock) (first axis = 45.70% and second axis = 15.80%) (Figure 24) diatom communities showed a distinct difference between the acute (black circle) and chronic (blue square) exposure groups. The diatom community sampled from the sediment (first axis = 38.86% and second axis = 15.53%) (Figure 25) also indicates a difference in the diatom communities between the acute and chronic exposure groups. However, the difference between the benthic (sediment) community and epiphytic and benthic (rock) communities is that all the acute and chronic exposures except for the acute control and 500 µgPt/L exposures were grouped much closer to each other. The biplot also suggests a substantial difference as the diversity and species composition of the diatom community indicate a significant change over time. Furthermore, the results showed that the main driver of change for all diatom communities was represented by the time stated on the first axis (X-Axis), followed by the dose concentration indicated by the second axis (Y-axis). The biplot represented the diversity and species composition of the diatom communities over time. A list of the diatom species used for the PCA can be found in Annexure B Table B1.

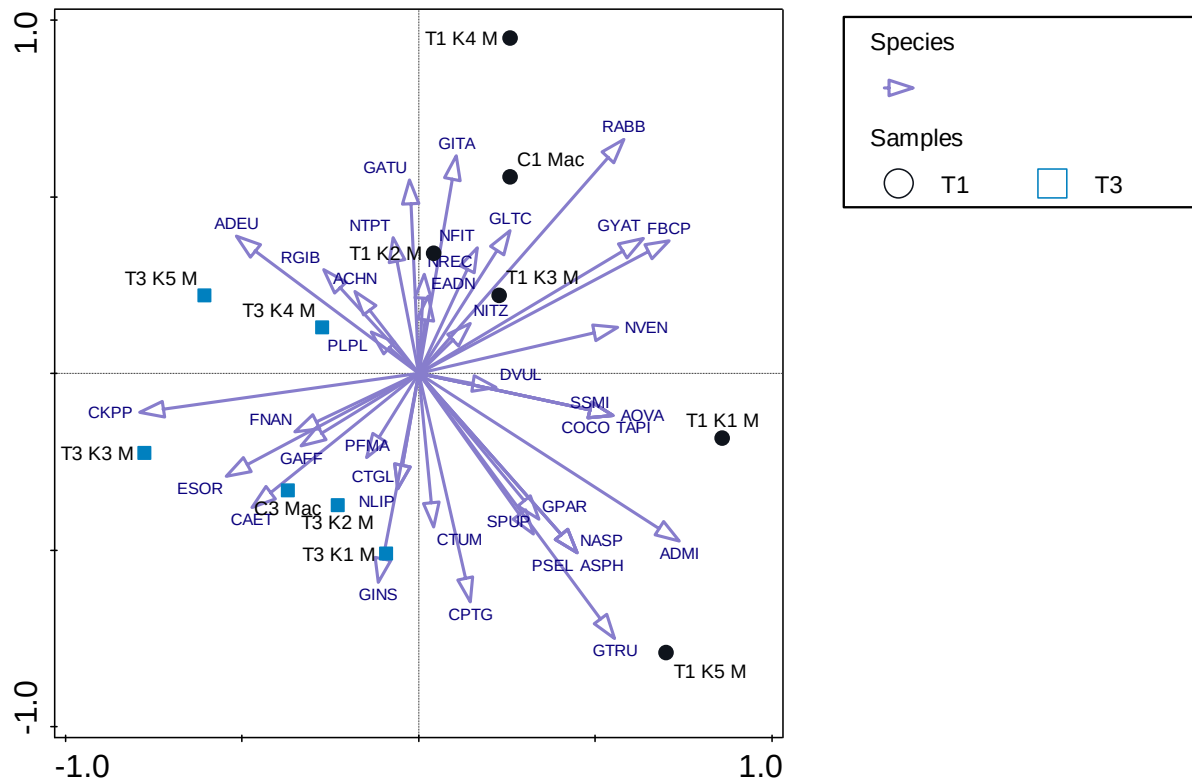


Figure 23: Principal Component Analyses (PCA) of the 48 hour (black) and eight week (blue) epiphytic diatom communities. The biplot explains the diatom species composition throughout the exposure time with 39 species. The first axis defines 26.98% of the data variation, and the second axis explains 16.98%. With T indicating the sample time, K indicating the respective exposure concentrations, and M indicating that the sample was collected from the macrophytes. Full species names for respective abbreviations used in this figure can be found in the supplementary information in Table B1 in the annexure.

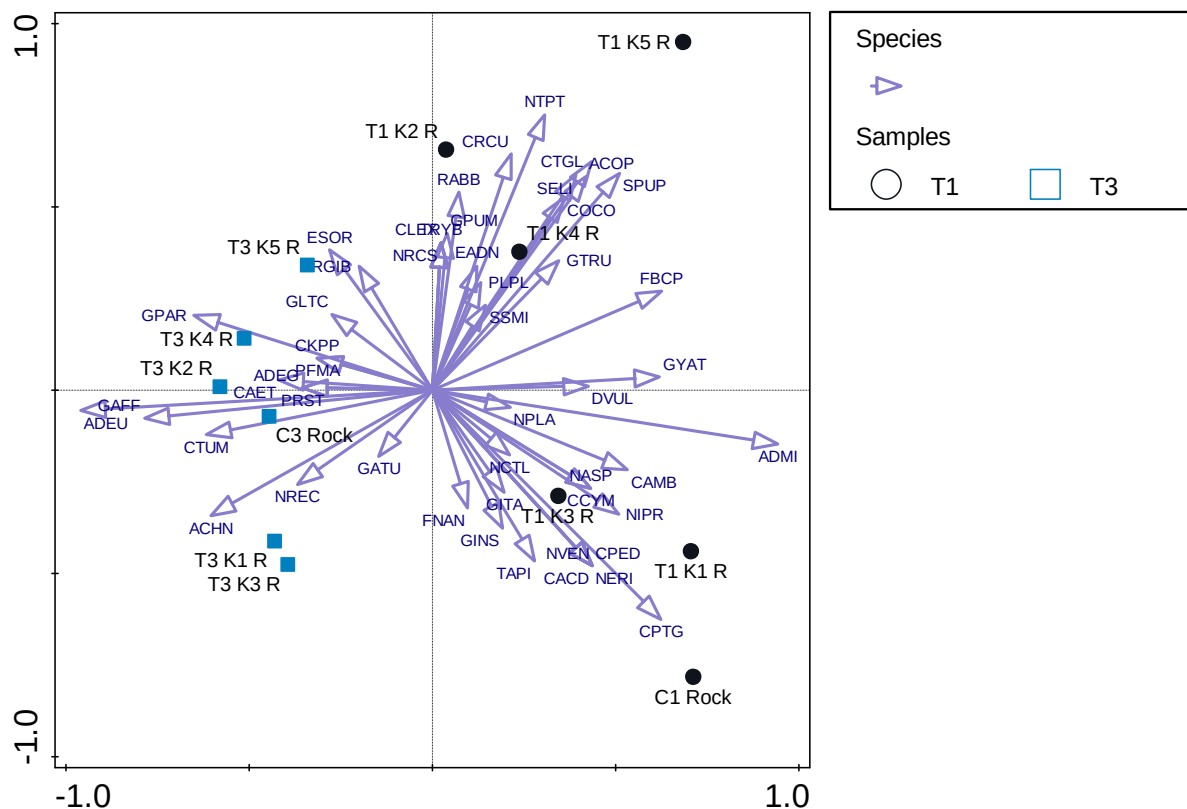


Figure 24: Principal Component Analyses (PCA) of the 48 hour (black) and eight week (blue) benthic diatom communities. The biplot presents the diatom species composition throughout the exposure time with 50 species. The first axis defines 45.70% of the data variation, and the second axis explains 15.80%. Full species names for respective abbreviations used in this figure can be found in the supplementary information in Table B1 in the annexure.

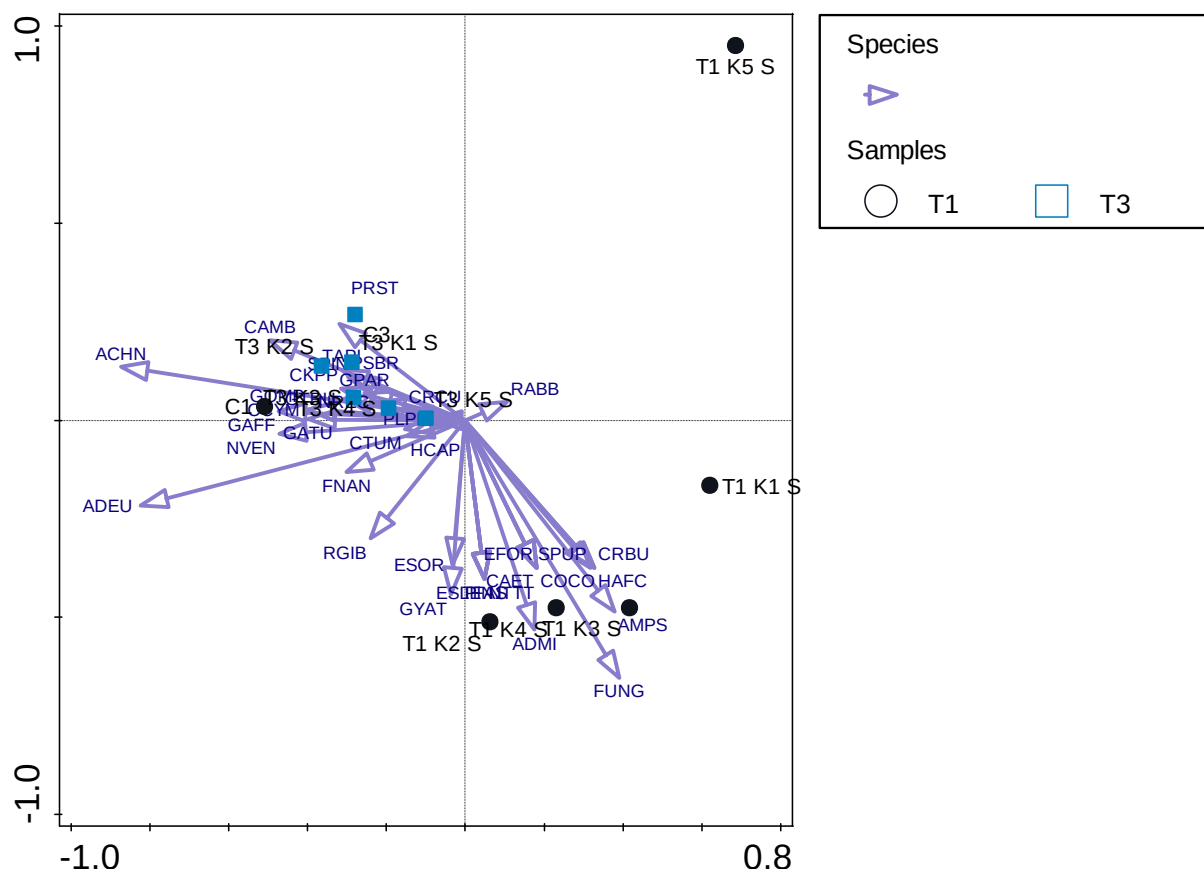


Figure 25: Principal Component Analyses (PCA) of the 48 hour (black) and eight week (blue) benthic diatom communities. The biplot presents the diatom species composition throughout the exposure time with 53 species. The first axis defines 38.86% of the data variation, and the second axis explains 15.53%. Full species names for respective abbreviations used in this figure can be found in the supplementary information in Table B1 in the annexure.

To visualise the effects of Pt on the different diatom community assemblages, a combined PCA was performed to evaluate the dis/similarities between the three samples (epithetic (black), benthic rock (blue), and benthic sediment (green)) sites and their communities (Figure 26). The results indicated that the differences observed between the epiphytic and benthic diatom communities were sample site-dependent (X-axis = 55.09%). The variation in each diatom community is explained by time (Y-Axis = 11.87%). The benthic (sediment) diatom community showed a definitive difference in community assemblage than the epiphytic and benthic (rock). The epiphytic diatom communities did not demonstrate a temporal difference from the benthic communities.

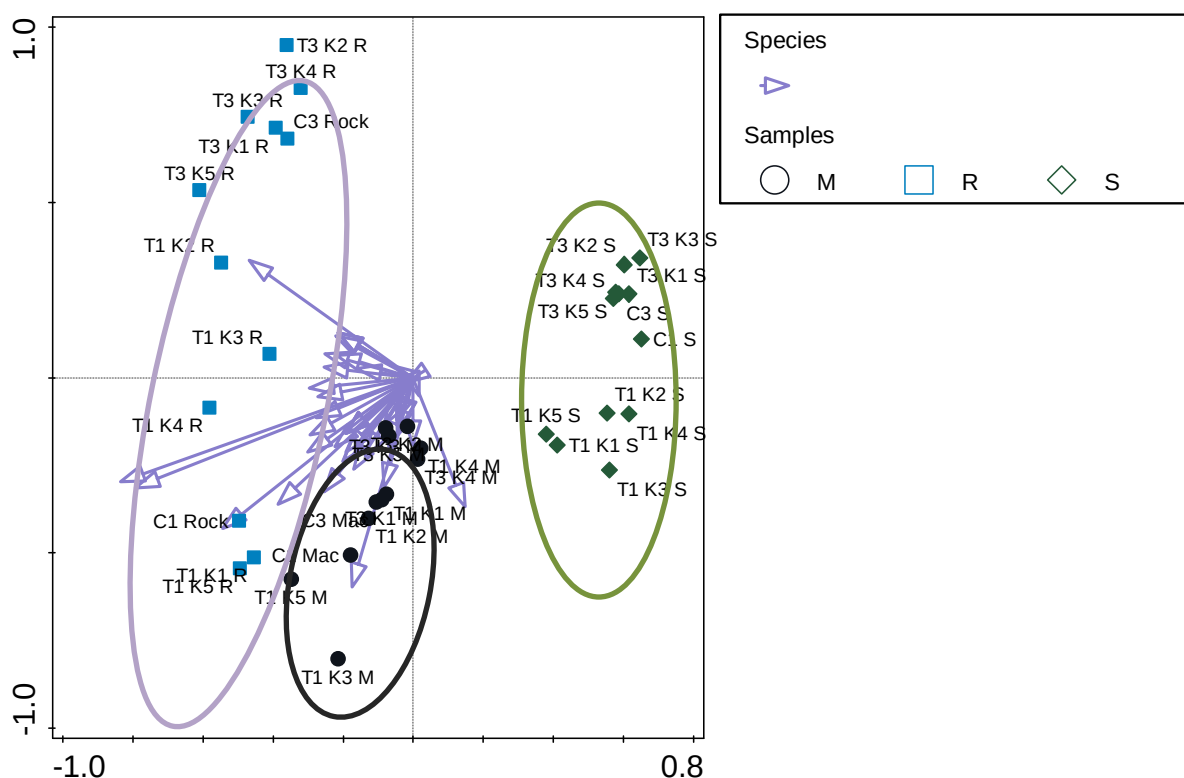


Figure 26: Principal Component Analyses (PCA) of the integrated multivariate analysis for the 48-hour and eight-week samples. The biplot presents the diatom species composition throughout the exposure time with 80 species. The first axis defines 55.09% of the data variation, and the second axis explains 11.88%. Full species names for respective abbreviations used in this figure can be found in the supplementary information in Table B1 in the annexure.

4.9 Principal Response Curve (PRC)

Effects of the Pt exposure concentrations on the diatom taxa over time can be seen in Figure 27 as a Principal Response Curve (PRC). The PRC weights were interpreted as follows; a positive PRC weight indicates a decrease in species numbers after exposure to the range of Pt, whereas a negative PRC weight suggests an increase in species numbers. *Craticula ambigua*, followed by *Gyrosigma attenuatum* and *Sellaphora pupula*, respectively, showed the highest PRC weight indicating that these species numbers decreased more in response to the Pt exposures when compared to the other species. However, results indicated that a total of 15 of the 25 species (species above the dotted line) showed a decrease in species numbers throughout the experimental time after being exposed to Pt. *Acnathes sp.*, followed by *Achnanthisidium eutrophilum* and *Gomphonema laticollum*, respectively, showed the lowest species weight out of the remaining ten species, indicating a noticeable increase in these species' numbers throughout the experimental time after being exposed to Pt.

All the exposure groups, except for the 500 µgPt/L, showed an increase in the diatom community duration of the study. It was noted that the 0.1 and 10 µgPt/L exposures showed that diatom communities increased at a slower rate than the 1 and 100 µgPt/L diatom communities, indicating that the 0.1 and 10 µgPt/L exposures had a potentially harmful effect on these diatom communities. The 500 µgPt/L exposure showed no changes in the diatom community as the acute and chronic exposure groups showed a species weight of 0.0 PRC. The Monte Carlo permutation test results indicated that Pt exposure had no significant ($p=0.934$) effects on any diatom communities over time.

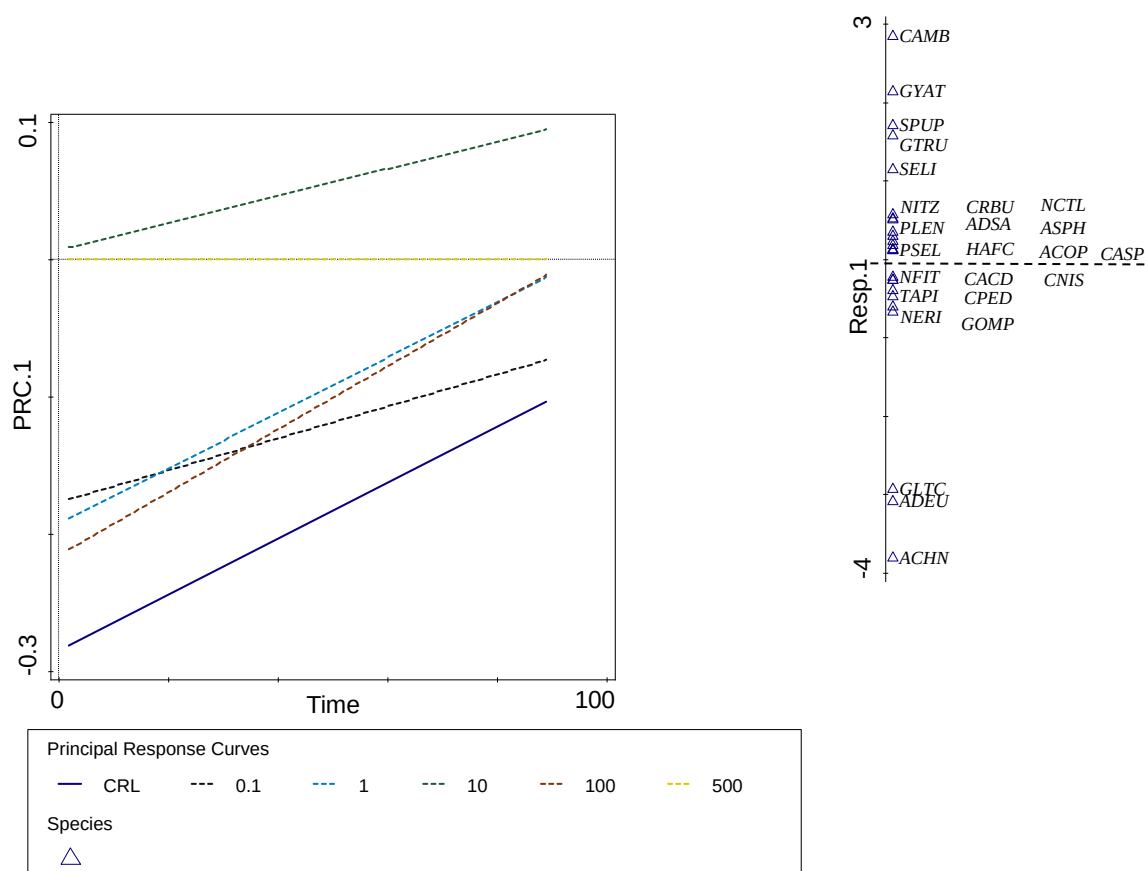


Figure 27: Principal response curve (PRC) indicating the effects of the different Pt concentrations on the Diatom community. The first axis explains 20.1% of the variance with the 25 best fitting Species. A complete species list can be found in Table B5 with supplementary information in table B2 of the annexure.

4.10 Diatom deformities after eight weeks of Pt exposure

Deformities (referred to as diatom teratologies) were identified in epiphytic and benthic diatom communities of the chronic exposure groups (Figure 29). The teratology percentage (%) was calculated by determining the rate of diatom teratologies found within each diatom community using $(\text{total teratologies}) / (\text{total individuals assessed}) \times 100 = \% \text{ teratologies}$. Teratology significance was set at $>1\%$ of the total diatom community considered. In total, 25 type 1 (diatom

valve (frustule)) teratologies were identified from four 500 $\mu\text{gPt/L}$ chronic exposure samples, resulting in a significant number of diatom teratologies, with 1.025% of teratologies identified for the epiphytic diatom community and a 1.875% of teratologies for the benthic diatom community.

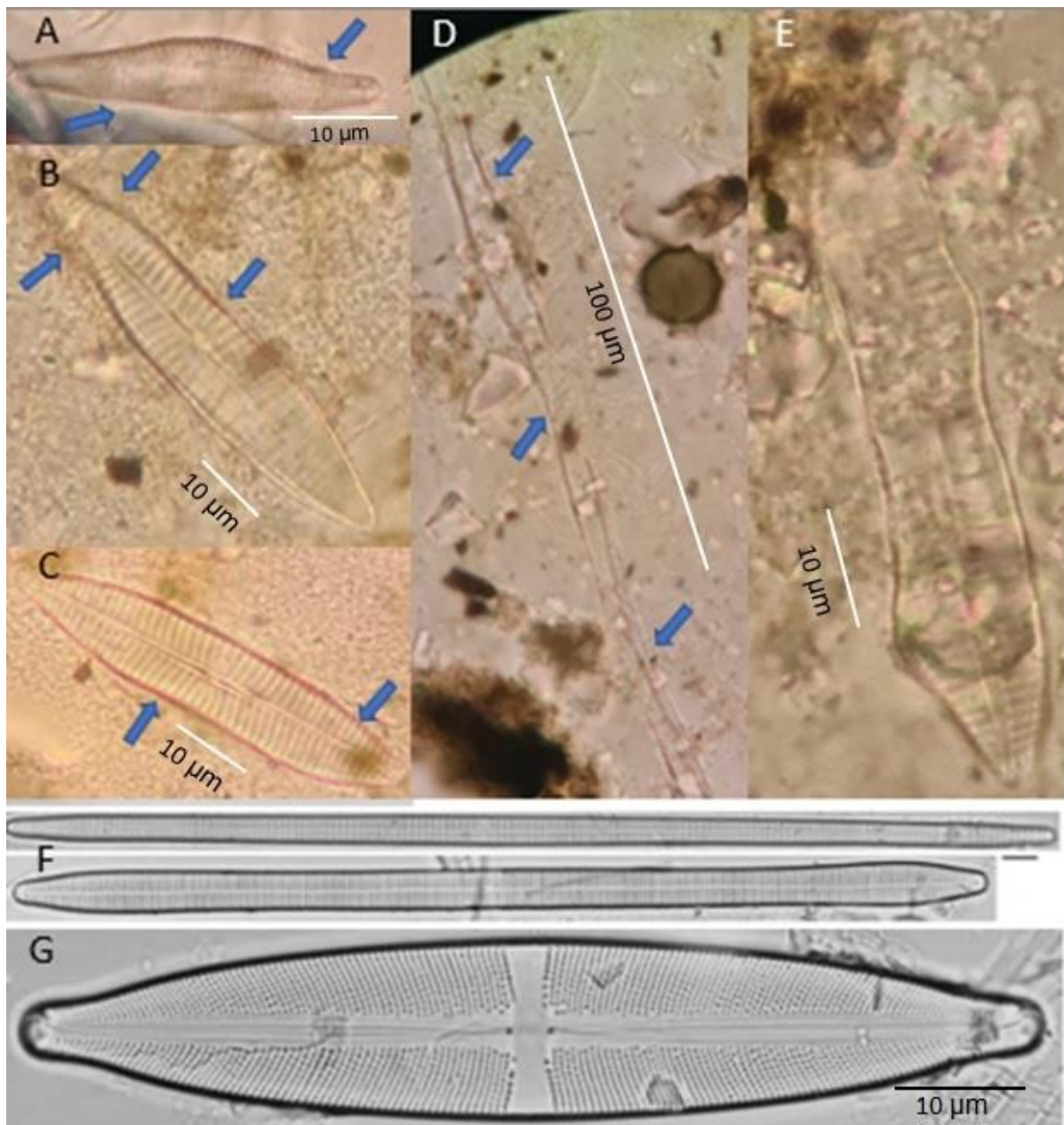


Figure 28: Diatom teratologies that were identified in the epiphytic and benthic chronic 500 $\mu\text{gPt/L}$ Pt exposure groups with blue arrows indicating frustule teratologies for (A) unidentifiable pennate species, (B) deformed *Navicula tripunctata*, (C) deformed *Navicula tripunctata*, (D) deformed *Fragilaria biceps*, (E) unidentifiable pennate species when compared to, (F) normal appearance of *Fragilaria biceps* and (G) normal symmetric pennate specimen.

CHAPTER 5: DISCUSSION

5.1 Water Quality

Microcosms were used to assess the partitioning of Pt to different environmental compartments. Static microcosms provided a semi-controlled environment where biotic and abiotic compartments were exposed to a range of Pt concentrations and monitored over time. With the successful utilisation of the variety of diatom taxa, macrophyte species, and mimicked aquatic environmental compartments, microcosms proved to be a valuable tool to characterise the behaviour of Pt and its potential impacts on aquatic communities.

Due to the experimental design, the microcosms were exposed to various environmental factors that potentially alter and simulate natural water quality parameters. The water quality was monitored as a variation in nutrient levels, and physicochemical parameters are known to be major drivers of change in the aquatic environment with a potential influence on the behaviour of metals (Erasmus, 2020). Past studies that utilised static microcosms found that established biotic communities (e.g., macrophytes, macroinvertebrates, and diatoms) tend to deplete the nutrients (Nitrates, nitrites, ammonium, and ammonia) and oxygen concentration within microcosms (de Necker, 2019; Radu *et al.*, 2015; Talling, 2001). Nutrients are used by aquatic biota and plants as food and for various biological processes (Dallas and Day 2004; Mirzahasanlou *et al.*, 2019; Radu *et al.*, 2015; Wiegner and Seitzinger, 2001). The same trend was observed in this study during the first four weeks of exposure. However, after four weeks, the abundance of *Charophyta* sp. declined remarkably (Pers. Obs. A.C. Smit), leading to an increase in nutrient and turbidity levels. Due to the nature of microcosms having no additional nutrient inputs from the environment, the disruption of plant nutrient cycling, decomposition of plant material and other organic material in the sediment explained the increase of nutrients and the turbidity levels (Asaeda *et al.*, 2000; Geurts *et al.*, 2010). The rise in salinity (measured through EC, TDS, and Cl⁻) throughout the study period was due to water evaporation, leading to increased concentrations of salts (Boersma *et al.*, 2014; Batzer *et al.*, 2016; de Necker, 2019). The overall pH of the water during the project was slightly alkaline, ranging between 8-9, due to Potchefstroom's natural groundwater (pH of 8.60) used to maintain the water levels of microcosms (Maguvu *et al.*, 2020). Therefore, the variation in pH observed between exposure groups is also the result of the increased salinity and Cl⁻ levels due to water evaporation. The COD indicates the oxygen required for the oxidation of organic material in the water body and indicates the presence of metal pollutants and other stressors in a water body. The presence of contaminants will result in higher COD due to oxidative stress (Boyles, 1997). It was found that the COD in this study showed the opposite as COD decreased over time. This can be explained by the enclosed nature of the microcosms since no additional input of organic material, or

supplementary metal exposures (other than the experimental exposures) took place throughout the experimental period that could have resulted in an altered COD level (Bhuiyan *et al.*, 2016).

Based on the South African water quality guidelines for environmental water quality, all physicochemical parameters and nutrient levels were within the prescribed criteria. They were indicative of an oligotrophic system. The temperature (varying more than 10% from the daily average temperature) and chlorine (being more than 5 µg/L) measurements (DWAF, 1996) were found to deviate from the TWQR. The temperature fluctuations observed during the project were due to weather patterns and seasonality, and this was as expected as the microcosms were exposed to the natural elements. Overall, the temperature slowly declined from March to July due to the winter months in the Southern hemisphere.

5.2 The accumulation of Pt in the water and sediment

According to laboratory-based PGE exposures completed previously, one of the major impediments is the loss of dose metal concentrations in the water phase (Brand *et al.*, 2019; Zimmermann and Sures, 2018). Apart from active biological uptake, non-biological processes affect Pt concentrations in the exposure environment, especially the precipitation and accumulation of Pt to tank surfaces and mussel shells (Sures and Zimmermann, 2007). The same impediments applied to the present study. Platinum concentrations found in the water samples showed that Pt was present in the water column throughout the experimental period and that recovered aqueous Pt concentrations were found to be dose concentration dependant. However, none of the sample concentrations reflected the exact dose concentration irrespective of the sample time, indicating a loss of dose material due to compartmentalisation to other environmental compartments.

Furthermore, Pt concentrations in the water column decreased over time as a significant difference in metal concentration was visible between the exposure times. With the highest concentration of Pt in the aqueous solution found in the microcosms with the highest dose concentration (500 µgPt/L), the exposure study conducted by Brand *et al.*, (2019) found that water concentrations for Pt ranged between 0.006 – 2.6 ngPt/L when exposures were done with concentrations ranging from 0.1 to 1000 µgPt/L. These results indicate that the current study's findings are correspondent to existing literature. When considering the enclosed nature of this study, Pt dose concentration lost from the water phase, (and the fact that biotic removal was less than 10% in all exposure groups) can be ascribed to non-biological processes (e.g., precipitation, partitioning) (Brand *et al.*, 2019; Ruchter *et al.*, 2015).

It was further confirmed as detectable Pt concentrations were found in the sediment sample for all the exposure concentrations. According to multiple studies, sediments tends to accumulate higher concentrations (between 3-14 µg/L Pt in soil with a maximum of 0.9 ng/L in water samples) of Pt compared to other aquatic environment compartments due to the high levels of organic carbon found in the sediment layer and metal precipitation from the water column (Eller *et al.*, 1989; Lustig *et al.*, 1998; Moldovan *et al.*, 2001; Moldovan *et al.*, 2003; Bowles and Gize, 2005; Cobelo-Garcia *et al.*, 2007; Cobelo-Garcia *et al.*, 2015; Frazzoli *et al.*, 2007; Sures and Zimmermann, 2007; Matthey, 2012; Ruchter *et al.*, 2015; Zimmermann and Sures, 2018; Erasmus, 2020; Dias-Morales *et al.*, 2021). Although the accumulation concentrations for 0.1, 1, 10 µgPt/L were less than expected, the 100 and 500 µgPt/L results still indicated that compartmentalisation to the sediment did take place and was consistent with existing literature.

However, only the Pt concentrations recovered from 100 and 500 µgPt/L exposures were found to be dose concentration dependant. For the 100 µgPt/L exposure, no change in Pt concentration over time indicated that Pt saturation did not occur at this exposure concentration. For the duration of the experiment, the sediment Pt concentration of 500 µgPt/L exposure decreased over time, acting as a source of Pt in the aquatic environment (Ruchter *et al.*, 2015), indicating that saturation of the sediment compartment occurred.

When comparing these two environmental compartments with existing literature, the results obtained were expected and reported by other researchers, as stated earlier. It was found that 100 µgPt/L aqueous Pt concentrations tended to partition very fast in the aquatic environment, where the 500 µgPt/L is slower to partition as the Pt was more abundant and that higher accumulation concentrations were found in the compartments of the 500 µgPt/L concentrations. However, according to the results of Pt concentrations found, the sediment exposed to the 500 µgPt/L showed a tendency to adsorb Pt faster with higher Pt concentrations compared to the sediment exposed to the 100 µgPt/L exposure groups. Indicating that higher Pt concentrations have the potential to accumulate faster to the sediment and could have a more severe impact on bottom-dwelling benthic organisms.

5.3 The accumulation of Pt in the macrophytes and artificial mussels

Macrophytes were selected as a sampling compartment because aquatic plants are primary producers in the aquatic environment and form the all-important first link in the food chain. The

ability of plants to absorb Pt from the soil may pose a significant threat to aquatic ecosystem health (Feichtmeier and Leopold, 2015; Miranda *et al.*, 2022). According to Pawlak *et al.* (2014), Pt concentrations in the soil or sediment can be absorbed into terrestrial plants via biological

pathways with decreasing accumulation concentrations in order of root>stem>leaves. The Pt source can also substantially influence exposures, affecting uptake and biologically available routes. Soluble Pt can be absorbed much quicker than particle-bound Pt, and the size of the particle-bound Pt and the binding strength thereof can enhance the uptake of Pt (Zimmermann *et al.*, 2015). In a study conducted by Shah *et al.*, (2010), heavy metal toxicity in plants was found to be dependent on the bioavailability and solubility of the metal at hand. Furthermore, in the case of excessive metal uptake or accumulation, elevated levels of a heavy metal may replace essential metals leading to pigment and enzyme abnormalities affecting plant growth and causing oxidative stress (Martins *et al.*, 2004; Shah *et al.*, 2010; DalCorso, 2012; Gupta *et al.*, 2013). Gupta *et al.*, 2013 stated that macrophytes such as the *Charophyta sp.* are known to be good indicators of heavy metal pollution since these macrophyte species are rooted in the sediment and, as such, are more sensitive than floating plants. Heavy metals such as chrome (Cr) are known to have disruptive effects on *Charophyta sp.* with exposures greater than 400 µg/L being toxic, resulting in macrophyte death (Gupta *et al.*, 2013). This forms a critical basis to explain the deteriorating population of *Charophyta sp.* observed for only the 500 µgPt/L exposures. As this study is conducted in an aquatic environment, other aspects have to be considered as part of the uptake dynamics in the plants, such as aqueous Pt concentrations and sediment Pt concentrations. This is because metal accumulation in plants can be linked to Pt deposition on external plant surfaces as well as various other uptake mechanisms. The macrophyte species used for this study differed in the sense that they were either completely submerged in the water column and anchored to the sediment (*Charophyta sp.*) or anchored to rocks and completely exposed (roots, leaves, and stem) to the aqueous Pt concentrations (*F. antipyretica*).

The Pt dose was administered to the water column, thus as with the sediment, partitioning/accumulation of Pt had to take place for the plants to have a detectible Pt concentration. Platinum accumulation in plants increased as exposure concentrations increased, thus indicating that aquatic macrophytes actively accumulated Pt. Pawlak *et al.*, (2014) state that different plant species vary significantly in their ability to accumulate Pt concentrations due to various biological activities (metabolic pathways). When considering the different Pt concentrations recovered from the macrophyte samples for this study, there was a distinct difference between the two macrophyte species used. The reasons behind this could be where they grow and possibly their metabolic pathways responsible for Pt uptake (Niemela *et al.*, 2007). These aspects were consistent with the results as it was determined that *F. antipyretica* had higher concentrations of Pt than that of *Charophyta sp.* This suggests that *F. antipyretica* will potentially be a good indicator of aqueous Pt contamination within an aquatic ecosystem, even at low exposure concentrations (Siebert *et al.*, 1996; Martins *et al.*, 2004). Since these plant species are adapted to absorb nutrients directly from the water column (Wersal and Madsen, 2011).

There is no information available on the maximum concentration of Pt that can be absorbed to plant material, and as such, it is difficult to conclude whether these compartments became saturated or not or whether they could potentially accumulate higher levels of Pt. (Miranda *et al.*, 2022). According to Pawlak *et al.* (2014), it is known that aquatic plants do accumulate Pt. Still, there is no data available on the rate at which the maximum concentration of any metal will absorb into an aquatic plant. However, it was only stated that metal accumulation tends to be dose-dependent, indicating that the same trend is visible for the macrophytes as suggested by Ruchter *et al.* (2015) as for the water column.

5.4 Bioavailability of Platinum to aquatic biota.

The next biological compartment of interest is the bioavailability of Pt since there is limited data on the bioavailability and bioaccumulation of Pt to animals under environmental conditions. Most papers addressing this issue describe research performed in the laboratory (Zimmerman *et al.*, 2015; Brand *et al.*, 2019). Bioaccumulation of toxic substances within aquatic organisms signifies a significant ecological occurrence and reflects the biological availability of pollutants in an ecosystem (Binelli *et al.*, 2015). Thus, aquatic invertebrates have an essential ecological role since they could, in theory, diffuse pollutants throughout the food web (Baun *et al.*, 2008). Specific characteristics make these organisms a valuable option as a biological model. An example is bivalves (mussels) which serve as sentinel organisms to monitor pollutants in the aquatic environment due to their filter-feeding, bottom-dwelling nature, and ability to bioaccumulate metals from the environment, thus providing a time-dependent reflection of pollution in the given setting (Roesijadi *et al.*, 1984; Sericano, 1993).

A correspondence between recovered concentrations and dose concentrations was found when looking at the results obtained for the AM sampled. The AMs are meant to mimic the biological environment. However, these devices do not have any feeding or biological processes confirming that the difference in Pt concentration led to the accumulation of Pt over a concentration gradient and thus reflect the potential bioavailability of Pt across a range of concentrations present in the aquatic environment (Gonzalez-Ray *et al.*, 2011). This result was as expected due to the difference in Pt concentrations in the Chelex[®] beads in the AMs and water column. It has been observed that the degree of accumulation of metal in the AM showed a positive relation since Pt accumulation increased with increasing exposure concentrations over time (Zimmermann *et al.*, 2015). In the aquatic setting, the accumulation of pollutants occurs at all levels simultaneously, which means that Pt accumulation can occur through ingestion of organic compounds, plants, and other aquatic organisms and via absorption of Pt dissolved in the water column within the same time frame (Zimmermann *et al.*, 2015; Labuschagne *et al.*, 2021). According to Vannini *et al.* (2011) and Schmid *et al.* (2007 & 2018), Pt is a known inducer of reactive oxygen stress (ROS)

in aquatic invertebrates and human lung epithelial cells, as demonstrated in toxicity studies when exposed to Pt (II) and Pt (IV), respectively (Schmid *et al.*, 2007). Metal bioaccumulation in the tissue of aquatic organisms can lead to ROS production, leading to oxidative stress, which inhibits the cells' ability to function correctly (Kono and Fridovich, 1982; Lushchak, 2011; McCord, 2000). When considering the fact that PGEs are known to have similar modes of action and cause stress in biota, the assumption can be made that Pt may have the same bioaccumulation characteristics and similar effects on biota as Pd (Zimmerman *et al.*, 2017).

5.5 Diatom assemblages

Diatoms form an integral part of the aquatic ecosystem as they are primary producers with a cosmopolitan abundance and play a key role in aquatic ecosystem function (Pandey *et al.*, 2017 & 2018). Knowledge of their sensitivity to a range of environmental factors and their rapid response to changes in these factors sparked the idea of a diatom-based water quality monitoring assessment resulting in the development of several diatom indices (Lavoie *et al.*, 2006; Coste *et al.*, 2009; Lavoie *et al.*, 2014; Mirzahasanlou *et al.*, 2019). Diatom communities have been used successfully to assess the past and present ecosystem integrity, water quality, and the impact of a wide range of pollutants (metals and pesticides) on aquatic ecosystems (Debenest *et al.*, 2010; Morin *et al.*, 2012; Lavoie *et al.*, 2017).

Diatom indices indicated an overall poor (eutrophic) ecosystem status for both the 48-hour and eight-week samples. Used in conjunction with the water quality monitoring, the observed changes in aquatic nutrients and physicochemical parameters resulted in little to no variation, change, or deterioration of the ecosystem status as water quality indicated an oligotrophic ecosystem (DWAF, 1996). Therefore, it can be stated that Pt can be considered the main driver of the results obtained from the diatom community assemblage assessment.

Diversity indices revealed that the diatom community assemblages did not change significantly for the epithetic or benthic sample sites over the eight-week exposure period. However, after considering the combined results, an interesting trend emerged. Considering the total species and species richness, there was no indication of the change within the diatom community in response to the Pt exposure since most of these indices are based on how close in numbers species are. Rather, communities changed with regard to the species that were present and which were dominant at that specific sample time. Meaning that when *Acnantes* *sp.* died off, it would be replaced with another species, such as *Achnanthidium minutissimum* (pollution tolerant), resulting in a significant shift within the diatom community structure. This occurrence will not be reported by most of the diatom indices (Tlili *et al.*, 2016; Oguma *et al.*, 2017). When an aquatic community is exposed to metal contamination, the change in the environment will

select species that can tolerate metal or pollutant-related stress, ultimately replacing sensitive species that tend to decrease or disappear depending on the type of exposure (Oguma *et al.*, 2017). This fact forms the conceptual basis of the Pollution-induced community tolerance (PICT) concept developed by Blanck *et al.*, (1988). The PICT occurs when the structure of a stressed community is rearranged to increase the overall community tolerance to the toxicant. The PICT concept explains the results of the diatom indices (BDI, GDI, and TDI) and Principal Response Curve (PRC) analyses, which indicated an initial negative effect on the diatom community whereafter the community abundance increased or stabilised. The PICT also corresponds to the pollution tolerant taxa and pollution sensitive indices. The pollution sensitivity index (IPS) indicated equal levels of pollution sensitivity for each sample site for both sampling times, confirming the acute and chronic effects of Pt.

Furthermore, the pollution tolerant taxa index (%PT) results showed a marginal increase in diatom taxa that are pollution tolerant. Especially for the benthic (sediment) community, a significant increase for the 0.1, 10, 100, and 500 µgPt/L exposures indicates a change in the diatom community structure between the 48-hour and 8-week samples. The epiphytic and benthic (rock) communities also indicated increased pollution tolerant taxa in the 0.1, 1, 10, and 100 µgPt/L exposures.

Furthermore, PCA analyses of each of the three-diatom communities indicated a change between the initial and chronic sample groups based on their community structure. The benthic (sediment) diatom community showed a different result than that of the epiphytic and benthic (rock) PCA, as a distinct change in species dominance within the community was observed. However, the changes observed in the PCA's relates to the %PT results. The %PT and PCA results indicated that the benthic (sediment) diatom communities experienced a significant negative response to Pt exposure (chronic exposure) over time. Sediment is known to accumulate high levels of metals and tends to act as an environmental sink for metal pollutants due to high levels of organic matter in the sediment (Zereini and Wiseman, 2015; Alméjija *et al.*, 2017; Erasmus *et al.*, 2020; Diaz-Morales *et al.*, 2021). The ecotoxicological characteristics of metals, in general, suggest that a

metal pollutant should have a more adverse effect on the community assemblages found in and on the sediment since higher levels of metal pollutants will be found in this environmental compartment. As for the epiphytic and benthic (rock) diatom communities, Pt is also the likely driver of change since the Pt concentration present in the aqueous phase is already in the most soluble stage and biologically available.

Metal pollutant stress can be challenging to characterise. However, diatoms can reflect responses to stress through abnormalities in their cell walls (Falasco *et al.*, 2009a; Falasco *et al.*, 2009b;

Morin *et al.*, 2012; Gonçalves *et al.*, 2019). Diatoms are well known for their unique and species-specific silica cell walls and the complex biological pathways involved in forming these cell walls. These cell walls are known to be sensitive to changes in water quality associated with metal and other toxic pollutants, resulting in diatom teratologies (Morin *et al.*, 2016). However, diatoms must be addressed at different levels of organisation (individual cell to community assemblage) to effectively assess a substance's potential effect on the diatom community. According to existing literature, metal exposure is known to cause a malfunction of the cells metabolic processes (respiration, nutrient cycling, and efficiency) (Bertrand *et al.*, 2001; Husaini and Rai, 1991), alteration of reproductive method (sexual or vegetative) (Cattaneo *et al.*, 1998; Joux-Arab *et al.*, 2000) and an increase in cell mortality and immobility (Torres *et al.*, 1998). Ultimately, metals can affect community assemblages by reducing cell numbers and causing a decline in cell size within a given species (Falasco *et al.*, 2009b; Gautam *et al.*, 2017). During the identification of the diatom species, a total of 25 diatom teratologies were observed in four separate 500 µgPt/L exposure samples, to the extent where the valve symmetry and overall shape were affected. These teratologies are believed to be the direct result of Pt exposure and can consequently lead to the deterioration of the general community assemblage. The presence of diatom teratologies can adversely affect the adaptability and fitness of an individual cell to thrive and reproduce (Lavoie *et al.*, 2017), leading to a less diverse and weakened diatom community (Lavoie *et al.*, 2017).

Currently, there is no available information on the uptake mechanisms or pathways that Pt follows to have a disruptive effect on cell wall formation. However, a considerable amount of ecotoxicological research done on metals like copper (Cu), nickel (Ni), palladium (Pd), and gold (Au) exposures on diatoms by Robinson *et al.*, (1997), Chakraborty *et al.*, (2006), Morin *et al.*, (2012 & 2016), Mu *et al.*, (2017), and Gautam *et al.*, (2017), suggests that due to the molecular weight and interaction characteristics of known metals and the effect they have on diatoms, it can be hypothesized that Pt is most probably bioaccumulated in diatoms by the same uptake mechanisms found for Cu, Ni, Pd, and Au. Diatoms are known to have the ability to take up

inorganic nutrients (e.g., ammonium, iron (Fe), and copper (Cu)) through the cell membrane. These nutrients are transported through the pores of the cell wall and into the cell membrane in the dissolved ionic form via action potentials (Losic, 2017). A recent study examined the newly discovered reduction-dependent Cu uptake pathway in oceanic diatoms at low and high Cu²⁺ levels (Kong and Price, 2020). Two subcellular pathways were identified for the uptake of Cu²⁺ through the cell membrane at low and high exposure concentrations. At low-level exposure of Cu²⁺ the main uptake route was the biological mediated Cu⁺ transporter (path 1), allowing Cu⁺ to enter the cell, with a low-affinity transport of Cu²⁺ via the divalent metal transport (path 2).

However, at High Cu^{2+} exposure concentrations, the leading uptake route was through the divalent metal transport (path 2), facilitating the influx of unusable biological Cu^{2+} , negatively influencing the biological mediated Cu^+ transporter (path 1). This leads to a balance disturbance between the Cu^{2+} and the Cu^+ concentration on a subcellular level. The higher concentration of Cu can negatively influence cellular metabolism and might lead to frustule deformation (Goncalves *et al.*, 2019; Park *et al.*, 2020).

As no significant fluctuation in water quality or water nutrients was reported, the possibility of an environmental stressor other than the Pt exposure concentrations was minimised. This document is the first to state the possible effect of Pt metal exposure on individual diatoms and diatom communities. These results indicate that diatoms can be of crucial importance for further ecotoxicological and bioassessments to determine the responses of aquatic ecosystems to Pt pollution.

CHAPTER 6: CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

This research study aimed to gain knowledge regarding the characteristics and behaviour of Pt through the introduction of a known Pt concentration to a semi-controlled aquatic environment. Further, this study aimed to research the potential effects of Pt pollution on the aquatic ecosystem by assessing the environmental compartments and epiphytic and benthic diatom communities. As it was hypothesised that (1) the compartmentalisation and accumulation of Pt in the sediment, water, macrophytes, and artificial mussels (AM) will be dependent on the amount of Pt introduced to the aquatic environment and that (2) an increase of Pt in the aquatic environment will result in a decrease in pollution sensitive taxa and an increase in pollution tolerant taxa (PT%), causing a disruptive effect on the aquatic diatom community assemblages.

The various environmental and biological compartments exposed to Pt indicated that Pt compartmentalisation and accumulation did occur over the eight-week experimental time. From the samples collected, Pt concentrations in the water column and macrophytes indicated that Pt compartmentalisation for all the exposures was dose-dependent as the recovered Pt concentrations increased as the dose concentrations increased. The macrophyte, *Charophyta* sp., was more sensitive to metal pollution than *F. antipyretica* due to its rooted nature. Only the 100 and 500 µgPt/L sediment indicated a Pt compartmentalisation and accumulation trend, corresponding to previous study results. The assessment of the AM was based on the accumulation potential of Pt through the diffusion coefficient, corresponding with the findings of another study, indicating that even without biological activity (feeding, metabolism), Pt will accumulate to aquatic organisms (such as live mussels). The compartmentalisation and accumulation of Pt to the different environmental compartments in the aquatic ecosystem supported the study's first hypothesis and was found to be dependent on the amount of Pt standard solution introduced to the aquatic environment.

Only the chronic Pt exposure resulted in diatom teratologies and an increase in PT% for the diatom community assemblage. The data, however, did not determine the precise time interval when the teratologies occurred. Since the lifecycle of a diatom is between two and three weeks, this appearance could have happened earlier than the eight-week samples. With this said, diatom teratologies only occurred at high concentrations. However, the results and the discussion indicate that some of the diatom indices and communities did indicate changes at concentrations as low as 0.1 µgPt/L. This suggests that environmentally relevant concentrations of Pt can have disruptive effects on the diatom community; therefore, the second hypothesis can be accepted.

6.2 Recommendations for future studies

- It was expected that the sediment would accumulate Pt concentrations that reflect the dose concentration based on previous studies. It will be beneficial if future studies focus on Pt concentrations lower than 100 µgPt/L in the aquatic environment to form a more holistic understanding of the chronic effects of these concentrations in the aquatic environment.
- This study was based on a single exposure event per microcosm, simulating a single contamination event per microcosm. Aquatic ecosystems near mining sites and smelters are frequently exposed to Pt contamination. To address this, future studies investigating the toxicity and disruptive effects of Pt on the aquatic environment focusing on the semi field-based study with multiple exposures should be undertaken to simulate an environment that is repeatedly exposed to Pt and assess the impact of repeated Pt exposures on the aquatic environment.
- Metals characteristics and behaviour in the aqua phase are affected by pH changes. Since this study was conducted in a slightly alkaline environment, future studies focusing on the characteristics of Pt in a more acidic environment should be undertaken to assess the behaviour of Pt in these environments.
- This study found that macrophytes tend to be more reflective of Pt in the aquatic environment. Future studies focusing on the different macrophyte species can deliver insight into Pt concentration in different compartments due to their location and nutrient cycling methods.
- Diatom teratologies were observed in this study; the data, however, did not determine the precise time interval when the teratologies occurred. The lifecycle of a diatom is between two and three weeks which means that this appearance could have occurred earlier than the eight-week samples. Thus, future studies might indicate the specific time frame in which teratologies occur with samples taken at more frequent time intervals.
- Although this study indicated that Pt could cause diatom teratologies, only the PT% index showed a significant change in diatom assemblages. This result, combined with the unknown pathways Pt follows to cause these teratologies, calls for further research in this field.
- During this study, an interesting concept on the PGE behaviour in the aquatic ecosystem in the sense that, in combination, PGEs could cause synergistic effects, which could cause more severe consequences than a single PGE exposure. This consequence could be because one metal inhibits an organism's defence- and repair mechanisms, while another could freely bind and act with endogenous biological ligands. However, this has not yet

been tested, and future studies are needed to confirm the suspicion that multiple metal exposures cause higher toxicities.

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ANNEXURE A: Physicochemical water variables

Table A-1: Physicochemical (in situ) water quality variables measured for the different exposure groups (mean value used) only for the three sampling times (48 hours, four weeks and eight weeks).

Sample	Date	pH	Salinity (PSU)	Total dissolved solids (PPM)	Electrical Conductivity (uS.cm ⁻¹)	Temperature (C°)
Control	29/05/2019	8.47	245	359.33	515.66	14.36
0.1	29/05/2019	8.37	273	401	573	14.46
1	29/05/2019	8.40	297.66	435.66	623	14.26
10	29/05/2019	8.36	287.33	422	605	14.4
100	29/05/2019	8.30	291	430	614.66	14.46
500	29/05/2019	8.13	302.33	443.33	640.66	14.46
Control	26/06/2019	8.76	311.33	454.33	663.33	7.2
0.1	26/06/2019	8.79	338.66	495	711.66	6.83
1	26/06/2019	8.88	353.33	515.66	742.33	7.06
10	26/06/2019	8.74	350	507	727.33	7.1
100	26/06/2019	8.66	342.33	496.66	714.66	6.8
500	26/06/2019	8.61	266.33	468.66	699.66	7
Control	24/07/2019	8.84	407.66	587.66	843	5.6
0.1	24/07/2019	8.94	421	611	875	5.6
1	24/07/2019	8.91	430	623.66	892.66	5.7
10	24/07/2019	8.83	427.66	620	897.33	5.6
100	24/07/2019	8.74	467.66	671.33	962.66	5.9
500	24/07/2019	8.66	474	681.33	978.33	6

Figure A-2: Weekly water quality data showing the average pH readings for each exposure group over the experimental time.

Date	Water quality monitoring pH					
	500 µgPt/L	100 µgPt/L	10 µgPt/L	1 µgPt/L	0.1 µgPt/L	Control
27/05/2019	7.35	7.856667	7.93	8.266667	8.16	8.083333
29/05/2019	8.13	8.306667	8.36	8.403333	8.373333	8.473333
03/06/2019	8.453333	8.41	8.49	8.536667	8.486667	8.566667
10/06/2019	8.74	8.543333	8.656667	8.736667	8.673333	8.596667
24/06/2019	8.616667	8.663333	8.746667	8.88	8.79	8.766667
01/07/2019	8.593333	8.566667	8.5	8.886667	8.76	8.686667
08/07/2019	8.733333	8.556667	8.77	8.876667	8.91	8.75
15/07/2019	8.626667	8.616667	8.686667	8.846667	8.773333	8.723333
22/07/2019	9.023333	9.113333	8.966667	9.173333	9.183333	9.126667
24/07/2019	8.66	8.74	8.83	8.91	8.946667	8.84

Figure A-3: Weekly water quality data showing the average Salinity readings for each exposure group over the experimental time.

Date	Water quality monitoring Salinity (uS.cm ⁻¹)					
	500 µgPt/L	100 µgPt/L	10 µgPt/L	1 µgPt/L	0.1 µgPt/L	Control
27/05/2019	285.6667	275.3333	273.3333	278.6667	255	229.6667
29/05/2019	302.3333	291	287.3333	297.6667	273	245
03/06/2019	319.3333	306.3333	296.6667	309	282	251.6667
10/06/2019	341.3333	322	314.3333	324.3333	301.6667	265
24/06/2019	266.3333	342.3333	350	353.3333	338.6667	311.3333
01/07/2019	351.3333	378.3333	367.3333	367.3333	357.6667	335.3333
08/07/2019	419	410.3333	399.3333	392.6667	379.3333	367.6667

15/07/2019	420.3333	423.3333	404	399.6667	386.3333	378.6667
22/07/2019	453	457	432.6667	423.3333	418	402
24/07/2019	474	467.6667	427.6667	430	421	407.6667

Figure A-4: Weekly water quality data showing the average Total Dissolved Solids readings for each exposure group over the experimental time.

Date	Water quality monitoring Total Dissolved solids (ppm)					
	500 µgPt/L	100 µgPt/L	10 µgPt/L	1 µgPt/L	0.1 µgPt/L	Control
27/05/2019	421.6667	405.3333	403.6667	413	378	339.3333
29/05/2019	443.3333	430	422	435.6667	401	359.3333
03/06/2019	468.6667	447.6667	433.6667	453	415.3333	369.3333
10/06/2019	498.6667	469.3333	460	473	440	389.3333
24/06/2019	484.3333	496.6667	507	515.6667	495	454.3333
01/07/2019	519	552.6667	535.3333	539	522.3333	492
08/07/2019	608.3333	597	580	571	552	535.6667
15/07/2019	610.6667	612.6667	586	578	566	550
22/07/2019	657	657.6667	625.3333	610.6667	605.6667	583.6667
24/07/2019	681.3333	671.3333	620	623.6667	611	587.6667

Figure A-5: Weekly water quality data showing the average Electrical Conductivity readings for each exposure group over the experimental time.

Date	Water quality monitoring Electrical Conductivity (uS.cm ⁻¹)					
	500 µgPt/L	100 µgPt/L	10 µgPt/L	1 µgPt/L	0.1 µgPt/L	Control
27/05/2019	610	584.6667	579	598.3333	546.3333	487.3333
29/05/2019	640.6667	614.6667	605	623	573	515.6667
03/06/2019	674	641	623.3333	650.6667	596.6667	531.3333

10/06/2019	716.3333	672	659.6667	680.6667	632	558
24/06/2019	699.6667	714.6667	727.3333	742.3333	711.6667	663.3333
01/07/2019	753.6667	793	767.6667	772.6667	746	702
08/07/2019	872.3333	859.3333	831.6667	815.6667	787.3333	769.3333
15/07/2019	879	871.3333	839.3333	827.6667	812.3333	788.3333
22/07/2019	944	943.3333	895.3333	875.3333	864.6667	836
24/07/2019	978.3333	962.6667	897.3333	892.6667	875	843

Figure A-6: Weekly water quality data showing the average temperature readings for each exposure group over the experimental time.

Date	Water quality monitoring Temperature (°C)					
	500 µgPt/L	100 µgPt/L	10 µgPt/L	1 µgPt/L	0.1 µgPt/L	Control
27/05/2019	610	584.6667	579	598.3333	546.3333	487.3333
29/05/2019	640.6667	614.6667	605	623	573	515.6667
03/06/2019	674	641	623.3333	650.6667	596.6667	531.3333
10/06/2019	716.3333	672	659.6667	680.6667	632	558
24/06/2019	699.6667	714.6667	727.3333	742.3333	711.6667	663.3333
01/07/2019	753.6667	793	767.6667	772.6667	746	702
08/07/2019	872.3333	859.3333	831.6667	815.6667	787.3333	769.3333
15/07/2019	879	871.3333	839.3333	827.6667	812.3333	788.3333
22/07/2019	944	943.3333	895.3333	875.3333	864.6667	836
24/07/2019	978.3333	962.6667	897.3333	892.6667	875	843

Table A-7: Nutrient concentrations measured for each individual site over the experimental time

Sample	Date	Turbidity (FAU)	TH (mg/L)	NH4+ (mg/L)	NO3 (mg/L)	NO2 (mg/L)	Cl- (mg/L)	PO4 (mg/L)	COD (mg/L)
Control A	29/05/2019	12	108	0.12	1.7	0.02	19.4	0.3	22
Control B	29/05/2019	6	108	0.13	1.7	0.02	12	0.47	15
Control C	29/05/2019	5	99	0.06	2.3	0.02	8.7	0.22	7.2
0.1A	29/05/2019	8	130	0.14	1.9	0.02	9	0.4	23.2
0.1B	29/05/2019	5	127	0.18	1.7	0.02	11	0.81	20
0.1C	29/05/2019	7	158	0.05	2	0.02	9.2	0.21	12
1A	29/05/2019	7	150	0.08	2.6	0.02	13	0.34	37.8
1B	29/05/2019	9	128	0.07	1.8	0.02	8.7	0.19	6.4
1C	29/05/2019	8	167	0.1	1.9	0.02	11	0.11	6.6
10A	29/05/2019	5	142	0.18	1.8	0.03	11.6	1.11	22.1
10B	29/05/2019	6	135	0.07	1.9	0.02	9.5	0.13	7.2
10C	29/05/2019	6	135	0.07	1.7	0.02	9.4	0.17	9.8
100A	29/05/2019	7	149	0.07	1.6	0.02	14.1	1.43	23.5
100B	29/05/2019	6	149	0.07	1.8	0.02	12.1	0.31	5
100C	29/05/2019	6	129	0.2	2.3	0.02	12.5	0.2	5.6
500A	29/05/2019	4	168	0.08	1.7	0.02	19.6	0.42	23.9
500B	29/05/2019	4	144	0.09	1.8	0.02	19.4	0.25	6.8
500C	29/05/2019	4	159	0.07	1.9	0.02	19.6	0.19	10.2
Control A	26/06/2019	5	124	0.06	1.7	0.02	16.4	0.37	22.3

Control B	26/06/2019	5	116	0.04	2.2	0.02	17.5	2.29	10.6
Control C	26/06/2019	5	142	0.06	2.4	0.02	16.3	0.47	8.2
0.1A	26/06/2019	5	141	0.06	1.7	0.02	15.8	2.54	21.3
0.1B	26/06/2019	4	157	0.05	3.2	0.02	16.8	0.12	7
0.1C	26/06/2019	4	181	0.05	2.4	0.04	16.2	0.12	9.8
1A	26/06/2019	4	136	0.04	1.9	0.02	15.5	1.44	20.3
1B	26/06/2019	5	179	0.04	2.5	0.04	18.7	0.16	12.2
1C	26/06/2019	4	195	0.05	2.3	0.02	15.7	0.13	9.5
10A	26/06/2019	4	171	0.05	2	0.02	17.4	0.09	15
10B	26/06/2019	5	136	0.07	4.5	0.03	14.8	0.29	3.3
10C	26/06/2019	4	151	0.06	2.5	0.02	15	0.63	5.1
100A	26/06/2019	4	151	0.04	1.9	0.02	17.4	0.1	22.7
100B	26/06/2019	5	165	0.05	2.4	0.05	17.1	0.13	5
100C	26/06/2019	5	148	0.06	1.8	0.02	17.2	0.13	7.6
500A	26/06/2019	4	161	0.04	2.1	0.03	23.5	0.11	24.6
500B	26/06/2019	4	159	0.07	1.8	0.02	24.3	0.12	12.7
500C	26/06/2019	4	139	0.05	2.2	0.02	18.8	0.22	5
Control A	24/07/2019	7	109	0.07	5.7	0.03	15	0.27	18
Control B	24/07/2019	9	110	0.05	4.5	0.02	17	0.26	15
Control C	24/07/2019	6	102	0.05	4.1	0.02	111	0.23	7.2
0.1A	24/07/2019	8	132	0.04	4.7	0.02	30	0.57	13.2
0.1B	24/07/2019	9	129	0.14	4.5	0.02	31	0.48	15.50

0.1C	24/07/2019	7	150	0.07	4.9	0.02	27	2.84	12
1A	24/07/2019	5	155	0.06	4.9	0.02	103	0.29	29.3
1B	24/07/2019	5	132	0.03	4.1	0.02	32	0.32	6.4
1C	24/07/2019	8	167	0.05	7.3	0.01	39	0.37	6.6
10A	24/07/2019	7	146	0.07	5.6	0.03	42	0.77	19.2
10B	24/07/2019	4	138	0.04	5.3	0.03	28	0.41	7.2
10C	24/07/2019	6	136	0.04	6	0.02	25	0.43	9.8
100A	24/07/2019	11	153	0.06	4.4	0.02	29	0.36	21.5
100B	24/07/2019	9	150	0.07	6.8	0.03	23	0.4	5
100C	24/07/2019	7	135	0.06	8.3	0.03	21	0.32	5.6
500A	24/07/2019	6	170	0.04	4.3	0.03	28	0.28	22.3
500B	24/07/2019	6	147	0.05	4.8	0.02	24	0.26	6.8
500C	24/07/2019	8	159	0.06	5.9	0.02	18	0.33	10.2

ANNEXURE B: Diatom taxa and counts for each of the sites for initial and chronic sample times

Table B-1: List of diatom taxa representing the diatom community in the microcosms.

Species	Code	Species	Code
<i>Acnantes</i> sp J.B.M. Bory de St. Vincent	ACHN	<i>Gomphonema laticollum</i> Reichardt	GLTC
<i>Amphora copulata</i> (Kütz) Schoeman & Archibald	ACOP	<i>Gomphonema</i> sp C.G. Ehrenberg	GOMP
<i>Achnanthidium exiguum</i> (Grunow) Czarnecki	ADEG	<i>Gomphonema parvulum</i> (Kützing) Kützing	GPAP
<i>Achnanthidium eutrophilum</i> (Lange-Bertalot) Lange-Bertalot	ADEU	<i>Gomphonema pumilum</i> (Grunow) Reichardt & Lange-Bertalot	GPUM
<i>Achnanthidium minutissimum</i> (Kützing) Czarnecki	ADMI	<i>Gomphonema truncatum</i> Ehr.	GTRU
<i>Achnanthidium saprophilum</i> (Kobayasi et Mayama) Round & Bukhtiyarova	ADSA	<i>Gyrosigma attenuatum</i> (Kützing) Rabenhorst	GYAT
<i>Amphora</i> sp	AMPS	<i>Hantzschia amphioxys</i> (Ehr.) Grunow fo. <i>capitata</i> O. Muller	HAFC
<i>Amphora ovalis</i> (Kützing) Kützing	AOVA	<i>Hippodonta capitata</i> (Ehr.) Lange-Bert. Metzeltin & Witkowski	HCAP
<i>Anomoeoneis sphaerophora</i> (Ehr.) Pfister	ASPH	<i>Navicula</i> sp.	NASP
<i>Craticula acidoclinata</i> Lange-Bertalot & Metzeltin	CACD	<i>Navicula clementis</i> Grun. var. <i>linearis</i> Brander ex Hustedt in Schmidt & al.	NCTL
<i>Caloneis aequatorialis</i> Hust.v. <i>transitoria</i> Manguin ex Kociolek & Reiers	CAET	<i>Navicula erifuga</i> Lange-Bertalot	NERI
<i>Craticula ambigua</i> (Ehrenberg) Mann	CAMB	<i>Nitzschia filiformis</i> (W.M. Smith) Van Heurck	NFIT
<i>Cymbella aspera</i> (Ehrenberg) H. Peragallo	CASP	<i>Nitzschia pura</i> Hustedt	NIPR
<i>Cymboplectra amphicephala</i> Krammer	CBAM	<i>Nitzschia</i> sp A.H. Hassall	NITZ
<i>Cymboplectra naviculiformis</i> (Auerswald) Krammer var. <i>laticapitata</i> Krammer	CBNL	<i>Nitzschia linearis</i> (Agardh) W.M. Smith var. <i>impressa</i> (Hustedt) Lange-Bertalot	NLIP
<i>Cymbella cymbiformis</i> Agardh	CCYM	<i>Navicula placentula</i> (Ehr.) Kützing	NPLA
<i>Cymbella leptoceros</i> var <i>excisa</i> (Petit) Cleve	CLEX	<i>Navicula recens</i> (Lange-Bertalot) Lange-Bertalot	NRCS
<i>Cymbella neocistula</i> Krammer var. <i>islandica</i> Krammer	CNIS	<i>Nitzschia recta</i> Hantzsch in Rabenhorst	NREC
<i>Cymbella kappii</i> (Cholnoky) Cholnoky	CKPP	<i>Navicula rostellata</i> Kützing	NROS
<i>Cocconeis</i> sp C.G. Ehrenberg	COCO	<i>Navicula tripunctata</i> (O.F. Müller) Bory	NTPT

<i>Cocconeis pediculus</i> Ehrenberg	CPED	<i>Navicula tripunctata</i> Bory forme teratogene	NTTT
<i>Cocconeis placentula</i> Ehrenberg	CPTG	<i>Navicula veneta</i> Kützing	NVEN
<i>Craticula buder</i> (Hustedt) Lange-Bertalot	CRBU	<i>Pinnularia borealis</i> Ehrenberg f. <i>rectangularis</i> Carlson	PBRT
<i>Craticula cuspidata</i> (Kützing) Mann	CRCU	<i>Planothidium frequentissimum</i> var. <i>magnum</i> (Straub) Lange-Bertalot	PFMA
<i>Cymatopleura solea</i> (Brebisson) W. Smith var. <i>apiculata</i> (W. Smith) Ralfs	CSAP	<i>Pinnularia</i> sp.	PIN1
<i>Cymbella turgidula</i> Grunow 1875 in A. Schmidt & al. var. <i>turgidula</i>	CTGL	<i>Planothidium engelbrechtii</i> (Choln.) Round & Bukhtiyarova	PLEN
<i>Cymbella tumida</i> (Brebisson) Van Heurck	CTUM	<i>Placoneis placentula</i> f. <i>lanceolata</i> (Grun. in Cl. & Grun.) Bukhtiyarova	PLPL
<i>Diatoma vulgare</i> Bory	DVUL	<i>Planothidium rostratum</i> (Oestrup) Lange-Bertalot	PRST
<i>Epithemia adnata</i> (Kützing) Brebisson	EADN	<i>Pseudostaurosira brevistriata</i> (Grun. in Van Heurck) Williams & Round	PSBR
<i>Eunotia formica</i> Ehrenberg	EFOR	<i>Pinnularia subcapitata</i> Gregory var. <i>elongata</i> Krammer	PSEL
<i>Encyonema silesiacum</i> (Bleisch in Rabh.) D.G. Mann	ESLE	<i>Rhoicosphenia abbreviata</i> (C. Agardh) Lange-Bertalot	RABB
<i>Epithemia sores</i> Kützing	ESOR	<i>Rhopalodia gibba</i> (Ehr.) O. Muller var. <i>gibba</i>	RGIB
<i>Fragilaria biceps</i> (Kützing) Lange-Bertalot	FBCP	<i>Staurosira elliptica</i> (Schumann) Williams & Round	SELI
<i>Fragilaria capucina</i> Desm. ssp. <i>rumpens</i> (Kütz.) Lange-Bert. ex Bukht.	FCRP	<i>Staurosirella pinnata</i> (Ehr.) Williams & Round	SPIN
<i>Fragilaria nanana</i> Lange-Bertalot	FNAN	<i>Sellaphora pupula</i> (Kützing) Mereschkovsky	SPUP
<i>Fragilaria ungeriana</i> Grunow	FUNG	<i>Stauroneis smithii</i> Grunow	SSMI
<i>Gomphonema affine</i> Kützing	GAFF	<i>Tryblionella apiculata</i> Gregory	TAPI
<i>Gomphonema acuminatum</i> (Kütz.) Rabh. var. <i>turris</i> (Ehr.) Wolle	GATU	<i>Tabularia fasciculata</i> (Agardh) Williams et Round	TFAS
<i>Gomphonema insigne</i> Gregory	GINS	<i>Tryblionella</i> sp. W. Smith	TRYB
<i>Gomphonema italicum</i> Kützing	GITA		

Table B-2: Diatom community results asper OMNIDIA for the epiphytic and benthic diatoms after 48 hours and eight

	T1 Con M	T1 K1 M	T1 K2 M	T1 K3 M	T1 K4 M	T1 K5 M	T1 Con R	T1 K1 R	T1 K2 R	T1 K3 R	T1 K4 R	T1 K5 R	T1 Con S	T1 K1 S	T1 K2 S	T1 K3 S	T1 K4 S	T1 K5 S	T3 Con M	T3 K1 M	T3 K2 M	T3 K3 M	T3 K4 M	T3 K5 M	T3 Con R	T3 K1 R
ACHN	162	246	182	132	270	132	119	151	101	190	129	117	402	232	262	260	204	213	245	196	201	188	170	219	248	225
AMPS	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	43	14	0	0	0	0	0	0	0	0	0
ACOP	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ADEU	157	186	170	172	231	60	0	0	0	0	0	0	408	271	384	317	380	161	168	220	269	199	292	212	0	0
ADEG	0	0	0	0	0	0	0	0	0	0	0	0	0	21	0	7	0	0	0	0	0	0	0	0	0	0
ADMI	0	37	0	0	0	52	134	114	47	86	16	104	0	0	56	23	0	0	0	0	0	0	0	0	0	0
ADEU	0	0	0	0	0	0	56	46	163	88	131	24	0	0	0	0	0	0	0	0	0	0	0	0	248	327
AOVA	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ASPH	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CACD	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CAET	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	31	0	28	3	0	5	0	0	1	0
CKPP	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	38	0	0	121	16	36	0	0
CAMB	0	0	0	0	0	0	0	9	0	4	0	3	0	0	0	0	0	0	0	0	0	0	0	0	2	1
CCYM	0	0	0	0	0	0	0	16	0	0	0	0	8	0	0	0	0	0	0	0	0	0	0	0	0	0
CBAM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	23	5	32	0	0	0	0	0	0	0	0
COCO	0	10	0	0	0	0	0	0	0	0	53	33	0	0	0	0	2	0	0	0	0	0	0	0	0	0
CBNL	0	0	0	0	0	0	0	0	0	0	0	0	0	6	0	0	0	0	0	0	0	0	0	0	0	0
CPTG	328	307	269	371	259	367	433	390	270	312	343	272	89	182	83	129	77	124	361	388	363	313	241	278	270	317
CTGL	0	0	0	0	0	0	0	0	0	0	21	25	0	0	0	0	0	0	0	7	0	0	0	0	0	0
CLEX	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CPED	0	0	0	0	0	0	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CRBU	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	18	0	0	0	0	0	0	0	0	0	0
CKPP	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CRCU	0	0	0	0	0	0	0	0	8	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CTUM	6	13	38	14	0	7	8	0	8	0	0	12	0	5	0	11	27	7	6	13	26	17	16	0	45	25
CSAP	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0
DVUL	9	4	2	7	0	3	7	0	6	35	0	15	8	43	21	0	0	0	4	2	2	0	2	8	0	1
EADN	0	0	3	0	0	0	0	8	35	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ESOR	0	0	0	0	0	0	0	0	96	0	0	0	0	0	14	0	0	0	5	0	12	1	0	3	5	18
EFOR	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	30	0	0	0	0	0	0	0	0	0
ESLE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	19	0	0	0	0	0	0	0	0	0	0	0
FBCP	42	33	59	74	59	71	34	69	67	21	7	61	0	49	42	60	53	29	11	19	19	14	37	17	8	21
FCRP	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0	36	28	0	0	0	0	0	0	0	0
FNAN	0	0	6	0	0	2	29	0	14	0	0	9	52	0	0	0	23	0	0	0	0	75	0	0	0	0
FUNG	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8	25	25	0	0	0	0	0	0	0	0	0
GAFF	30	0	20	4	0	32	0	3	10	0	7	4	0	0	6	0	18	0	0	22	28	19	52	33	41	31
GATU	8	3	12	13	6	1	8	16	0	0	0	0	46	0	0	4	0	0	9	7	0	1	8	9	3	0
GINs	32	7	28	15	0	111	39	71	5	42	41	15	20	55	32	28	77	46	44	33	26	8	47	41	55	50
GITA	18	5	0	0	6	0	3	39	12	0	0	0	64	0	0	0	0	25	0	0	0	2	3	4	0	3
GLTC	49	2	64	5	23	28	33	0	35	0	14	9	18	36	18	3	0	15	12	6	5	1	12	10	38	9
GPAR	0	14	0	9	22	25	0	0	7	0	0	0	0	0	0	0	0	0	5	29	9	3	7	2	18	1

GTRU	0	20	0	0	0	23	0	0	0	26	36	19	0	0	0	0	0	0	4	8	2	0	0	0	0	6
GYAT	37	14	41	25	19	15	14	23	8	48	0	36	51	15	51	36	12	10	10	1	2	3	4	0	0	4
GOMP	0	0	0	0	0	0	0	0	0	0	0	0	7	0	0	0	0	0	0	0	0	0	0	0	0	0
GPUM	0	0	0	0	0	0	0	0	72	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HAFC	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
HCAP	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0
NASP	0	0	0	0	0	24	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
NCTL	0	0	0	0	0	0	0	0	0	24	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
NERI	0	0	0	0	0	0	38	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
NIPR	0	0	0	0	0	0	1	19	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
NPLA	0	0	0	0	0	0	0	0	18	56	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
NRCS	0	0	0	0	0	0	0	0	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
NFIT	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
NTPT	0	0	0	0	0	0	0	0	0	0	0	0	0	264	58	157	114	51	0	0	0	0	0	0	0	0
NITZ		0	0	16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
NLIP	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0
NVEN	0	0	0	0	0	0	0	0	0	0	0	0	20	0	0	0	49	0	0	0	0	0	0	0	0	0
NREC	0	0	0	0	17	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	16	0	0	0	0	0
NTTT	0	0	0	0	0	0	0	0	0	0	0	0	0	0	63	0	0	0	0	0	0	0	0	0	0	0
NTPT	172	159	206	245	164	119	97	167	132	99	223	251	0	0	0	0	0	0	209	194	143	107	196	217	141	110
NVEN	18	44	0	0	0	0	89	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PIN1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	35	0	0	0	0	0	0	0	0	0	0	0
PBRT	0	0	0	0	0	0	0	0	0	0	0	0	0	7	0	0	0	0	0	0	0	0	0	0	0	0
PLEN	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0	0
PFMA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	16	0	0	0	0	0
PLPL	11	0	0	0	0	0	0	0	0	0	39	4	0	0	0	0	0	0	0	1	3	1	2	0	0	0
PSEL	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PRST	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PSBR	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RABB	110	65	78	97	84	61	38	0	27	26	47	72	0	11	11	0	7	6	39	36	37	42	63	42	47	32
RGBB	8	14	22	1	32	7	0	0	42	108	21	59	0	0	24	0	6	0	0	10	10	52	32	67	17	19
SPUP	0	0	0	0	0	35	0	3	0	0	4	21	0	0	0	0	0	0	0	0	0	0	0	2	0	0
SSMI	0	8	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
TAPI	0	4	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
SELI	0	0	0	0	0	0	0	0	0	0	0	25	2	0	7	0	0	51	0	0	0	0	0	0	0	0
TRYB	0	0	0	0	0	0	0	0	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SPIN	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SPUP	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	53	0	0	0	0	0	0	0	0	0	0
TFAS	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0

Table B-3: One-way ANOVA results for the metal analyses in the different environmental compartments. With the significant differences indicating a difference in Platinum concentration over time

Sample	Exposure concentration	One way ANOVA	
		P value	F value
Water	Control	0.584	0.588
	0.1	<0.001	125.8
	1	0.175	2.357
	10	<0.001	212.7
	100	<0.001	202.2
	500	0.997	0.003
Sediment	Control	0.177	2.342
	0.1	0.100	3.448
	1	0.670	0.427
	10	0.234	1.862
	100	0.004	15.13
	500	0.437	0.952
Macrophyte <i>Charophyta sp</i>	Control	0.758	0.289
	0.1	0.160	2.524
	1	0.016	8.901
	10	0.989	0.010
	100	0.092	3.628
	500	0.015	9.103
Macrophyte <i>antipyretica</i>	Control	0.962	0.038
	0.1	0.847	0.170
	1	0.500	0.778
	10	0.377	1.150

	100	0.070	0.070
	500	0.327	1.351
Artificial Mussels	Control	0.614	0.529
	0.1	0.936	0.066
	1	0.871	0.140
	10	0.993	0.006
	100	0.737	0.328
	500	0.421	1

Table B-4: One -way ANOVA results for the diatom community indexes

Figure	<i>P and F value</i>	A	B	C	D	E	F
Pielou's Evenness Index (J')	P	0.799	0.6137	0.6288	0.9737	0.6506	0.9773
	F	0.4594	0.7312	0.7079	0.1569	0.6748	0.1467
Shannon Index H'(loge)	P	0.9093	0.9572	0.0706	0.7626	0.5063	0.9408
	F	0.2903	0.198	2.744	0.5117	0.9099	0.2327
Simpson diversity index	P	0.8138	0.7734	0.1503	0.9162	0.5714	0.9592
	F	0.438	0.4962	2.004	0.2783	0.7985	0.1936
Total species(S)	P	0.6017	0.8813	0.0728	0.2213	0.2957	0.9043
	F	0.75	0.3362	2.712	1.649	1.389	0.2987
Species richness(d)	P	0.6013	0.8812	0.0772	0.2213	0.3524	0.9141
	F	0.7506	0.3365	2.653	1.649	1.234	0.282
BDI	P	0.8179	0.6658	0.6448	0.967	0.6487	0.9333
	F	0.432	0.652	0.6836	0.1747	0.6777	0.2472
GDI	P	0.6873	0.8764	0.9439	0.2213	0.5063	0.981
	F	0.6204	0.358	0.2265	1.649	0.9099	0.1349
TDI	P	0.982	0.9896	0.4088	0.7871	0.5576	0.9709
	F	0.1317	0.10217	1.102	0.4766	0.8213	0.1646
%PT	P	0.4678	0.2481	0.6854	0.6513	0.4582	0.9022
	F	0.9814	1.546	0.6232	0.6738	1	0.3024
IPS	P	0.5535	0.663	0.6545	0.6345	0.6452	0.992
	F	0.8282	0.6563	0.669	0.6692	0.6829	0.0917

Table B-5: The 25 Diatom taxa used for the PRC calculation

Species	Code	Species	Code
<i>Acnantes</i> sp J.B.M. Bory de St. Vincent	ACHN	<i>Gomphonema</i> sp C.G. Ehrenberg	GOMP
<i>Amphora copulata</i> (Kütz) Schoeman & Archibald	ACOP	<i>Gyrosigma attenuatum</i> (Kützing) Rabenhorst	GYAT
<i>Achnanthidium eutrophilum</i> (Lange-Bertalot) Lange-Bertalot	ADEU	<i>Hantzschia amphioxys</i> (Ehr.) Grunow fo. <i>capitata</i> O. Muller	HAFC
<i>Achnanthidium saprophilum</i> (Kobayasi et Mayama) Round & Bukhtiyarova	ADSA	<i>Navicula clementis</i> Grun.var. <i>linearis</i> Brander ex Hustedt in Schmidt & al.	NCTL
<i>Anomoeoneis sphaerophora</i> (Ehr.) Pfitzer	ASPH	<i>Navicula erifuga</i> Lange-Bertalot	NERI
Bertalot & Metzeltin	CACD	<i>Nitzschia filiformis</i> (W.M. Smith) Van Heurck	NFIT
<i>Craticula ambigua</i> (Ehrenberg) Mann	CAMB	<i>Nitzschia</i> sp A.H. Hassall	NITZ
<i>Cymbella aspera</i> (Ehrenberg) H. Peragallo	CASP	<i>Planothidium engelbrechtii</i> (Choln.) Round & Bukhtiyarova	PLEN
<i>Cymbella neocistula</i> Krammer var. <i>islandica</i> Krammer	CNIS	<i>Pinnularia subcapitata</i> Gregory var. <i>elongata</i> Krammer	PSEL
<i>Cocconeis pediculus</i> Ehrenberg	CPED	<i>Staurosira elliptica</i> (Schumann) Williams & Round	SELI
<i>Craticula buderii</i> (Hustedt) Lange-Bertalot	CRBU	<i>Sellaphora pupula</i> (Kützing) Mereschkowksy	SPUP
<i>Gomphonema laticollum</i> Reichardt	GLTC	<i>Tryblionella apiculata</i> Gregory	TAPI
<i>Gomphonema truncatum</i> . Ehrenberg.	GTRU		